Difco Manual

11th Edition

Important Notice

- Product labels/package inserts take precedence over the formula or instructions listed in the manual.
- Generic names may be substituted for trade names in the ingredient list, providing more information regarding animal origin, i.e. “pancreatic digest of casein” instead of Casitone.
- During 1999, catalog numbers will be changed to meet new UCC/EAN128 labeling requirements. Please visit us at **www.bd.com/microbiology** for more information.

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Foreword

This edition of the DIFCO MANUAL, the eleventh published since 1927, has been extensively revised and rewritten. The purpose of the Manual is to provide information about products used in microbiology. The Manual has never been intended to replace any official compendium or the many excellent standard text books of scientific organizations or individual authors.

Difco is perhaps best known as the pioneer in bacteriological culture media. Numerous times one will find the trademarks Difco® or Bacto® preceding the names of materials used by scientists in their published papers. Because Difco products have been readily available worldwide longer than any others, Difco products have become the common-language reagents of the microbiological community. Standardized products readily available worldwide are essential for corroborative studies demanded by rigorous science.

Recommendation and approval have been extended to our products by the authors of many standard text books and by the committees on methods and procedures of scientific societies throughout the world. Difco products continue to be prepared according to applicable standards or accepted formulae. It is expected that they will be used only by or under the supervision of microbiologists or other professionals qualified by training and experience to handle pathogenic microorganisms. Further, it is expected that the user will be throughly familiar with the intended uses of the formulations and will follow the test procedures outlined in the applicable official compendia and standard text books or procedures manual of the using laboratory.

Grateful acknowledgment is made of the support we have received from microbiologists throughout the world. It is our desire to continue and extend our services to the advancement of microbiology and related sciences.

Difco Laboratories
Division of Becton Dickinson and Company

Introduction

Microbiology, through the study of bacteria, emerged as a defined branch of modern science as the result of the monumental and immortal research of Pasteur and Koch. In 1876, Robert Koch, for the first time in history, propagated a pathogenic bacterium in pure culture outside the host's body. He not only established *Bacillus anthracis* as the etiological agent for anthrax in cattle, but he inaugurated a method of investigating disease which ushered in the golden age of medical bacteriology.

Early mycologists, A. de Bary and O. Brefeld, and bacteriologist, R. Koch and J. Schroeter, pioneered investigations of pure culture techniques for the colonial isolation of fungi and bacteria on solid media. Koch, utilizing state-of-the-art clear liquid media which he solidified with gelatin, developed both streak and pour plate methods for isolating bacteria. Gelatin was soon replaced with agar, a solidifying agent from red algae. It was far superior to gelatin in that it was resistant to microbial digestion and liquefaction.

The capability of Koch to isolate disease-producing bacteria on solidified culture media was further advanced by manipulating the cultural environment using meat extracts and infusions so as to reproduce, as closely as possible, the infected host's tissue. The decade immediately following Koch's epoch-making introduction of solid culture media for the isolation and growth of bacteria ranks as one of the brightest in the history of medicine because of the number, variety, and brilliance of the discoveries made in that period. These discoveries, which, as Koch himself expressed it, came "as easily as ripe apples fall from a tree," were all dependent upon and resulted from the evolution of correct methods for the *in vitro* cultivation of bacteria.

The fundamental principles of pure culture isolation and propagation still constitute the foundation of microbiological practice and research. Nevertheless, it has become more and more apparent that a successful attack upon problems unsolved is closely related to, if not dependent upon, a thorough understanding of the subtle factors influencing bacterial metabolism. With a suitable culture medium, properly used, advances in microbiology are more readily made than when either the medium or method of use is inadequate. The microbiologist of today is, therefore, largely concerned with the evolution of methods for the development and maintenance of microbial growth upon which an understanding of their unique and diversified biological and biochemical characteristics can be investigated. To this end, microbiologists have developed innumerable enrichment culture techniques for the isolation and cloning of microorganisms with specific nutritional requirements. These organisms and their unique characteristics have been essential to progress in basic biological research and modern applied microbiology.

The study of microorganisms is not easy using microscopic single cells. It is general practice to study pure cultures of a single cell type. In the laboratory, microbiological culture media are utilized which contain various nutrients that favor the growth of particular microorganisms in pure cultures. These media may be of simple and defined chemical composition or may contain complex ingredients such as digests of plant and animal tissue. In particular, the cultivation of bacteria is dependent upon nutritional requirements which are known to vary widely. Autotrophic bacteria are cultivated on chemically defined or synthetic media while heterotrophic bacteria, for optimal growth, may require more complex nutrients such as peptones, meat or yeast extracts. These complex mixtures of nutrients readily supply fastidious heterotrophic bacteria with vitamins and other growth-promoting substances necessary for desired cultivation. The scientific literature abounds with descriptions of enriched, selective and differential culture media necessary for the proper isolation, recognition and enumeration of various bacterial types.

Almost without exception whenever bacteria occur in nature, and this is particularly true of the pathogenic forms, nitrogenous compounds and carbohydrates are present. These are utilized in the maintenance of growth and for the furtherance of bacterial activities. So complex is the structure of many of these substances, however, that before they can be utilized by bacteria they must be dissimilated into simpler compounds then assimilated into cellular material. Such metabolic alterations are affected by enzymatic processes of hydrolysis, oxidation, reduction, deamination, etc., and are the result of bacterial activities of primary and essential importance. These changes are ascribed to the activity of bacterial enzymes which are both numerous and varied. The processes involved, as well as their end-products, are exceedingly complex; those of fermentation, for example, result in the production of such end-products as acids, alcohols, ketones, and gases including hydrogen, carbon dioxide, methane, etc. The study of bacterial metabolism, which defines the organized chemical activities of a cell, has led to the understanding of both catabolic or degradative activities and anabolic or synthetic activities. From these studies has come a better understanding of the nutritional requirements of bacteria, and in turn, the development of culture media capable of producing rapid and luxuriant growth, both essential requisites for the isolation and study of specific organisms.

Studies to determine the forms of carbon, hydrogen, and nitrogen which could most easily be utilized by bacteria for their development were originally carried on by Naegeli[1] between 1868 and 1880, and were published by him in the latter year. Naegeli's report covered the use of a large variety of substances including carbohydrates, alcohols, amino acids, organic nitrogen compounds, and inorganic nitrogen salts.

The first reference to the use of peptone for the cultivation of microorganisms is that made by Naegeli in the report referred to above, when in 1879, he compared peptone and ammonium tartrate. Because of its content amino acids and other nitrogenous compounds which are readily utilized by bacteria, peptone soon became one of the most important constituents of culture media, as it still remains. In the light of our present knowledge, proteins are known to be complex compounds composed of amino acids joined together by means of the covalent peptide bond linkage. When subjected to hydrolysis, proteins yield polypeptides of various molecular sizes, metapeptones, proteoses, peptones and peptides, down to the level of simple amino acids. The intermediate products should be considered as classes of compounds, rather than individual substances, for there exists no sharp lines of demarcation between the various classes. One group shades by imperceptible degrees into the next. All bacteriological peptones, thus, are mixtures of various products of protein hydrolysis. Not all the

products of protein decomposition are equally utilizable by all bacteria. In their relation to proteins, bacteria may be divided into two classes; those which decompose naturally occurring proteins, and those which require simpler nitrogenous compounds such as peptones and amino acids.

The relation of amino acids to bacterial metabolism, and the ability of bacteria to use these compounds, have been studied by many workers. Duval,[2,3] for example, reports that cysteine and leucine are essential in the cultivation of *Mycobacterium leprae*. Kendall, Walker and Day[4] and Long[5] reported that the growth of *M. tuberculosis* is dependent upon the presence of amino acids. Many other workers have studied the relation of amino acids to the growth of other organisms, as for example, Hall, Campbell, and Hiles[6] to the meningococcus and *Streptococcus*; Cole and Lloyd[7] and Cole and Onslow[8] to the gonococcus; and Jacoby and Frankenthal[9] to the influenza bacillus. More recently Feeley, et al.[34] demonstrated that the nonsporeforming aerobe, *Legionella pneumophila* requires L-cysteine · HCI for growth on laboratory media. Indispensable as amino acids are to the growth of many organisms, certain of them in sufficient concentration may exert an inhibitory effect upon bacterial development.

From the data thus far summarized, it is apparent that the problem of bacterial metabolism is indeed complicated, and that the phase concerned with bacterial growth and nutrition is of the utmost practical importance. It is not improbable that bacteriological discoveries such as those with *Legionella pneumophila* await merely the evolution of suitable culture media and methods of utilizing them, just as in the past important discoveries were long delayed because of a lack of similar requirements. Bacteriologists are therefore continuing to expend much energy on the elucidation of the variations in bacterial metabolism, and are continuing to seek methods of applying, in a practical way, the results of their studies.

While the importance of nitrogenous substances for bacterial growth was recognized early in the development of bacteriological technique, it was also realized, as has been indicated, that bacteria could not always obtain their nitrogen requirements directly from protein. It is highly desirable, in fact essential, to supply nitrogen in readily assimilable form, or in other words to incorporate in media proteins which have already been partially broken down into their simpler and more readily utilizable components. Many laboratory methods, such as hydrolysis with alkali,[10] acid,[11,12,13] enzymatic digestion,[8,14,15,16,17,18] and partial digestion of plasma[10] have been described for the preparation of protein hydrolysates.

The use of protein hydrolysates, particularly gelatln and casein, has led to especially important studies related to bacterial toxins by Mueller, et al.[20-25] on the production of diphtheria toxin; that of Tamura, et al.[25] of toxin of *Clostridium welchii*; that of Bunney and Loerber[27,28] on scarlet fever toxin, and of Favorite and Hammon[29] on *Staphylococcus* enterotoxin. In addition, the work of Snell and Wright[30] on the microbiological assay of vitamins and amino acids was shown to be dependent upon the type of protein hydrolysate utilized. Closely associated with research on this nature are such studies as those of Mueller[31,32] on pimelic acid as a growth factor for *Corynebacterium diphtheriae*, and those of O'Kane[33] on synthesis of riboflavin by staphylococci. More recently, the standardization of antibiotic susceptibility testing has been shown to be influenced by peptones of culture media. Bushby and Hitchings[35] have shown that the antimicrobial activities of trimethoprim and sulfamethoxazole are influenced considerably by the thymine and thymidine found in peptones of culture media.

In this brief discussion of certain phases of bacterial nutrition, we have attempted to indicate the complexity of the subject and to emphasize the importance of continued study of bacterial nutrition. Difco Laboratories has been engaged in research closely allied to this problem in its broader aspects since 1914 when Bacto Peptone was first introduced. Difco dehydrated culture media, and ingredients of such media, have won universal acceptance as useful and dependable laboratory adjuncts in all fields of microbiology.

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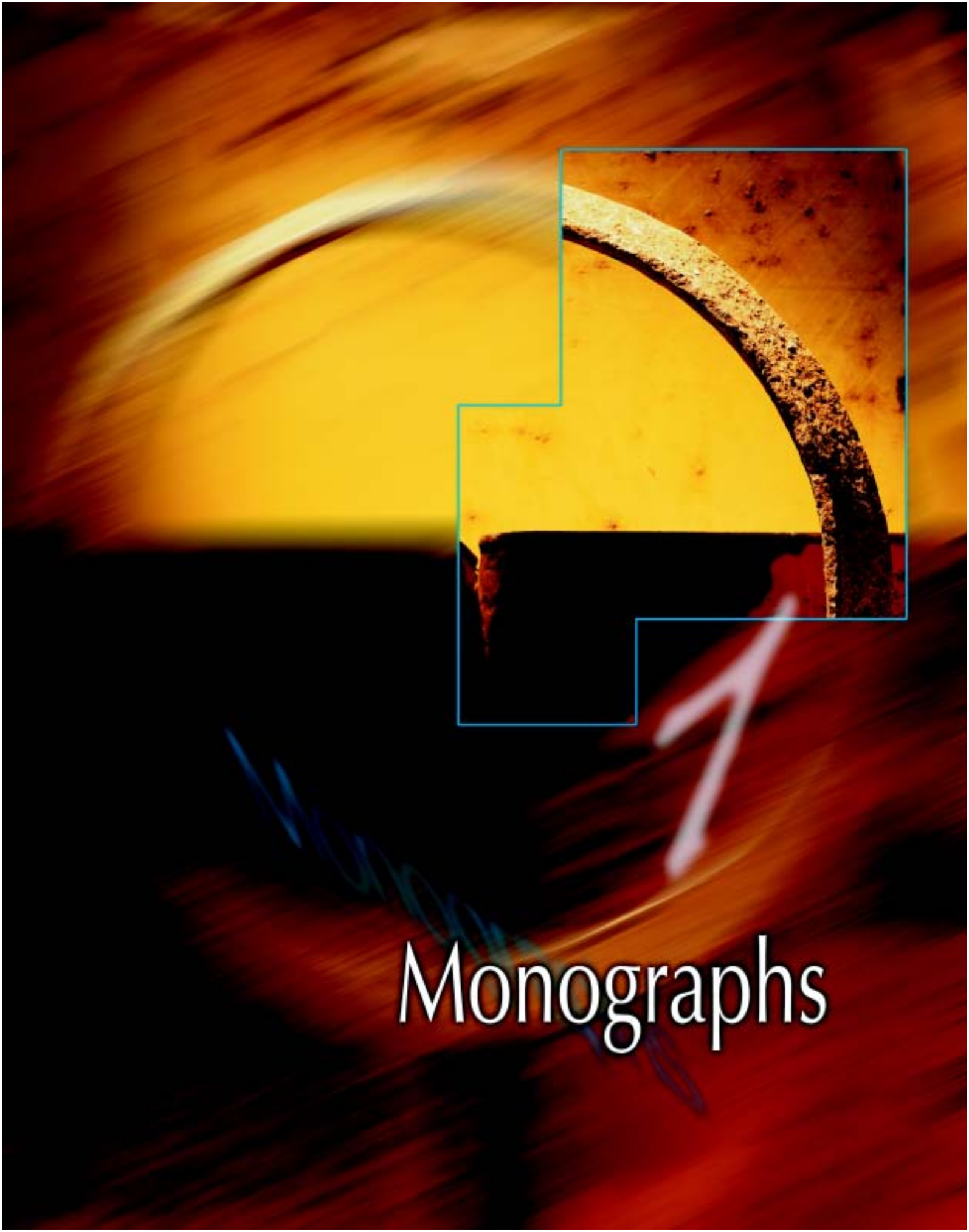
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Monographs

History of Difco Laboratories

Original Difco Laboratories Manufacturing facility.

Difco Laboratories, originally known as Ray Chemical, was founded in 1895. This company produced high quality enzymes, dehydrated tissues and glandular products to aid in the digestion process. Ray Chemical acquired Digestive Ferments Company, a company that specialized in producing digestive enzymes for use as bacterial culture media ingredients. The experience of processing animal tissues, purifying enzymes and performing dehydration procedures created a smooth transition to the preparation of dehydrated culture media. In 1913, the Digestive Ferments Company moved to Detroit, Michigan, and dropped the name, Ray Chemical.

After 1895, meat and other protein digests were developed to stimulate growth of bacteria and fungi. The extensive research performed on the analysis of pepsin, pancreatin and trypsin (and their digestive processes) led to the development of Bacto® Peptone. Bacto Peptone, first introduced in 1914, was used in the bacteriological examination of water and milk as a readily available nitrogen source. Bacto Peptone has long been recognized as the standard peptone for the preparation of bacteriological culture media.

The development of Proteose Peptone, Proteose Peptone No. 2 and Proteose Peptone No. 3 was the result of accumulated information that no single peptone is the most suitable nitrogen source for growing fastidious bacteria. Proteose Peptone was developed for use in the preparation of diphtheria toxin of high and uniform potency. Bacto Tryptose was originally formulated to provide the growth requirements of Brucella. Bacto Tryptose was also the first peptone prepared that did not require the addition of infusions or other enrichments for the isolation and cultivation of fastidious bacteria.

The Digestive Ferments Company began the preparation of diagnostic reagents in 1923. Throughout the development of products used in the diagnosis of syphilis and other diseases, Difco worked closely with and relied on the direct involvement of expert scientists in the field. Bacto Thromboplastin, the first manufactured reagent used in coagulation studies, was developed in the early 1930s. This product was another in a long line of many "firsts" for Difco Laboratories.

In 1934, the Digestive Ferments Company chose an acronym, "Difco," to rename the company. The focus of Difco Laboratories was to develop new and improved culture media formulations.

After World War II, the microbiology and health care fields expanded rapidly. Difco focused on the development of microbiological and immunological products to meet this growing demand. In the 1940s, Difco pursued the challenging task of producing bacterial antisera and antigens. Lee Laboratories, a subsidiary, remains one of the largest manufacturers of bacterial antisera. Additional "firsts" for Difco Laboratories came in the 1950s with the development of C Reactive Protein Antiserum, Treponemal Antigen and Antistreptolysin Reagents.

Throughout the 1950s and 1960s, Difco continued to add products for clinical applications. Bacto Blood Cultures Bottles were developed to aid in the diagnosis and treatment of sepsis. Difco Laboratories pioneered in the preparation of reagents for in vitro propagation and maintenance of tissue cells and viruses.

With the discovery of penicillin, a brand new branch of microbiology was born. Difco initiated developmental research by preparing antibiotic disks for use in a "theorized" disk diffusion procedure. The result was Bacto Sensitivity Disks in 1946, followed by Dispens-O-Discs™ in 1965.

In the 1960s, Difco Laboratories became the largest manufacturer of microbiological culture media by acquiring the ability to produce agar. Difco offers the same premier "gold standard," Bacto Agar, today.

Bactrol™ Disks were introduced by Difco Laboratories in 1972. Bactrol Disks are water-soluble disks containing viable microorganisms of known cultural, biochemical and serological characteristics used for quality control testing. Bactrol Disks became the first of many products manufactured by Difco for use in quality control.

In 1983, Difco purchased the Paul A. Smith Company, later to be known as Pasco®. A semi-automated instrument, the Pasco MIC/ID System, is used for bacterial identification and sensitivity testing. The Pasco Data Management System can be used in industrial and clinical laboratories, either alone or as a back up to automated systems.

In 1992, ESP®, an automated continuous monitoring blood culture system, was introduced. ESP was the first blood culture system to detect both gas production and consumption by organism growth. The technology continued with ESP Myco, an adaptation to the system that allowed for growth, detection and susceptibility testing of mycobacteria species. The ESP clinical system was sold to AccuMed International in 1997.

In 1995, Difco Laboratories celebrated 100 years in business. In 1995, Difco was the first U.S. microbiology company to receive ISO 9001 certification. The International Organization for Standardization (ISO) verifies that Difco Laboratories maintains quality standards for the worldwide microbiology industry.

In 1997, Difco Laboratories, the "industrial microbiology leader," was purchased by the "clinical microbiology leader," Becton Dickinson Microbiology Systems, to form the largest microbiology company in the world. Together, Becton Dickinson Microbiology Systems and Difco Laboratories look forward to an even stronger future with our combined commitment to serving microbiologists worldwide.

History of Microbiology and Culture Media

The science of microbiology evolved from a series of significant discoveries. The Dutch microscopist, Anton van Leeuwenhoek, was the first to observe bacteria while examining different water sources. This observation was published in 1676 by the Royal Society in London. Anton van Leeuwenhoek was also the first to describe the parasite known today as *Giardia lamblia*. In 1667, the discovery of filamentous fungi was described by Robert Hooke.

After microorganisms were visually observed, their growth or reproduction created a major controversy. The conflict was over the spontaneous generation theory, the idea that microorganisms will grow spontaneously. This controversy continued for years until Louis Pasteur's renowned research. Pasteur realized that the theory of spontaneous generation must be refuted for the science of microbiology to advance. The controversy remained even after Pasteur's successful experiment using heat-sterilized infusions.

Two important developments were required for the science of microbiology to evolve. The first was a sophisticated microscope; the second was a method for culturing microorganisms. Compound microscopes were developed in Germany at the end of the sixteenth century but it was not until the early nineteenth century that achromatic lenses were developed, allowing the light in the microscope to be focused.

In 1719, Leeuwenhoek was the first to attempt differentiation of bacteria by using naturally colored agents such as beet juice. In 1877, Robert Koch used methylene blue to stain bacteria. By 1882, Robert Koch succeeded in staining the tubercle bacillus with methylene blue. This landmark discovery was performed by using heat to penetrate the stain into the organism. Two years later Hans Christian Gram, a Danish pathologist, developed the Gram stain. The Gram stain is still widely used in the differentiation of gram-positive and gram-negative bacteria.

In 1860, Pasteur was the first to use a culture medium for growing bacteria in the laboratory. This medium consisted of yeast ash, sugar and ammonium salts. In 1881, W. Hesse used his wife's agar (considered an exotic food) as a solidifying agent for bacterial growth.

The study of fungi and parasites lagged behind other microorganisms. In 1839, ringworm was the first human disease found to be caused by fungi, followed closely by the recognition of *Candida albicans* as the cause of thrush. It was not until 1910 that Sabouraud introduced a medium that would support the growth of pathogenic fungi. The interest of scientists in studying fungi was often related to crop protection. There continues to be a close connection between mycology and botany today.

By 1887, a simple device called the Petri dish revolutionized microbiology. With the invention of the Petri dish, the focus turned to culture media formulations. With all the research being performed, scientists began to replace gelatin with agar because it was resistant to microbial digestion and liquefaction.

Early years at Difco Laboratories.

The study of immunity began after the discovery of the tubercle bacillus by Robert Koch. With this acclaimed discovery, the involvement of bacteria as agents of disease became evident. The first rational attempts to produce artificial active immunity were by Pasteur in 1880 during his work with cholera.

Antibiotics had a dramatic beginning with the famous discovery of penicillin by Alexander Fleming in 1928. Fleming found a mold spore that accidentally landed on a culture of staphylococci. It was not until the late 1930s that scientists could purify penicillin and demonstrate its antibacterial effects. Commercial production of penicillin began as a combined wartime project between the United States and England. This project was the beginning of the fermentation industry and biotechnology.

Around 1930, certain growth factors, including factor X and V, were shown to be important in bacterial nutrition. In the early 1950s, most of the vitamins were also characterized as co-enzymes. This detailed information lead scientists to develop an understanding of biochemical pathways.

A "booming" development of microbiology began after World War II. Molecular biology, biotechnology and the study of genetics were fields of extraordinary growth. By 1941, the study of microbiology and genetics came together when *Neurospora crassa*, a red bread mold, was used to study microbial physiology. The study of bacterial genetics moved dramatically forward during the 1940s following the discovery of antibiotic resistance. The birth of molecular biology began in 1953 after the publication by Watson and Crick of the structure of DNA.

In 1953, viruses were defined by Luria as "submicroscopic entities, capable of being introduced into specific living cells and of reproducing inside such cells only". The work of John Enders on culturing viruses lead to the development of vaccines. Enders

demonstrated that a virus could be grown in chick embryos and would lose its ability to cause disease after successive generations. Using this technique, Salk developed the polio vaccine.

One organism that has made a great contribution to molecular biology is *Escherichia coli*. In 1973, Herbert Boyer and Stanley Cohen produced recombinant DNA through plasmid transformation. The researchers found that the foreign gene not only survived, but copied the genetic material. This study and similar others started a biotechnology revolution that has gained momentum over the years.

In the 1980s, instrumentation entered the microbiology laboratory. Manual procedures could be replaced by fully automated instruments for bacterial identification, susceptibility testing and blood culture procedures. Immunoassays and probe technologies are broadening the capabilities of the microbiologist.

With rapid advances in technologies and instrumentation, the basic culture media and ingredients listed in this Manual remain some of the most reliable and cost effective tools in microbiology today.

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Microorganism Growth Requirements

Microorganism growth on culture media depends on a number of important factors:

- Proper nutrients must be available.
- Oxygen or other gases must be available, as required.
- Moisture is necessary.
- The medium must have an appropriate pH.
- Proper temperature relations must prevail.
- The medium must be free of interfering bioburden.
- Contamination must be prevented.

A satisfactory microbiological culture medium must contain available sources of:

- Carbon,
- Nitrogen,
- Inorganic phosphate and sulfur,
- Trace metals,
- Water,
- Vitamins.

These were originally supplied in the form of meat infusion. Beef or yeast extracts frequently replace meat infusion in culture media. The addition of peptones, which are digests of proteins, provides readily available sources of nitrogen and carbon.

The pH of the culture medium is important for microorganism growth. Temperature is another important parameter: mesophilic bacteria and fungi have optimal growth at temperatures of 25-40°C; thermophilic ("heat loving") organisms grow only at temperatures greater than 45°C; psychrophilic ("cold loving") organisms require temperatures below 20°C. Human pathogenic organisms are generally mesophiles.

Common Media Constituents

Media formulations are developed on the ability of bacteria to use media components.

CONSTITUENTS	SOURCE
Amino-Nitrogen	Peptone, protein hydrolysate, infusions and extracts
Growth Factors	Blood, serum, yeast extract or vitamins, NAD
Energy Sources	Sugar, alcohols and carbohydrates
Buffer Salts	Phosphates, acetates and citrates
Mineral Salts and Metals	Phosphate, sulfate, magnesium, calcium, iron
Selective Agents	Chemicals, antimicrobials and dyes
Indicator Dyes	Phenol red, neutral red
Gelling agents	Agar, gelatin, alginate, silica gel

Media Ingredients

Peptone, protein hydrolysates, infusions and extracts are the major sources of nitrogen and vitamins in culture media. Peptones are water-soluble ingredients derived from proteins by hydrolysis or digestion of the source material, e.g. meat, milk.

Carbohydrates are employed in culture media as energy sources and may be used for differentiating genera and identifying species.

Buffers maintain the pH of culture media.

Selective Agents include Bile Salts, dyes and antimicrobial agents. **Bile Salts and desoxycholate** are selective for the isolation of gram-negative microorganisms, inhibiting gram-positive cocci.

Dyes and indicators are essential in the preparation of differential and selective culture media. In these formulations, dyes act as bacteriostatic agents, inhibitors of growth or indicators of changes in acidity or alkalinity of the substrate.

Antimicrobial agents are used in media to inhibit the growth of bacteria, yeasts and fungi.

Solidifying agents, including agar, gelatin and albumin, can be added to a liquid medium in order to change the consistency to a solid or semisolid state.

Environmental Factors in Culture Media

Atmosphere

Most bacteria are capable of growth under ordinary conditions of oxygen tension. Obligate aerobes require the free admission of oxygen, while anaerobes grow only in the absence of atmospheric oxygen. Between these two groups are the microaerophiles, which develop best under partial anaerobic conditions, and the facultative anaerobes, which are capable of growing in the presence or absence of oxygen. Anaerobic conditions for growth of microorganisms are obtained in a number of ways:

- Addition of small amounts of agar to liquid media;
- Addition of fresh tissue to the medium;
- Addition of a reducing substance to the medium; e.g., sodium thioglycollate, thioglycollic acid and L-cystine;
- Displacement of the air by carbon dioxide;
- Absorption of the oxygen by chemicals;
- Inoculation into the deep layers of solid media or under a layer of oil in liquid media.

Many microorganisms require an environment of 5-10% CO_2. Levels greater than 10% are often inhibitory due to a decrease in pH as carbonic acid forms. Culture media vary in their susceptibility to form toxic oxidation products if exposed to light and air.

Water Activity

Proper moisture conditions are necessary for continued luxuriant growth of microorganisms. Organisms require an aqueous environment and must have “free” water. “Free” water is not bound in complex structure and is necessary for transfer of nutrients and toxic waste products. Evaporation during incubation or storage results in loss of “free” water and reduction of colony size or total inhibition of organism growth.

Protective Agents and Growth Factors

Calcium carbonate, soluble starch and charcoal are examples of protective agents used in culture media to neutralize and absorb toxic metabolites produced by bacterial growth.

NAD (V factor) and hemin (X factor) are growth factors required by certain bacteria; e.g., *Haemophilus* species, and for enhanced growth of *Neisseria* species.

Surfactants, including Tween® 80, lower the interfacial tension around bacteria suspended in the medium. This activity permits more rapid entry of desired compounds into the bacterial cell and can increase bacterial growth.

Culture Media Ingredients – Agars

History

Agar was discovered in 1658 by Minora Tarazaemon in Japan.[1] According to legend, this Japanese innkeeper threw surplus seaweed soup into the winter night and noticed it later transformed into a gel by the night’s freezing and the day’s warmth.[2] In 1882, Koch was the first to use agar in microbiology.[3,4] Walter Hesse, a country doctor from Saxony, introduced Koch to this powerful gelling agent.[5] Hesse had learned about agar from his wife, Fanny Hesse, whose family had contact with the Dutch East Indies where agar was being used for jellies and jams.[3,5,6] The term ‘agar-agar’ is a Malaysian word that initially referred to extracts from Eucheuma, which yields carrageenan, not agar.[5]

By the early 1900s, agar became the gelling agent of choice instead of gelatin. Agar was found more suitable because it remained solid at the temperatures required for growth of human pathogens and was resistant to breakdown by bacterial enzymes.

Production of agar in the United States was started just before the beginning of World War II as a strategic material.[5] In the 1940s, bacteriological-grade agar manufactured by the American Agar Company of San Diego, California, served as reference agar for the evaluation of the characteristics of other culture media components, such as peptones.[5]

Characteristics

Agar is a phycocolloid, a water-soluble polysaccharide, extracted from a group of red-purple marine algae (Class *Rhodophyceae*) including *Gelidium*, *Pterocladia* and *Gracilaria*. These red-purple marine algae are widely distributed throughout the world in temperate zones.

Agar is derived from a group of red-purple marine algae as pictured above.

For Difco Agars, *Gelidium* is the preferred source of agar. The most important properties of agar are:[5]

- Good transparency in solid and gel forms to allow identification of colony type;
- Consistent lot-to-lot gel strength that is sufficient to withstand the rigors of streaking but not so stiff that it affects diffusion characteristics;
- Consistent gelling (32-40°C) and melting (approximately 85°C) temperatures, a property known as hysteresis;
- Essential freedom from metabolically useful chemicals such as peptides, proteins and fermentable hydrocarbons;
- Low and regular content of electronegative groups that could cause differences in diffusion of electropositive molecules (e.g., antibiotics, nutrients);
- Freedom from toxic substances (bacterial inhibitors);
- Freedom from hemolytic substances that might interfere with normal hemolytic reactions in culture media;
- Freedom from contamination by thermophilic spores.

Agars are normally used in final concentrations of 1-2% for solidifying culture media. Smaller quantities of agar (0.05-0.5%) are used in culture media for motility studies (0.5% w/v) and growth of anaerobes (0.1%) and microaerophiles.[2]

The Manufacturing Process

Difco Laboratories selects the finest *Gelidium* marine algae from world sources and requires algae harvested from water where the temperature is both constant and temperate. Bacto Agar and Agar Granulated are produced from an Ice Agar purification process. Agar is insoluble in cold water but is colloidally dispersible in water above 90°C.[2] When an agar gel is frozen, the agar skeleton contracts toward the center of the mass as a membrane, leaving ice as a separate phase.[2]

Through a variety of processes, the agar is extracted from the *Gelidium*, resulting in a liquid agar that is purified. The liquid agar is first gelled and then frozen, causing the soluble and suspended contaminants to be trapped in the frozen water. The ice is then washed from the agar, eliminating the contaminants. The Ice Agar process results in greater consistency and freedom from interposing contaminants when used in microbiological procedures.

Product Applications

Bacto Agar is optimized for beneficial calcium and magnesium content. Detrimental ions such as iron and copper are reduced. Bacto Agar is recommended for clinical applications, auxotrophic studies, bacterial and yeast transformation studies and bacterial molecular genetics applications.[7,8]

Agar Flake is recommended for use in general bacteriological purposes. The quality is similar to Bacto Agar. The flakes are more wettable than the granules found in Bacto Agar.

Agar Granulated is qualified to grow recombinant strains of *Escherichia coli* (HB101) and *Saccharomyces cerevisiae*. Agar Granulated may be used for general bacteriological purposes where clarity is not a strict requirement. This agar was developed to address the special needs of the Biotechnology Industry for large scale applications.

Noble Agar is the purest form of Difco agar. It is washed extensively and bleached to remove extraneous material. The result is a white powder in dry form, clear and colorless in solution and when solidified in plates. This agar is suitable for immunodiffusion studies, for use in some electrophoretic applications and as a substrate for mammalian and plant tissue culture.

Agar Technical is suitable for many general bacteriological applications. This agar is not as highly processed as other Difco agars and has lower technical specifications. This agar is not recommended for growth of fastidious organisms.

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Culture Media Ingredients – Peptones and Hydrolysates

Typical fermentation process.

History

Peptones were originally described by Naegeli in 1879.[3] In this report, Naegeli compared peptone and ammonium tartrate. With the rich amino acid and nitrogen compounds readily utilized by bacteria, peptone soon became one of the most important constituents of culture media. The importance of peptone as a nutritive source was demonstrated by Klinger.[4]

Bacto Peptone was introduced commercially in 1914, and became the standard peptone for the preparation of bacteriological culture media. The development of Bacto Proteose Peptone, Bacto Proteose Peptone No. 2 and Bacto Proteose Peptone No. 3 resulted from accumulated information that no single peptone is the most suitable nitrogen source for culturing fastidious bacteria. Extensive investigations were undertaken at Difco Laboratories using peptic digests of animal tissue prepared under varying digestion parameters. Bacto Tryptone was developed by Difco Laboratories while investigating a peptone particularly suitable for the elaboration of indole by bacteria.

Other non-chemically defined ingredients, including Bacto Liver, Bacto Beef Heart for Infusion and Bacto Yeast Extract can serve as nitrogen or carbon sources. Infusions of meat were first employed as nutrients in culture media. It was discovered that for many routine procedures in the preparation of culture media, extracts have the advantage of greater ease in preparation, uniformity and economy than infusions.

Protein Biochemistry

Proteins consist of amino acids joined together by means of the covalent peptide bond linkage. When the bonds are hydrolyzed, proteins yield polypeptides of various molecular sizes, proteoses, peptones and peptides down to the level of simple amino acids. Bacteriological peptones are mixtures of various products of protein hydrolysis, organic nitrogen bases, inorganic salts and trace elements.

Preparation of Peptones

The composition of peptones varies with the origin and the method of preparation. Some common sources of peptone include:

Meat (fresh, frozen or dried)
Fish (fresh, dried)
Casein
Gelatin
Keratin (horn, hair, feathers)
Ground Nuts
Soybean Meal
Cotton Seed
Sunflower Seeds
Microorganisms (yeasts, algae, bacteria)
Guar Protein
Blood
Corn Gluten
Egg Albumin

Demineralized water is added to these protein sources to form a thick suspension. The digestion process follows with an acid or enzyme. Acid and alkaline hydrolyses are performed by boiling the protein with mineral acids or strong alkalis at increased pressure to raise the temperature of the reaction. This procedure can decrease the vitamin content of the protein and a portion of the amino acid content.

Digestion with proteolytic enzymes is performed at lower temperatures and normal atmospheric pressure. This process is often less harmful to the protein and amino acids. Microbial Proteoses, Papain, Pancreatin and Pepsin are used most often by Difco Laboratories in the manufacture of peptones.

The peptone suspension is then centrifuged and filtered. The suspension is concentrated to approximately 67% total solids and the product now appears as a syrup. This peptone syrup is spray dried and packaged.

Infusions and Extracts

The water-soluble fractions of materials such as muscle, liver, yeast cells and malt are usually low in peptides but contain valuable extractives such as vitamins, trace metals and complex carbohydrates.[5] It is common practice to combine infusions and peptones to obtain the best of both products.[5] Bacto Yeast Extract, Bacto Malt Extract, Bacto Beef Heart for Infusion and Bacto Beef Extract are examples of extracts and infusions manufactured by Difco Laboratories for use in the preparation of culture media.

Peptone Performance

The quality and performance of peptones, infusions and extracts are very dependent on the freshness or preservation of the raw materials.[5] Extensive quality control testing is performed on all peptones and other culture media ingredients during the manufacturing process and on the final product. Certificates of Analysis supply information from the manufacturer on lot specific final testing of a product.

A typical analysis was performed on Difco peptones and hydrolysates to aid in the selection of products for research or production needs when specific nutritional characteristics are required. The specifications for the typical analysis include:

- Physical characteristics
- Nitrogen content
- Amino acids
- Inorganics
- Vitamins
- Biological testing

The quality of peptones and culture media ingredients is truly assessed by their ability to support adequate growth of various microorganisms when incorporated into the medium.[6] The nature of peptones, infusions and extracts will then play a major role in the growth performance properties of the medium and, in turn, advance the science of microbiology.[6]

Media Ingredients

Autolyzed Yeast

Autolyzed Yeast is a desiccated product containing both the soluble and insoluble portions of autolyzed bakers' yeast. Autolyzed Yeast is recommended for the preparation of yeast supplements used in the microbiological assay of riboflavin and pantothenic acid.[7,8,] Autolyzed Yeast provides vitamins, nitrogen, amino acids and carbon in microbiological culture media.

Beef
Beef Heart for Infusion

Beef and Beef Heart for Infusion provide nitrogen**,** amino acids and vitamins in microbiological culture media. Beef is desiccated, powdered, fresh lean beef, prepared especially for use in beef infusion media. Large quantities of beef are processed at one time to secure a uniform and homogenous product. Beef Heart for Infusion is prepared from fresh beef heart tissue and is recommended for preparing heart infusion media. Beef Heart for Infusion is processed from large volumes of raw material, retaining all the nutritive and growth stimulating properties of fresh tissues.

Beef Extract
Beef Extract, Desiccated

Beef Extract and Beef Extract, Desiccated are replacements for infusion of meat. Beef Extract and Beef Extract, Desiccated provide nitrogen, vitamins, amino acids and carbon in microbiological culture media. Beef Extract is standard in composition and reaction and generally used to replace infusion of meat. In culture media, Beef Extract is usually employed in concentration of 0.3%. Beef Extract, Desiccated, the dried form of Beef Extract, was developed to provide a product for ease of use in handling. Beef Extract is in the paste form. The products are to be used in a 1 for 1 substitution.

Bile Salts
Bile Salts No. 3

Bile Salts and Bile Salts No. 3 are used as selective agents for the isolation of gram-negative microorganisms, inhibiting gram-positive cocci. Bile is derived from the liver. The liver detoxifies bile salts by conjugating them to glycine or taurine. A bile salt is the sodium salt of a conjugated bile acid. Bile Salts and Bile Salts No. 3 contain bile extract standardized to provide inhibitory properties for selective media. Bile Salts No. 3 is a modified fraction of bile acid salts, providing a refined bile salt. Bile Salts No. 3 is effective at less than one-third concentration of Bile Salts.

Casamino Acids
Casamino Acids, Technical
Casamino Acids, Vitamin Assay

Casamino Acids, Casamino Acids, Technical and Casamino Acids, Vitamin Assay are derived from acid hydrolyzed casein. Casein is a milk protein and a rich source of amino acid nitrogen. Casamino Acids, Casamino Acids, Technical and Casamino Acids, Vitamin Assay are added to media primarily because of their organic nitrogen and growth factor components; their inorganic components also play a vital role.[9] Casamino Acids is recommended for use with microbiological cultures that require a completely hydrolyzed protein as a nitrogen source. In Casamino Acids, hydrolysis is carried on until all the nitrogen in the casein is converted to amino acids or other compounds of relative chemical simplicity. The hydrolysis of Casamino Acids, Technical is carried out as in the preparation of Casamino Acids, but the sodium chloride and iron content have not been decreased to the same extent. Casamino Acids, Vitamin Assay is an acid digest of casein specially treated to markedly reduce or eliminate certain vitamins. It is recommended for use in microbiological assay media and in growth promotion studies.

Casein Digest

Casein Digest is an enzymatic digest of casein, providing a distinct source of amino acids for molecular genetics media. Casein Digest is used as a nitrogen and amino acid source for microbiological culture media. Casein Digest is similar to N-Z Amine A. This product is digested under conditions different from other enzymatic digests of casein, including Tryptone and Casitone.

Casitone

Casitone is a pancreatic digest of casein. Casitone is recommended for preparing media where an enzymatic hydrolyzed casein is desired. Casein is a rich source of amino acid nitrogen. This product is used to support the growth of fastidious microorganisms and its high tryptophan content makes it valuable for detecting indole production.

Fish Peptone No. 1

Fish Peptone No. 1 is a non-mammalian, non-animal peptone used as a nitrogen source in microbiological culture media. Fish Peptone No. 1 is a non-bovine origin peptone, to reduce Bovine Spongiform Encephalopathy (BSE) risk. This peptone was developed by Difco Laboratories for pharmaceutical and vaccine production and can replace any peptone, depending on the organism and production application.

Gelatin

Gelatin is a protein of uniform molecular constitution derived chiefly by the hydrolysis of collagen.[10] Collagens are a class of albuminoids found abundantly in bones, skin, tendon, cartilage and similar tissues of animals.[10] Gelatin is used in culture media to detect gelatin liquifaction by bacteria and as a nitrogen and amino acid source.

Gelatone

Gelatone is a pancreatic digest of gelatin, deficient in carbohydrates. Gelatone is used as a media ingredient for fermentation studies and, alone, to support the growth of non-fastidious microorganisms. Gelatone is in granular form for convenience in handling and is distinguished by a low cystine and tryptophan content.

Liver

Liver is prepared from large quantities of carefully trimmed fresh beef liver. Liver is a desiccated powder of beef liver. The nutritive factors of fresh liver tissue are retained in infusion prepared from Liver. Liver is used as a source of nitrogen, amino acids and vitamins in microbiological culture media. The reducing substances contained in liver create an anaerobic environment, necessary to support the growth of anaerobes. One hundred thirty-five (135) grams of desiccated Liver are equivalent to 500 grams of fresh liver.

Malt Extract

Malt Extract is obtained from barley, designed for the propagation of yeasts and molds. Malt Extract is particularly suitable for yeasts and molds because it contains a high concentration of carbohydrates, particularly maltose. This product is generally employed in concentrations of 1-10%. Malt Extract provides carbon, protein and nutrients for the isolation and cultivation of yeasts and molds in bacterial culture media.

Neopeptone, Difco

Neopeptone is an enzymatic digest of protein. Neopeptone contains many peptide sizes in combination with vitamins, nucleotides, minerals and other carbon sources. Neopeptone is particularly well suited in supplying the growth requirements of fastidious bacteria. This peptone is extremely valuable in media for the cultivation of pathogenic fungi. Growth of these microorganisms is rapid and colony formation is uniform and typical.

Oxgall

Oxgall is manufactured from large quantities of fresh bile by rapid evaporation of the water content. Bile is composed of fatty acids, bile acids, inorganic salts, sulphates, bile pigments, cholesterol, mucin, lecithin, glycuronic acids, porphyrins and urea. The use of Oxgall ensures a regular supply of bile and assures a degree of uniformity impossible to obtain with fresh materials. It is prepared for use in selective media for differentiating groups of bile tolerant bacteria. Oxgall is used as a selective agent for the isolation of gram-negative microorganisms, inhibiting gram-positive bacteria. The major components of Oxgall are taurocholic and glycocholic acids.

Peptamin

Peptamin, referred to as Peptic Digest of Animal Tissue, complies with the US Pharmacopeia XXIII (USP).[11] Peptamin provides nitrogen, amino acids, vitamins and carbon in microbiological culture media. Diluting and rinsing solutions, Fluid A and Fluid D, contain 0.1% Peptamin.

Peptone, Bacto
Peptone Bacteriological, Technical

Bacto Peptone and Peptone Bacteriological, Technical are enzymatic digests of protein and rich nitrogen sources. Bacto Peptone was introduced in 1914 and became the standard peptone for the preparation of culture media. Peptone Bacteriological, Technical can be used as the nitrogen source in microbiological culture media when a standardized peptone is not essential. Both peptones have a high peptone and amino acid content and only a negligible quantity of proteoses and more complex nitrogenous constituents.

Proteose Peptone
Proteose Peptone No. 2
Proteose Peptone No. 3

The development of Proteose Peptone, Proteose Peptone No. 2 and Proteose Peptone No. 3 is the result of accumulated information demonstrating that no single peptone is the most suitable nitrogen source for culturing fastidious bacteria. Proteose Peptone is an enzymatic digest of protein high in proteoses. Many factors account for the suitability of Proteose Peptone for the culture of fastidious pathogens, including the nitrogen components, buffering range and the high content of proteoses. Proteose Peptone No. 2 and Proteose Peptone No. 3 are enzymatic digests of protein. Proteose Peptone No. 2 is used for producing bacterial toxins and is suitable for media of nutritionally less-demanding bacteria. Proteose Peptone No. 3 is a modification of Proteose Peptone, adapted for use in the preparation of chocolate agar for propagation of *Neisseria* species and chocolate tellurite agar for *Corynebacterium diphtheriae.*

Sodium Deoxycholate
Sodium Taurocholate

Sodium Desoxycholate is the sodium salt of desoxycholic acid. Since Sodium Desoxycholate is a salt of a highly purified bile acid, it is used in culture media in lower concentrations than in naturally occurring bile. Sodium Taurocholate is the sodium salt of a conjugated bile acid. Sodium Taurocholate contains about 75% sodium taurocholate in addition to other naturally occurring salts of bile acids. Sodium Desoxycholate and Sodium Taurocholate, like other bile salts, are used as selective agents in microbiological culture media. They are used to aid in the isolation of gram- negative microorganisms, inhibiting gram-positive organisms and spore forming bacteria.

Soytone

Soytone is an enzymatic digest of soybean meal. The nitrogen source in Soytone contains the naturally occurring high concentrations of vitamins and carbohydrates of soybean.

TC Lactalbumin Hydrolysate
TC Yeastolate

TC Lactalbumin Hydrolysate is an enzymatic digest of lactalbumin for use as an enrichment in tissue culture media. Lactalbumin is a protein derived after removal of casein from milk. TC Yeastolate is a desiccated, clarified, water soluble portion of autolyzed fresh yeast prepared and certified for use in tissue culture procedures. TC Yeastolate is a source of vitamin B complex.

Tryptone Peptone

Tryptone Peptone is a pancreatic digest of casein used as a nitrogen source in culture media. Casein is the main protein of milk and is a rich source of amino acid nitrogen. Tryptone Peptone is rich in tryptophan, making it valuable for use in detecting indole production.[12] The absence of detectable levels of carbohydrates in Tryptone Peptone makes it a suitable peptone in differentiating bacteria on the basis of their ability to ferment various carbohydrates.

Tryptose

Tryptose is a mixed enzymatic hydrolysate with distinctive nutritional properties. The digestive process of Tryptose results in assorted peptides, including those of higher molecular weight. Tryptose was originally developed as a peptone particularly adapted to the growth requirements of *Brucella*.

Yeast Extract
Yeast Extract, Technical

Yeast Extract and Yeast Extract, Technical are water soluble portions of autolyzed yeast containing vitamin B complex. Yeast Extract is an excellent stimulator of bacterial growth and used in culture media. The autolysis is carefully controlled to preserve the naturally occurring B-complex vitamins. Yeast Extract is generally employed in the concentration of 0.3-0.5%, with improved filterability at 20%. Yeast Extract, Technical is used in bacterial culture media when a standardized yeast extract is not essential. Yeast Extract, Technical was developed to demonstrate acceptable clarity and growth promoting characteristics. Yeast Extract and Yeast Extract, Technical also provide vitamins, nitrogen, amino acids and carbon in microbiological culture media.

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Media Preparation

The preparation of culture media from dehydrated media requires accuracy and attention to preparation. The following points are included to aid the user in successful and reproducible preparation of culture media.

Dehydrated Media and Ingredients

- Store in a cool (15-30°C), dark and dry area unless otherwise specified.
- Note date opened.
- Check expiry (applied to intact container).
- Verify that the physical characteristics of the powder are typical.

Glassware / Plasticware

- Use high quality, low alkali borosilicate glass.
- Avoid detergent residue.
- Check for alkali or acid residue with a few drops of brom thymol blue pH indicator (yellow is acidic; blue is alkaline).
- Use vessels at least 2-3 times the volume of medium.
- Discard (recycle) etched or chipped glassware.
- Do not used etched glassware.

Equipment

- Use measuring devices, scales, pH meters, autoclaves and other equipment that are frequently and accurately calibrated.

Water

- Use distilled or deionized water.
- pH 5.5-7.5.

Dissolving the Medium

- Accurately weigh the appropriate amount of dehydrated medium.
- Dissolve the medium completely.
- Agitate the medium while dissolving.
- Take care to not overheat. Note media that are very sensitive to overheating. Overheated media will frequently appear darker. Do not heat in a microwave.

Sterilization

- The autoclave set-temperature should be 121°C.
- Routine autoclave maintenance is important. Ask manufacturer to check for “hot” and “cold” spots.
- The recommended 15 minute sterilization assumes a volume of 1 liter or less. Larger volumes may require longer cycles. Check with your autoclave manufacturer for recommended load configurations.
- Quantities of media in excess of two liters may require an extended autoclave time to achieve sterilization. Longer sterilization cycles can cause nutrient concentration changes and generation of inhibitory substances.

Adding Enrichments and Supplements

- Enrichments and supplements tend to be heat sensitive.
- Cool medium to 45-55°C in a waterbath prior to adding enrichments or supplements.
- Ensure adequate mixing of the basal medium with enrichments or supplements by swirling to mix thoroughly.
- Sterile broths may be cooled to room temperature before adding enrichment.

pH

- Commercial dehydrated media are designed to fall within the specified pH range *after* steam sterilization. The pH tends to fall approximately 0.2 units during steam sterilization.
- For filter sterilization, adjust the pH, if necessary, prior to filtering.
- Avoid excessive pH adjustments.

Dispensing Media

- Ensure gentle mixing during dispensing.
- Cool the medium to 50-55°C prior to dispensing to reduce water evaporation.
- Dispense quickly.
- If using an automatic plate dispenser, dispense general purpose media before dispensing selective media.
- Immediately recover or recap tubes to reduce the chance of contamination. Leave Petri dish covers slightly open for 1-2 hours to obtain a dry surface.

Storage and Expiry

- In general, store steam-sterilized plated media inverted in a plastic bag or other container in a dark refrigerator for up to 1-2 weeks.

Quality Control

- For media prepared in-house, each lot of every medium must be tested.
- Maintain Quality Control Organisms appropriately.
- Maintain appropriate records.
- Report deficiencies to the manufacturer.

The following table is a troubleshooting guide to assist in the preparation of reliable culture media.

PROBLEM	A	B	C	D	E	F	G	H	OTHER CAUSES
Abnormal color of medium	•	•	•						
Incorrect pH	•	•	•	•	•	•	•		Storage at high temperature Hydrolysis of ingredients pH determined at wrong temperature
Nontypical precipitate	•	•	•	•	•	•			
Incomplete solubility					•				Inadequate heating Inadequate convection in a too small flask
Darkening or carmelization	•			•	•	•			
Toxicity		•	•						Burning or scorching
Tract substances (Vitamins)		•							Airborne or environmental sources of vitamins
Loss of gelation property				•	•	•		•	Hydrolysis of agar due to pH shift Not boiling medium
Loss of nutritive value or selective or differential properties	•		•	•	•	•	•	•	Burning or scorching Presence of strong electrolytes, sugar solutions, detergents, antiseptics, metallic poisons, protein materials or other substances that may inhibit the inoculum
Contamination									Improper sterilization Poor technique in adding enrichments and pouring plates Not boiling agar containing medium

Key

A Deteriorated Dehydrated Medium
B Improperly Washed Glassware
C Impure Water
D Incorrect Weighing
E Incomplete Mixing
F Overheating
G Repeated Remelting
H Dilution by a Too Large Inoculum

Media Sterilization

Sterilization is any process or procedure designed to entirely eliminate viable microorganisms from a material or medium. Sterilization should not be confused with disinfection, sanitization, pasteurization or antisepsis which are intended to inactivate microorganisms, but may not kill all microorganisms present. Sterilization can be accomplished by the use of heat, chemicals, radiation or filtration.[1]

Sterilization with Heat[1]

The principal methods of thermal sterilization include 1) moist heat (saturated steam) and 2) dry heat (hot air) sterilization. Heat kills microorganisms by protein denaturation and coagulation. Moist heat has the advantage of being more rapid and requiring lower temperatures than dry heat. Moist heat is the most popular method of culture media sterilization. When used correctly, it is the most economical, safe and reliable sterilization method.

Moist Heat Sterilization

Water boils at 100°C, but a higher temperature is required to kill resistant bacterial spores in a reasonable length of time. A temperature range of 121-124°C for 15 minutes is an accepted standard condition for sterilizing up to one liter of culture medium. The definition of "autoclave at 121°C for 15 minutes" refers to the temperature of the contents of the container being held at 121°C for 15 minutes, not to the temperature and time at which the autoclave has been set.[2] The steam pressure of 15 pounds per square inch at this temperature aids in the penetration of the heat into the material being sterilized. If a larger volume is to be sterilized in one container, a longer period should be employed. Many factors can affect sterility assurance, including size and contents of the load and the drying and cooling time. Certain products may decompose at higher temperature and longer cycles. For this reason, it is important that all loads be properly validated.

The basic principles for validation and certification of a sterilizing process are enumerated as follows:[3]

1. Establish that the processing equipment has the capability of operating within the required parameters.

2. Demonstrate that the critical control equipment and instrumentation are capable of operating within the prescribed parameters for the process equipment.
3. Perform replicate cycles representing the required operational range of the equipment and employing actual or simulated product. Demonstrate that the processes have been carried out within the prescribed protocol limits and, finally, that the probability of microbial survival in the replicate processes completed is not greater than the prescribed limits.
4. Monitor the validated process during routine operation. Periodically as needed, requalify and recertify the equipment.
5. Complete the protocols and document steps 1-4, above.

For a complete discussion of process validation, refer to appropriate references.

Ensuring that the temperature is recorded correctly is vital. The temperature must reach all parts of the load and be maintained for the desired length of time. Recording thermometers are employed for the chamber and thermocouples may be buried inside the load.

For best results when sterilizing culture media, plug tubes or flasks of liquids with nonabsorbent cotton or cap loosely. Tubes should be placed in racks or packed loosely in baskets. Flasks should never be more than two-thirds full. It is important to not overload the autoclave chamber and to place contents so that there is a free flow of steam around the contents. After sterilizing liquids, the chamber pressure must be reduced slowly to atmospheric pressure. This allows the liquid to cool below the boiling point at atmospheric pressure before opening the door to prevent the solution from boiling over.

In autoclave operation, all of the air in the chamber must be expelled and replaced by steam; otherwise, "hot spots" and "cold spots" will occur. Pressure-temperature relations of a properly operated autoclave are shown in the table below.

Pressure-Temperature Relations in Autoclave[4]
(Figures based on complete replacement of air by steam)

PRESSURE IN POUNDS	TEMPERATURE (°C)	TEMPERATURE (°F)
5	109	228
10	115	240
15	121	250
20	126	259
25	130	267
30	135	275

Over-sterilization or prolonged heating will change the composition of the medium. For example, carbohydrates are known to break down in composition upon overheating. Over-sterilizing media can cause a number of problems, including:

- Incorrect pH;
- A decrease in the gelling properties of agar;
- The development of a nontypical precipitate;
- Carmelization or darkening of the medium;
- Loss of nutritive value;
- Loss of selective or differential properties.

There are certain media (e.g., Hektoen Enteric Agar and Violet Red Bile Agar) that should not be autoclaved. To dissolve these media formulation, heat to boiling to dissolve completely. It is important to follow all label directions for each medium. Media supplements should be sterile and added aseptically to the sterilized medium, usually at 45-55°C.

Dry Heat Sterilization[1]

Dry heat is employed for materials such as metal instruments that could be corroded by moist heat, powders, ointments and dense materials that are not readily penetrated by steam. Because dry heat is effective only at considerably higher temperatures and longer times than moist heat, dry heat sterilization is restricted to those items that will withstand higher temperatures. The dry heat time for sterilization is 120 minutes at 160°C.

Chemical Sterilization[1]

Chemical sterilization employs gaseous and liquid sterilants for certain medical and industrial instruments. The gases include ethylene oxide, formaldehyde and beta-propiolactone. The liquid sterilants include glutaraldehyde, hydrogen peroxide, peracetic acid, chlorine dioxide and formaldehyde. Chemical sterilization is not employed in the preparation of culture media. For a complete discussion of this topic, consult appropriate references.

Radiation Sterilization[1]

Radiation sterilization is an optional treatment for heat-sensitive materials. This includes ultraviolet light and ionizing radiation.

Ultraviolet light is chemically active and causes excitation of atoms within the microbial cell, particularly the nucleic acids, producing lethal mutations. This action stops the organism from reproducing. The range of the ultraviolet spectrum that is microbiocidal is 240-280 nm. There is a great difference in the susceptibility of organisms to ultraviolet radiation; *Aspergillus niger* spores are 10 times more resistant than *Bacillus subtilis* spores, 50 times more resistant than *Staphylococcus aureus* and *Escherichia coli*, and 150 times more resistant than influenza virus.

Because most materials strongly absorb ultraviolet light, it lacks penetrating power and its applications are limited to surface treatments. Much higher energy, 100 to millions of times greater, is generated by ionizing radiations. These include gamma-rays, high energy X-rays and high energy electrons.

Ionizing radiation, unlike ultraviolet rays, penetrates deeply into atoms, causing ionization of the electrons. Ionizing radiation may directly target the DNA in cells or produce active ions and free radicals that react indirectly with DNA.

Gamma radiation is used more often than x-rays or high-energy electrons for purposes of sterilization. Gamma rays are generated by

radioactive isotopes, cobalt-60 being the usual source. Gamma radiation requires many hours of exposure for sterilization. Validation of a gamma irradiation procedure includes:[4]

- Establishment of article materials compatibility;
- Establishment of product loading pattern and completion of dose mapping in the sterilization container;
- Establishment of timer setting;
- Demonstration of the delivery of the required sterilization dose.

The advantages of sterilization by irradiation include low chemical reactivity, low measurable residues, and few variables to control.[3] Gamma irradiation is used for treating many heat-sensitive products that can also be treated by gaseous sterilization, including medical materials and equipment, pharmaceuticals, biologicals, certain prepared media and laboratory equipment.

Sterilization by Filtration[1,3]

Filtration is a useful method for sterilizing liquids and gases. Filtration excludes microorganisms rather than destroying them. Two major types of filters may be used, depth filters and membrane filters.

The membrane filter screens out particles, while the depth filter entraps them. Membrane filters depend largely on the size of the pores to determine their screening effectiveness. Electrostatic forces are also important. A membrane filter with an average pore size of 0.8 µm will retain particulate matter as small as 0.05 µm. For removing bacteria, a pore size of 0.2 µm is commonly used. For retention of viruses and mycoplasmas, pore sizes of 0.01-0.1 µm are recommended. Cocci and bacilli range in size from about 0.3 to 1 µm in diameter. Most viruses are 0.02-0.1 µm, with some as large as 0.25 µm.

Rating the pore size of filter membranes is by a nominal rating that reflects the capability of the filter membrane to retain microorganisms of size represented by specified strains. Sterilizing filter membranes are membranes capable of retaining 100% of a culture of 10^7 microorganisms of a strain of *Pseudomonas diminuta* (ATCC® 19146) per square centimeter of membrane surface under a pressure of not less than 30 psi. These filter membranes are nominally rated 0.22 µm or 0.2 µm. Bacterial filter membranes (also known as analytical filter membranes), which are capable of retaining only larger microorganisms, are labeled with a nominal rating of 0.45 µm.

Membrane filters are used for the commercial production of a number of pharmaceutical solutions and heat-sensitive injectables. Serum for use in bacterial and viral culture media are often sterilized by filtration, as well as some sugars that are unstable when heated. Membrane filtration is useful in testing pharmaceutical and medical products for sterility.

Sterility Assurance[1]

Sterility Assurance is the calculated probability that a microorganism will survive sterilization. It is measured as the SAL, Sterility Assurance Level, or "degree of sterility". For sterility assurance, *Bacillus stearothermophilus* which contains steam heat-resistant spores is employed with steam sterilization at 121°C.

Testing Sterilizing Agents[1,5]

Sterilization by moist heat (steam), dry heat, ethylene oxide and ionizing radiation is validated using biological indicators. The methods of sterilization and their corresponding indicators are listed below:

STERILIZATION METHOD	BIOLOGICAL INDICATOR
Steam	*Bacillus stearothermophilus*
Dry heat	*Bacillus subtilis* var. *niger*
Ethylene oxide	*Bacillus subtilis* var. *globigii*
Filtration	*Pseudomonas diminuta*

For moist heat sterilization, paper strips treated with chemicals that change color at the required temperature may be used.

The heat-resistant spores of *B. stearothermophilus* are dried on paper treated with nutrient medium and chemicals. After sterilization, the strips are incubated for germination and growth, and a color change indicates whether they have or have not been activated. Spore strips should be used in every sterilization cycle.

Glossary[1,6]

Bioburden is the initial population of living microorganisms in the product or system being considered.

Biocide is a chemical or physical agent intended to produce the death of microorganisms.

Calibration is the demonstration that a measuring device produces results within specified limits of those produced by a reference standard device over an appropriate range of measurements.

Death rate is the rate at which a biocidal agent reduces the number of cells in a microbial population that are capable of reproduction. This is determined by sampling the population initially, during and following the treatment, followed by plate counts of the surviving microorganisms on growth media.

D value stands for decimal reduction time and is the time required in minutes at a specified temperature to produce a 90% reduction in the number of organisms.

Microbial death is the inability of microbial cells to metabolize and reproduce when given favorable conditions for reproduction.

Process validation is establishing documented evidence that a process does what it purports to do.

Sterility Assurance Level is generally accepted when materials are processed in the autoclave and attain a 10^{-6} microbial survivor probability; i.e., assurance of less than one chance in one million that viable microorganisms are present in the sterilized article.[3]

Sterilization process is a treatment process from which the probability of microorganism survival is less than 10^{-6}, or one in a million.

Thermal Death Time and **Thermal-Chemical Death Time** are terms referring to the time required to kill a specified microbial population upon exposure to a thermal or thermal-chemical sterilizing agent under specified conditions. A typical thermal death time value with highly resistant spores is 15 minutes at 121°C for steam sterilization.

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Quality Control Organisms

Bacteria Control Strain Source

An integral part of quality control testing includes quality control organisms. Microorganisms should be obtained from reputable sources, for example, the American Type Culture Collection (ATCC®) or other commercial sources.

Maintenance / Frozen Stock Cultures

If using commercial stock cultures, follow the manufacturer's recommendations for growth and maintenance.

To prepare frozen stock cultures of *Staphylococcus* species, *Streptococcus* species, Enterobacteriaceae and *Pseudomonas aeruginosa*:

1. Reconstitute the stock culture, if necessary.
2. Inoculate multiple plates of a general purpose medium (e.g., TSA or blood agar).
3. Incubate plates for 18-24 hours in an appropriate atmosphere and at the recommended temperature.
4. Check for purity and correct colony morphology.
5. If necessary, verify biochemical tests.
6. Remove sufficient growth from a confluent area to prepare a 0.5 McFarland standard (1-2 x 10^8 CFU/ml). For fastidious organisms, adjust to a 1 McFarland.
7. Suspend the growth in 50-100 ml of cryoprotective medium, e.g., Tryptic Soy Broth with 10-15% Glycerol, Skim Milk or sterile defibrinated sheep blood.
8. Dispense 0.5-1.0 ml into sterile glass or plastic freezing vials. Prepare enough vials for one year of storage. Assume only one freeze/thaw cycle per vial. Assume at least one fresh culture every four weeks.
9. Store vials at or below -50°C (freezer) for one year. Organisms will keep longer (indefinitely) if stored in an ultra low temperature freezer or in a liquid nitrogen tank.

To use a frozen culture:

1. Thaw the vial quickly.
2. Use the culture directly or subculture.
3. Discard any unused cell suspension.

Working Cultures

Prepare no more than three serial subcultures from a frozen stock culture.

1. Inoculate an agar slant or plate with the frozen stock culture and incubate overnight.
2. Store the working culture at 2-8°C or at room temperature for up to four weeks.
3. Check for purity and appropriate colony morphology.

OR

1. Use the frozen stock culture directly as a working culture.

Maintain anaerobic cultures in Cooked Meat Medium or another suitable anaerobic medium. Alternatively, use frozen anaerobic cultures.

Test Procedure

1. Inoculate an agar plate from the "working culture".
2. Incubate overnight.
3. Suspend 3-5 isolated colonies with typical appearance in a small volume (0.5-1.0 ml) of TSB. Incubate 4-5 hours in an appropriate atmosphere and temperature.
4. Adjust the turbidity to 0.5 McFarland and 0.08-0.1 absorbance units at 625 nm.

OR

1. Adjust an overnight culture to a 0.5 McFarland.
2. Plate 0.01 ml of the specimen to confirm a colony count of 1-2 x 10^8 CFU/ml. If using a frozen culture, confirm the appropriate density.

To Test Cultural Response

Non-Selective Media

Dilute the cell suspension 1:100 in normal saline or purified water. Inoculate each plate with 0.01 ml to give 1-2 x 10^4 CFU/plate. Reduce the inoculum ten fold, if necessary, to obtain isolated colonies.

Selective Media and Tubed Media

Dilute the cell suspension 1:10 in normal saline or purified water. Streak each plate with 10.01 ml of the suspension to provide 1-2 x 10^5 CFU/plate. Reduce the inoculum ten fold, if necessary, to avoid overwhelming some selective media.

Results

For general-purpose media, sufficient, characteristic growth and typical colony morphology should be obtained with all test strains. For selective media, growth of designated organisms is inhibited and adequate growth of desired organisms is obtained. Color and hemolytic reaction criteria must be met.

Reference

National Committee for Clinical Laboratory Standards. 1996. Quality assurance for commercially prepared microbiological culture media, 2nd ed. Approved standard. M22-A2, vol. 16, no. 16. National Committee for Clinical Laboratory Standards, Wayne, PA.

Typical Analysis

"Typical" chemical compositions have been determined on media ingredients. The typical analysis is used to select products for research or production needs when specific nutritional characteristics are required. The specifications for the typical analysis include:

- Physical characteristics,
- Nitrogen content,
- Amino acids,
- Inorganics,
- Vitamins, and
- Biological testing.

All values are presented as weight/weight; % = g/100 g.

Glossary

Ash

The higher the ash content, the lower the clarity of the prepared ingredient. The ash content includes sodium chloride, sulfate, phosphates, silicates and metal oxides. Acid-insoluble ash is typically from silicates found in animal fodder.

Moisture

Lower moisture levels (<5%) are preferred. Higher moisture levels in dehydrated ingredients may reduce stability. In the presence of high moisture and high ambient temperatures, chemical interactions will cause darkening of the product and falling pH. These characteristics indicate product deterioration.

Nitrogen

Total Nitrogen: Total nitrogen is usually measured by the Kjeldhal digestion or titration method. Not all organic nitrogen is nutritive. Percent (%) nitrogen x 6.25 ≈ % proteins, peptides or amino acids present.

Amino Nitrogen: The amino nitrogen value shows the extent of protein hydrolysis by measuring the increase in free amino groups. This is a nutritionally meaningful value.

pH

Changes in pH from specified values, either after storage or processing, indicate deterioration. These changes are usually accompanied by darkening of the end product. Hydrolysates vary in their pH resistance according to their inherent buffering (phosphate) capacity.

Phosphates

High-phosphate ingredients may be unsuitable for pH indicator media due to the inherent buffering of phosphates. However, phosphates do aid in gas production, which can be enhanced by deliberate addition of sodium phosphate.

Sodium Chloride

The NaCl content may reflect significant pH adjustments during processing, e.g., acid hydrolysates. (See Ash).

Trace Metals

Trace metals can directly antagonize antimicrobial activity in vitro or impact toxin production (e.g., *C. diphtheriae* toxin production is

maximal at low concentrations, 0.1- 0.2 mg/l, and inhibited at high concentrations). Chelating agents (e.g., citrate) may be added to culture media to sequester trace metals and clarify the media.

Antigenic Schema for *Salmonella*

Update of the Kauffmann-White Schema[1]

The Centers for Disease Control has modified the Kauffmann-White antigenic schema originally proposed by Ewing.[1-3] The updated schema are used with Difco *Salmonella* Antisera as an aid in the serological identification of *Salmonella*.

All of the *Salmonella* serovars belong to two species, *S. bongori* containing 18 serovars and *S. enterica* containing the remaining 2300-plus serovars which are divided among six subspecies.[1] The six subspecies of *S. enterica* are:

S. enterica subsp. *enterica* (I or 1)
S. enterica subsp. *salamae* (II or 2)
S. enterica subsp. *arizonae* (IIIa or 3a)
S. enterica subsp. *diarizonae* (IIIb or 3b)
S. enterica subsp. *houtenae* (IV or 4)
S. enterica subsp. *indica* (VI or 6)

The legitimate species name for the above strains is *S. choleraesuis*. However, this name may be confused with the serotype named "choleraesuis." At the International Congress for Microbiology in 1986, the International Subcommittee for Enterobacteriaceae agreed to adopt the species name "*S. enterica*."[4] LeMinor and Popoff[5] published a request to the Judicial Commission to use *S. enterica* as the official species name. The Judicial Commission ruled that *S. choleraesuis* is the legitimate name.[6,7] *S. enterica* is used in many countries and is favorably accepted as the species name.[3,8] The Centers for Disease Control has adopted this designation until the problem of naming this species is resolved.[1]

Nomenclature and classification of these bacteria are ever changing.[9] *Salmonella* and the former *Arizona* should be considered a single genus, *Salmonella*.[10] All serovars in subspecies *enterica* are named. Serovars in other subspecies (except some in subspecies *salamae* and *houtenae*) are not named. It is recommended that laboratories report named *Salmonella* serovars by name and unnamed serovars by antigenic formula and subspecies. For the most recent information on nomenclature, consult appropriate references.[1,3,9,10,12]

Serotypes of *Salmonella* are defined based on the antigenic structure of both somatic or cell wall (O) antigens and flagellar (H) antigens. The antigenic formula gives the O antigen(s) first followed by the H antigen(s). The major antigens are separated by colons and the components of the antigens separated by commas. For example, the antigenic formula for *Salmonella typhimurium* is *Salmonella* 1,4,5,12:i:1,2. This means that the strain has O antigen factors 1,4,5 and 12, the flagella phase 1 antigen I, and flagella phase 2 antigens 1 and 2.

Complete identification of *Salmonella* requires cultural isolation, biochemical characterization and serotyping. Any serological results obtained before biochemical identification must be considered as presumptive identification only. Consult Reference 1 and other appropriate references for complete identification of *Salmonella*.[1,3,9,11-14]

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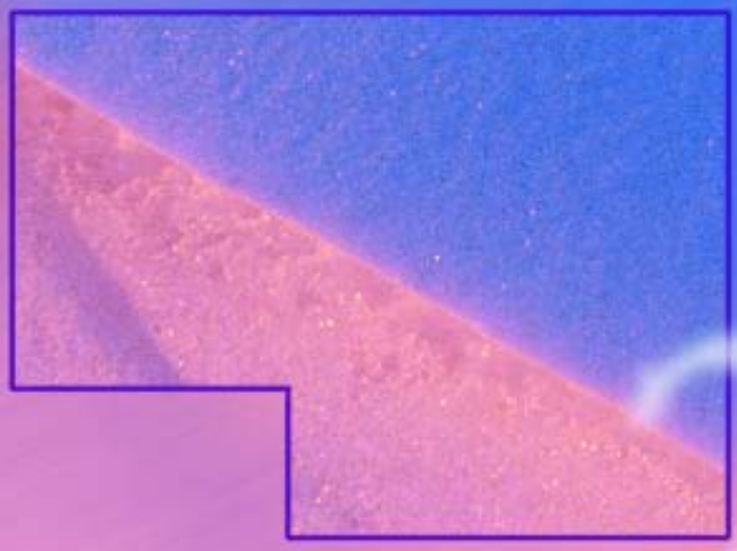

Culture Media and Ingredients, Dehydrated

Agar

Bacto® Agar · Agar Flake · Agar, Granulated · Agar Noble Agar Bacteriological Technical

Intended Use

Bacto® Agar is a solidifying agent in which extraneous matter, pigmented portions and salts have been reduced to a minimum. Bacto® Agar is used in preparing microbiological culture media.

Agar Flake is a solidifying agent used in preparing microbiological culture media.

Agar, Granulated is a solidifying agent used in preparing microbiological culture media.

Agar Noble is a solidifying agent that is essentially free of impurities. It is used in electrophoretic and nutritional procedures and in preparing microbiological culture media when increased purity is required.

Agar Bacteriological Technical is a solidifying agent used in preparing microbiological culture media. Although Agar Bacteriological Technical has wider quality control parameters than other bacteriological agars, solubility, gelation temperature and solidity are carefully monitored to permit its use.

Summary and Explanation

Agar is a phycocolloid extracted from a group of red-purple marine algae (Class Rhodophyceae) including *Gelidium, Pterocladia* and *Gracilaria*. *Gelidium* is the preferred source for Difco agars. Impurities, debris, minerals and pigment are reduced to specified levels during manufacture.

Agar was first suggested for microbiological purposes in 1881 by Fannie Hesse.[1, 2] By the early 1900s, agar became the gelling agent of choice over gelatin because agar remains firm at growth temperatures

User Quality Control

Identity Specifications

	BACTO® AGAR	AGAR FLAKE	AGAR, GRANULATED	AGAR NOBLE	AGAR BACTERIOLOGICAL TECHNICAL
Dehydrated Appearance:	Very light beige, free flowing, homogeneous, granules.	Off-white to light beige, free flowing, flakes.	Very light beige to light tan, free flowing homogeneous, granules.	White to off-white, free flowing,homogeneous, fine granules.	Very light to medium beige, free flowing, homogeneous.
Solution 1.5% solution soluble in distilled or deionized water upon boiling	Solution is very light amber; very slightly to slightly opalescent. Clarity is less than 10 Nephelometric turbidity units.	Solution is very light to light amber, very slightly to slightly opalescent.	Solution is very light to medium amber, very slightly opalescent to opalescent.	Solution is colorless, clear to very slightly opalescent.	Solution is very light to medium amber, opalescent.
Loss on Drying (LOD)	16-20%	Less than or equal to 20%	Less than or equal to 20%	Less than or equal to 20%	Less than or equal to 20%
Ash[6]	Less than or equal to 6.5%	2-5.2%	Less than or equal to 6.5%	Less than or equal to 2%	Less than or equal to 6.5%
Calcium µg/g (ppm)	300-3,000 ppm	Less than or equal to 3,400 ppm	Less than or equal to 3,000 ppm	100-2,600 ppm	Less than or equal to 3,000 ppm
Magnesium µg/g (ppm)	50-1,000 ppm	Less than or equal to 1,850 ppm	Less than or equal to 1,000 ppm	0-750 ppm	Less than or equal to 1,300 ppm
Melting Point	83-89°C	Greater than or equal to 85°C	83-89°C	Greater than or equal to 85°C	Greater than or equal to 85°C
Gelation Point	32-39°C	32-39°C	32-39°C	32-39°C	32-39°C

Cultural Response

Prepare the agar formulation of Nutrient Broth (0003) or LB Broth, Miller (0446) by adding 1.5% agar. Sterilize and pour plates. Inoculate with 100-1,000 CFU of the indicated test organisms and incubate at 35 ± 2°C for 18-24 hours. Record recovery.

	BACTO® AGAR	AGAR FLAKE	AGAR, GRANULATED	AGAR NOBLE	AGAR BACTERIOLOGICAL TECHNICAL
Nutrient Broth with:					
Escherichia coli ATCC® 25922*	Good	Good		Good	Good
Staphylococcus aureus ATCC® 25923*	Good	Good		Good	Good
LB Broth, Miller with:					
Escherichia coli ATCC® 33694 (HB101)			Good		
Saccharomyces cerevisiae ATCC® 9763			Good		

*These cultures are available as Bactrol™ Disks and should be used as directed in the Bactrol Disks Technical Information.

Can of Bacto Agar

for many pathogens. Agar is also generally resistant to a breakdown by bacterial enzymes. The use of agar in microbiological media significantly contributed to the advance of microbiology, paving the way for pure culture isolation and study.

Agar is a gel at room temperature, remaining firm at temperatures as high as 65°C.[3] Agar melts at approximately 85°C, a different temperature from that at which it solidifies, 32-40°C. This property is known as hysteresis. Agar is generally resistant to shear forces; however, different agars may have different gel strengths or degrees of stiffness.

Agar is typically used in a final concentration of 1-2% for solidifying culture media. Smaller quantities (0.05-0.5%) are used in media for motility studies (0.5% w/v) and for growth of anaerobes (0.1%) and microaerophiles.[3]

Specifications for bacteriological grade agar include good clarity, controlled gelation temperature, controlled melting temperature, good diffusion characteristics, absence of toxic bacterial inhibitors, and relative absence of metabolically useful minerals and compounds.

Product Applications

Bacto® Agar is optimized for beneficial calcium and magnesium content. Detrimental ions such as iron and copper are reduced. Bacto® Agar is recommended for clinical applications, auxotrophic studies, bacterial and yeast transformation studies, and bacterial molecular genetics applications.[4, 5]

Agar Flake is recommended for general bacteriological purposes. The quality is similar to Bacto® Agar. However, the flakes are more easily wetted than the granules found in Bacto® Agar.

Agar, Granulated is qualified for culturing recombinant strains of *Escherichia coli* (HB101) and *Saccharomyces cerevisiae*. Agar, Granulated may be used for general bacteriological purposes where clarity is not a strict requirement.

Noble Agar is extensively washed and bleached. This agar should be used for applications where extreme clarity and high purity are required. Noble Agar is suitable for immunodiffusion, some electrophoretic applications, and as a substrate for mammalian or plant tissue culture.

Agar Bacteriological Technical is suitable for many bacteriological applications. This agar is not highly processed, has broader technical specifications than other Difco agars, and is not recommended for growth of fastidious organisms.

Typical Analysis

	BACTO® AGAR	AGAR, GRANULATED	AGAR NOBLE	AGAR BACTERIOLOGICAL TECHNICAL
Physical Characteristics				
Ash (%)	3.6	3.4	1.3	4.1
Color	lt. beige	lt. beige	off white	lt beige
Texture	granular free-flowing	granular free-flowing	fine granular free flowing	granular free-flowing
Clarity, 1.5% Soln (NTU)	4.3	5.3	3.7	26.2
Loss on Drying (%)	17.3	12.2	16.0	18.2
pH, 1.5% Soln	6.5	6.6	5.7	6.9
Gel Strength (g/cm^2)	600	560	700	613
Gelation Point(°C)	35°C	35°C	35°C	36°C
Melting Point (°C)	88°C	88°C	87°C	88°C
Biological Testing (CFU/g)				
Spore Count	<1,000	<1,000	<1,000	4,300
Standard Plate Count	<1,000	<1,000	<1,000	2,725
Inorganics (%)				
Calcium	0.179	0.133	0.015	0.110
Chloride	0.021	<0.005	<0.050	0.172
Cobalt	<0.001	<0.001	<0.001	<0.001
Copper	<0.001	<0.001	<0.001	<0.001
Iron	0.002	0.003	<0.001	0.002
Lead	<0.001	<0.001	<0.001	<0.001
Magnesium	0.068	0.041	0.002	0.093
Manganese	<0.001	<0.001	<0.001	<0.001
Nitrate	<0.005	<0.005	<0.050	<0.005
Phosphate	<0.005	0.010	<0.050	0.015
Potassium	0.121	0.079	0.022	0.124
Sodium	0.837	0.776	0.335	0.932
Sulfate	1.778	1.710	0.663	0.367
Sulfur	0.841	0.868	0.333	0.646
Tin	<0.001	<0.001	<0.001	<0.001
Zinc	<0.001	<0.001	<0.001	<0.001

Precautions

1. For Laboratory and Manufacturing Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store dehydrated agar below 30°C. Dehydrated agar is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use the product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bacto® Agar
Agar Flake
Agar, Granulated
Agar Noble
Agar Bacteriological Technical

Materials Required But Not Provided

Materials vary depending on the application.

Method of Preparation

Method of preparation varies depending on the application.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Bacto® Agar, Agar Flake, Agar, Granulated, Agar Noble or Agar Bacteriological Technical.

Results

Refer to appropriate references and procedures for results.

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Packaging

Bacto® Agar	100 g	0140-15
	1 lb	0140-01
	2 kg	0140-07
	10 kg	0140-08
Agar Flake	500 g	0970-17
Agar, Granulated	500 g	0145-17
	2 kg	0145-07
	10 kg	0145-08
Agar Noble	100 g	0142-15
	500 g	0142-17
Agar Bacteriological Technical	500 g	0812-17
	2 kg	0812-07
	10 kg	0812-08

Bacto® 2xYT

Intended Use

Bacto 2xYT is used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

2xYT is a nutritionally rich growth medium designed for growth of recombinant strains of *Escherichia coli*. This medium is also used for propagation of M13 bacteriophage for sequencing and phage display research.[1-3] The components of 2xYT provide nitrogen and growth factors that allow bacteriophage to reproduce in large quantities without exhausting the host. *E. coli* grows more rapidly in this rich medium because it provides amino acids, nucleotide precursors, vitamins and other metabolites that the cell would otherwise have to synthesize.[2]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear.
Reaction of 3.1% Solution 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare 2xYT per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli (C600)	23724	100-300	Good
Escherichia coli (JM103)	39403	100-300	Good
Escherichia coli (JM107)	47014	100-300	Good
Escherichia coli (HB101)	33694	100-300	Good
Escherichia coli (DH-1)	33849	100-300	Good
Escherichia coli (DH-5)	53868	100-300	Good

The cultures listed are the minimum that should be used for performance testing.

Principles of the Procedure

Tryptone and Yeast Extract provide the necessary nutrients and cofactors required for excellent growth of *E. coli*. Sodium Chloride is included to provide a suitable osmotic environment.

Formula

2xYT

Formula Per Liter

Bacto Tryptone	16 g
Bacto Yeast Extract	10 g
Sodium Chloride	5 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

2xYT

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Dissolve 31 grams in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Please consult appropriate references for recommended test procedures.[1-3]

Results

Growth is evident in the form of turbidity.

References

1. **Sambrook, J., E. F. Fritsch, and T. Maniatis**. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl**. 1994. Current protocols in molecular biology, vol 1. Current Protocols, New York, N.Y.
3. **Davis, L. G., M. D. Dibner, and J. F. Battey**. 1986. Basic methods in molecular biology. Elsevier, New York, N.Y.

Packaging

2xYT	500 g	0440-17-0

Bacto® A-1 Medium

Intended Use

Bacto A-1 Medium is used for detecting fecal coliforms in water.

Also Known As

A-1 Medium is also referred to as A-1 Broth.

Summary and Explanation

Since the early 1900s enumeration of coliform organisms, specifically *E. coli*, has been used to determine water purity. Elevated-temperature, most-probable-number (MPN) methods are routinely used for the analysis of water and food samples for the presence of fecal coliforms. One limiting factor in using *E. coli* is the length of time required for complete identification.[1] A-1 Medium was formulated to hasten the recovery of *E. coli* and reduce the incidence of false positive cultures.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, lumpy.
Solution:	3.15% solution, soluble in distilled or deionized water on boiling. Solution is light amber, opalescent immediately after sterilization. Solution is light amber, clear, may have flocculent precipitate upon cooling.
Prepared Medium:	(When cooled to room temperature) - Light amber, clear, flocculent precipitate may be present.
Reaction of 3.15% Solution at 25°C:	pH 6.9 ± 0.1

Cultural Response

Prepare A-1 Medium per label directions. Prepare tubes by placing fermentation vials and 10 ml amounts of medium into tubes. Inoculate and incubate at 35 ± 2°C for 3 hours. Transfer tubes to a 44.5°C waterbath for 21 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU(APPROX.)	GROWTH
Bacillus subtilis†	6633	100	none
Enterobacter aerogenes	13048*	100	poor to good/may produce gas
Enterococcus faecalis	19433*	100	none to poor
Escherichia coli	25922*	100	good/with gas production
Escherichia coli	13762	100	good/with gas production

The cultures listed are the minimum that should be used for performance testing.
†*Bacillus subtilis* is available as Subtilis Spore Suspension.

Uninoculated tube

Escherichia coli ATCC® 25922 with fermentation vial

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disk Technical Information.

In 1972 Andrews and Presnell developed A-1 Medium. A-1 Medium recovers *E. coli* from estuarine water in 24 hours instead of 72 hours, and in greater numbers without the preenrichment step.[2] Using a 3-hour preincubation step for the enumeration of coliforms in chlorinated wastewater gave results that were statistically comparable to those obtained in the two-step MPN technique.[3]

A-1 Medium can be used in a single-step procedure for the detection of fecal coliforms in source water, seawater, treated wastewater and foods. Prior enrichment in a presumptive medium is not required.[4] A-1 Medium conforms to standard methods for the isolation of fecal coliforms in water and foods.[4,5,6]

Principles of the Procedure

Tryptone provides the nitrogen, vitamins, minerals and amino acids in A-1 Medium. Lactose is the carbon source and, in combination with Salicin, provides energy for organism growth. Sodium Chloride maintains the osmotic balance of the medium. Triton X-100 is a surfactant.

Formula

A-1 Medium

Formula Per Liter	
Bacto Tryptone	20 g
Bacto Lactose	5 g
Sodium Chloride	5 g
Bacto Salicin	0.5 g
Triton X-100	1 ml

Final pH 6.9 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store prepared medium in the dark at room temperature for no longer than 7 days.[4]

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet the specifications for identity and performance.

Procedure

Materials Provided

A-1 Medium

Materials Required But Not Provided

Glassware
Fermentation vials
Autoclave
Incubator (35°C)
Waterbath (44.5°C)
Test tubes
Distilled or deionized water

Method of Preparation

1. Suspend 31.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 10 minutes.

NOTE: For 10 ml water samples, prepare double-strength medium to ensure the ingredient concentrations are not reduced below those of the standard medium.[4]

Specimen Collection and Preparation

Obtain and process specimens according to the procedures established by laboratory policy or standard methods.[4,5,6]

Test Procedure

1. Inoculate tubes of A-1 Medium as directed in standard methods.[4,5,6]
2. Incubate at 35 ± 0.5°C for 3 hours.
3. Transfer tubes to a water bath at 44.5 ± 0.2°C and incubate for an additional 21 ± 2 hours.
4. Maintain water level in bath above level of liquid in inoculated tubes.

Results[5]

Gas production in the inverted vial, or dissolved gas that forms fine bubbles when slightly agitated, is a positive reaction indicating the presence of fecal coliforms. Calculate fecal coliform densities using MPN tables from standard methods.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Fecal coliform counts are usually greater than *E. coli* counts.[5]
3. Interpretation of test procedure using A-1 Medium requires understanding of the microflora of the specimen.[5]

References

1. **Andrews, W. H., C. D. Diggs, and C. R. Wilson.** 1975. Evaluation of a medium for the rapid recovery of *Escherichia coli* from shellfish. Appl. Microbiol. **29**:130- 131.
2. **Andrews, W. H., and M. W. Presnell.** 1972. Rapid recovery of *Escherichia coli* from estuarine water. Appl. Microbiol. **23**:521-523.
3. **Standridge, and Delfino.** 1981. Appl. Environ. Microbiol. **42**:918.
4. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.)**. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
6. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

A-1 Medium	500 g	1823-17

Bacto® AC Broth
Bacto AC Broth w/o Dextrose

Intended Use

Bacto AC Broth is used for cultivating a wide variety of microorganisms and for the sterility testing of turbid or viscous solutions and other materials not containing mercurial preservatives.

Bacto AC Broth w/o Dextrose is used, with the addition of a carbohydrate, for cultivating a wide variety of microorganisms.

Summary and Explanation

AC Broth and AC Broth w/o Dextrose possess growth-promoting properties for voluminous growth of a wide variety of microorganisms. Christensen[1] and Malin and Finn[2] reported that AC Medium does not exhibit the toxicity shown by media containing sodium thioglycollate.

User Quality Control

Identity Specifications

AC Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.4% solution, soluble in distilled or deionized water. Solution is medium to dark amber, clear to very slightly opalescent.
Prepared Tubes:	Light to medium amber, clear to very slightly opalescent.
Reaction of 3.4% Solution at 25°C:	pH 7.2 ± 0.2

AC Broth w/o Dextrose

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.92% solution, soluble in distilled or deionized water. Solution is medium to dark amber, clear to very slightly opalescent.
Prepared Tubes:	Medium to dark amber, clear to very slightly opalescent.
Reaction of 2.92% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare AC Broth or AC Broth w/o Dextrose per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Corynebacterium diphtheriae Type *mitis*	8024	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Several early studies reported on the wide variety of organisms able to grow on AC Medium.[3,4,5] AC Broth is suitable for use in the detection of obligately aerobic contaminants in biologicals and other products. AC Broth and AC Broth w/o Dextrose are also useful in the isolation and cultivation of many common pathogenic and saprophytic aerobes.[6] The media can be used to test the sterility of biologicals and solutions that do not contain mercurial preservatives. Fluid Thioglycollate Medium should be employed for the sterility testing of solutions containing mercurial preservatives.

AC Broth w/o Dextrose has the same formula as AC Broth except that the dextrose is omitted, allowing for the addition of other carbohydrates if desired.

Principles of the Procedure

Proteose Peptone No. 3, Beef Extract, and Malt Extract provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Vitamins and cofactors required for growth as well as additional sources of nitrogen and carbon are provided by Yeast Extract. Dextrose is included in AC Broth as a carbon energy source. Ascorbic Acid is added to clarify the solution.

Formula

AC Broth

Formula Per Liter

Bacto Proteose Peptone No. 3	20 g
Bacto Beef Extract	3 g
Bacto Yeast Extract	3 g
Bacto Malt Extract	3 g
Bacto Dextrose	5 g
Ascorbic Acid	0.2 g

Final pH 7.2 ± 0.2 at 25°C

AC Broth w/o Dextrose

Formula Per Liter

Bacto Proteose Peptone No. 3	20 g
Bacto Beef Extract	3 g
Bacto Yeast Extract	3 g
Bacto Malt Extract	3 g
Ascorbic Acid	0.2 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated media below 30°C. The dehydrated media are very hygroscopic. Keep container tightly closed.

AC Broth

Store prepared medium at 15-30°C. After prolonged storage, reheat in flowing steam or a boiling water bath for a few minutes to drive off dissolved gases. Cool without agitation.

AC Broth w/o Dextrose

Store prepared medium at 15-30°C.

Expiration Date

The expiration date applies to the products in their intact containers when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

AC Broth
AC Broth w/o Dextrose

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend appropriate amount of medium in 1 liter distilled or deionized water:
   AC Broth - 34 grams;
   AC Broth w/o Dextrose - 29.2 grams.
2. If necessary, warm slightly to dissolve completely.
3. Dispense as desired. Autoclave at 121° C for 15 minutes.
   If the medium is not used the same day it is sterilized, place in flowing steam or a boiling water bath for a few minutes to drive off dissolved gases. Allow to cool without agitation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. When reheating prepared media to drive off dissolved gases do not overheat because this may result in decreased growth.

References

1. Paper read at New York Meeting. Am. Pub. Health Assoc., 1944.
2. **Malin, B., and R. K. Finn.** 1951. The use of a synthetic resin in anaerobic media. J. Bacteriol. **62**:349-350.
3. **Reed, G. B., and J. H. Orr.** 1943. Cultivation of anaerobes and oxidative-reduction potentials. J. Bacteriol. **45**:309-320.
4. **Schneiter, R., J. E. Dunn, and B. H. Caminita.** 1945. Studies in connection with the selection of a satisfactory culture medium for bacterial air sampling. Pub. Health Reports **60**:789-806.
5. **Kolb, R. W., and R. Schneiter.** 1950. The germicidal and sporicidal efficacy of methyl bromide for *Bacillus anthracis*. J. Bacteriol. **59**:401-412.
6. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 13-14. Williams & Wilkins, Baltimore, MD.

Packaging

AC Broth	500 g	0317-17
AC Broth w/o Dextrose	10 kg	0599-08

Bacto® APT Agar
Bacto APT Broth

Intended Use

Bacto APT Agar is used for cultivating heterofermentative lactobacilli and other organisms requiring high thiamine content. It is also used for maintaining stock cultures of *Lactobacillus viridescens* ATCC® 12706 used in the assay of thiamine.

Bacto APT Broth is used for culturing *Lactobacillus viridescens* ATCC 12706 used in the assay of thiamine. It is also used for cultivating heterofermentative lactobacilli and other organisms requiring high thiamine content.

Also Known As

All Purpose Tween

Summary and Explanation

Evans and Niven[1] investigated cultivating the heterofermentative lactobacilli that cause the faded or greenish discoloration of cured meat products, while Deibel, Evans and Niven[2] investigated thiamine requiring bacteria, specifically *Lactobacillus viridescens*. Their formulations led to the development of APT Agar and APT Broth.

The lactic acid bacteria, a group of acid producing bacteria, include the genera *Streptococcus*, *Leuconostoc*, *Pediococcus* and *Lactobacillus*.[3] These organisms are widespread in nature and are associated with bacterial spoilage of foods such as dairy, meat and vegetable products.[3] One use of APT Agar and APT Broth is for cultivating these heterofermentative lactic acid bacteria from food products.[3]

APT Agar and APT Broth are also used in the microbiological assay of thiamine. In the assay, APT Agar is the maintenance medium that preserves the viability and sensitivity of *Lactobacillus viridescens* ATCC 12706. APT Broth is used for growing *Lactobacillus viridescens* ATCC 12706 and preparing the inoculum.

Principles of the Procedure

APT Agar and APT Broth contain Tryptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate. The Manganese Chloride, Magnesium Sulfate and Ferrous Sulfate provide ions used in replication by lactobacilli. Sorbitan Monooleate Complex is a source of fatty acids required by lactobacilli. Bacto Agar is the solidifying agent in APT Agar.

Formula

APT Agar

Formula Per Liter

Bacto Yeast Extract	7.5 g
Bacto Tryptone	12.5 g
Bacto Dextrose	10 g
Sodium Citrate	5 g
Thiamine Hydrochloride	0.001 g
Sodium Chloride	5 g
Dipotassium Phosphate	5 g
Manganese Chloride	0.14 g
Magnesium Sulfate	0.8 g
Ferrous Sulfate	0.04 g
Sorbitan Monooleate Complex	0.2 g
Bacto Agar	15 g

Final pH 6.7 ± 0.2 at 25°C

User Quality Control

Identity Specifications

APT Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.12%, soluble in distilled or deionized water on boiling. Solution, upon cooling, is medium amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Medium:	Medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 6.12% Solution at 25°C:	pH 6.7 ± 0.2

APT Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.62%, soluble in distilled or deionized water with slight heating. Solution, upon cooling, is light to medium amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Medium:	Light to medium amber, clear to very slightly opalescent without significant precipitate.
Reaction of 4.62% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare APT Agar and APT Broth per label directions. Inoculate and incubate at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus fermentum	9338	100-1,000	good
Lactobacillus viridescens	12706	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

APT Broth

Formula Per Liter

Bacto Yeast Extract	7.5 g
Bacto Tryptone	12.5 g
Bacto Dextrose	10 g
Sodium Citrate	5 g
Thiamine Hydrochloride	0.001 g
Sodium Chloride	5 g
Dipotassium Phosphate	5 g
Manganese Chloride	0.14 g
Magnesium Sulfate	0.8 g
Ferrous Sulfate	0.04 g
Sorbitan Monooleate Complex	0.2 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

APT Agar
APT Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water:
   APT Agar: 61.2 grams;
   APT Broth: 46.2 grams.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Avoid overheating.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

For maintaining stock cultures of *Lactobacillus viridescens* ATCC® 12706 prepare a stab inoculation. Prepare stock cultures in triplicate at monthly intervals. One of the transfers is saved for the

preparation of stock cultures. The others are used to prepare inoculum in APT Broth for assay as needed. Following incubation at 35-37°C for 24-48 hours, store stock cultures at 2-8°C.

Results

Refer to appropriate references and procedures for results.

References

1. **Evans, J. B., and C. F. Niven, Jr.** 1951. Nutrition of the heterofermentative lactobacilli that cause greening of cured meat products. J. Bact. **62**:599-603.
2. **Deibel, R. H., J. B. Evans, and C. F. Niven, Jr.** 1957. Microbiological assay for thiamine using *Lactobacillus viridescens*. J. Bact. **74**:818-821.
3. **Vedamuthu, E. R., M. Raccach, B. A. Glatz, E. W. Seitz, and M. S. Reddy.** 1992. Acid-producing microorganisms, p. 225-238. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

APT Agar	500 g	0654-17
	2 kg	0654-07
	10 k	0654-08
APT Broth	500 g	0655-17

Bacto® Acetate Differential Agar

Intended Use

Bacto Acetate Differential Agar is used for differentiating microorganisms of the *Shigella* genus from those of the *Escherichia* genus.

Also Known As

Acetate Differential Agar is also known as Sodium Acetate Agar.

Summary and Explanation

Although classified taxonomically as different species for clinical reasons, *Shigella* species and *E. coli* are essentially the same genus and species. Their DNA relatedness is high, they are difficult to differentiate biochemically, and they cross-react serologically.[1] One way they can be differentiated is by using a medium containing sodium acetate as a sole source of carbon. Many strains of *E. coli* are able to use acetate as a carbon source, whereas typical cultures of *Shigella* are unable to grow.

Trabulsi and Ewing[2] developed Acetate Differential Agar by substituting sodium acetate for sodium citrate in their basal medium, Simmons Citrate Agar. They demonstrated that none of the *Shigella* tested grew on the Acetate Differential Agar. A large percentage of *E. coli* strains, belonging to various O antigen groups, did use the acetate within 2 to 7 days of incubation.

The majority of *Salmonella, Citrobacter, Klebsiella, Enterobacter* and *Serratia* groups use acetate and grow on Acetate Differential Agar within 1 to 7 days. *Proteus* and *Providencia* groups, however, fail to grow on the medium. Several standard methods list Acetate

User Quality Control

Identity Specifications

Dehydrated Appearance:	Medium yellowish-tan to light green, free-flowing, homogeneous.
Solution:	2.92% solution, soluble in distilled or deionized water on boiling. Solution is emerald green, slightly opalescent.
Prepared Medium:	Emerald green to green, slightly opalescent.
Reaction of 2.92% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Prepare Acetate Differential Agar per label directions. Inoculate the medium and incubate at 35 ± 2°C for 2-7 days. Acetate utilization is indicated by a color change of the slant from green to blue.

ORGANISMS	ATCC®	GROWTH	APPEARANCE
Escherichia coli	25922*	good	blue
Shigella sonnei	25931*	poor to good	green

The organisms listed are the minimum that should be used for performance testing.

Uninoculated tube — *Escherichia coli* ATCC® 25922

Differential Agar as a possible medium for the differentiation of *Enterobacteriaceae*.[2,3,4]

Principles of the Procedure

Acetate Differential Agar consists of a mixture of salts and sodium acetate as a sole source of carbon. Brom Thymol Blue is added to detect the alkaline products resulting from acetate utilization. Mono Ammonium Phosphate and Dipotassium Phosphate provide buffering capability. Bacto Agar is a solidifying agent.

Formula

Acetate Differential Agar

Formula Per Liter

Sodium Acetate	2 g
Magnesium Sulfate	0.1 g
Sodium Chloride	5 g
Mono Ammonium Phosphate	1 g
Dipotassium Phosphate	1 g
Bacto Brom Thymol Blue	0.08 g
Bacto Agar	20 g

Final pH 6.7 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Acetate Differential Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
0.85% NaCl solution

Method of Preparation

1. Suspend 29.2 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Dispense into tubes to allow a 10 mm butt and a 30 mm slant.
4. Autoclave at 121°C for 15 minutes.
5. Allow tubes to cool in a slanted position to give the recommended butt and slant size.

Test Procedure

1. Inoculate agar slant surfaces with 16-18 hour cultures emulsified in 1 ml of 0.85% sodium chloride solution.
2. Incubate aerobically at 35 ± 2°C for at least 7 days; read daily, examining for a change in the color of the medium from green to blue.

Results

Positive: Blue
Negative: Green

Limitations of the Procedure

1. Some strains of *E. coli* and nonmotile, anaerogenic *E. coli* (*Alkalescens-Dispar*) grow slowly or not at all and, thus, may give a false-negative reaction.
2. Further biochemical, physiological and serological tests are required to differentiate species.
3. False-positive results may occur from a too heavy inoculum.
4. MacFaddin[5] suggests that correct results occur only when some syneresis fluid is present in the bottom of the tube (junction of the slant and butt).

References

1. **Gray, L. D.** 1995. *Escherichia*, *Salmonella*, *Shigella*, and *Yersinia*, pp. 450-456. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
3. **Andrews, W. H., G. A. June, and P. S. Sherrod.** 1995. *Shigella*, p. 6.01-6.06. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 17-20. Williams & Wilkins, Baltimore, MD.

Packaging

Acetate Differential Agar	500 g	0742-17

Bacto® Actinomycete Isolation Agar
Bacto Glycerol

Intended Use

Bacto Actinomycete Isolation Agar is used with added glycerol for isolating and cultivating actinomycetes from soil and water.

Bacto Glycerol is used in preparing microbiological culture media.

Summary and Explanation

Although some genera are important to human medicine, most of the actinomycetes are part of the indigenous flora of soil, water, and vegetation. Actinomycetes may impart a musty odor to water or a muddy flavor to fish.[2] Actinomycetes can cause massive growths which will form a thick foam in the activated sludge process, causing a disruption in wastewater treatment.[3,4] Actinomycetes are gram positive, acid-fast cells, growing as filaments that may branch and may form irregularly shaped rods and cocci.

Olsen[1] formulated Actinomycete Isolation Agar for isolating and cultivating actinomycetes from soil and water. The formula, supplemented with Glycerol, is a highly purified fermentable alcohol used occasionally for differentiating certain bacteria and in media for isolating and culturing fastidious bacteria.

Principles of the Procedure

Actinomycete Isolation Agar contains Sodium Caseinate which is a source of nitrogen. Asparagine is an amino acid and a source of organic nitrogen. Sodium Propionate is a substrate used in anaerobic fermentation. Dipotassium Phosphate provides buffering capability to maintain pH balance. Magnesium Sulfate and Ferrous Sulfate provide sources of sulfates and metallic ions. Bacto Agar is the solidifying agent. The added Glycerol is a source of carbon.

User Quality Control

Identity Specifications

Actinomycete Isolation Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.2% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, opalescent to opaque with precipitation.
Prepared Medium:	Medium amber, opalescent.
Reaction of 2.2% Solution with 0.5% Glycerol at 25°C:	pH 8.1 ± 0.2

Cultural Response

Prepare Actinomycete Isolation Agar per label directions with the addition of 0.5% Glycerol. Inoculate and incubate at 30 ± 2°C for up to 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Streptomyces achromogenes	12767	100-1,000	good
Streptomyces albus	3004	100-1,000	good
Streptomyces lavendulae	8664	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Formula

Actinomycete Isolation Agar

Formula Per Liter

Sodium Caseinate	2 g
Asparagine	0.1 g
Sodium Propionate	4 g
Dipotassium Phosphate	0.5 g
Magnesium Sulfate	0.1 g
Ferrous Sulfate	0.001 g
Bacto Agar	15 g

Final pH 8.1 ± 0.2 at 25°C

Glycerol

Not applicable

Precautions

1. For Laboratory Use.
2. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Glycerol at 15-30°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Actinomycete Isolation Agar
Glycerol

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (30°C)

Method of Preparation

1. Suspend 22 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Add 5 grams Glycerol.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs. Transport immediately to the laboratory, in accordance with recommended guidelines.
2. Process each specimen as appropriate for that specimen.

Test Procedure

Inoculate medium and incubate at 30°C for up to 72 hours.

Results

Refer to appropriate references and procedures for results.

References

1. **Olsen, E. H.** 1960. Personal Communication.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg**. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Lechevalier, H. A.** 1975. Actinomycetes of sewage-treatment plants. Environ. Protection Technol. Ser., EPA-600/2-75-031, U. S. Environmental Protection Agency, Cincinnati, OH.
4. **Lechevalier, M. P., and H. A. Lechevalier.** 1974. *Nocardia amarae*, sp. nov., an actinomycete common in foaming activated sludge. Int. J. Syst. Bacteriol. **24**:278.

Packaging

Actinomycete Isolation Agar	100 g	0957-15
	500 g	0957-17
Glycerol	100 g	0282-15
	500 g	0282-17

Bacto® Agar Medium No. F

Intended Use

Bacto Agar Medium No. F is a selective medium used for detecting *Enterobacteriaceae* and other gram-negative bacteria in pharmaceutical products.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.15% solution, soluble in distilled or deionized water on boiling. Solution is reddish-purple, slightly opalescent.
Prepared Medium:	Reddish-purple, slightly opalescent without a precipitate.
Reaction of 5.15% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Agar Medium No. F per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	COLONY DESCRIPTION
Escherichia	11775	100-1,000	good	reddish-purple, may have a slight precipitate around the colonies
Salmonella gallinarum	9184	100-1,000	good	reddish-purple, may have a slight precipitate around the colonies
Staphylococcus aureus	6538	1,000-2,000	inhibited	–

The cultures listed are the minimum that should be used for performance testing.

Summary and Explanation

Agar Medium No. F is based on the formula for Agar Medium F (Agar Medium with Bile, Crystal Violet, Neutral Red and Glucose) described in DAB, 10th Edition. Agar Medium No. F is recommended for use in the detection of *Enterobacteriaceae* and other gram-negative bacteria in pharmaceuticals.[1]

Principles of the Procedure

Agar Medium No. F, based on Violet Red Bile Agar and Violet Red Bile Glucose Agar, uses Sodium Cholate instead of the Bile Salts No. 3 used in Violet Red Bile Agar and Violet Red Bile Glucose Agar. Carbon and nitrogen sources required for growth of a variety of organisms are provided by Bacto Peptone and Yeast Extract. Selectivity is due to the presence of Crystal Violet and Sodium Cholate which markedly to completely inhibit growth of gram-positive microorganisms. Bacto Agar is the solidifying agent.

Differentiation is based on the fermentation of Dextrose and Lactose. Organisms growing in this medium that can ferment dextrose, such as members of the family *Enterobacteriaceae*, produce a localized pH drop which, followed by absorption of the Neutral Red, imparts a reddish-purple color to the colony. A zone of precipitated Sodium Cholate may also be present due to this drop in pH. These reactions are further intensified in those organisms that can ferment both lactose and dextrose.

Formula

Agar Medium No. F

Formula Per Liter

Bacto Peptone	7 g
Bacto Yeast Extract	3 g
Bacto Lactose	10 g
Bacto Dextrose	10 g

Sodium Chloride	5 g
Sodium Cholate	1.5 g
Neutral Red	0.03 g
Crystal Violet	0.002 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Agar Medium No. F

Materials Required But Not Provided

Lactose Broth
Enterobacteriaceae Enrichment Broth Mossel (EE Broth Mossel)
Flasks with closures
Distilled or deionized water
Incubator (35°C)
Polysorbate 20 or Polysorbate 80

Method of Preparation

1. Suspend 51.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Sterilize by steaming for 30 minutes. Do Not Autoclave.

Specimen Collection and Preparation

1. Collect samples in sterile containers and transport immediately to the laboratory following recommended guidelines.[1,2]
2. Process each sample using procedures appropriate for that sample.[1,2]

Test Procedure[1,2]

1. Pre-enrich the sample in Lactose Broth. If the sample is insoluble in water, add 0.1 ml of polysorbate 20 or polysorbate 80 to the Lactose Broth.
2. Homogenize the mixture and incubate at 35 ± 2°C for 2-5 hours.
3. Transfer 1 ml of enriched Lactose Broth to 100 ml of EE Broth Mossel (Enterobacteriaceae Enrichment Broth-Mossel).
4. Incubate at 35 ± 2°C for 24-48 hours.
5. Subculture all enrichment broth cultures showing growth onto Agar Medium No. F.
6. Incubate at 35 ± 2°C for 18-24 hours.
7. Examine plates for the presence of presumptive *Enterobacteriaceae* colonies.

Results

Colonies of the family *Enterobacteriaceae* are reddish-purple in color and are generally surrounded by a zone of precipitated bile salt. Growth of gram-positive organisms is markedly to completely suppressed. Further biochemical testing is necessary to confirm the presence and identification of *Enterobacteriaceae*. Consult appropriate references for further information on identification of *Enterobacteriaceae*.[3,4]

References

1. **DAB, 10th Edition.** 1991. V.2 Biology, V.2.1.8 Proving Certain Microorganisms, VIII.10 Media (Microbiological Pollution), Frankfurt/Main.
2. **British Pharmacopoeia, Volume II, Appendix XVI.** 1988. HMSO, London.
3. **Farmer, J. J.** 1995. *Enterobacteriaceae*: introduction and identification. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Agar Medium No. F	500 g	0666-17

Amino Acid Assay Media

Bacto® Lysine Assay Medium · Bacto Methionine Assay Medium Bacto Cystine Assay Medium

Intended Use

Bacto Lysine Assay Medium is used for determining lysine concentration by the microbiological assay technique.

Bacto Methionine Assay Medium is used for determining methionine concentration by the microbiological assay technique.

Bacto Cystine Assay Medium is used for determining L-cystine concentration by the microbiological assay technique.

Also Known As

Lysine Assay Medium, Methionine Assay Medium and Cystine Assay Medium are also referred to as Amino Acid Assay Media.

Summary and Explanation

Amino Acid Assay Media are prepared for use in the microbiological assay of amino acids. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose.
2. Inoculum Media: To condition the test culture for immediate use.
3. Assay Media: To permit quantitation of the amino acid under test. They contain all the factors necessary for optimal growth of the test organism except the single essential amino acid to be determined.

Amino Acid Assay Media are prepared according to the formulations of Steel et al.[1] They are used in the microbiological assay of amino acids using *Pediococcus acidilactici* ATCC® 8042 as the test organism.

Principles of the Procedure

Lysine Assay Medium, Methionine Assay Medium and Cystine Assay Medium contain all the factors essential for the growth of *Pediococcus acidilactici* ATCC® 8042, except the amino acid under assay. The addition of the amino acid in specified increasing concentrations gives a growth response by the test organism.

Formula

Lysine Assay Medium, Methionine Assay Medium, or Cystine Assay Medium

All amino acid assay media contain the following formula. Omit the particular amino acid to be assayed from the medium.

Formula Per Liter		
Bacto Dextrose	50	g
Sodium Acetate	40	g
Ammonium Chloride	6	g
Monopotassium Phosphate	1.2	g
Dipotassium Phosphate	1.2	g
Magnesium Sulfate	0.4	g
Ferrous Sulfate	20	mg
Manganese Sulfate	40	mg
Sodium Chloride	20	mg
Adenine Sulfate	20	mg
Guanine Hydrochloride	20	mg
Uracil	20	mg
Xanthine	20	mg
Thiamine Hydrochloride	1	mg
Pyrodoxine Hydrochloride	2	mg
Pyridoxamine Hydrochloride	600	mg
Pyridoxal Hydrochloride	600	mg
Calcium Pantothenate	1	mg
Riboflavin	1	mg
Nicotinic Acid	2	mg
p-Aminobenzoic Acid	200	µg
Biotin	2	µg
Folic Acid	20	µg
Glycine	0.2	g
DL-Alanine	0.4	g
Bacto Asparagine	0.8	g
L-Aspartic Acid	0.2	g
L-Proline	0.2	g
DL-Serine	0.1	g
DL-Tryptophane	80	mg
L-Cystine	0.1	g
L-Glutamic Acid	0.6	g
L-Histidine Hydrochloride	0.124	g
DL-Phenylalanine	0.2	g
DL-Threonine	0.4	g
L-Tyrosine	0.2	g
DL-Valine	0.5	g
L-Lysine Hydrochloride	0.5	g
DL-Methionine	0.2	g
DL-Isoleucine	0.5	g
DL-Leucine	0.5	g
L-Arginine Hydrochloride	0.484	g

Final pH 6.7 ± 0.2 at 25ºC

User Quality Control

Identity Specifications

Lysine Assay Medium, Methionine Assay Medium, or Cystine Assay Medium

Dehydrated Appearance:	White to off-white, homogeneous, may have a tendency to clump.
Solution:	5.25% (single strength) and 10.5% (double strength) solution, soluble in distilled or deionized water upon boiling. Solution (single strength) is light to medium amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Medium:	Single strength-light to medium amber, clear.
Reaction of 5.25% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare Lysine Assay Medium, Methionine Assay Medium and Cystine Assay Medium per label directions. These media will support the growth of *Pediococcus acidilactici* ATCC® 8042 when supplemented with the appropriate amino acid. Test Lysine Assay Medium by creating a standard curve using L-Lysine at 0 to 300 µg per 10 ml. Test Methionine Assay Medium by creating a standard curve using DL-Methionine at 0 to 60 µg per 10 ml. Test Cystine Assay Medium by creating a standard curve using L-Cystine at 0 to 50 µg per 10 ml.

The test organism listed is the minimum used for performance testing.

Precautions

1. For Laboratory Use.
2. Great care to avoid contamination of media or glassware must be taken in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware is heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
3. **Methionine Assay Medium and Cystine Assay Medium**

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust.

Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Kidney, Bladder.

FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

4. Take precautions to keep sterilizing and cooling conditions uniform throughout the assay.
5. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated media at 2-8ºC. The dehydrated medium is very hygroscopic and may be stored in a container with calcium chloride or other desiccant. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Lysine Assay Medium or
Methionine Assay Medium or
Cystine Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Pediococcus acidilactici* ATCC® 8042
Sterile tubes, optically standardized
Centrifuge
Spectrophotometer (660 nm)
L-Lysine HCl
DL-Methionine
L-Cystine
Sterile 0.85% NaCl

Method of Preparation

Lysine Assay Medium, Methionine Assay Medium, and Cystine Assay Medium

1. Suspend 10.5 grams in 100 ml distilled or deionized water.
2. Boil for 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedure. The samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Stock Culture and Inoculum

Stock cultures of *Pediococcus acidilactici* ATCC® 8042 are prepared by stab inoculation into tubes of Lactobacilli Agar AOAC or Micro Assay Culture Agar. Incubate cultures at 35-37°C for 24 hours. Store stock cultures at 2-8°C. Make transfers at monthly intervals in triplicate.

The inoculum for assay is prepared by subculturing the test organism into 10 ml Lactobacilli Broth AOAC or Micro Inoculum Broth. Incubate at 35-37ºC for 16-24 hours. After incubation, centrifuge the cells under aseptic conditions and decant the liquid supernatant. Wash the cells 3 times with 10 ml sterile 0.85% NaCl solution. After the third wash, resuspend the cells in 10 ml sterile 0.85% NaCl solution. Dilute the 10 ml cell suspension with the appropriate amount of sterile 0.85% NaCl solution. (See Table 1 below.) One drop of the diluted inoculum suspension is used to inoculate each of the assay tubes.

Amino Acid Solution

Prepare stock solutions of each amino acid as described in Table 1. If the DL form is used, twice the concentration of the amino acid is required. Prepare the stock solutions fresh daily.

Increasing amounts of the standard or the unknown and sufficient distilled or deionized water to give a total volume of 10 ml per tube, are added to the tubes containing 5 ml of the rehydrated medium. The appropriate volumes of the standards and their final concentrations are listed in the table.

Measure the growth response turbidimetrically or titrimetrically. Turbidimetric readings are made after incubation at 35-37°C for 16-20 hours. Titrimetric readings are made after incubation at 35-37ºC for 72 hours.

It is essential that a standard curve be constructed each time an assay is run. Conditions of autoclaving and temperature of incubation that influence the standard curve readings cannot always be duplicated.

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.

Table 1. *Preparation of inoculum dilution, amino acid stock and working solution.*

ASSAY MEDIUM	TEST CULTURE	PREPARATION OF INOCULUM DILUTION (CELL SUSPENSION + (STERILE 0.85% NaCl)	PREPARATION OF AMINO ACID STOCK SOLUTION (AMINO ACID)	+ (DISTILLED H_2O)	STANDARD WORKING SOLUTION (STOCK SOLUTION) + (DISTILLED H_2O)	VOLUME OF STANDARD WORKING SOLUTION (ml/10 ml TUBE)	FINAL AMINO ACID CONCENTRATION µg/10 ml
Cystine Assay Medium	*Pediococcus acidilactici* ATCC® 8042	1 ml + 19 ml	L-cystine	1 g + 100 ml + 1 ml HCl heated, then cooled, add up to 1,000 ml	1 ml + 99 ml	0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5	0.0, 5, 10, 15, 20, 25, 30, 40, 50
Lysine Assay Medium	*Pediococcus acidilactici* ATCC® 8042	1 ml + 19 ml	L-lysine	6 g + 1,000 ml	1 ml + 99 ml	0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5	0.0, 30, 60, 90, 120, 150, 180, 240, 300
Methionine Assay Medium	*Pediococcus acidilactici* ATCC® 8042	1 ml + 19 ml	DL-methionine	1.2 g + 1,000 ml	1 ml + 99 ml	0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5	0.0, 6, 12, 18, 24, 30, 36, 48, 60

2. Determine the amount of amino acid at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of amino acid in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average. Use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. **Steel, Sauberlich, Reynolds, and Baumann.** 1949. J. Biol. Chem. **177**:533.

Packaging

Lysine Assay Medium	100 g	0422-15*
Methionine Assay Medium	100 g	0423-15*
Cystine Assay Medium	100 g	0467-15*

*Store at 2-8°C

Bacto® Anaerobic Agar

Intended Use

Bacto Anaerobic Agar is used for cultivating anaerobic microorganisms.

Summary and Explanation

Brewer[1] described a special Petri dish cover that allowed surface growth of anaerobes and microaerophiles without anaerobic equipment. The microorganisms were grown on an agar-based medium having a low oxidation-reduction potential. Anaerobic Agar is a modification of Brewer's original formula. This medium is suitable for standard plating procedures used in cultivating anaerobic bacteria.[2,3,4]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	5.8% solution, soluble in distilled or deionized water on boiling. Light amber, slightly opalescent. As the medium cools, it becomes green due to aeration.
Prepared Medium:	Light green, slightly opalescent.
Reaction of 5.8% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Anaerobic Agar per label directions. Inoculate the medium and incubate at 35 ±2°C under anaerobic conditions for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacteroides fragilis	25285*	100-1,000	good
Clostridium perfringens	13124*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Anaerobic bacteria cause a variety of infections in humans, including otitis media, oral infections, endocarditis, meningitis, wound infections following bowel surgery or trauma, and bacteremia.[5,6] Anaerobic bacteria are the predominant flora colonizing the skin and mucous membranes of the body.[3] Anaerobes vary in their sensitivity to oxygen and nutritional requirements.[2] Anaerobic bacteria lack cytochromes and thus are unable to use oxygen as a terminal electron acceptor.[3]

Principles of the Procedure

Casitone provides the nitrogen, vitamins and amino acids in Anaerobic Agar. Dextrose is a carbon source. Sodium Chloride maintains the osmotic equilibrium. Sodium Thioglycollate and Sodium Formaldehyde Sulfoxylate are reducing agents. Methylene Blue serves as an indicator of anaerobiosis with a blue color indicating the presence of oxygen. Bacto Agar is the solidifying agent.

Formula

Anaerobic Agar

Formula Per Liter

Bacto Casitone	20 g
Sodium Chloride	5 g
Bacto Dextrose	10 g
Bacto Agar	20 g
Sodium Thioglycollate	2 g
Sodium Formaldehyde Sulfoxylate	1 g
Methylene Blue	0.002 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious material.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Anaerobic Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes
Brewer Anaerobic Petri dish covers (optional)

Method of Preparation

1. Suspend 58 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense as desired.

Specimen Collection and Preparation

Anaerobic bacteria are overlooked or missed unless the specimen is properly collected and transported to the laboratory.[2] Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

Standard Petri Dishes:[2]

1. Inoculate a properly obtained specimen onto the medium and streak to obtain isolated colonies.
2. Immediately incubate anaerobically at 35°C.
3. Examine at 24 hours if incubating plates in an anaerobic chamber. Examine at 48 hours if incubating plates in an anaerobic jar or anaerobic pouch.
4. Extended incubation may be necessary to recover some anaerobes.

Brewer Anaerobic Agar Plates:

1. Dispense 50-60 ml of Anaerobic Agar into a standard Petri dish. For best results use porous tops to obtain a dry surface.
2. Inoculate the surface of the medium by streaking; avoid the edges of the plates.
3. Replace the standard Petri dish lid with a sterile Brewer anaerobic Petri dish cover. The cover should not rest on the Petri dish bottom. The inner glass ridge should seal against the uninoculated periphery of the agar. It is essential that the sealing ring inside the cover is in contact with the medium. This seal must not be broken before the end of the incubation period. A small amount of air is caught over the surface of the medium; however, the oxygen in this space reacts with reducing agents in the medium to form an anaerobic environment.
4. Incubate aerobically as desired.

For a complete discussion on anaerobic and microaerophilic bacteria from clinical specimens, refer to the appropriate procedures outlined in the references.[2,3,4] For the examination of anaerobic bacteria in food, refer to Standard Methods.[7,8,9]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Clinical specimens must be obtained properly and transported to the laboratory in a suitable anaerobic transport container.[2]
3. The microbiologist must be able to verify quality control of the medium and determine whether the environment is anaerobic.[2]
4. The microbiologist must perform aerotolerance testing on each isolate recovered to ensure that the organism is an anaerobe.[2]
5. Methylene blue is toxic to some anaerobic bacteria.

References

1. **Brewer, J. H.** 1942. A new Petri dish and technique for use in the cultivation of anaerobes and microaerophiles. Science **95**:587.
2. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Etiological agents recovered from clinical material, p. 474-503. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Balows, A., W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.).** 1991. Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
6. **Smith, L. D. S.** 1975. The pathogenic anaerobic bacteria, 2nd ed. Charles C. Thomas, Springfield, Il.
7. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithesburg, MD.
8. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
9. **Marshall, R. T. (ed.).** 1992. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Packaging

Anaerobic Agar	500 g	0536-17

Bacto® Antibiotic Assay Media

Bacto Antibiotic Medium 1 · Bacto Antibiotic Medium 2 Bacto Antibiotic Medium 3 · Bacto Antibiotic Medium 4 Bacto Antibiotic Medium 5 · Bacto Antibiotic Medium 8 Bacto Antibiotic Medium 9 · Bacto Antibiotic Medium 10 Bacto Antibiotic Medium 11 · Bacto Antibiotic Medium 12 Bacto Antibiotic Medium 19

Intended Use

Bacto Antibiotic Assay Media are used for determining antibiotic potency by the microbiological assay technique.[1,6,7]

User Quality Control

Identity Specifications

Antibiotic Medium 1

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	3.05% solution, soluble in distilled or deionized water upon boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 3.05% Solution at 25°C:	pH 6.55 ± 0.05

Antibiotic Medium 2

Dehydrated Appearance:	Light tan, homogeneous, free-flowing.
Solution:	2.55% solution, soluble in distilled or deionized water upon boiling; light- medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light-medium amber, slightly opalescent.
Reaction of 2.55% Solution at 25°C:	pH 6.55 ± 0.05

Antibiotic Medium 3

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	1.75% solution, soluble in distilled or deionized water; light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear.
Reaction of 1.75% Solution at 25°C:	pH 7.0 ± 0.05

continued on following page

Also Known As

DIFCO PRODUCT NAME	GROVE AND RANDALL[8]	USP[1]	21 CFR[6]	AOAC[7]
Antibiotic Medium 1	Penassay Seed Agar	Medium 1	Medium 1	Agar Medium A
Antibiotic Medium 2	Penassay Base Agar	Medium 2	Medium 2	Agar Medium C
Antibiotic Medium 3	Penassay Broth	Medium 3	Medium 3	Broth Medium A
Antibiotic Medium 4	Yeast Beef Agar	–	Medium 4	Agar Medium B
Antibiotic Medium 5	Streptomycin Assay Agar	Medium 5	Medium 5	Agar Medium E
Antibiotic Medium 8	–	Medium 8	Medium 8	Agar Medium D
Antibiotic Medium 9	Polymyxin Base Agar	Medium 9	Medium 9	–
Antibiotic Medium 10	Polymyxin Seed Agar	Medium 10	Medium 10	–
Antibiotic Medium 11	Neomycin Assay Agar	–	Medium 11	Agar Medium J
Antibiotic Medium 12	–	–	–	–
Antibiotic Medium 19	–	Medium 19	Medium 19	–

Summary and Explanation

The activity (potency) of an antibiotic can be demonstrated under suitable conditions by its inhibitory effect on microorganisms.[1] Reduction in antimicrobial activity may reveal changes not demonstrated by chemical methods.[1] Antibiotic assays are performed by the cylinder plate method and the turbidimetric "tube" assay. The cylinder plate method, first described by Abraham et al.[2] for the assay of penicillin, was later modified by Foster and Woodruff[3] and by Schmidt and Moyer[4] et al.

Antibiotic Assay Media are prepared according to the specifications of the U.S. Pharmacopeia (USP) XXIII[1], European Pharmacopeia, Code of Federal Regulations (21CFR[6]) and the Association of Official Analytical Chemists (AOAC)[7]. The Antibiotic Media are identified numerically and also, where applicable, with names assigned by Grove and Randall in Assay Methods of Antibiotics.[8] Antibiotic Medium 19 corresponds to the use described in Outline of Details for Official Microbiological Assays of Antibiotics.[9]

The use of standardized culture media and careful control of all test conditions are fundamental requisites in the microbiological assay of antibiotics in order to achieve satisfactory test results.

Principles of the Procedure

Cylinder Plate Assay

This method is based on the diffusion of an antibiotic solution from a cylinder placed on the surface of an inoculated agar medium. The diameter of a zone of inhibition after incubation depends, in part, on the concentration or activity of the antibiotic. This method is used in the assay of commercial preparations of antibiotics, as well as in the quantitative determination of antibiotics in body fluids, animal feeds and other materials.

Turbidimetric Assay

The turbidimetric method is based on the inhibition of growth of a microbial culture in a fluid medium containing a uniform solution of an antibiotic.[1] Turbidimetric determinations have the advantage of requiring a short incubation period, providing test results after 3 or 4 hours. However, the presence of solvents or other inhibitory materials may influence turbidimetric assays more markedly than cylinder plate assays. Use of this method is appropriate only when test samples are clear.

User Quality Control cont.

Antibiotic Medium 4

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.65% solution, soluble in distilled or deionized water on boiling; light amber, very slightly opalescent.
Prepared Medium:	Light amber, very slightly to slightly opalescent.
Reaction of 2.65% Solution at 25°C:	pH 6.55 ± 0.05

Antibiotic Medium 5

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.55% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 2.55% Solution at 25°C:	pH 7.9 ± 0.1

Antibiotic Medium 8

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.55% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 2.55% Solution at 25°C:	pH 5.85 ± 0.05

Antibiotic Medium 9

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in distilled or deionized water upon boiling; light to medium amber, slightly opalescent, may have a slight flocculent precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent with slight flocculent precipitate.
Reaction of 5.0% Solution at 25°C:	pH 7.25 ± 0.05

Antibiotic Medium 10

Dehydrated Appearance:	Beige, homogeneous, moist with a tendency to clump.
Solution:	5.2% solution, soluble in distilled or deionized water upon boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 5.2% Solution at 25°C:	pH 7.25 ± 0.05

Antibiotic Medium 11

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	3.05% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 3.05% Solution at 25°C:	pH 8.0 ± 0.1

Antibiotic Medium 12

Dehydrated Appearance:	Tan, homogeneous, free-flowing.
Solution:	6.25% solution, soluble in distilled or deionized water upon boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 6.25% Solution at 25°C:	pH 6.1 ± 0.1

Antibiotic Medium 19

Dehydrated Appearance:	Light tan, homogeneous, free-flowing.
Solution:	6.0% solution, soluble in distilled or deionized water upon boiling; medium amber, very slightly to slightly opalescent.
Prepared Medium:	Medium amber, slightly opalescent.
Reaction of 6.0% Solution at 25°C:	pH 6.1 ± 0.1

continued on following page

User Quality Control cont.

Cultural Response

Antibiotic Medium 1
Antibiotic Medium 2
Prepare Antibiotic Medium 1 or Antibiotic Medium 2 per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Staphylococcus aureus	6538P	30-300	good

Antibiotic Medium 3
Prepare Antibiotic Medium 3 per label directions. Inoculate and incubate at 35 ± 2°C for up to 24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Enterococcus faecium	10541	approx. 10^7	good
Escherichia coli	10536	approx. 10^7	good
Klebsiella pneumoniae	10031	approx. 10^7	good
Staphylococcus aureus	6538P	approx. 10^7	good

Antibiotic Medium 4
Prepare Antibiotic Medium 4 per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Micrococcus luteus	9341	30-300	good

Antibiotic Medium 5
Antibiotic Medium 8
Prepare Antibiotic Medium 5 or Antibiotic Medium 8 per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Bacillus subtilis	6633	30-300	good

Antibiotic Medium 9
Antibiotic Medium 10
Prepare Antibiotic Medium 9 or Antibiotic Medium 10 per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Bordetella bronchiseptica	4617	30-500	good

Antibiotic Medium 11
Prepare Antibiotic Medium 11 per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Micrococcus luteus	9341	30-300	good
Staphylococcus epidermidis	12228	30-300	good

Antibiotic Medium 12
Antibiotic Medium 19
Prepare Antibiotic Medium 12 or Antibiotic Medium 19 per label directions. Inoculate and incubate at 30 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH*
Saccharomyces cerevisiae	2601	30-300	good

The cultures listed are the minimum that should be used for performance testing.

*When tested in an appropriate antibiotic assay procedure in parallel with a previously approved lot of material, inhibition of growth should produce the specified zones and be comparable to the previously approved lot.[6]

Formula

Antibiotic Medium 1 (Penassay Seed Agar)

Formula Per Liter

Bacto Beef Extract	1.5 g
Bacto Yeast Extract	3 g
Bacto Casitone	4 g
Bacto Peptone	6 g
Bacto Dextrose	1 g
Bacto Agar	15 g

Final pH 6.55 ± 0.05 at 25°C

Antibiotic Medium 2 (Penassay Base Agar)

Formula Per Liter

Bacto Beef Extract	1.5 g
Bacto Yeast Extract	3 g
Bacto Peptone	6 g
Bacto Agar	15 g

Final pH 6.55 ± 0.05 at 25°C

Antibiotic Medium 3 (Penassay Broth)

Formula Per Liter

Bacto Beef Extract	1.5 g
Bacto Yeast Extract	1.5 g
Bacto Peptone	5 g
Bacto Dextrose	1 g
Sodium Chloride	3.5 g
Dipotassium Phosphate	3.68 g
Monopotassium Phosphate	1.32 g

Final pH 7.0 ± 0.05 at 25°C

Antibiotic Medium 4 (Yeast Beef Agar)

Formula Per Liter

Bacto Beef Extract	1.5 g
Bacto Yeast Extract	3 g
Bacto Peptone	6 g
Bacto Dextrose	1 g
Bacto Agar	15 g

Final pH 6.55 ± 0.05 at 25°C

Antibiotic Medium 5 (Streptomycin Assay Agar)

Formula Per Liter

Bacto Beef Extract	1.5 g
Bacto Yeast Extract	3 g
Bacto Peptone	6 g
Bacto Agar	15 g

Final pH 7.9 ± 0.1 at 25°C

Antibiotic Medium 8

Formula Per Liter

Bacto Beef Extract	1.5 g
Bacto Yeast Extract	3 g
Bacto Peptone	6 g
Bacto Agar	15 g

Final pH 5.85 ± 0.05 at 25°C

Antibiotic Medium 9 (Polymyxin Base Agar)

Formula Per Liter

Bacto Casitone	17 g
Soytone	3 g
Bacto Dextrose	2.5 g

Sodium Chloride ..... 5 g
Dipotassium Phosphate ..... 2.5 g
Bacto Agar ..... 20 g
Final pH 7.25 ± 0.05 at 25°C

Antibiotic Medium 10 (Polymyxin Seed Agar)
Formula Per Liter
Bacto Casitone ..... 17 g
Soytone ..... 3 g
Bacto Dextrose ..... 2.5 g
Sodium Chloride ..... 5 g
Dipotassium Phosphate ..... 2.5 g
Bacto Agar ..... 12 g
Polysorbate 80 ..... 10 g
Final pH 7.25 ± 0.05 at 25°C

Antibiotic Medium 11 (Neomycin Assay Agar)
Formula Per Liter
Bacto Beef Extract ..... 1.5 g
Bacto Yeast Extract ..... 3 g
Bacto Casitone ..... 4 g
Bacto Peptone ..... 6 g
Bacto Dextrose ..... 1 g
Bacto Agar ..... 15 g
Final pH 7.95 ± 0.05 at 25°C

Antibiotic Medium 12
Formula Per Liter
Bacto Beef Extract ..... 2.5 g
Bacto Yeast Extract ..... 5 g
Bacto Peptone ..... 10 g
Bacto Dextrose ..... 10 g
Sodium Chloride ..... 10 g
Bacto Agar ..... 25 g
Final pH 6.1 ± 0.1 at 25°C

Antibiotic Medium 19
Formula Per Liter
Bacto Peptone ..... 9.4 g
Bacto Beef Extract ..... 2.4 g
Bacto Yeast Extract ..... 4.7 g
Bacto Dextrose ..... 10 g
Sodium Chloride ..... 10 g
Bacto Agar ..... 23.5 g
Final pH 6.1 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store dehydrated Antibiotic Media (except Antibiotic Medium 10) below 30°C. Store dehydrated Antibiotic Medium 10 at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Antibiotic Medium 1
Antibiotic Medium 2
Antibiotic Medium 3
Antibiotic Medium 4
Antibiotic Medium 5
Antibiotic Medium 8
Antibiotic Medium 9
Antibiotic Medium 10
Antibiotic Medium 11
Antibiotic Medium 12
Antibiotic Medium 19

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Sterile tubes
Waterbath
Test organisms
Maintenance medium for test organisms
Cylinder Plate Assay: Petri dishes 20 x 100 mm with suitable covers
Stainless steel or porcelain cylinders
Turbidimetric Assay: Glass or plastic tubes

Selection of Media for the Microbiological Assay of Antibiotics[1,6]

Antibiotic	Assay Method	Organism	ATCC®	Maintenance Medium	Inoculum Medium	Cylinder Plate Base Layer	Cylinder Plate Seed Layer	Turbidimetric Assay Medium
Amikacin	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Amoxicillin	Cylinder Plate	*Micrococcus luteus*	9341	1	1	11	11	
Amphotericin B	Cylinder Plate	*Saccharomyces cerevisiae*	9763	19	19		19	
Ampicillin	Cylinder Plate	*Micrococcus luteus*	9341	1	1	11	11	
Bacitracin	Cylinder Plate	*Micrococcus luteus*	7468	1	1	2	1	
Bacitracin	Cylinder Plate	*Micrococcus luteus*	10240**	1	1	1	1	
Capreomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1	1			3

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Selection of Media for the Microbiological Assay of Antibiotics[1,6] cont.

Antibiotic	Assay Method	Organism	ATCC®	Maintenance Medium	Inoculum Medium	Cylinder Plate Base Layer	Cylinder Plate Seed Layer	Turbidimetric Assay Medium
Carbenicillin	Cylinder Plate	*Pseudomonas aeruginosa*	25619	1	1	9	10	
Cefaclor	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cefadroxil	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cefamandole	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cefazolin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cefotaxime	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cefoxitin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cephalexin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cephaloglycin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cephaloridine	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cephalothin	Cylinder Plate	*Staphylococcus aureus*	6538P*	1	1	2	1	
Cephapirin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Cephradine	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Chloramphenicol	Turbidimetric	*Escherichia coli*	10536	1	1			3
Chlortetracycline	Cylinder Plate	*Bacillus cereus*	11778**	1		8	8	
Chlortetracycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Chlortetracycline	Turbidimetric	*Staphylococcus aureus*	9144**		3			3
Clindamycin	Cylinder Plate	*Micrococcus luteus*	9341	1	1	11	11	
Cloxacillin	Cylinder Plate	*Staphylococcus aureus*	6538P*	1	1	2	1	
Colistimethate, sodium	Cylinder Plate	*Bordetella bronchiseptica*	4617	1	1	9	10	
Colistin	Cylinder Plate	*Bordetella bronchiseptica*	4617	1	1	9	10	
Cyclacillin	Cylinder Plate	*Micrococcus luteus*	9341	1	1	11	11	
Cycloserine	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Dactinocycin	Cylinder Plate	*Bacillus subtilis*	6633	1	1	5	5	
Demeclyocycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Dicloxacillin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Dihydro-	Cylinder Plate	*Bacillus subtilis*	6633	1	1	5	5	
streptomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1	1			3
Doxycycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Erythromycin	Cylinder Plate	*Micrococcus luteus*	9341	1	1	11	11	
Erythromycin	Cylinder Plate	*Micrococcus luteus*	9341**	1 or 3	1 or 3		11	
Gentamicin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	1	11	11	
Gramicidin	Turbidimetric	*Enterococcus faecium*	10541	3	3			3
Hygromycin B	Cylinder Plate	*Bacillus subtilis*	6633**			5	5	
Kanamycin	Turbidimetric	*Staphylococcus aureus*	6538P	1	1			3
Kanamycin B	Cylinder Plate	*Bacillus subtilis*	6633	1	1	5	5	
Lincomycin	Cylinder Plate	*Micrococcus luteus*	9341**	1 or 3	1 or 3	5	11	
Lincomycin	Turbidimetric	*Staphylococcus aureus*	6538P	1	1			3
Meclocycline	Turbidimetric	*Staphylococcus aureus*	6538P	1	1			3
Methacycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Methicillin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	

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Selection of Media for the Microbiological Assay of Antibiotics[1,6] cont.

Antibiotic	Assay Method	Organism	ATCC®	Maintenance Medium	Inoculum Medium	Cylinder Plate Base Layer	Cylinder Plate Seed Layer	Turbidimetric Assay Medium
Mitomycin	Cylinder Plate	*Bacillus subtilis*	6633	1	1	8	8	
Nafcillin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Natamycin	Cylinder Plate	*Saccharomyces cerevisiae*	9763	19	19		19	
Neomycin	Cylinder Plate	*Staphylococcus aureus*	6538P***	1	1	11	11	
Neomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1	1			3
Netilmicin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	1	11	11	
Novobiocin	Cylinder Plate	*Micrococcus luteus*	9341**	1 or 3	1 or 3	2	2	
Novobiocin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	1	2	1	
Nystatin	Cylinder Plate	*Saccharomyces cerevisiae*	2601	19	19		19	
Oleandomycin	Cylinder Plate	*Micrococcus luteus*	9341**	1 or 3	1 or 3		11	
Oleandomycin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	1	11	11	
Oxacillin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Oxytetracyline	Cylinder Plate	*Bacillus cereus*	11778**	1			8	
Oxytetracycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Paromomycin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	1	11	11	
Penicillin G	Cylinder Plate	*Staphylococcus aureus*	6538P*	1	1	2	1	
Penicillin V	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	2	1	
Plicomycin	Cylinder Plate	*Staphylococcus aureus*	6538P	1	1	8	8	
Polymyxin B	Cylinder Plate	*Bordetella bronchiseptica*	4617	1	1	9	10	
Procaine Penicillin	Cylinder Plate	*Micrococcus luteus*	9341**	1 or 3	1 or 3	1	4	
Rifampin	Cylinder Plate	*Bacillus subtilis*	6633	1	1	2	2	
Rolitetracycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Sisomicin	Cylinder Plate	*Staphylococcus epidermidis*	12228	1	1	11	11	
Spectinomycin	Turbidimetric	*Escherichia coli*	10536	1	1			3
Streptomycin	Cylinder Plate	*Bacillus subtilis*	6633	1	1	5	5	
Streptomycin	Cylinder Plate	*Bacillus subtilis*	6633**	32		5	5	
Streptomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1	1			3
Tetracycline	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Tobramycin	Turbidimetric	*Staphylococcus aureus*	6538P*	1	1			3
Troleandomycin	Turbidimetric	*Klebsiella pneumoniae*	10031	1	1			3
Tyrothricin	Turbidimetric	*Enterococcus faecium*	10541	3	3			3
Vancomycin	Cylinder Plate	*Bacillus subtilis*	6633	1	1	8	8	

* For USP methods, use *Staphylococcus aureus* ATCC® 29737.
** Specified by AOAC for Drugs in Feeds.
*** For USP methods, use *Staphylococcus epidermidis* ATCC® 12228.

Method of Preparation

1. Suspend the appropriate amount of medium in 1 liter distilled or deionized water:
   Antibiotic Medium 1 - 30.5 grams;
   Antibiotic Medium 2 - 25.5 grams;
   Antibiotic Medium 3 - 17.5 grams;
   Antibiotic Medium 4 - 26.5 grams;
   Antibiotic Medium 5 - 25.5 grams;
   Antibiotic Medium 8 - 25.5 grams;
   Antibiotic Medium 9 - 50 grams;
   Antibiotic Medium 10 - 52 grams;
   Antibiotic Medium 11 - 30.5 grams;
   Antibiotic Medium 12 - 62.5 grams;
   Antibiotic Medium 19 - 60 grams.
2. Boil to dissolve completely (except Antibiotic Medium 3, which dissolves without boiling).

3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45-50°C.
5. Antibiotic Medium 11, only: To alter the pH, add 1N HCl or 1N NaOH to the medium at 45-50°C.
6. Dispense as appropriate.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Organism Preparation

Maintain stock cultures on agar slants and make transfers at 1- or 2-week intervals. Prepare the inoculum for assay by washing growth from a fresh 24-48 hour agar slant using sterile distilled water, saline or Antibiotic Medium 3 and further dilute the culture to obtain the desired organism concentration. In some turbidimetric assays, a 18- to 24-hour culture of the test organism in Antibiotic Medium 3, diluted to obtain the optimal number of organisms, is used.

When *Bacillus subtilis* is used as the test organism, inoculate it on Antibiotic Medium 1 and incubate at 37°C for 1 week, wash spores from the agar surface, and heat the spores at 56°C for 30 minutes. Wash the spores 3 times in distilled water, heat again at 65°C for 30 minutes, and then dilute to the optimal concentration. This inoculum preparation should produce a sharp zone in the assay.

Antibiotic Medium modified by the addition of 300 mg manganese sulfate ($MnSO_4 \cdot H_2O$) per liter often aids the sporulation of *B. subtilis* and may be used in preparing the spore suspension. A standardized spore suspension prepared from *B. subtilis* ATCC® 6633 is available as Bacto Subtilis Spore Suspension.

When *B. cereus var. mycoides* is required, inoculate the organism on Antibiotic Medium 1 and incubate at 30°C for 1 week. Wash and prepare the spores as for *B. subtilis*, above. A standardized spore suspension of *B. cereus var. mycoides* is available as Bacto Cereus Spore Suspension.

Cylinder Plate Assay

Use 20 x 100 mm Petri dishes with sufficient depth so that cylinders used in the assay will not be pushed into the medium by the cover. Porcelain covers glazed on the outside, only, are recommended.

Use stainless steel or porcelain assay cylinders having the following dimensions (± 0.1 mm): 8 mm outside diameter, 6 mm inside diameter and 10 mm long.[1] Carefully clean the cylinders to remove all residues, using an occasional acid bath, i.e., with approximately 2N nitric acid or with chromic acid.[1] Four or six cylinders are generally used per plate, evenly spaced on a 2.8 cm radius.

To assure accurate assays, work on a level surface to obtain uniformly thick base and seed layers in the Petri dish. Allow the base layer to solidify and then overlay the seed layer containing a proper concentration of the test organism. The amount of medium in the layers varies for different antibiotics, with most assays specifying a 21 ml base layer and a 4 ml seed layer. In any case, dishes with flat bottoms are required to assure complete coverage of the bottom of the dish when small amounts of base medium are used. Tilt the plate to obtain even coverage of the base layer by the seed layer and allow it to solidify in a level position. Plates should be used the same day as prepared.

Turbidimetric Assay

Use glass or plastic test tubes (i.e., 16 x 125 mm or 18 x 150 mm) that are relatively uniform in length, diameter and thickness and substantially free from surface blemishes.[1] Tubes that will be placed in the spectrophotometer should be matched and free of scratches or blemishes.[1] Clean the tubes thoroughly to remove all antibiotic residues and traces of cleaning solution and, prior to subsequent use, sterilize tubes that have been previously used.[1]

Prepare working dilutions of the antibiotic reference standards in specific concentrations. To a 1 ml quantity of each solution in a suitable tube, add 9 ml of inoculated broth, as required. Prepare similar solutions of the assay materials containing approximately the same amounts of antibiotic activity and place in tubes. Incubate the tubes for 3-4 hours at the required temperature, generally in a water bath. At the end of the incubation period, stop growth by adding 0.5 ml of 1:3 formalin. Determine the amount of growth by measuring light transmittance with a suitable spectrophotometer. Determine the concentration of the antibiotic by comparing the growth obtained with that given by reference standard solutions.

For a complete discussion of antibiotic assay methods, refer to appropriate procedures outlined in the references.[1,5,6,7]

Results

Refer to appropriate procedures for results.[1,5,6,7]

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

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4. **Schmidt, W. H., and A. J. Moyer**. 1944. Penicillin. I. Methods of assay. J Bacteriol. **47**:199.
5. **European Pharmacopoeia.** 1994. Council of Europe, 2nd ed. Maisonneuve S. A. Sainte-Ruffine, FR.
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7. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
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9. **Kirshbaum, A., and B. Arret.** 1967. Outline of details for official microbiological assays of antibiotics. J. Pharm. Sci. **56**:512.

Packaging

Antibiotic Medium 1	500 g	0263-17
	2 kg	0263-07
	10 kg	0263-08
Antibiotic Medium 2	500 g	0270-17
	10 kg	0270-08
Antibiotic Medium 3	500 g	0243-17
	2 kg	0243-07
Antibiotic Medium 4	500 g	0244-17
Antibiotic Medium 5	500 g	0277-17
Antibiotic Medium 8	500 g	0667-17
Antibiotic Medium 9	500 g	0462-17
Antibiotic Medium 10	500 g	0463-17
Antibiotic Medium 11	500 g	0593-17
Antibiotic Medium 12	500 g	0669-17
Antibiotic Medium 19	500 g	0043-17

Bacto® Aseptic Commissioning Medium

Intended Use

Bacto Aseptic Commissioning Medium is a fluid medium used in validating aseptic packing lines.

Summary and Explanation

Aseptic Commissioning Medium is a basic medium in which growth can be demonstrated by either acid or gas production. It is ideally suited for validating and commissioning aseptic packing and filling lines.

Principles of Procedure

Peptone and Yeast Extract provide basic nutrients. Sucrose is a carbohydrate source. Phenol Red is a pH indicator. Sodium Chloride maintains the osmotic balance.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige to pink, free-flowing, homogeneous.
Solution:	1.75% solution, soluble in distilled or deionized water on warming, orange-red, clear.
Prepared Medium:	Orange-red, clear.
Reaction of 1.75% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare the medium per label directions. Inoculate test organisms into tubes with fermentation vials and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID	GAS
Bacillus cereus	14579	100-1,000	good	+	–
Enterobacter aerogenes	13048*	100-1,000	good	+	+
Escherichia coli	25922*	100-1,000	good	+**	+ or –
Staphylococcus aureus	25923*	100-1,000	good	+	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

**May revert to alkaline after prolonged incubation.

Formula

Aseptic Commissioning Medium

Formula Per Liter

Peptone	5 g
Yeast Extract	2.5 g
Sucrose	5 g
Phenol Red	5 mg
Sodium Chloride	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Aseptic Commissioning Medium

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water

Method of Preparation

1. Suspend 17.5 grams in 1 liter distilled or deionized water.
2. Heat gently to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Test Procedure

1. Dispense reconstituted medium into the packing line upstream of the sterilization process.
2. Incubate final packs at 30°C, as appropriate, for up to 7 days.

Results

Gas production is demonstrated by swelling of the pack and acid production by a color change of the medium to yellow. Growth is indicated by turbidity in the medium.

Packaging

Aseptic Commissioning Medium	500 g	1862-17
	5 kg	1862-03

Bacto® Azide Blood Agar Base

Intended Use

Bacto Azide Blood Agar Base is used for isolating streptococci and staphylococci; for use with blood in determining hemolytic reactions.

Also Known As

"Blood Agar Base" may be abbreviated as BAB.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.3% solution, soluble in distilled or deionized water upon boiling. Light to medium amber, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent without precipitate.
	With 5% blood, cherry red, opaque.
Reaction of 3.3% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Azide Blood Agar Base per label directions, enrich with 5% sterile defibrinated blood. Inoculate prepared medium and incubate at 35 ± 2°C. Read plates for growth, hemolysis, colony size at 18-24 and 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HEMOLYSIS
Enterococcus faecalis	19433*	100-1,000	good	alpha/ gamma
Escherichia coli	25922*	1,000-2,000	inhibited	–
Staphylococcus aureus	25923*	100-1,000	good	beta
Staphylococcus epidermidis	12228*	100-1,000	good	gamma
Streptococcus pneumoniae	6305	100-1,000	good	alpha
Streptococcus pyogenes	19615*	100-1,000	good	beta

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Summary and Explanation

In 1933, Edwards[1] used a liquid medium containing Crystal Violet and Sodium Azide as a selective broth in the isolation of mastitis streptococci. Snyder and Lichstein[2,3] reported that 0.01% Sodium Azide in blood agar prevented the swarming of Proteus species, and permitted the isolation of streptococci from mixed bacterial populations. Packer[4] modified Edwards' medium and prepared Infusion Blood Agar containing 1:15,000 Sodium Azide and 1:500,000 Crystal Violet for the study of bovine mastitis. Mallmann, Botwright and Churchill[5] reported that Sodium Azide exerted a bacteriostatic effect on gram negative bacteria. The Azide Blood Agar Base formulation was based on the work of these researchers.

Azide Blood Agar Base is used in the isolation of gram positive organisms from clinical and non-clinical specimens. Azide Blood Agar Base can be supplemented with 5-10% sheep, rabbit or horse blood for isolating, cultivating and determining hemolytic reactions of fastidious pathogens.

Principles of the Procedure

Tryptose and Beef Extract provide nitrogen, vitamins, carbon and amino acids. Sodium Chloride maintains osmotic balance. Sodium Azide is the selective agent, suppressing the growth of gram negative bacteria. Bacto Agar is the solidifying agent.

Supplementation with 5-10% blood provides additional growth factors for fastidious microorganisms, and is used to determine hemolytic patterns of bacteria.

Formula

Azide Blood Agar Base

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	3 g
Sodium Chloride	5 g
Sodium Azide	0.2 g
Bacto Agar	15 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep

container tightly closed. TARGET ORGAN(S): Cardiovascular, Lungs, Nerves.

FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Azide Blood Agar Base

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Sterile defibrinated blood (optional)
Sterile Petri dishes

Method of Preparation

1. Suspend 33 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50°C.
4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Collect specimens in sterile containers or with sterile swabs. Transport immediately to the laboratory in accordance with recommended guidelines outlined in the references.

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions demonstrating both oxygen-stable and oxygen-labile streptolysins.[6]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.

Results

Examine plates for growth and hemolytic reactions after 18-24 and 40-48 hours of incubation. Four different types of hemolysis on blood agar media can be described:[7]

a. Alpha (α)-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony, causing a greenish discolorization of the medium.

b. Beta (β)-hemolysis is the lysis of red blood cells, resulting in a clear zone surrounding the colony.

c. Gamma (γ)-hemolysis indicates no hemolysis. No destruction of red blood cells occurs, and there is no change in the medium.

d. Alpha-prime (α′)-hemolysis is a small zone of complete hemolysis that is surrounded by area of partial lysis.

Limitations of the Procedure

1. Nutritional requirements of organisms vary. Strains may be encountered that fail to grow or grow poorly on this medium.
2. Azide Blood Agar Base is intended for selective use and should be inoculated in parallel with nonselective media.
3. Hemolytic patterns of streptococci grown on Azide Blood Agar Base are somewhat different than those observed on ordinary blood agar. Sodium azide enhances hemolysis. Alpha and beta zones may be extended.[4]
4. Hemolytic patterns may vary with the source of animal blood or base medium used.[6]

References

1. **Edwards, S. J.** 1933. The diagnosis of *Streptococcus mastitis* by cultural methods. J. Comp. Pathol. Ther. **46**:211.
2. **Snyder, M. L., and H. C. Lichstein.** 1940. Sodium azide as an inhibition substance of gram-negative bacteria. J. Infect. Dis. **67**:113.
3. **Lichstein, H. C., and M. L. Snyder.** 1941. The inhibition of the spreading growth of *Proteus* and other bacteria to permit the isolation of associated streptococci. J. Bacteriol. **42**:653.
4. **Packer, R. A.** 1943. The use of sodium azide (NaN_3) as an inhibition substance of gram-negative bacteria. J. Infect. Dis. **67**:113.
5. **Mallmann, Botwright, and Churchill.** 1943. J. Bacteriol. **46**:343.
6. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
7. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Packaging

Azide Blood Agar Base	500 g	0409-17
	10 kg	0409-08

Bacto® Azide Dextrose Broth

Intended Use

Bacto Azide Dextrose Broth is used for cultivating streptococci in water and wastewater.

Summary and Explanation

The formula for Azide Dextrose Broth originated with Rothe at the Illinois State Health Department.[1] In a comparative study, Mallmann and Seligmann[2] investigated the detection of streptococci in water and wastewater using Azide Dextrose Broth. Their work supported use of the medium in determining the presence of streptococci in water, wastewater, shellfish and other materials. Azide Dextrose Broth has also been used for primary isolation of streptococci from foodstuffs[3,4] and other specimens of sanitary significance as an indication of fecal contamination.

Azide Dextrose Broth is specified for use in the presumptive test of water and wastewater for fecal streptococci by the Multiple-Tube Technique.[5]

Principles of the Procedure

Azide Dextrose Broth contains Beef Extract and Tryptose as sources of carbon, nitrogen, vitamins and minerals. Dextrose is a fermentable carbohydrate. Sodium Chloride maintains the osmotic balance of the medium. Sodium Azide inhibits cytochrome oxidase in gram-negative bacteria.

Group D streptococci grow in the presence of azide, ferment glucose, and cause turbidity.

Formula

Azide Dextrose Broth

Formula Per Liter

Bacto Beef Extract	4.5 g
Bacto Tryptose	15 g
Bacto Dextrose	7.5 g
Sodium Chloride	7.5 g
Sodium Azide	0.2 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Cardiovascular, Lungs, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.47% (single strength) and 6.94% (double strength) solution, soluble in distilled or deionized water. Single-strength solution is light to medium amber, clear to very slightly opalescent; double-strength solution is medium to dark amber, clear.
Prepared Medium:	Light to medium amber, clear (single strength).
Reaction of 3.47% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Azide Dextrose Broth per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus faecalis	19433*	100-1,000	good
Escherichia coli	25922*	1,000-2,000	inhibited

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube — *Enterococcus faecalis* ATCC® 29212

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Azide Dextrose Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Tubes with closures
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 34.7 grams in 1 liter distilled or deionized water. Rehydrate with proportionally less water when liquid inocula will exceed 1 ml.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Presumptive Test Procedure[5]

1. Inoculate a series of Azide Dextrose Broth tubes with appropriately graduated quantities of sample. Use sample quantities of 10 ml or less. Use double-strength broth for 10 ml inocula. Consult an appropriate reference for suggested sample sizes.[5]
2. Incubate inoculated tubes at 35 ± 2°C for 20-48 hours.
3. Examine each tube for turbidity at the end of 24 ± 2 hours. If no turbidity is evident, reincubate and read again at the end of 48 ± 3 hours.

Results

A positive test is indicated by turbidity (cloudiness) in the broth. A negative test remains clear.

All Azide Dextrose Broth tubes showing turbidity after 24- or 48-hours incubation must be subjected to the Confirmed Test Procedure. Consult appropriate references for details of the Confirmed Test Procedure[5] and further identification of *Enterococcus*.[5,6]

Limitations of the Procedure

1. Azide Dextrose Broth is used to detect presumptive evidence of fecal contamination. Further biochemical testing must be done for confirmation.
2. For inoculum sizes of 10 ml or larger, use double strength medium to prevent dilution of ingredients.[5,6]

References

1. **Rothe**. 1948. Illinois State Health Department.
2. **Mallmann, W. L., and E. B. Seligmann.** 1950. A comparative study of media for the detection of streptococci in water and sewage. Am. J. Public Health **40**:286.
3. **Larkin, E. P., W. Litsky, and J. E. Fuller.** 1955. Fecal streptococci in frozen foods. I. A bacteriological survey of some commercially frozen foods. Appl. Microbiol. **3:**98.
4. **Splittstoesser, D. F., R. Wright, and G. J. Hucker.** 1961. Studies on media for enumerating enterococci in frozen vegetables. Appl. Microbiol. **9:**303.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Azide Dextrose Broth	500 g	0387-17

Bacto® B_{12} Assay Medium USP

Intended Use

Bacto B_{12} Assay Medium USP is used for determining vitamin B_{12} concentration by the microbiological assay technique.

Also Known As

USP is an abbreviation for United States Pharmacopeia.

Summary and Explanation

Vitamin Assay Media are used in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test.

B_{12} Assay Medium USP is used in the microbiological assay of vitamin B_{12} according to the procedures of the Vitamin B_{12} Activity Assay in USP[1] and the Cobalamin (Vitamin B_{12} Activity) Assay in AOAC.[2] *Lactobacillus delbrueckii* subsp. *lactis* ATCC® 7830 (*Lactobacillus leichmannii*) is the test organism used in this procedure.

Principles of the Procedure

B_{12} Assay Medium USP is a vitamin B_{12}-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *L. delbrueckii* subsp. *lactis* ATCC® 7830. To obtain a standard curve, USP Cyanocobalamin Reference is added in specified increasing concentrations giving a growth response that can be measured titrimetrically or turbidimetrically.

Formula

B_{12} Assay Medium USP

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	15 g
Bacto Dextrose	40 g
Bacto Asparagine	0.2 g
Sodium Acetate	20 g
Ascorbic Acid	4 g
L-Cystine	0.4 g
DL-Tryptophane	0.4 g
Adenine Sulfate	20 mg
Guanine Hydrochloride	20 mg
Uracil	20 mg
Xanthine	20 mg
Riboflavin	1 mg
Thiamine Hydrochloride	1 mg
Biotin	10 µg
Niacin	2 mg
p-Aminobenzoic Acid	2 mg
Calcium Pantothenate	1 mg
Pyridoxine Hydrochloride	4 mg
Pyridoxal Hydrochloride	4 mg
Pyridoxamine Hydrochloride	800 µg
Folic Acid	200 µg
Monopotassium Phosphate	1 g
Dipotassium Phosphate	1 g
Magnesium Sulfate	0.4 g
Sodium Chloride	20 mg
Ferrous Sulfate	20 mg
Manganese Sulfate	20 mg
Sorbitan Monooleate Complex	2 g

Final pH 6.0 ± 0.1 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light to light beige, homogeneous, with a tendency to clump.
Solution:	4.25% (single strength) or 8.5% (double strength) solution, soluble in distilled or deionized water on boiling for 2-3 minutes. Light amber, clear, may have a slight precipitate (single strength).
Prepared Medium:	(Single strength) very light to light amber, clear, may have a slight precipitate.
Reaction of 4.25% Solution at 25°C:	pH 6.0 ± 0.1

Cultural Response

Prepare B_{12} Assay Medium USP per label directions. Prepare a standard curve using USP Cyanocobalamin Reference Standard at levels of 0.0 to 0.25 ng per 10 ml. The medium supports the growth of *L. delbrueckii* subsp. *lactis* ATCC® 7830 when supplemented with cyanocobalamin (vitamin B_{12}).

Precautions

1. For Laboratory Use.
2. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
3. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.
4. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

B_{12} Assay Medium USP

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus delbrueckii* subsp. *lactis* ATCC® 7830
Lactobacilli Agar AOAC or B_{12} Culture Agar USP
Lactobacilli Broth AOAC or B_{12} Inoculum Broth USP
Sterile 0.85% saline
Distilled or deionized water
Spectophotometer or nephelometer
B12 Culture Agar USP
B12 Inoculum Broth USP
Cyanocobalamin USP (vitamin B_{12})

Method of Preparation

1. Suspend 8.5 grams in 100 ml distilled or deionized water.
2. Heat to boiling for 2-3 minutes to dissolve completely.
3. Distribute 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 5 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assay, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Follow assay procedures as outlined in USP[1] or AOAC.[2] Use levels of B_{12} in the preparation of the standard curve according to these

references. It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. Generally satisfactory results are obtained with B_{12} at the following levels: 0.0, 0.025, 0.05, 0.075, 0.1, 0.125, 0.15, 0.2 and 0.25 ng per assay tube (10 ml).

Stock cultures of *L. delbrueckii* subsp. *lactis* ATCC® 7830 are prepared by stab inoculation into 10 ml of B_{12} Culture Agar USP or Lactobacilli Agar AOAC. After 16-24 hours incubation at 35-37°C, the cultures are kept refrigerated. The inoculum for assay is prepared by subculturing a stock culture of *L. delbrueckii* subsp. *lactis* into 10 ml of B_{12} Inoculum Broth USP. For a complete discussion on B_{12} Culture Agar USP and B_{12} Inoculum Broth USP, refer to USP.[1]

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average and use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. For successful results to these procedures, all conditions of the assay must be followed precisely.
3. Aseptic technique should be used throughout the assay procedure.
4. The use of altered or deficient media may cause mutants having different nutritional requirements and will not give a satisfactory response.

References

1. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention Inc., Rockville, MD.
2. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

B_{12} Assay Medium USP	100 g	0457-15

Bacto® B_{12} Culture Agar USP
Bacto B_{12} Inoculum Broth USP

Intended Use

Bacto B_{12} Inoculum Broth USP is used for preparing the inoculum of *Lactobacillus delbrueckii* subsp. *lactis* ATCC® 7830 used in the Vitamin B_{12} Activity Assay.

Bacto B_{12} Culture Agar USP is used for cultivating *L. delbrueckii* subsp. *lactis* ATCC 7830 used in the Vitamin B_{12} Activity Assay.

Also Known As

USP is an abbreviation for United States Pharmacopeia.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose.
2. Inoculum Media: To condition the test culture for immediate use.
3. Assay Media: To permit quantitation of the vitamin under test. They contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Lactobacillus species grow poorly on non-selective culture media and require special nutrients. Mickle and Breed[2] reported the use of tomato juice in culture media for lactobacilli. Kulp,[3] while investigating the use of tomato juice on bacterial development, found that growth of *Lactobacillus acidophilus* was enhanced.

B_{12} Culture Agar USP is recommended for maintaining stock cultures of *L. delbrueckii* subsp. *lactis* ATCC 7830 (*Lactobacillus leichmannii*) for use in the Vitamin B_{12} Activity Assay according to US Pharmacopeia (USP).[1]

B_{12} Inoculum Broth USP is used for preparing the inoculum of *L. delbrueckii* subsp. *lactis* ATCC 7830 in the microbiological assay of vitamin B_{12} according to USP.[1]

Principles of the Procedure

Proteose Peptone No. 3 provides the nitrogen and amino acids in B_{12} Culture Agar USP and B_{12} Inoculum Broth USP. Yeast Extract is the vitamin source in the formulas. Tomato Juice is added to create the proper acidic environment. Dextrose is the carbon source, and Sorbitan Monooleate Complex acts an emulsifier. Potassium Phosphate Dibasic acts as the buffering agent in B_{12} Inoculum Broth USP, and Monopotassium Phosphate is the buffering agent in B_{12} Culture Agar USP. Bacto Agar is the solidifying agent in B_{12} Culture Agar USP.

Formula

B_{12} Culture Agar USP

Formula Per Liter

Tomato Juice	100 ml
Bacto Proteose Peptone No. 3	7.5 g
Bacto Yeast Extract	7.5 g
Bacto Dextrose	10 g
Monopotassium Phosphate	2 g
Sorbitan Monooleate Complex	1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.1 at 25°C

B_{12} Inoculum Broth USP

Formula Per Liter

Tomato Juice	100 ml
Bacto Proteose Peptone No. 3	7.5 g
Bacto Yeast Extract	7.5 g
Bacto Dextrose	10 g
Sorbitan Monooleate Complex	0.1 g
Potassium Phosphate Dibasic	2 g

Final pH 6.8 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

B_{12} Culture Agar USP

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.7% solution, soluble in distilled or deionized water upon boiling. Solution is light to medium amber, opalescent when hot, slightly opalescent with flocculent precipitate when cooled.
Prepared Medium:	Light to medium amber, slightly opalescent, may have a slight flocculent precipitate.
Reaction of 4.7% Solution at 25°C:	pH 6.8 ± 0.1

B_{12} Inoculum Broth USP

Dehydrated Appearance:	Tan, homogeneous, tendency to clump.
Solution:	3.2% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, opalescent when hot, clear when cooled to room temperature.
Prepared Medium:	Medium amber, clear.
Reaction of 3.2% Solution at 25°C:	pH 6.8 ± 0.1

Cultural Response

B_{12} Culture Agar USP or B_{12} Inoculum Broth USP

Prepare B_{12} Culture Agar USP or B_{12} Inoculum Broth USP per label directions. Inoculate medium with test organism and incubate at 35 ± 2°C for 16-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus delbrueckii subsp. *lactis*	7830	300-1,000	good

The culture listed is the minimum that should be used for performance testing.

3. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used.

Storage

Store the dehydrated media at 2-8°C. The dehydrated media are very hygroscopic. Keep containers tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

B_{12} Culture Agar USP
B_{12} Inoculum Broth USP

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Distilled or deionized water
Inoculating needle

Method of Preparation

1. Suspend the appropriate amount of medium in 1 liter distilled or deionized water:

   B_{12} Culture Agar USP 47 grams

   B_{12} Inoculum Broth USP 32 grams
2. Boil to dissolve completely. (B_{12} Culture Agar)
3. Dispense 10 ml amounts into tubes.
4. Autoclave at 121°C for 15 minutes.
5. Allow tubes of B_{12} Culture Agar to cool in an upright position.

Stock Culture

1. Prepare stock cultures in triplicate in sterile B_{12} Culture Agar USP.
2. Inoculate the tubes using a straight wire inoculating needle.
3. Incubate cultures for 16-24 hours at any temperature between 30-40°C, but held constant within ± 0.5°C.
4. Store at 2-8°C.
5. Before using a fresh culture for assay, make no fewer than 10 successive transfers of the culture in a 2 week period.
6. Prepare stab cultures at least three times each week and do not use a culture for preparing assay inoculum if over 4 days old.

Inoculum

Prepare inoculum as described in USP.[1]

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assay, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

For a complete discussion of vitamin assay methodology, refer to appropriate procedures outlined in USP.[1]

Results

For test results of vitamin assay procedures refer to USP.[1]

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. For successful results of these procedures, all conditions of the assay must be followed precisely.
3. Aseptic technique should be used throughout the assay procedure.
4. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.

References

1. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention Inc. Rockville, MD.
2. **Mickle, and Breed**. 1925. Technical Bulletin 110, NY State Agriculture Ex. Station.
3. **Kulp, J. W. L., and V. White.** 1932. Modified medium for plating *Lactobacillus acidophilus*. Science **76**:17.

Packaging

B_{12} Culture Agar USP	100 g	0541-15*
B_{12} Inoculum Broth USP	100 g	0542-15*

*Store at 2-8°C

Bacto® BAGG Broth

Intended Use

Bacto BAGG Broth is used for presumptively identifying and confirming fecal streptococci.

Also Known As

Buffered Azide Glucose Glycerol Medium

Summary and Explanation

In developing Buffered Azide Glucose Glycerol (BAGG) Medium, Hajna[1] modified the formula of SF Broth as specified by Hajna and Perry.[2] Hajna found that adding glycerol to SF Medium enhanced dextrose fermentation by *Enterococcus faecalis*. Decreasing the concentration of brom cresol purple allowed for easier detection of a color change within 24 hours. The BAGG Broth formulation made the original SF Medium more useful in testing for fecal contamination of water and other materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige with a slight green tint, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water containing 0.5% glycerol. Solution is purple, clear.
Prepared Tubes:	Purple, clear.
Reaction of 3.6% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Prepare BAGG Broth per label directions. Inoculate tubes in duplicate and incubate at 35 ± 2°C and 45 ± 1.0°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID PRODUCTION
Enterococcus faecalis	19433*	100-1,000	good	+ (yellow)
Enterococcus faecium	27270	100-1,000	good	+ (yellow)
Escherichia coli	25922*	1,000-2,000	markedly to completely inhibited	–
Streptococcus pyogenes	19615*	1,000-2,000	markedly to completely inhibited	–

Uninoculated tube — *Enterococcus faecalis* ATCC® 29212

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

BAGG Broth contains Tryptose as a source for carbon, nitrogen, vitamins and minerals. Dextrose is a fermentable carbohydrate. Sodium Chloride maintains the osmotic balance of the medium. Sodium Azide inhibits gram-negative bacteria. Brom Cresol Purple is a pH indicator.

Enterococci grow in the presence of azide and ferment glucose, producing an acid pH that changes the color of the medium.

Formula

BAGG Broth

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	5 g
Dipotassium Phosphate	4 g
Monopotassium Phosphate	1.5 g
Sodium Chloride	5 g
Sodium Azide	0.5 g
Bacto Brom Cresol Purple	0.015 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. HARMFUL. HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Cardiovascular, Lungs, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

BAGG Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Glycerol
Tubes with closures
Autoclave
Incubators (35 ± 2°C, 45 ± 1°C)

Method of Preparation

1. Dissolve 36 grams in 1 liter distilled or deionized water containing 5 ml glycerol. Rehydrate with proportionally less water when liquid inocula will exceed 1 ml.
2. Dispense into tubes with closures.
3. Autoclave at 114 -118°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure[1-3]

1. Inoculate duplicate tubes with sample. Use single-strength medium for inocula of 1 ml or less. Use double-strength medium for inocula of 10 ml.
2. Incubate one set of tubes at 35 ± 2°C for 18-48 hours. Incubate the second set at 45 ± 1°C for 18-48 hours.
3. Read tubes for growth and acid production.

Results[1-3]

1. A positive test is indicated by the production of a yellow color (acid) throughout the medium. This result is presumptive evidence of the presence of fecal streptococci. Further testing must be performed to confirm this result. Consult appropriate references for further identification of *Enterococcus*.[3]
2. A negative result is indicated by no change in the medium (purple color).

Limitations of the Procedure

1. The concentration of the medium must be adjusted to the inoculum size. Refer to discussion in Test Procedure.

References

1. **Hajna, A. A.** 1951. A buffered azide glucose-glycerol broth for presumptive and confirmative tests for fecal streptococci. Pub. Health Lab. **9**:80-81.
2. **Hajna, A. A., and C. A. Perry.** 1943. A comparative study of presumptive and confirmative media for bacteria of the coliform group and for fecal streptococci. Am. J. Pub. Health **33**:550-556.
3. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

BAGG Broth	500 g	0442-17

Bacto® BG Sulfa Agar · Bacto SBG Enrichment
Bacto SBG Sulfa Enrichment

Intended Use

Bacto BG Sulfa Agar is used for isolating *Salmonella*.

Bacto SBG Enrichment and Bacto SBG Sulfa Enrichment is used for enriching *Salmonella* prior to isolation procedures.

Also Known As

BG is an abbreviation for Brilliant Green and SBG is an abbreviation for Selenite Brilliant Green.

Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella* in domesticated animals. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness.[1] The illness results from consumption of raw, undercooked or improperly processed foods contaminated with *Salmonella*. Many of these cases of *Salmonella*-related gastroenteritis are due to improper handling of poultry products. Various poultry products are routinely monitored for *Salmonella* before their distribution for human consumption, but in many instances, contaminated food samples elude detection.

BG Sulfa Agar is a highly selective medium. Osborne and Stokes[2] added 0.1% sodium sulfapyridine to Brilliant Green Agar to enhance the selective properties of this medium for *Salmonella*. This formula is recommended as a selective isolation medium for *Salmonella* following enrichment. It is also recommended for direct inoculation with primary specimens for *Salmonella* isolation.

For food testing, BG Sulfa Agar has been used for detection of *Salmonella* in low and high moisture foods.[3,4] It has also been used for detecting *Salmonella* in feeds and feed ingredients.[5] This medium is recommended when testing foods for *Salmonella* following USDA guidelines.[6,7]

SBG Enrichment and SBG Sulfa Enrichment are prepared according to the formulas described by Stokes and Osborne.[8] The researchers found that whole egg and egg yolk reduced the selective properties of selenite brilliant green enrichment.[2] They also found that the addition of sulfapyridine restored these selective properties.[2]

SBG Enrichment and SBG Sulfa Enrichment are selective enrichments for the isolation of *Salmonella* species, especially from egg products. The shell and the contents of the egg at the time of oviposition are generally sterile or harbor very few microorganisms.[9,10,11] Contamination of the shell occurs afterwards from nesting material, floor litter, and avian fecal matter.[12] Salmonellae are of most concern in egg products.[12]

User Quality Control

Identity Specifications

BG Sulfa Agar

Dehydrated Appearance:	Pink, free flowing, homogeneous.
Solution:	5.9% solution, soluble in distilled or deionized water on boiling. Solution is very dark amber, very slightly to slightly opalescent.
Prepared Plates:	Dark reddish-amber, slightly opalescent.
Reaction of 5.9% Solution at 25°C:	pH 6.9 ± 0.2

SBG Enrichment

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.37% solution, soluble in distilled or deionized water; green, opalescent with slight precipitate.
Prepared Medium:	Green, opalescent without significant precipitation.
Reaction of 2.37% Solution at 25°C:	pH 7.2 ± 0.2

SBG Sulfa Enrichment

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.42% solution, soluble in distilled or deionized water; green, opalescent without significant precipitation.
Prepared Medium:	Green, opalescent without significant precipitation.
Reaction of 2.42% Solution at 25°C:	pH 7.2 ± 0.2

continued on following page

Principles of the Procedure

In BG Sulfa Agar, Proteose Peptone and Yeast Extract provide nitrogen, vitamins and minerals. Lactose and Sucrose are the sources of carbohydrates in the medium. Brilliant Green and Sodium Pyridine are complementary in inhibiting gram-positive bacteria and most gram-negative bacilli other than *Salmonella* spp. Phenol Red is the pH indicator that turns the medium a yellow color with the formation of acid when lactose and/or sucrose is fermented. Bacto Agar is a solidifying agent.

Bacto Peptone provides the nitrogen, minerals and amino acids in SBG Enrichment and SBG Sulfa Enrichment. Yeast Extract is the vitamin source. D-Mannitol is the carbon source to stimulate organism growth. The phosphates acts as buffers in the enrichments. Sodium Taurocholate, Sodium Selenite and Brilliant Green are the selective agents. The selective agents are used to inhibit gram positive organisms and enteric bacteria other than *Salmonella*. Sodium Sulfapyridine is added in SBG Sulfa Enrichment to increase selectivity.

Formula

BG Sulfa Agar

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Proteose Peptone No. 3	10 g
Bacto Lactose	10 g
Bacto Saccharose	10 g
Sodium Sulfapyridine	1 g
Sodium Chloride	5 g
Bacto Agar	20 g
Brilliant Green	0.0125 g
Bacto Phenol Red	0.08 g

Final pH 6.9 ± 0.2 at 25°C

SBG Enrichment

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Peptone	5 g
Bacto D-Mannitol	5 g
Sodium Taurocholate	1 g
Sodium Selenite	4 g
Dipotassium Phosphate	2.65 g
Monopotassium Phosphate	1.02 g
Bacto Brilliant Green	0.005 g

Final pH 7.2 ± 0.2 at 25°C

SBG Sulfa Enrichment

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Peptone	5 g
Bacto D-Mannitol	5 g
Sodium Taurocholate	1 g
Sodium Sulfapyridine	0.5 g
Sodium Selenite	4 g
Dipotassium Phosphate	2.65 g
Monopotassium Phosphate	1.02 g
Bacto Brilliant Green	0.005 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. **BG Sulfa Agar**
   For Laboratory Use.

   SBG Enrichment, SBG Sulfa Enrichment
   For Laboratory Use.

2. **SBG Enrichment**

   VERY TOXIC. VERY TOXIC BY INHALATION AND IF SWALLOWED. DANGER OF CUMULATIVE EFFECTS. (EC) IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Kidney, Liver, Spleen.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed, induce vomiting; seek medical advice immediately and show this container or label.

   SBG Sulfa Enrichment

   VERY TOXIC. VERY TOXIC BY INHALATION AND IF SWALLOWED. DANGER OF CUMULATIVE EFFECTS. (EC)

Cultural Response

BG Sulfa Agar

Prepare BG Sulfa Agar per label directions. Inoculate and incubate the plates at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLOR OF COLONIES/MEDIUM
Enterococcus faecalis	29212*	1,000-2,000	none	–/no change
Escherichia coli	25922*	100-1,000	none to poor	yellow-green
Salmonella typhimurium	14028*	100-1,000	good	pink-white/red

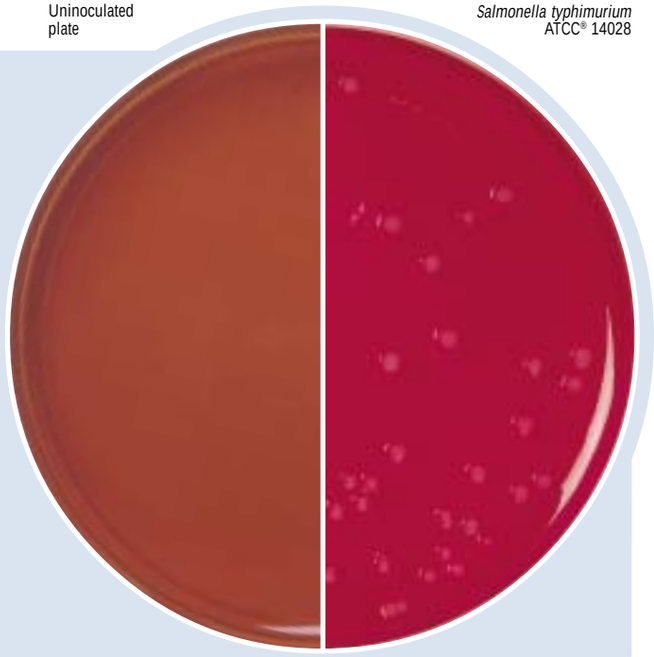
SBG Enrichment and SBG Sulfa Enrichment

Prepare SBG Enrichment and SBG Sulfa Enrichment per label directions. Inoculate tubes with the test organisms. Incubate inoculated medium at 35 ± 2°C for 18-24 hours. After incubation, subculture onto prepared plates of MacConkey Agar.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR ON MACCONKEY
Escherichia coli	25922*	100-1,000	none to poor	pink, if any
Salmonella typhimurium	14028*	100-1,000	good	colorless
Shigella sonnei	9290	100-1,000	poor to fair	colorless

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Kidney, Liver, Spleen.

FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed, induce vomiting; seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium and enrichments below 30°C. The dehydrated products are very hygroscopic. Keep container tightly closed.

Store prepared BG Sulfa Agar plates at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

BG Sulfa Agar
SBG Enrichment
SBG Sulfa Enrichment

Materials Required But Not Provided

Flasks with closures
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50°C)
Petri dishes
Incubator (35°C)
Sterile test tubes

Method of Preparation

BG Sulfa Agar

1. Suspend 59 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Avoid overheating which will decrease selectivity.
4. Cool to 45-50°C in a waterbath.
5. Dispense into sterile Petri dishes.

SBG Enrichment and SBG Sulfa Enrichment

1. Dissolve the appropriate amount of medium in 1 liter distilled or deionized water:

SBG Enrichment	23.7grams/liter
SBG Sulfa Enrichment	24.2 grams/liter

2. Boil gently for 5-10 minutes.
3. Avoid overheating. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

For information about specimen preparation and inoculation of food samples, consult appropriate references.[7,12]

Results

BG Sulfa Agar

The typical *Salmonella* colonies appear as pink-white to red opaque colonies surrounded by a brilliant red medium. The few lactose and/or sucrose fermenting organisms that grow are readily differentiated due to the formation of a yellow-green colony surrounded by an intense yellow-green zone. BG Sulfa Agar is not suitable for the isolation of *S. typhi* or *Shigella*; however, some strains of *S. typhi* may grow forming red colonies.

SBG Enrichment and SBG Sulfa Enrichment

Examine prepared media for growth. Positive tubes should be subcultured onto prepared media for isolation and identification of bacteria.

Limitations of the Procedure

1. On BG Sulfa Agar colonies of *Salmonella* sp. vary from red to pink to white depending on length of incubation and strain.[13]
2. BG Sulfa Agar is normally orange-brown in color; however, on incubation, it turns bright red and returns to normal color at room temperature.[13]
3. *S. typhi* does not grow adequately on BG Sulfa Agar. *Shigella* sp. do not grow on BG Sulfa Agar.[13]
4. Do not sterilize BG Sulfa Agar longer than 15 minutes; longer periods decrease the selectivity of the medium.
5. Since BG Sulfa Agar is highly selective, it is recommended that less selective media, such as MacConkey Agar, be used simultaneously.
6. SBG Enrichment and SBG Sulfa Enrichment should be used in conjunction with selective prepared medium for bacterial identification.

References

1. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall (ed.), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. **Osborn, W. W., and J. L. Stokes.** 1955. A modified selenite brilliant green medium for the isolation of *Salmonella* from egg products. Appl. Microbiol. **3:**295-299.
3. **D'Aoust, J. Y., C. Maishment, D. M. Burgener, D. R. Conley, A. Loit, M. Milling, and U. Purvis.** 1980. Detection of *Salmonella* in refrigerated preenrichment and enrichment broth cultures. J. Food Prot. **43:**343-345.
4. **D'Aoust, J. Y.** 1984. Effective enrichment-plating conditions for detection of *Salmonella* in foods. J. Food Prot. **47:**588-590.
5. **D'Aoust, J. Y., A. Sewell, and A. Boville.** 1983. Rapid cultural methods for detection of *Salmonella* in feeds and feed ingredients. J. Food Prot. **46:**851-855.
6. **Moats, W. A.** 1981. Update on *Salmonella* in foods: selective plating media and other diagnostic media. J. Food Prot. **44:**375-380.

7. **Federal Register.** 1996. Pathogen reduction; hazard analysis and critical point (HACCP) systems; final rule. Fed. Regis. **61:**38917-38925.
8. **Osborn, W. W., and J. L. Stokes.** 1955. Appl. Microbiol. **3**:217.
9. **Brooks, J. and D. J. Taylor.** 1955.Rep. Rd. Invest., Bd. 60, H. M. S. O. London, England.
10. **Forsythe, R. H., J. C. Ayres, and J. L. Radlo.** 1953. Factors affecting the microbiological populations of shell eggs. Food Technol. **7**:49.
11. **Stadelman, W. J., A. I. Ikeme, R. A. Roop, and S. E. Simmons.** 1982. Thermally processed hard-cooked eggs. Poultry Science **61**:388.
12. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
13. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

BG Sulfa Agar	500 g	0717-17
SBG Enrichment	500 g	0661-17
SBG Sulfa Enrichment	500 g	0715-17

Bacto® Baird-Parker Agar Base Bacto EY Tellurite Enrichment

Intended Use

Bacto Baird-Parker Agar Base is used with Bacto EY Tellurite Enrichment in isolating and enumerating staphylococci in foods and other materials.

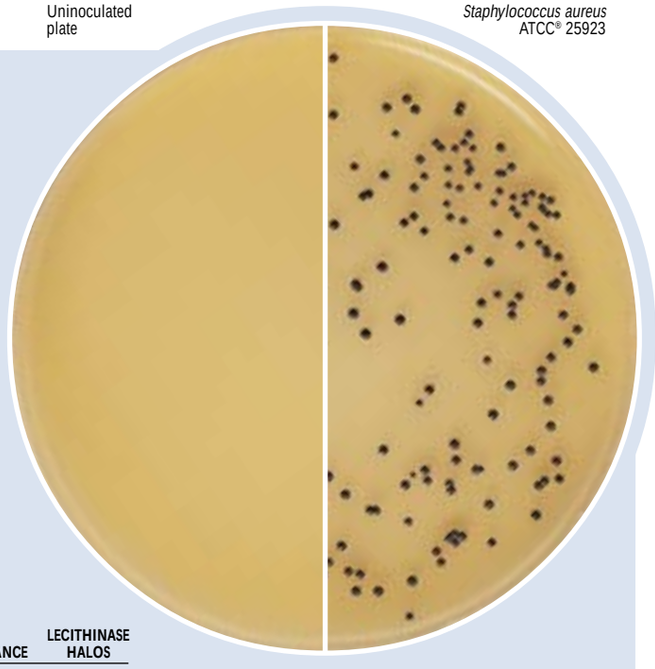
Also Known As

Baird-Parker is also known as Egg Tellurite Glycine Pyruvate Agar (ETGPA) based on its composition.

EY Tellurite Enrichment is also known as Egg Yolk Tellurite Enrichment.

Summary And Explanation

The formulation of Baird-Parker Agar was published in 1962.[1] It is a selective medium for isolation and presumptive identification of coagulase-positive staphylococci.



User Quality Control

Identity Specifications

Baird Parker Agar Base

Dehydrated Appearance: Light tan, free-flowing, homogeneous.

Solution: 6.3% solution, soluble in distilled or deionized water on boiling; light to medium amber, slightly opalescent.

Prepared Medium (Final): Yellow, opalescent.

Reaction of 6.3% Solution at 25°C: pH 6.9 + 0.1

EY Tellurite Enrichment

Appearance: Canary yellow, opaque suspension with a resuspendable precipitate.

Cultural Response

Baird-Parker Agar Base, EY Tellurite Enrichment

Prepare Baird-Parker Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	LECITHINASE HALOS
Bacillus subtilis	6633	1,000	poor to fair	brown	–
Escherichia coli	25922*	1,000	none	–	N/A
Proteus mirabilis	25933	1,000	good	brown	–
Staphylococcus aureus	25923*	100	good	black	+
Staphylococcus aureus	6538	100	good	black	+
Staphylococcus epidermidis	14990	100	poor to good	black	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Baird-Parker Agar is widely used and is included in many Standard Methods procedures for testing foods, dairy products and other materials.[2,3,4,5,6] Coagulase-positive staphylococci can grow and reproduce in cosmetic products and these should be tested using standard microbiological methods.[4] In 1995. the American Public Health Association (APHA) published proposed procedures for testing swimming pools for coagulase-positive staphylococci.[7]

Principles of the Procedure

Baird-Parker Agar Base contains Tryptone and Beef Extract as carbon and nitrogen sources for general growth. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Glycine and Sodium Pyruvate stimulate growth of staphylococci. The selectivity of the medium is due to Lithium Chloride and Potassium Tellurite (provided in EY Tellurite Enrichment) which suppress growth of organisms other than staphylococci. The differentiation of coagulase-positive staphylococci depends on the Potassium Tellurite and Egg Yolk (provided in the EY Tellurite Enrichment). Staphylococci that contain lecithinase break down the Egg Yolk and cause clear zones around the colonies. An opaque zone of precipitation may form due to lipase activity. Reduction of Potassium Tellurite, also a characteristic of coagulase-positive staphylococci, causes blackening of the colonies. Bacto Agar is the solidifying agent.

Formula

Baird-Parker Agar Base

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Beef Extract	5 g
Bacto Yeast Extract	1 g
Glycine	12 g
Sodium Pyruvate	10 g
Lithium Chloride	5 g
Bacto Agar	20 g

Final pH 6.9 ± 0.1 at 25°C

EY Tellurite Enrichment

Egg yolk emulsion containing Potassium Tellurite.

Precautions

1. For Laboratory Use.
2. **Baird Parker Agar Base**

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Kidneys, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store Baird Parker Agar Base below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Store EY Tellurite Enrichment at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Baird-Parker Agar Base
EY Tellurite Enrichment

Materials Required but not Provided

Flask with closure
Distilled or deionized water
Autoclave
Petri dishes
Waterbath (45-50°C)
Incubator (35°C)

Method of Preparation

1. Suspend 63 grams Baird-Parker Agar Base in 950 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45-50°C.
5. Warm EY Tellurite Enrichment to 45-50°C and mix thoroughly to resuspend the precipitate.
6. Aseptically add 50 ml of prewarmed enrichment to the medium. Mix thoroughly.

Specimen Collection and Preparation

Certain foods and other materials may require repair-selective enrichment if injured cells are suspected or selective enrichment if raw food materials or nonprocessed foods containing large numbers of competing microorganisms are being tested.[2] Consult standard references for specific instructions for the type of material being tested.[2,3,4,5]

Test Procedure

1. Prepare dilutions of test samples if indicated by standard procedure.[2,3,4,5]
2. Transfer 1 ml of sample to each of 3 Baird-Parker Agar plates and distribute over the surface using a sterile, bent glass rod.
3. Allow the inoculum to be absorbed by the medium (about 10 minutes) before inverting the plates.
4. Incubate at 35-37°C for 45-48 hours.
5. Examine plates having 20-200 colonies, counting colonies typical of *S. aureus.*

Results

Coagulase-positive staphylococci produce black, shiny, convex colonies with entire margins and clear zones, with or without an opaque zone, around the colonies.

Coagulase-negative staphylococci produce poor or no growth. If growth occurs, colonies are black; clear or opaque zones are rare.

Most other organisms are inhibited or grow poorly. If growth occurs, colonies are light to brown-black with neither clear nor opaque zones.

Limitations of the Procedure

Baird-Parker Agar is selective for coagulase-positive staphylococci but other bacteria may grow. Microscopic examination and biochemical tests will differentiate coagulase-positive staphylococci from other microorganisms.

References

1. **Baird-Parker, A. C.** 1962. An improved diagnostic and selective medium for isolating coagulase-positive staphylococci. J. Appl. Bacteriol. **25**:12-19.
2. **Lancette, G. A., and S. R. Tatini**. 1992. *Staphylococcus aureus*, p. 533-550. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall, (ed.), Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
4. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Andrews, W. H.** 1995. Microbial Methods, p. 1-119. Official methods of analysis of AOAC International, 16th ed. AOAC International. Arlington, VA.
6. **United States Pharmacopeial Convention.** 1995. The United States Pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
7. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Recreational waters, p. 9.26-9.27. *In* Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Packaging

Baird-Parker Agar Base	100 g	0768-15
	500 g	0768-17
	2 kg	0768-07
	10 kg	0768-08
EY Tellurite Enrichment	6 x 100 ml	0779-73

Bacto® Beef Extract
Bacto Beef Extract, Desiccated

Intended Use

Bacto Beef Extract and Bacto Beef Extract, Desiccated are used in preparing microbiological culture media.

Summary and Explanation

Beef Extract is prepared and standardized for use in microbiological culture media, where it is generally used to replace infusions of meat. Culture media containing Beef Extract have been recommended for use in the bacteriological examination of water, milk and other materials where having media of uniform composition is important.

Beef Extract has been employed by many investigators. Bedell and Lewis[1] used it in their medium for the study of non-sporulating anaerobes of the intestinal tract. Hutner[2] used a medium containing Beef Extract as a stock broth in the study of nutritional needs of streptococci. Beef Extract is the formula of Potato Infusion Agar for the cultivation of *Brucella*. Fletcher Medium Base, Starch Agar, Dextrose Agar, Dextrose Broth and CLED Agar all contain Beef Extract to enhance the growth of bacteria. Antibiotic Assay media specified by US Pharmacopeia[3] includes Beef Extract in the formula. Several media containing Beef Extract are recommended in standard methods for multiple applications.[4,5,6]

In culture media, Beef Extract is usually employed in concentrations of 0.3%. Concentrations may vary slightly according to the requirements of individual formulas, but do not often exceed 0.5%. Beef Extract may be relied upon for biochemical studies, particularly fermentation reactions, because of its independence from fermentable substances that would interfere with the accuracy of such determinations.

Beef Extract, Desiccated, the dried form of Beef Extract, was developed to provide a product for ease of use in handling. Beef Extract is in the paste form. The products are to be used in a one for one substitution, however variations tend to be formulation specific and require actual performance testing.

Principles of the Procedure

Beef Extract and Beef Extract, Desiccated are replacements for infusion of meat. Beef Extract and Beef Extract, Desiccated provide nitrogen, vitamins, amino acids and carbon in several formulations of microbiological culture media.

Typical Analysis

	BEEF EXTRACT	BEEF EXTRACT, DESICCATED
Physical Characteristics		
Ash (%)	24.1	10.2
Clarity, 1% Soln (NTU)	116.8	1.7
Filterability (g/cm^2)	0.1	0.6
Loss on Drying (%)	77.2	2.5
pH, 1% Soln	5.4	6.9
Carbohydrate (%)		
Total	0.2	<0.1
Nitrogen Content (%)		
Total Nitrogen	11.2	14.0
Amino Nitrogen	3.8	2.2
AN/TN	33.8	15.7

	BEEF EXTRACT	BEEF EXTRACT, DESICCATED
Amino Acids (%)		
Alanine	2.54	8.96
Arginine	1.39	5.66
Aspartic Acid	1.67	4.30
Cystine	0.18	0.17
Glutamic Acid	6.01	12.55
Glycine	4.14	16.25
Histidine	4.94	2.50
Isoleucine	0.53	1.45
Leucine	1.00	3.63
Lysine	1.45	3.27
Methionine	0.30	1.08
Phenylalanine	<0.01	2.00
Proline	2.16	9.58
Serine	0.90	2.10
Threonine	0.67	1.42
Tryptophan	0.05	0.32
Tyrosine	1.99	1.03
Valine	0.86	2.62

User Quality Control

Identity Specifications

Beef Extract

Dehydrated Appearance: Medium to dark brown paste.

Solution: 0.3% solution - soluble in distilled or deionized water upon warming. Light to medium amber in color, clear, no precipitate.

Reaction of 0.3% Solution at 25°C: pH 6.9 ± 0.2

Beef Extract, Desiccated

Dehydrated Appearance: Medium to dark brown, free-flowing, homogeneous powder.

Solution: 0.3% solution - soluble in distilled or deionized water at a 0.3% concentration. 0.3% solution is light to medium amber in color, clear without a precipitate.

Reaction of 0.3% Solution at 25°C: pH 6.6-7.4

Cultural Response

Beef Extract

Prepare a sterile solution of 0.3% Beef Extract or Beef Extract, Desiccated, and 0.5% Bacto Peptone. Adjust the pH to 6.9-7.1. Inoculate tubes with the test organisms, incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Salmonella typhimurium	14028*	100-1,000	good
Staphylococcus aureus	25923*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

	BEEF EXTRACT	BEEF EXTRACT, DESICCATED
Inorganics (%)		
Calcium	0.068	0.018
Chloride	1.284	1.576
Cobalt	<0.001	<0.001
Copper	<0.001	0.001
Iron	<0.001	0.001
Lead	<0.001	<0.001
Magnesium	0.239	0.022
Manganese	<0.001	<0.001
Phosphate	5.458	0.345
Potassium	5.477	1.994
Sodium	2.315	2.774
Sulfate	0.629	0.829
Sulfur	0.707	0.661
Tin	<0.001	<0.001
Zinc	<0.001	0.002
Vitamins (µg/g)		
Biotin	0.1	0.1
Choline (as Choline Chloride)	1171.5	1300.0
Cyanocobalamin	0.5	<0.1
Folic Acid	3.3	0.6
Inositol	4113.2	2100.0
Nicotinic Acid	774.7	138.1
PABA	20.0	40.5
Pantothenic Acid	91.0	8.7
Pyridoxine	7.3	2.8
Riboflavin	0.4	<0.1
Thiamine	<0.1	<0.1
Thymidine	1093.4	111.3
Biological Testing (CFU/g)		
Coliform	negative	negative
Salmonella	negative	negative
Spore Count	299	585
Standard Plate Count	117	690
Thermophile Count	33	28

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated product below 30°C. The dehydrated ingredient is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Beef Extract
Beef Extract, Desiccated

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Beef Extract or Beef Extract, Desiccated in the formula of the medium being prepared. Add Beef Extract or Beef Extract, Desiccated as required.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Beef Extract or Beef Extract, Desiccated.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on prepared medium.
2. Formula allowances may be required due to the lower sodium chloride concentration of Beef Extract, Desiccated.

References

1. **Bedell and Lewis.** 1938. J. Bacteriol. **36**:567.
2. **Hutner.** 1938. J. Bacteriol. **35**:429.
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4. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Beef Extract	500 g	0126-17
Beef Extract, Desiccated	500 g	0115-17
	10 kg	0115-08

Bacto® BiGGY Agar

Intended Use

Bacto BiGGY Agar is used for isolating and differentiating *Candida* spp.

Also Known As

BiGGY Agar is an abbreviation for Bismuth Glucose Glycine Yeast Agar. BiGGY Agar is also referred to as Nickerson Agar and Nickerson Candida Elective Agar.

Summary and Explanation

BiGGY Agar is a modification of the formula described by Nickerson.[1,2] This medium was developed while studying sulfite reduction of *Candida* species. Nickerson described BiGGY Agar as a selective and differential medium for the isolation of *Candida albicans*. *C. albicans* can be differentiated from other *Candida* species based on colony morphology.

Candidiasis is the most frequently encountered opportunistic fungal infection.[3] It is caused by a variety of species of *Candida*, with *Candida albicans* being the most frequent etiological agent, followed by *Candida tropicalis* and *Candida (Torulopsis) glabrata*.[3] *Candida* species can be present in clinical specimens as a result of environmental contamination, colonization or actual disease process.[4]

Principles of the Procedure

Yeast Extract provides the nitrogen, vitamins and amino acids in BiGGY Agar. Glycine is used to stimulate growth. Dextrose is the carbon source. *Candida* species reduce bismuth sulfite, and colonies become brown to black in color. Bismuth Sulfite Indicator is also used as a selective agent against bacteria, often present as normal flora. Bacto Agar is used as the solidifying agent.

Formula

BiGGY Agar

Formula Per Liter

Bacto Yeast Extract	1 g
Glycine	10 g
Bacto Dextrose	10 g
Bismuth Sulfite Indicator	8 g
Bacto Agar	20 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

BiGGY Agar

Materials Required But Not Provided

Glassware
Incubator (30°C)
Waterbath (optional)
Sterile Petri dishes

Method of Preparation

1. Suspend 49 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. Avoid overheating. DO NOT AUTOCLAVE.
3. Evenly disperse the flocculent precipitate when dispensing.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion on the isolation and identification of yeast species refer to the procedures described in appropriate references.[3,4]

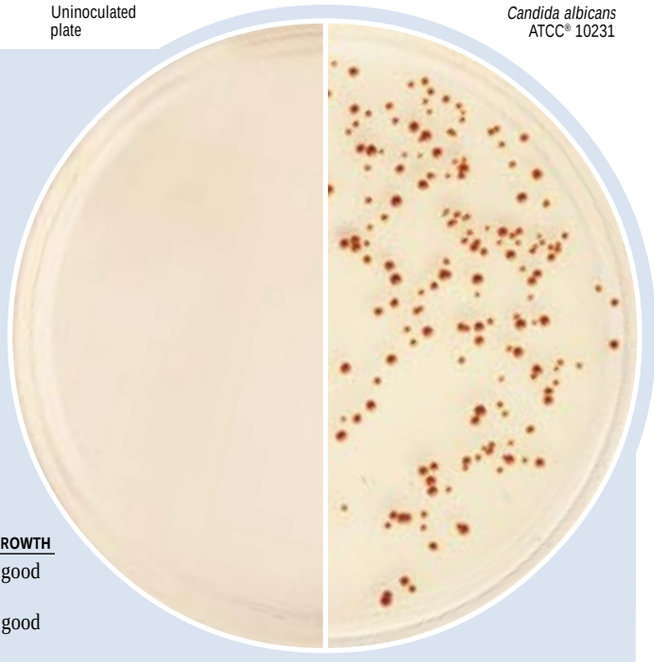
Results

Colony morphology according to Nickerson[2] after 48 hours of incubation on BiGGY Agar:

C. albicans	Intensely brown-black colonies with slight mycelial fringe, medium sized, no diffusion.
C. tropicalis	Discrete dark brown colonies with black centers and sheen, medium sized, diffuse blackening of the surrounding medium after 72 hours of incubation.
C. pseudotropicalis	Large, dark reddish-brown colonies, flat with slight mycelial fringe.
C. krusei	Large flat wrinkled colonies with silvery black top, brown edge and yellow halo.
C. parakrusei	Medium sized flat wrinkled colonies with red dish-brown color and yellow mycelial fringe.
C. stellatoidea	Medium size, flat, dark brown colonies; very light mycelial fringe.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Pigmented bacterial and yeast-like fungi are usually inhibited on BiGGY Agar. They can be differentiated by microscopic examination, if necessary. Dermatophytes and molds seldom appear and are easily recognized by development of aerial mycelia.[5]
3. Further growth characteristic and biochemical tests are needed to differentiate yeasts, particularly identification of *Candida* species.[5]



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.9% solution, soluble upon boiling in distilled or deionized water. Solution is very light to light amber, opalescent with a flocculent dispersable precipitate.
Prepared Medium:	Very light to light amber, opalescent with a flocculent precipitate.
Reaction of 4.9% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare BiGGY Agar per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	COLONY DESCRIPTION	GROWTH
Candida albicans	10231	100-1,000	brown to black, no diffusion into medium, no sheen	good
Candida kefyr	4135	100-1,000	reddish brown, flat colonies, no diffusion	good
Candida tropicalis	750	100-1,000	brown to black, sheen, black diffusion into medium	good
Escherichia coli	25922*	1,000-2,000	–	markedly inhibited

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

4. It is recommended that BiGGY Agar be prepared fresh, just prior to use.[1,2]
5. Do not use slants because the reactions are unsatisfactory.[1,2]

References

1. **Nickerson, W. J.** 1947. Biology of pathogenic fungi. The Chronica Botanica Co., Waltham, MA.
2. **Nickerson, W. J.** 1953. Reduction of inorganic substances by yeasts. I. Extracellular reduction of sulfite by species of *Candida*. J. Infect. Dis. **93**:43.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
4. **Warren, N. G., and K. C. Hazen.** 1995. *Candida*, *Cryptococcus*, and other yeasts of medical importance, p. 723-737. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover and R. H. Yolken (ed.)., Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 65-68. Williams & Wilkins, Baltimore, MD.

Packaging

BiGGY Agar	100 g	0635-15
	500 g	0635-17

Bacto® Bile Esculin Agar Base
Bacto Bile Esculin Agar

Intended Use

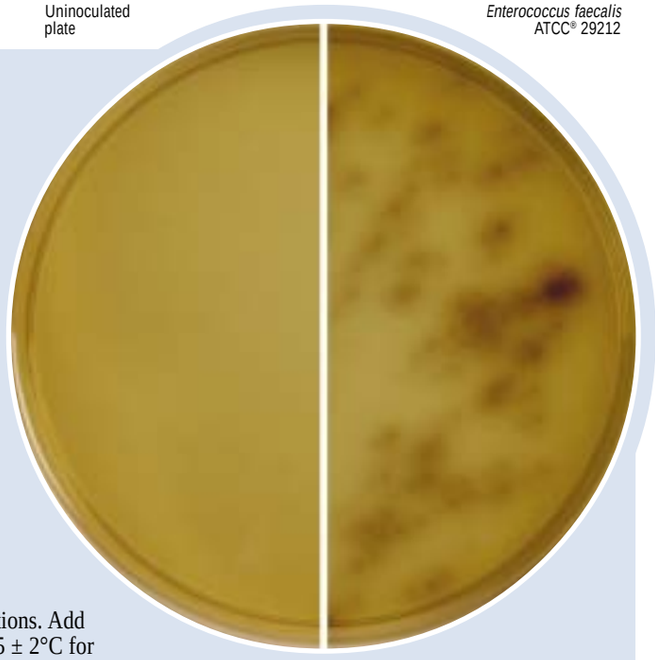
Bacto Bile Esculin Agar Base (with added esculin) and Bacto Bile Esculin Agar are differential media used for isolating and presumptively identifying group D streptococci.

Also Known As

Bile Esculin Agar is also known as Bile Esculin Medium (BEM). The spelling, aesculin, is often seen in literature.

Summary and Explanation

Bile Esculin Agar Base and Bile Esculin Agar are prepared according to the formulation described by Swan[1] and further evaluated by



User Quality Control

Identity Specifications

Dehydrated Appearance:	Greenish, light to medium beige, homogeneous, free-flowing.
Solution:	6.3% solution Bile Esculin Agar Base; 6.4% solution Bile Esculin Agar: soluble in distilled or deionized water on boiling. Solutions are medium to dark amber, slightly opalescent; media with esculin have a bluish cast.
Prepared Plates:	Greenish to medium amber, slightly opalescent; media with esculin have a bluish cast.
Reaction of Solution at 25°C:	6.3% solution Bile Esculin Agar Base; 6.4% solution Bile Esculin Agar: pH 6.6 ± 0.2

Cultural Response

Prepare Bile Esculin Agar Base or Bile Esculin Agar per label directions. Add 0.1% esculin to Bile Esculin Agar Base. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ESCULIN HYDROLYSIS
Enterococcus faecalis	29212*	100-1,000	good	+
Streptococcus pyogenes	19615*	2,000-10,000	inhibited	–

+ = positive, blackening of medium – = negative, no change

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Facklam and Moody.[2] Rochaix[3] first noted the value of esculin hydrolysis in the identification of enterococci. Meyer and Schönfeld[4] added bile to the esculin medium and demonstrated that 61 of 62 enterococci strains were able to grow and hydrolyze esculin, while the other streptococci could not.

Molecular taxonomic studies of the genus *Streptococcus* have placed enterococci, previously considered group D streptococci, in the distinct genus *Enterococcus*.[6] Streptococci with Lancefield group D antigen include the nonhemolytic species *Streptococcus bovis*.[7] The ability to hydrolyze esculin in the presence of bile is a characteristic of enterococci and group D streptococci.

Swan[1] compared the use of an esculin medium containing 40% bile salts with the Lancefield serological method of grouping. He reported that a positive reaction on the bile esculin medium correlated with a serological group D precipitin reaction. Facklam and Moody,[2] in a comparative study of tests used to presumptively identify group D streptococci, found that the bile esculin test provided a reliable means of identifying group D streptococci and differentiating them from non-group D streptococci. Facklam[5] further confirmed the usefulness of Bile Esculin Agar in another study differentiating enterococci/group D streptococci from non-group D streptococci.

Lindell and Quinn[8] showed that the medium is also useful in the differentiation of the *Klebsiella-Enterobacter-Serratia* group from other *Enterobacteriaceae.* Edberg et al.[9] recommended the medium for routine testing of the *Enterobacteriaceae* in order to differentiate *Klebsiella-Enterobacter-Serratia* spp. Bile Esculin Agar is listed in standard procedures for the microbiological examination of food products.[10-13]

Principles of the Procedure

Organisms positive for esculin hydrolysis hydrolyze the glycoside esculin to esculetin and dextrose. The esculetin reacts with the ferric citrate to form a dark brown or black complex. Oxgall (bile) is used to inhibit gram-positive bacteria other than enterococci. Beef Extract and Bacto Peptone provide the carbon and nitrogen sources required for growth of a wide variety of organisms. Bacto Agar is the solidifying agent.

Formula

Bile Esculin Agar

Formula Per Liter	
Bacto Beef Extract	3 g
Bacto Peptone	5 g
Esculin	1 g
Bacto Oxgall	40 g
Ferric Citrate	0.5 g
Bacto Agar	15 g

Final pH 6.6 ± 0.2 at 25°C

Bile Esculin Agar Base

Formula Per Liter	
Bacto Beef Extract	3 g
Bacto Peptone	5 g
Bacto Oxgall	40 g
Ferric Citrate	0.5 g
Bacto Agar	15 g

Final pH 6.6 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Bile Esculin Agar Base**:

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable gloves and eye/face protection. Use only in well ventilated areas. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   Bacto Bile Esculin Agar:

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Lungs.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bile Esculin Agar Base
Bile Esculin Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Esculin (to be added to Bile Esculin Agar Base)
Filter-sterilized horse serum (optional)
Petri dishes
Tubes with closures

Method of Preparation

1. Suspend the specidied amount of medium in 1 liter distilled or deionized water:

   Bile Esculin Agar Base - 63 grams

   Bile Esculin Agar - 64 grams

2. Heat to boiling to dissolve completely.
3. **Bile Esculin Agar Base**, only: Add 1 gram (or another desired amount) of Esculin and mix thoroughly.
4. Autoclave at 121°C for 15 minutes. Overheating may cause darkening of the media.
5. Cool to 50-55°C.
6. If desired, aseptically add 50 ml of filter-sterilized horse serum. Mix thoroughly.
7. Dispense as desired.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. The bile esculin test was originally formulated to identify enterococci. However, the properties of growth on 40% bile media and esculin hydrolysis are characteristics shared by most strains of Group D streptococci.[14] The bile esculin test should be used in combination with other tests to make a positive identification. Facklam[14] and Facklam et al.[15] recommend a combination of the bile esculin test and salt tolerance (growth in 6.5% NaCl). *Streptococcus bovis* will give a positive reaction on Bile Esculin Agar, but unlike *Enterococcus* spp., it cannot grow on 6.5% NaCl or at 10°C.[16]
2. Bile Esculin Agar should be considered a differential medium, but with the addition of sodium azide (which inhibits gram-negative bacteria) the medium can be made more selective (see Bile Esculin Azide Agar).
3. Occasional viridans strains will be positive on Bile Esculin Agar or will display reactions that are difficult to interpret.[17] Of the viridans group, 5 to 10% may be able to hydrolyze esculin in the presence of bile.[16]
4. Use a light inoculum when testing *Escherichia coli* on Bile Esculin Agar. Wasilauskas[18] suggests that the time required for an isolate to hydrolyze esculin is directly proportional to the size of the inoculum. For a tabulation of those *Enterobacteriaceae* that can hydrolyze esculin, refer to Farmer.[19]

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6. **Schleifer, K. H., and R. Kilpper-Balz.** 1987. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. Syst. Appl. Microbiol. **10**:1-19.
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Packaging

Bile Esculin Agar Base	500 g	0878-17
Bile Esculin Agar	100 g	0879-15
	500 g	0879-17
Esculin	10 g	0158-12

Bacto® Bile Esculin Azide Agar

Intended Use

Bacto Bile Esculin Azide Agar is used for isolating, differentiating and presumptively identifying group D streptococci.

Also Known As

Bile Esculin Azide (BEA) Agar conforms with Selective Enterococcus Medium (SEM) and Pfizer Selective Enterococcus Medium (PSE).

Summary and Explanation

Bile Esculin Azide Agar is a modification of the medium reported by Isenberg[1] and Isenberg, Goldberg and Sampson.[2] The formula modifies Bile Esculin Agar by adding sodium azide and reducing the concentration of bile. The resulting medium is more selective but still provides for rapid growth and efficient recovery of group D streptococci. Enterococcal streptococci were previously grouped in the genus *Streptococcus* with the Lancefield group D antigen. Molecular taxonomic studies have shown that enterococci were sufficiently different from other members of the genus *Streptococcus* to warrant the separate genus *Enterococcus*.[6] Other streptococci with the group D antigen exist in the genus *Streptococcus*, such as the non-hemolytic species *Streptococcus bovis*.[9]

The ability to hydrolyze esculin in the presence of bile is a characteristic of enterococci and group D streptococci. Esculin hydrolysis and bile tolerance, as shown by Swan[3] and by Facklam and Moody[4], permit the isolation and identification of group D streptococci in 24 hours. Sabbaj, Sutter and Finegold[5] evaluated selective media for selectivity, sensitivity, detection, and enumeration of presumptive group D streptococci from human feces. Bile Esculin Azide Agar selected for *S. bovis*, displayed earlier distinctive reactions, and eliminated the requirement for special incubation temperatures.

Brodsky and Schiemann[6] evaluated Pfizer Selective Enterococcus Medium (Bile Esculin Azide Agar) in the recovery of fecal streptococci from sewage effluent on membrane filters and found the medium to be highly selective for enterococci. Jensen[7] found that Bile Esculin Azide Agar supplemented with vancomycin combines differential and selective properties to rapidly isolate vancomycin-resistant enterococci from heavily contaminated specimens.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige to medium beige, free-flowing, homogeneous.
Solution:	5.7% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber with bluish cast, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Medium to dark amber with bluish cast, slightly opalescent.
Reaction of 5.7% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Prepare Bile Esculin Azide Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ESCULIN HYDROLYSIS
Enterococcus faecalis	29212*	100-1,000	good	positive, blackening of the medium
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition	negative, no color change

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Organisms positive for esculin hydrolysis hydrolyze the glycoside esculin to esculetin and dextrose. Esculetin reacts with ferric ammonium citrate to form a dark brown or black complex. Oxgall (bile) inhibits gram-positive bacteria other than enterococci, while sodium azide inhibits gram-negative bacteria. Tryptone and Proteose Peptone No. 3 provide nitrogen, vitamins and minerals. Yeast Extract provides vitamins and cofactors required for growth, as well as additional sources of nitrogen and carbon. Sodium chloride maintains the osmotic balance of the medium. Bacto Agar is the solidifying agent.

Formula

Bile Esculin Azide Agar

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Proteose Peptone No. 3	3 g
Bacto Tryptone	17 g
Bacto Oxgall	10 g
Bacto Esculin	1 g
Ferric Ammonium Citrate	0.5 g
Sodium Chloride	5 g
Sodium Azide	0.15 g
Bacto Agar	15 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. HARMFUL BY INHALATION AND IF SWALLOWED. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Cardiovascular, Lungs, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bile Esculin Azide Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Petri dishes
Horse Serum, filter sterilized (optional)

Method of Preparation

1. Suspend 57 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Overheating may cause darkening of the medium.
4. If desired, aseptically add 50 ml of filter-sterilized horse serum. Mix thoroughly.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

For isolation of group D streptococci, inoculate the sample onto a small area of one quadrant of a Bile Esculin Azide Agar plate and streak for isolation. This will permit development of discrete colonies. Incubate at 35°C for 18-24 hours. Examine for colonies having the characteristic morphology of group D streptococci.

Results

Group D streptococci grow readily on this medium and hydrolyze esculin, resulting in a dark brown color around the colonies after 18-24 hours incubation.

Limitations of the Procedure

1. *Staphylococcus aureus* and *Staphylococcus epidermidis* may exhibit growth on the medium (less than 1 mm, white-gray colonies), but they will show no action on the esculin.[2]
2. Other than the enterococci, *Listeria monocytogenes* consistently blackens the medium around colonies. After 18-24 hours, there may be a reddish to black-brown zone of hydrolysis surrounding pinpoint *Listeria* colonies. After 48 hours, white-gray pigmented colonies will be seen. *Listeria* do not attain the same degree of esculin hydrolysis displayed by enterococci in this short incubation period.[2]

References

1. **Isenberg, H. D.** 1970. Clin. Lab. Forum. July.
2. **Isenberg, H. D., D. Goldberg, and J. Sampson.** 1970. Laboratory studies with a selective enterococcus medium. Appl. Microbiol. **20**:433.
3. **Swan, A.** 1954. The use of bile-esculin medium and of Maxted's technique of Lancefield grouping in the identification of enterococci (group D streptococci). J. Clin. Pathol. **7**:160.
4. **Facklam, R. R., and M. D. Moody.** 1970. Presumptive identification of group D streptococci: The bile-esculin test. Appl. Microbiol. **20**:245.
5. **Sabbaj, J., V. L. Sutter, and S. M. Finegold.** 1971. Comparison of selective media for isolation of presumptive group D streptococci from human feces. Appl. Microbiol. **22**:1008.
6. **Brodsky, M. H., and D. A. Schiemann.** 1976. Evaluation of Pfizer selective enterococcus and KF media for recovery of fecal streptococci from water by membrane filtration. Appl. Environ. Microbiol. **31**:695-699.
7. **Jensen, B. J.** 1996. Screening specimens for vancomycin-resistant *Enterococcus*. Laboratory Medicine **27**:53-55.
8. **Schleifer, K. H., and R. Kilpper-Balz.** 1987. Molecular and chemotaxonomic approaches to the classification of streptococci, enterococci and lactococci: a review. Syst. Appl. Microbiol. **10**:1-19.
9. **Ruoff, K. L.** 1995. *Streptococcus. In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Bile Esculin Azide Agar	100 g	0525-15
	500 g	0525-17
	2 kg	0525-07

Bacto® Biotin Assay Medium

Intended Use

Bacto Biotin Assay Medium is used for determining biotin concentration by the microbiological assay technique.

Summary and Explanation

Vitamin Assay Media are used in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test. Assay media contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Biotin Assay Medium is prepared for use in the microbiological assay of biotin using *Lactobacillus plantarum* ATCC® 8014 as the test organism.

Principles of the Procedure

Biotin Assay Medium is a biotin-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *L. plantarum* ATCC® 8014. The addition of biotin standard in specified increasing concentrations gives a growth response by this organism that can be measured titrimetrically or turbidimetrically.

Formula

Biotin Assay Medium

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	12	g
Bacto Dextrose	40	g
Sodium Acetate	20	g
L-Cystine	0.2	g
DL-Tryptophane	0.2	g
Adenine Sulfate	20	mg
Guanine Hydrochloride	20	mg
Uracil	20	mg
Thiamine Hydrochloride	2	mg
Riboflavin	2	mg
Niacin	2	mg
Calcium Pantothenate	2	mg
Pyridoxine Hydrochloride	4	mg
p-Aminobenzoic Acid	200	µg
Dipotassium Phosphate	1	g
Monopotassium Phosphate	1	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20	mg
Ferrous Sulfate	20	mg
Manganese Sulfate	20	mg

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Take great care to avoid contamination of media or glassware for microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware, free from detergents and other chemicals, must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
3. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, homogeneous with a tendency to clump.
Solution:	3.75% (single strength) solution, soluble in distilled or deionized water on boiling 2-3 minutes. Light amber, clear, may have a slight precipitate.
Prepared Medium:	(Single strength) light amber, clear, may have slight precipitate.
Reaction of 3.75% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Biotin Assay Medium per label directions. Prepare a standard curve using biotin at levels of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1 ng per 10 ml. The medium supports the growth of *L. plantarum* ATCC® 8014 when prepared in single strength and supplemented with biotin.

4. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Biotin Assay Medium

Materials Required But Not Provided

Lactobacilli Agar AOAC
Centrifuge
Spectrophotometer
Biotin
Glassware
Autoclave
Sterile tubes
Stock culture of *Lactobacillus plantarum* ATCC® 8014
Sterile 0.85% saline
Distilled or deionized water

Method of Preparation

1. Suspend 7.5 grams in 100 ml distilled or deionized water.
2. Boil 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 5 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assay, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Stock Cultures

Stock cultures of the test organism, *L. plantarum* ATCC® 8014, are prepared by stab inoculation of Lactobacilli Agar AOAC. After 16-24 hours incubation at 35-37°C, the tubes are stored in the refrigerator. Transfers are made weekly.

Inoculum

Inoculum for assay is prepared by subculturing from a stock culture of *L. plantarum* ATCC® 8014 to 10 ml of single-strength Biotin Assay Medium supplemented with 0.5 ng biotin. After 16-24 hours incubation at 35-37°C, the cells are centrifuged under aseptic conditions and the supernatant liquid decanted. The cells are washed three times with 10 ml sterile 0.85% saline. After the third wash, the cells are resuspended in 10 ml sterile 0.85% saline and finally diluted 1:100 with sterile 0.85% saline. One drop of this suspension is used to inoculate each 10 ml assay tube.

Standard Curve

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. The standard curve is obtained by using biotin at levels of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.8 and 1 ng per assay tube (10 ml).

The concentration of biotin required for the preparation of the standard curve may be prepared by dissolving 0.1 gram of d-Biotin or equivalent in 1,000 ml of 25% alcohol solution (100 µg per ml). Dilute the stock solution by adding 2 ml to 98 ml of distilled water. This solution is diluted by adding 1 ml to 999 ml distilled water, giving a solution of 2 ng of biotin per ml. This solution is further diluted by adding 10 ml to 90 ml distilled water, giving a final solution of 0.2 ng of biotin per ml. Use 0.0, 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 ml of this final solution. Prepare the stock solution fresh daily.

Biotin Assay Medium may be used for both turbidimetric and titrimetric analysis. Before reading, the tubes are refrigerated for 15-30 minutes to stop growth. Turbidimetric readings should be made after 16-20 hours at 35-37°C. Titrimetric determinations are made after 72 hours incubation at 35-37°C. The most effective assay range, using Biotin Assay Medium, has been found to be between 0.1 ng and 1 ng biotin.

For a complete discussion of antibiotic assay methodology, refer to appropriate procedures outlined in the references.[1,2]

Results

Calculations

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average. Use the results only if two thirds of the values do not vary by more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results to these procedures, all conditions of the assay must be followed precisely.

References

1. **Federal Register.** 1992. Tests and methods of assay of antibiotics and antibiotic-containing drugs. Fed. Regist. **21**:436.100-436.106.
2. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. Biological tests and assay, p. 1690-1696. The United States Pharmacopeial Convention, Rockville, MD.

Packaging

Biotin Assay Medium	100 g	0419-15

Bacto® Bismuth Sulfite Agar

Intended Use

Bacto Bismuth Sulfite Agar is used for isolating *Salmonella* spp, particularly *Salmonella typhi*, from food and clinical specimens.

Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella* in domesticated animals. Infection with nontyphi *Salmonella* often causes mild, self-limiting illness.[1] Typhoid fever, caused by *S. typhi*, is characterized by fever, headache, diarrhea, and abdominal pain, and can produce fatal respiratory, hepatic, splenic, and/or neurological damage. These illnesses result from consumption of raw, undercooked or improperly processed foods contaminated with *Salmonella*. Many cases of *Salmonella*-related gastroenteritis are due to improper handling of poultry products. United States federal guidelines require various poultry products to be routinely monitored before distribution for human consumption but contaminated food samples often elude monitoring.

Bismuth Sulfite Agar is a modification of the Wilson and Blair[2-4] formula. Wilson[5,6] and Wilson and Blair[2-4] clearly showed the superiority of Bismuth Sulfite medium for isolation of *S. typhi*. Cope and Kasper[7] increased their positive findings of typhoid from 1.2 to 16.8% among food handlers and from 8.4 to 17.5% among contacts with Bismuth Sulfite Agar. Employing this medium in the routine laboratory examination of fecal and urine specimens, these same authors[8] obtained 40% more positive isolations of *S. typhi* than were obtained on Endo medium. Gunther and Tuft,[9] employing various media in a comparative way for the isolation of typhoid from stool and urine specimens, found Bismuth Sulfite Agar most productive. On Bismuth Sulfite Agar, they obtained 38.4% more positives than on Endo Agar, 33% more positives than on Eosin Methylene Blue Agar, and 80% more positives on Bismuth Sulfite Agar than on the Desoxycholate media. These workers found Bismuth Sulfite Agar to be superior to Wilson's original medium. Bismuth Sulfite Agar was stable, sensitive and easier to prepare. Green and Beard,[10] using Bismuth Sulfite Agar, claimed that this medium successfully inhibited sewage organisms. The value of Bismuth Sulfite Agar as a plating medium after enrichment has been demonstrated by Hajna and Perry.[11]

Since these earlier references to the use of Bismuth Sulfite Agar, this medium has been generally accepted as routine for the detection of most *Salmonella*. The value of the medium is demonstrated by the many references to the use of Bismuth Sulfite Agar in scientific publications, laboratory manuals and texts. Bismuth Sulfite Agar is used in microbial limits testing as recommended by the United States Pharmacopeia. In this testing, pharmaceutical articles of all kinds, from raw materials to the finished forms, are evaluated for freedom from *Salmonella* spp.[12]

For food testing, the use of Bismuth Sulfite Agar is specified for the isolation of pathogenic bacteria from raw and pasteurized milk, cheese products, dry dairy products, cultured milks, and butter.[1,13-15] The use of Bismuth Sulfite Agar is also recommended for use in testing clinical specimens.[16,17] In addition, Bismuth Sulfite Agar is valuable when investigating outbreaks of *Salmonella* spp., especially *S. typhi*.[18-20]

Bismuth Sulfite Agar is used for the isolation of *S. typhi* and other *Salmonella* from food, feces, urine, sewage and other infectious materials. The typhoid organism grows luxuriantly on the medium, forming characteristic black colonies, while gram-positive bacteria and members of the coliform group are inhibited. This inhibitory action of Bismuth Sulfite Agar toward gram-positive and coliform organisms permits the use of a much larger inoculum than possible with other media employed for similar purposes in the past. The use of larger inocula greatly increases the possibility of recovering the pathogens, especially when they are present in relatively small numbers. Small numbers of organisms may be encountered in the early course of the disease or in the checking of carriers and releases.

Principles of the Procedure

In Bismuth Sulfite Agar, Beef Extract and Bacto Peptone provide nitrogen, vitamins and minerals. Dextrose is an energy source. Disodium phosphate is a buffering agent. Bismuth sulfite indicator and brilliant green are complementary in inhibiting gram-positive bacteria and members of the coliform group, while allowing *Salmonella* to grow luxuriantly. Ferrous sulfate is for H_2S production. When H_2S is present, the iron in the formula is precipitated, giving positive cultures the characteristic brown to black color with metallic sheen. Agar is a solidifying agent.

Formula

Bismuth Sulfite Agar

Formula Per Liter

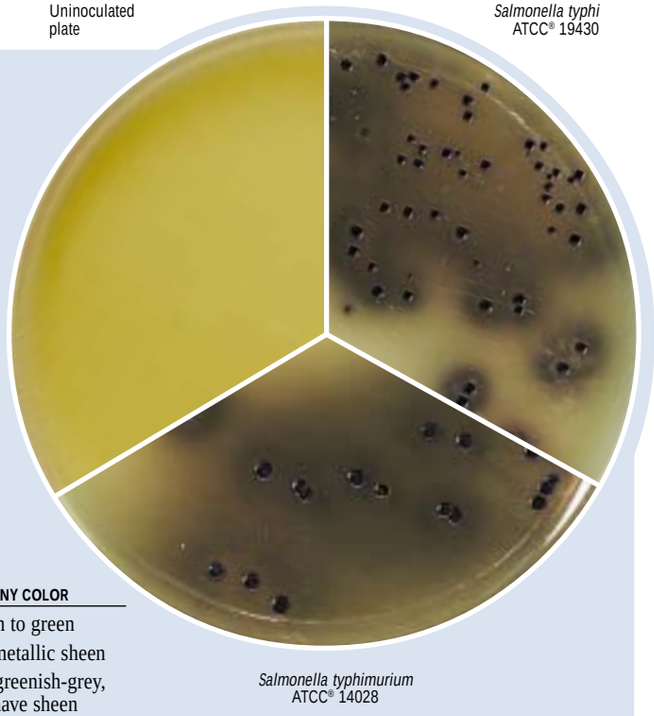
Bacto Beef Extract	5 g
Bacto Peptone	10 g
Bacto Dextrose	5 g
Disodium Phosphate	4 g
Ferrous Sulfate	0.3 g
Bismuth Sulfite Indicator	8 g
Bacto Agar	20 g
Brilliant Green	0.025 g

Final pH 7.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** MAY CAUSE SENSITIZATION BY INHALATION. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige to light green, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in distilled or deionized water on boiling. Solution is light green, opaque with a flocculent precipitate that must be dispersed by swirling contents of flask.
Prepared Plates:	Light grey-green to medium green, opaque with a flocculent precipitate.
Reaction of 5.2% solution at 25°C:	7.7 ± 0.2

Cultural Response

Prepare Bismuth Sulfite Agar per label directions.
Inoculate and incubate the plates at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC®	CFU	GROWTH	COLONY COLOR
Escherichia coli	25922*	1,000-2,000	partial inhibition	brown to green
Salmonella typhi	19430	100-1,000	good	black w/metallic sheen
Salmonella typhimurium	14028*	100-1,000	good	black or greenish-grey, may have sheen
Enterococcus faecalis	29212*	1,000-2,000	markedly inhibited	–

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared plates at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bismuth Sulfite Agar

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Waterbath (45-50°C)
Petri dishes
Incubator (35°C)

Method of Preparation

1. Suspend 52 grams in 1 liter distilled or deionized water.
2. Heat to boiling no longer than 1-2 minutes to dissolve. Avoid overheating. DO NOT AUTOCLAVE.
3. Cool to 45-50°C in a waterbath.
4. Gently swirl flask to evenly disperse the flocculent precipitate. Dispense into sterile Petri dishes.

   NOTE: Best results are obtained when the medium is dissolved and used immediately. The melted medium should not be allowed to solidify in flasks and remelted. Current references suggest that the prepared plated medium should be aged for one day before use.[13,21]

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[1,13-20]
2. Process each specimen, using procedures appropriate for that specimen or sample.[1,13-20]

Test Procedure

For isolation of *Salmonella* spp. from food, samples are enriched and selectively enriched. Streak 10 µl of selective enrichment broth onto Bismuth Sulfite Agar. Incubate plates for 24-48 hours at 35°C. Examine plates for the presence of *Salmonella* spp. Refer to appropriate references for the complete procedure when testing food samples.[1,13-15]

For isolation of *Salmonella* spp. from clinical specimens, inoculate fecal specimens and rectal swabs onto a small area of one quadrant of the Bismuth Sulfite Agar plate and streak for isolation. This will permit the development of discrete colonies. Incubate plates at 35°C. Examine at 24 hours and again at 48 hours for colonies resembling *Salmonella* spp.

For additional information about specimen preparation and inoculation of clinical specimens, consult appropriate references.[16-20]

Results

The typical discrete *S. typhi* surface colony is black and surrounded by a black or brownish-black zone which may be several times the size of the colony. By reflected light, preferably daylight, this zone exhibits a distinctly characteristic metallic sheen. Plates heavily seeded with *S. typhi* may not show this reaction except near the margin of the mass inoculation. In these heavy growth areas, this organism frequently appears as small light green colonies. This fact emphasizes the importance of inoculating plates so that some areas are sparsely populated with discrete *S. typhi* colonies. Other strains of *Salmonella* produce black to green colonies with little or no darkening of the surrounding medium.

Generally, *Shigella* spp. other than *S. flexneri* and *S. sonnei* are inhibited. *Shigella flexneri* and *Shigella sonnei* strains that do grow on this medium produce brown to green, raised colonies with depressed centers and exhibit a crater-like appearance.

E. coli is partially inhibited. Occasionally a strain will be encountered that will grow as small brown or greenish glistening colonies. This color is confined entirely to the colony itself and shows no metallic sheen. A few strains of *Enterobacter aerogenes* may develop on this medium, forming raised, mucoid colonies. *Enterobacter* colonies may exhibit a silvery sheen, appreciably lighter in color than that produced by *S. typhi*. Some members of the coliform group that produce hydrogen sulfide may grow on the medium, giving colonies similar in appearance to *S. typhi*. These coliforms may be readily differentiated because they produce gas from lactose in differential media, for example, Kligler Iron Agar or Triple Sugar Iron Agar. The hydrolysis of urea, demonstrated in Urea Broth or on Urea Agar Base, may be used to identify *Proteus* sp.

To isolate *S. typhi* for agglutination or fermentation studies, pick characteristic black colonies from Bismuth Sulfite Agar and subculture them on MacConkey Agar. The purified colonies from MacConkey Agar may then be picked to differential tube media such as Kligler Iron Agar, Triple Sugar Iron Agar or other satisfactory differential media for partial identification. All cultures that give reactions consistent with *Salmonella* spp. on these media should be confirmed biochemically as *Salmonella* spp. before any serological testing is performed. Agglutination tests may be performed from the fresh growth on the differential tube media or from the growth on nutrient agar slants inoculated from the differential media. The growth on the differential tube media may also be used for inoculating carbohydrate media for fermentation studies.

Limitations of the Procedure

1. It is important to streak for well isolated colonies. In heavy growth areas, *S. typhi* appears light green and may be misinterpreted as negative growth for *S. typhi*.[22]
2. *S. typhi* and *S. arizonae* are the only enteric organisms to exhibit typical brown zones on the medium. Brown zones are not produced by other members of the *Enterobacteriaceae*. However, *S. arizonae* is usually inhibited.[22]
3. Colonies on Bismuth Sulfite Agar may be contaminated with other viable organisms; therefore, isolated colonies should be subcultured to a less selective medium (e.g., MacConkey Agar).[22]
4. Typical *S. typhi* colonies usually develop within 24 hours; however, all plates should be incubated for a total of 48 hours to allow growth of all typhoid strains.[22]

5. DO NOT AUTOCLAVE. Heating this medium for a period longer than necessary to just dissolve the ingredients destroys its selectivity.

References

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Packaging

Bismuth Sulfite Agar	100 g	0073-15
	500 g	0073-17
	10 kg	0073-08

Bacto® Blood Agar Base
Bacto Blood Agar Base No. 2

Intended Use

Bacto Blood Agar Base is used for isolating and cultivating a wide variety of microorganisms and, with added blood, for cultivating fastidious microorganisms.

Bacto Blood Agar Base No. 2 is used for isolating and cultivating fastidious microorganisms with or without added blood.

Also Known As

Blood Agar Base is abbreviated as BAB, and may be referred to as Infusion Agar.

Summary and Explanation

Blood agar bases are typically supplemented with 5-10% sheep, rabbit or horse blood for use in isolating, cultivating and determining hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, blood agar bases can be used as general purpose media.

In 1919, Brown[1] experimented with blood agar formulations for the effects of colony formation and hemolysis; the growth of pneumococci was noticeably influenced when the medium contained peptone manufactured by Difco.

Blood Agar Base is a modification of Huntoon's[2] "Hormone" Medium with a slight acidic composition. Norton[3] found the pH of 6.8 to be advantageous in culturing streptococci and pneumococci. Blood Agar Base No. 2 is a nutritionally rich medium for maximum recovery of fastidious microorganisms.

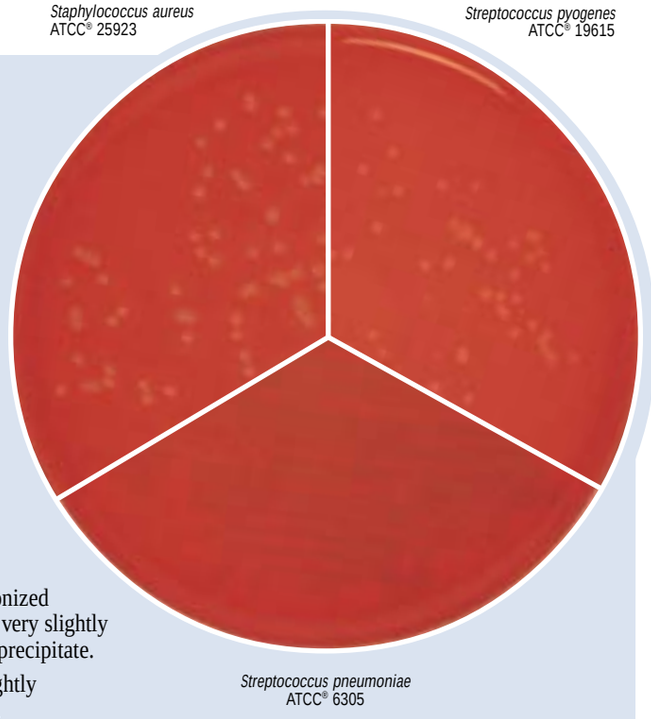
Blood Agar Base media are specified in Standard Methods[4,5,6] for food testing.

Principles of the Procedure

Blood Agar Base formulations have been prepared using specially selected raw materials to support good growth of a wide variety of fastidious microorganisms.

Infusion from Beef Heart and Tryptose provide nitrogen, carbon, amino acids and vitamins in Blood Agar Base. Proteose Peptone No. 3 is the nitrogen source for Blood Agar Base No. 2 while Yeast Extract and Liver Digest provide essential carbon, vitamin, nitrogen and amino acids sources. Both media contain Sodium Chloride to maintain osmotic balance and Bacto Agar as a solidifying agent. Blood Agar Bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of beta-hemolytic streptococci.[7]

Supplementation with blood (5-10%) provides additional growth factors for fastidious microorganisms and is the basis for determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood or type of base medium used.[8] Chocolate agar for isolating *Haemophilus* and *Neisseria* species can be prepared from Blood Agar Base No. 2 by supplementing the medium with 10% sterile defibrinated blood (chocolatized).



User Quality Control

Identity Specifications

Blood Agar Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Without blood -light to medium amber, slightly opalescent.
	With 5% sheep blood - cherry red, opaque.
Reaction of 4.0% Solution at 25°C:	pH 6.8 ± 0.2

Blood Agar Base No. 2

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.95% solution, soluble in distilled or deionized water upon boiling; medium to dark amber very slightly to slightly opalescent, without significant precipitate.
Prepared Medium:	Without blood-medium to dark amber, slightly opalescent, without significant precipitate.
	With 5% sheep blood-cherry red, opaque.
Reaction of 3.95% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Blood Agar Base or Blood Agar Base No. 2 per label directions with and without 5% sterile defibrinated sheep blood. Inoculate and incubate at 35 ± 2°C under approximately 10% CO_2 for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HEMOLYSIS
Escherichia coli	25922	100-1,000	good	–
Staphylococcus aureus	25923*	100-1,000	good	beta
Streptococcus pneumoniae	6305	100-1,000	good	alpha
Streptococcus pyogenes	19615*	100-1,000	good	beta

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

Blood Agar Base

Formula Per Liter

Beef Heart, Infusion from	500 g
Bacto Tryptose	10 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Blood Agar Base No. 2

Formula Per Liter

Bacto Proteose Peptone No. 3	15 g
Liver Digest	2.5 g
Bacto Yeast Extract	5 g
Sodium Chloride	5 g
Bacto Agar	12 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Blood Agar Base

Blood Agar Base No. 2

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile defibrinated blood
Sterile Petri dishes

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water:
   Blood Agar Base - 40 grams;
   Blood Agar Base No. 2 - 39.5 grams.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. To prepare chocolate agar, add 10% sterile defibrinated blood to Blood Agar Base No. 2 at 80°C. Mix well.
6. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory in accordance with recommended guidelines outlined in the references.

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to the activity of both oxygen-stable and oxygen-labile streptolysins.[8]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.

Results

Examine the medium for growth and hemolytic reactions after 18-24 and 48 hours incubation. Four types of hemolysis on blood agar media can be described:[9]

a. Alpha hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This causes a greenish discoloration of the medium.
b. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
c. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
d. Alpha-prime hemolysis (ὰ) is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Limitations of the Procedure

1. Blood Agar Base media are intended for use with blood supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.
2. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
3. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-hemolytic on horse, human and rabbit blood agar and alpha-hemolytic on sheep blood agar.[8]
4. Colonies of *Haemophilus haemolyticus* are beta-hemolytic on horse and rabbit blood agar and must be distinguished from colonies of beta-hemolytic streptococci using other criteria. The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of *H. haemolyticus*.[10]
5. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[8] For optimal performance, incubate blood agar base media under increased CO_2 or anaerobic conditions.

References

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Packaging

Blood Agar Base	100 g	0045-15
	500 g	0045-17
	2 kg	0045-07
Blood Agar Base No. 2	500 g	0696-17

Bacto® Bordet Gengou Agar Base

Intended Use

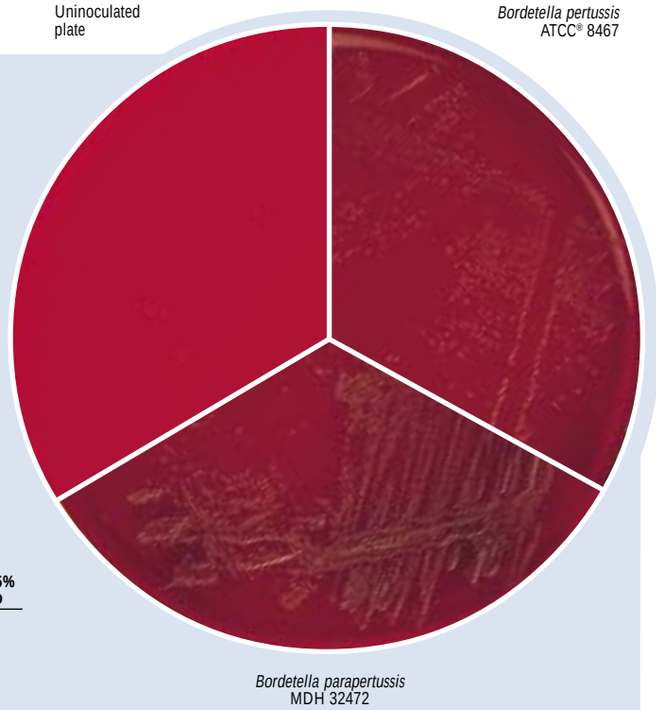
Bacto Bordet Gengou Agar Base is used with added blood for isolating *Bordetella pertussis* and other *Bordetella* species.

Also Known As

Bordet Gengou Agar Base is also referred to as B-G Agar Base and Bordet-Gengou Potato-Glycerol Agar.[1]

Summary and Explanation

Bordet Gengou Agar Base is a modification of the medium originally described by Bordet and Gengou[2] in 1906 for the cultivation of *Haemophilus pertussis*, now *Bordetella pertussis*. The original formula used a base medium consisting of 1% glycerol and potato extract with



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble upon boiling in distilled or deionized water containing 1% glycerol; light to medium amber, opalescent, may have a slight precipitate.
Prepared Medium:	Plain - Light to medium amber, opalescent, may have a precipitate. With 15% blood - Cherry red, opaque.
Reaction of 3.0% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare Bordet Gengou Agar Base enriched with 15% sterile defibrinated blood per label directions. Inoculate and incubate at 35 ± 2°C for 48-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH W/O BLOOD	GROWTH W/15% RABBIT BLOOD
Bordetella bronchiseptica	4617	30-300	good	good
Bordetella parapertussis	MDH 32472	30-300	poor to good	good
Bordetella pertussis	8467	30-300	poor to good	good

The cultures listed are the minimum that should be used for performance testing.

an equal volume of human or rabbit blood. The modified medium is prepared according to the formula recommended by the American Public Health Association.[3] Eldering and Kendrick[4] reported that the addition of 1% proteose peptone or neopeptone increased growth of *B. pertussis*, thereby increasing the yield of vaccine.

The genus *Bordetella* consists of four species: *Bordetella pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium*.[5] All *Bordetella* are respiratory pathogens, residing on the mucous membranes of the respiratory tract. *B. pertussis* and *B. parapertussis* are uniquely human pathogens. *B. pertussis* is the major cause of whooping cough or pertussis. *B. parapertussis* is associated with a milder form of the disease.[6] *B. bronchiseptica* is an opportunistic human pathogen associated with both respiratory and non-respiratory infections, often occurring in patients having close contact with animals.[5] *B. bronchiseptica* has not been reported to cause pertussis. There have been no reports of recovery of *B. avium* from humans.[5]

The "cough plate" method for the diagnosis of whooping cough was originally reported by Chievitz and Meyer.[7] This technique is no longer recommended. Nasopharyngeal washings or a nasopharyngeal swab (calcium alginate on a wire handle) should be collected within the first week of paroxysmal coughing.[8]

Principles of the Procedure

Infusion from Potato provides nitrogen, vitamins and amino acids. Glycerol is a carbon source. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent. The addition of blood provides essential growth requirements for *Bordetella* species.

Many factors will inhibit growth of *B. pertussis*, including fatty acids present in nasal secretions or cotton from the collection swab. Starch, present from the Potato Infusion, absorbs fatty acids.

Modified Bordet Gengou medium, enriched with 15-20% blood, yields typical *B. pertussis* growth. The colonies appear small, white, opaque and surrounded by a characteristic zone of hemolysis that is not sharply defined but merges diffusely into the medium. The zone of hemolysis is usually absent if 30% or more blood is added to the medium and cannot be seen on charcoal-containing media.[9] Sterile, defibrinated sheep or rabbit blood can be used in preparing the medium.

Formula

Bordet Gengou Agar Base

Formula Per Liter	
Potato, Infusion from	125 g
Sodium Chloride	5.5 g
Bacto Agar	20 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bordet Gengou Agar Base

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Sterile defibrinated blood
Sterile Petri dishes

Method of Preparation

1. Suspend 30 grams in 1 liter distilled or deionized water containing 10 grams of glycerol.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C. Aseptically add 15% sterile defibrinated sheep or rabbit blood. Mix well.
5. Dispense into sterile Petri dishes.

Specimen Collection and Preparation[9]

Specimens should be obtained during the early phases of the disease and prior to the convalescent stage and antimicrobial therapy. The specimen of choice is duplicate nasopharyngeal swabs. Direct plating of the specimen at bedside is recommended; when this is not possible, submerge both swabs into Regan-Lowe transport medium.

Test Procedure[9]

1. Roll one of the swabs over the primary inoculation area of the Bordet Gengou plate and streak for isolation. Return the swab to the transport medium. Incubate the transport medium for 48 hours. Plate the swabs onto a duplicate set of media.
2. Incubate the culture plates at 35°C for 5-7 days in a moist chamber. Increased CO_2 is not recommended. Growth of *B. pertussis* appears in 3-5 days. Other bordetellae species can appear in 1-3 days.
3. Nasopharyngeal specimens may contain staphylococci that produce a diffusible substance inhibitory to *B. pertussis* growth. For these specimens, use a plating medium with methicillin (2.5 µg/ml) or cephalexin (40 µg/ml) and a medium without antimicrobics.
4. Isolates suspected of being *B. pertussis* should be confirmed by using a specific antiserum in either the slide agglutination or fluorescent antibody staining techniques.[10]

Results

For a complete discussion on the isolation and identification of *Bordetella* species refer to the appropriate procedures outlined in the references.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

2. Some *Haemophilus* species will grow on *Bordetella* isolation media and may cross-react with *B. pertussis* antisera. It may be prudent to rule out X and V factor dependence.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol. 1, p. 86-92. Williams & Wilkins, Baltimore, MD.
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9. **Isenberg, H. D.** (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
10. **Koneman, E. W.** 1988. Color atlas and textbook of diagnostic microbiology, 3rd ed. J. B. Lippincott Company, Washington, D.C.

Packaging

Bordet Gengou Agar Base	100 g	0048-15
	500 g	0048-17

Bacto® Bovine Albumin 5%

Intended Use

Bacto Bovine Albumin 5% is used to enrich media for cultivating a large variety of microorganisms and tissue cells.

Also Known As

Bovine Albumin can be abbreviated as BSA.[1]

User Quality Control

Identity Specifications

Bovine Albumin 5%

Appearance:	Light amber, clear to very slightly opalescent.
Sterility Test:	Negative.
Reaction of Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Dubos Broth Base per label directions, substituting Bovine Albumin 5% for Dubos Medium Albumin. Inoculate and incubate at 35 ± 2°C under CO_2 for up to three weeks.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Mycobacterium intracellulare	13950	100-1,000	good
Mycobacterium tuberculosis H37Ra	25177	100-1,000	good
Mycobacterium tuberculosis H37Ra	27294	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Summary and Explanation

Davis and Dubos[2] recommended the use of bovine albumin at a final concentration of 0.5% in liquid media for culturing *Mycobacterium tuberculosis*. In this study, bovine albumin neutralized the toxicity of fatty acids and permitted more luxuriant growth of *M. tuberculosis*.

Ellinghausen and McCullough[3] used bovine albumin fraction V at a final concentration of 1% in liquid, semisolid and solid media for culturing leptospires. Morton et al.[4] demonstrated that 1% bovine albumin stimulated growth of *Mycoplasma* (PPLO).

Bovine Albumin can be added to normally sterile specimens, tissues and body fluids for direct inoculation onto culture media used for isolating mycobacteria. BSA is also used as an enrichment when contaminated specimens are digested.

Bovine Albumin 5%, modified with added sodium chloride and dextrose, is available as Dubos Medium Albumin.

Principles of the Procedure

Bovine Albumin 5% is a filter sterilized solution of Bovine Albumin Fraction V. BSA is suggested as a culture media enrichment because its buffering capacity and detoxifying effect on specimen sediment.[1] Bovine Albumin 5% also increases adhesion of the specimen to solid media.[1]

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.
3. Mycobacterial organisms are BioSafety Level 2 pathogens. The handling of clinical specimen material that is potentially infected with mycobacteria should be performed in a Class I or II biological safety cabinet (BSC).[1]

Storage

Store Bovine Albumin 5% at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bovine Albumin 5%

Materials Required But Not Provided

Materials vary depending on the specimen collected and the procedure performed.

Method of Preparation

Refer to the final concentration of Bovine Albumin in the procedure being used to inoculate specimens. A 0.2% solution of Bovine Albumin 5% is recommended for the inoculation of sterile and contaminated specimens when isolating mycobacteria.[1]

Specimen Collection and Preparation

Many different specimen types can be collected for mycobacterial cultures but the majority will be from the respiratory tract.[1] Tissues, body fluids, urine, blood and gastric aspirates can also be tested for the presence of mycobacteria. Refer to the procedures established by laboratory policy or to appropriate references for specific guidelines on specimen collection and processing.

Test Procedure

Sterile Specimens for the Isolation of Mycobacteria[1]

Normally sterile tissues may be ground in 0.2% BSA and inoculated directly in culture media. Concentrate body fluids before inoculation because they normally contain only a small number of mycobacteria. Centrifuge fluids at 3,000 x g and inoculate the sediment onto liquid or solid media. For a complete discussion of the inoculation of sterile specimens, refer to appropriate references.

Contaminated Specimens for the Isolation of Mycobacteria[1]

A concentration of 0.2% Bovine Albumin fraction V can be added to specimen sediment that has been digested and centrifuged by the NALC-NaOH digestion method. Using a separate sterile pipette for each tube, add 1-2 ml of 0.2% BSA, then resuspend the sediment with the pipette or by shaking the tube gently by hand.

Several digestion procedures exist. Consult appropriate references for a complete discussion on all digestion and decontamination methods and other testing procedures.

Results

All media should be examined closely for evidence of growth. Refer to the procedure established by laboratory policy or to appropriate references on typical growth patterns and confirmation tests.

Limitations of the Procedure

1. Bovine Albumin 5% is not recommended for use with Bactec® because BSA may delay detection times.[1]

References

1. **Nolte, F. S., and B. Metchock.** 1995. *Mycobacterium*, p. 400-437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
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4. **Morton, H. E., P. E. Smith, N. B. Williams, and C. F. Eickenberg.** 1951. Isolation of pleuropneumonia-like organisms from human saliva: A newly detected member of the oral flora. J. Dent. Res. **30**:415-422.

Packaging

Bovine Albumin 5%	12 x 20 ml	0668-64

Brain Heart Infusion Media

Bacto® Brain Heart Infusion · Bacto Brain Heart Infusion Agar Bacto Clostridium Difficile Antimicrobic Supplement CC Bacto Brain Heart CC Agar · Bacto Brain Heart Infusion w/PAB and Agar · Bacto Brain Heart Infusion w/o Dextrose

Intended Use

Bacto Brain Heart Infusion is used for cultivating fastidious microorganisms, including streptococci, pneumococci and meningococci.

Bacto Brain Heart Infusion Agar is used for cultivating fastidious microorganisms, especially fungi and yeasts, and, with added antibiotics, for isolating fungi.

Bacto Clostridium Difficile Antimicrobic Supplement CC is used with Brain Heart Infusion Agar in preparing Clostridium Difficile Agar.

Bacto Brain Heart CC Agar is used for isolating and cultivating fastidious fungi.

Bacto Brain Heart Infusion w/PAB and Agar is used for cultivating fastidious organisms, particularly from blood containing sulfonamides.

Bacto Brain Heart Infusion w/o Dextrose is used for cultivating fastidious organisms.

Also Known As

Brain Heart Infusion is abbreviated as BHI.

Summary and Explanation

In 1919, Rosenow[1] devised an excellent medium for culturing streptococci by supplementing dextrose broth with brain tissue. Hayden[2] revised Rosenow's procedure by adding crushed marble to the medium and reported favorable growth of organisms from dental pathogens. Brain Heart Infusion is a modification of the media described by Rosenow[1] and Hayden[2] in which infusion from calf brains has replaced the brain tissue and disodium phosphate has replaced the calcium carbonate buffer.

Brain Heart Infusion Agar is used for cultivating a variety of fastidious microorganisms, fungi and yeasts. This medium is used in combination with penicillin and streptomycin. Roseburg, Epps and Clark[3] reported that the isolation and cultivation of *Actinomyces israelii* was enhanced on Brain Heart Infusion with 2% agar compared with 1% dextrose infusion agar. Howell[4] used Brain Heart Infusion with the addition of 2% Bacto Agar and 10% sterile defibrinated horse blood for the cultivation of *Histoplasma capsulatum*.

Brain Heart Infusion Agar can be used with Clostridium Difficile Antimicrobic Supplement CC, a selective supplement containing lyophilized cycloserine and cefoxitin, for the preparation of Clostridium Difficile Agar. The complete medium is based on the formula of Willey and Bartlett[5] and recommended for use in the isolation of *Clostridium difficile* from fecal specimens. *C. difficile* is the major cause of antibiotic-associated diarrhea and pseudomembranous colitis.[6]

Brain Heart CC Agar is prepared with chloramphenicol and cycloheximide (Actidione) according to the formulation of Ajello et al.[7] and McDonough et al.[8] These selective agents restrict growth of bacteria and saprophytic fungi. Brain Heart CC Agar is used in the isolation of fungi that cause systemic disease, such as *Histoplasma capsulatum* and *Blastomyces dermatiditis*.

Brain Heart Infusion media are specified in several standard methods references for food testing.[9,10,11] Standard Methods for the Examination of Water and Wastewater recommends Brain Heart Infusion media in tests for the verification of fecal streptococci.[12]

Brain Heart Infusion is recommended by the National Committee for Clinical Laboratory Standards (NCCLS) for the preparation of inocula used in antimicrobial susceptibility tests.[13]

Brain Heart Infusion w/o Dextrose is a basal medium used with added carbohydrates for fermentation studies.

Modifications of BHI media include:[14]

- Brain Heart Infusion Agar with penicillin (20,000 U) and streptomycin (40 mg) for the selective isolation of pathogenic

User Quality Control

Identity Specifications

Brain Heart Infusion

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in distilled or deionized water; light to medium amber, clear without significant precipitate.
Prepared Medium:	Light to medium amber, clear without significant precipitate.
Reaction of 3.7% Solution at 25°C	pH 7.4 ± 0.2

Brain Heart Infusion Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in distilled or deionized water on boiling; light to medium amber, slightly opalescent to opalescent with a flocculent precipitate.
Prepared Medium:	Plain - light to medium amber, slightly opalescent with a precipitate. With 5% sheep blood-cherry red, opaque.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

Brain Heart CC Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in distilled or deionized water on boiling; medium amber, slightly opalescent without significant precipitate.
Prepared Medium:	Medium amber, slightly opalescent without a precipitate.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

Brain Heart Infusion w/PAB and Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in distilled or deionized water on boiling; light to medium amber, slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 3.8% Solution at 25°C:	pH 7.4 ± 0.2

Brain Heart Infusion w/o Dextrose

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water; light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear.
Reaction of 3.5% Solution at 25°C:	pH 7.4 ± 0.2

Clostridium Difficile Antimicrobic Supplement CC

Lyophilized Appearance:	White, homogeneous cake.
Solution:	Soluble in 5 ml sterile distilled or deionized water. Colorless, clear.
Microbial Limits Test:	Satisfactory (negative).
Reaction of Rehydrated Vial at 25°C:	pH 5.9-6.3

continued on following page

fungi from specimens heavily contaminated with bacteria and saprophytic fungi;

- Brain Heart Infusion with 3% sodium chloride for the isolation of *Vibrio parahaemolyticus*;
- Brain Heart Infusion with agar, yeast extract, sodium chloride, inactivated horse serum and penicillin for the cultivation of fastidious fungi;
- Brain Heart Infusion with casein to support the growth of *Serratia marcescens*;
- Brain Heart Infusion with 0.7% agar to support the growth of staphylococcal species for the production of enterotoxin; and,
- Brain Heart Infusion with rabbit serum and yeast extract for the cultivation of *Mycoplasma equirhinis.*

Principles of the Procedure

Infusion from Beef Heart, Calf Brains and Proteose Peptone provide nitrogen, carbon, sulfur and vitamins in Brain Heart Infusion media. Dextrose is a carbon energy source that facilitates organism growth. Sodium Chloride maintains the osmotic balance of the medium. Disodium Phosphate is a buffering agent. Bacto Agar is a solidifying agent.

The nutritionally rich broth formulation of Brain Heart Infusion supports growth of a variety of microorganisms, as does the medium when supplemented with agar and/or blood. BHI (broth) is often used as a blood culture medium and as a basal medium for metabolic tests, particularly for identifying streptococci.15 BHI with 0.5% Polysorbate 80 can be used for detecting *Mycobacterium avium-intracellulare* complex organisms and *M. tuberculosis* from blood cultures.[15]

Brain Heart Infusion Agar is used in the aminoglycoside and vancomycin screen test for resistant enterococci.[16] BHI Agar with 5-10% sheep blood and chloramphenicol (16 µg/ml) and gentamicin (5 µg/ml) will inhibit the growth of bacteria while allowing growth of dimorphic fungi.[15] This agar can be used as a primary plating medium

User Quality Control cont.

Cultural Response

Brain Heart Infusion (0037)
Brain Heart Infusion w/o Dextrose (0502)
Prepare the selected medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090*	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

Brain Heart Infusion Agar (0418)
Prepare medium with and without 5% sheep blood per label directions. Inoculate and incubate *Aspergillus* aerobically at 30 ± 2°C for 18-72 hours; incubate all other organisms aerobically at 35 ± 2°C with 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH PLAIN	GROWTH w/5% SHEEP BLOOD
Aspergillus niger	16404	100-1,000	good	good
Streptococcus pneumoniae	6305	100-1,000	good	good
Streptococcus pyogenes	19615	100-1,000	good	good
Staphylococcus aureus	25923*	100-1,000	good	good

Brain Heart Infusion w/PAB and Agar (0499)
Prepare medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours under appropriate atmospheric conditions.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacteroides fragilis	25285	100-1,000	good
Neisseria meningitidis	13090*	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

Brain Heart CC Agar (0483)
Prepare medium per label directions. Inoculate and incubate at 25 ± 2°C for up to 7 days

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	inhibited
Candida albicans	10231	100-1,000	fair to good
Escherichia coli	25922*	100-1,000	inhibited
Trichophyton mentagrophytes	9533	1 mm^2	good

Uninoculated tube | *Neisseria meningitidis* ATCC® 13090 | *Streptococcus pyogenes* ATCC® 19615
Brain Heart Infusion

Clostridium Difficile Antimicrobic Supplement CC
Prepare 500 ml Brain Heart Infusion Agar supplemented with 5% sterile sheep blood and 5 ml Clostridium Difficile Antimicrobic Supplement CC. Inoculate and incubate at 35 ± 2°C under anaerobic conditions for 24-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Clostridium difficile	17858	100-1,000	good
Clostridium difficile	9689	100-1,000	good
Clostridium perfringenes	13124*	1,000-2,000	markedly to completely inhibited
Enterococcus faecalis	33186	1,000-2,000	markedly to completely inhibited
Escherichia coli	25922*	1,000-2,000	markedly to completely inhibited

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disk Technical Information.

for the growth of fungi since it has been shown to yield better recovery than the previously recommended Sabouraud Dextrose Agar.[15] In Brain Heart CC Agar, chloramphenicol is used as a broad-spectrum antibiotic to inhibit a wide range of bacteria; cycloheximide inhibits saprophytic fungi. Sheep blood provides essential growth factors for fastidious fungi.

Clostridium Difficile Agar (Brain Heart Infusion Agar supplemented with 5% sheep blood or 7% horse blood and Clostridium Difficile Antimicrobic Supplement CC) improves the growth and recovery of *C. difficile*. Clostridium Difficile Agar markedly to completely inhibits most aerobic and anaerobic enteric organisms other than *C. difficile*.[5] The final concentration of cycloserine and cefoxitin in Clostridium Difficile Agar is 250 mcg/ml and 10 mcg/ml, respectively.

Brain Heart CC Agar can be supplemented with sheep blood (5-10%) for enrichment and gentamicin (5 mg/l) for additional selectivity.[14] McDonough et al[17] demonstrated that the temperature of incubation affects the sensitivity of some pathogenic fungi to antibiotics. Incubate the medium containing antibiotics at room temperature. The specimen source and the type of fungus suspected will indicate the isolation medium to be used. Both an antimicrobic-containing medium and a non-selective medium should be used on primary isolates with incubation at both 25°C and 37°C.

Brain Heart Infusion w/PAB and Agar contains p-aminobenzoic acid (0.05 g/l) to neutralize sulfonamides in the blood of patients receiving this therapy. This formulation will also inactivate streptomycin in the ratio of 10 ml of medium to 100 units of streptomycin. The addition of 0.1% agar to Brain Heart Infusion w/PAB and Agar provides optimum conditions for aerobic organisms, microaerophiles and obligate anaerobes.

Formula

Brain Heart Infusion

Formula Per Liter

Calf Brains, Infusion from	200 g
Beef Heart, Infusion from	250 g
Bacto Proteose Peptone	10 g
Bacto Dextrose	2 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 g

Final pH 7.4 ± 0.2 at 25°C

Brain Heart Infusion Agar

Formula Per Liter

Calf Brains, Infusion from	200 g
Beef Heart, Infusion from	250 g
Bacto Proteose Peptone	10 g
Bacto Dextrose	2 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C

Brain Heart Infusion w/o Dextrose

Formula Per Liter

Calf Brains, Infusion from	200 g
Beef Heart, Infusion from	250 g
Bacto Proteose Peptone	10 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 g

Final pH 7.4 ± 0.2 at 25°C

Brain Heart Infusion W/PAB and Agar

Formula Per Liter

Calf Brains, Infusion from	200 g
Beef Heart, Infusion from	250 g
Bacto Proteose Peptone	10 g
Bacto Dextrose	2 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 g
Bacto Agar	1 g
p-Aminobenzoic Acid	0.05 g

Final pH 7.4 ± 0.2 at 25°C

Brain Heart CC Agar

Formula Per Liter

Calf Brains, Infusion from	200 g
Beef Heart, Infusion from	250 g
Bacto Proteose Peptone	10 g
Bacto Dextrose	2 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 g
Bacto Agar	15 g
Chloramphenicol	50 mg
Cycloheximide	500 mg

Final pH 7.4 ± 0.2 at 25°C

Clostridium Difficile Antimicrobic Supplement CC

Formula per 5 ml

Cycloserine	125 mg
Cefoxitin	5 mg

Precautions

1. For Laboratory Use.
2. **Brain Heart CC Agar: HARMFUL.** HARMFUL BY INHALATION AND IF SWALLOWED. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. POSSIBLE RISK OF HARM TO THE UNBORN CHILD. Do not breathe dust. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Eyes/Ears, Cardiovascular, Muscles, Blood, Lymph Glands, Nerves, Urogenital.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed, induce vomiting; seek medical advice immediately and show this container or label.
3. **Brain Heart CC Agar**: Avoid overheating or holding the medium in the melted state. Doing so tends to reduce the selective properties of the medium.
4. When testing human serum, treat all specimens as infectious agents.
5. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Clostridium Difficile Antimicrobic Supplement CC at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Brain Heart Infusion
Brain Heart Infusion Agar
Clostridium Difficile Antimicrobic Supplement CC
Brain Heart CC Agar
Brain Heart Infusion w/PAB and Agar
Brain Heart Infusion w/o Dextrose

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Waterbath (optional)
Sterile defibrinated blood (optional)
Sterile Petri dishes
Sterile tubes
Anaerobic system for Clostridium Difficile Agar

Method of Preparation

Brain Heart Infusion Media

1. Suspend an appropriate amount of the selected medium in 1 liter distilled or deionized water:
   Brain Heart Infusion - 37 grams;
   Brain Heart Infusion Agar - 52 grams;
   Brain Heart CC Agar - 52 grams;
   Brain Heart Infusion w/PAB and Agar - 38 grams;
   Brain Heart Infusion w/o Dextrose - 35 grams.
2. If the medium contains agar (Brain Heart Infusion Agar, Brain Heart CC Agar and Brain Heart Infusion w/PAB and Agar), heat it to boiling to dissolve completely. Avoid overheating.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Clostridium Difficile Agar

1. Rehydrate and sterilize 500 ml of **Brain Heart Infusion Agar** per label directions. Cool to 45-50°C.
2. Aseptically rehydrate **Clostridium Difficile Antimicrobic Supplement CC** with 5 ml sterile distilled or deionized water. Invert the vial gently several times to dissolve the contents. Use immediately.
3. Aseptically add 5% sterile defibrinated sheep blood or 7% defibrinated horse blood and 5 ml of Clostridium Difficile Antimicrobic Supplement CC to the rehydrated medium.
4. Mix thoroughly, avoiding the formation of bubbles, and dispense into sterile Petri dishes.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Brain Heart Infusion Media.

Clostridium Difficile Agar

1. Inoculate a representative portion of the specimen directly onto the surface of a freshly prepared or previously reduced Clostridium Difficile Agar plate and streak for isolation. The inoculum should include mucous, blood or membranous material, if present.
2. Incubate at 35°C under anaerobic conditions.
3. Examine for growth after 24-48 hours incubation.

   For a complete discussion on the isolation and identification of *Clostridium difficile* refer to appropriate procedures in the references.[15,18,20]

Results

Clostridium Difficile Agar

After 24 hours incubation, colonies of *C. difficile* appear non-hemolytic, 1-3 mm in diameter, off-white to gray, flat and circular with an undulated edge. Colonies become larger (3-5 mm) after 48 hours incubation.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Certain pathogenic fungi may be inhibited by the antibiotics in Brain Heart CC Agar.[19]
3. Clostridium Difficile Antimicrobic Supplement CC is intended for use in the preparation of Clostridium Difficile Agar. Although this medium is selective for *C. difficile*, additional testing using pure cultures is necessary for complete identification. Consult appropriate references for further information.[15,18,20]
4. Suspected colonies of *C. difficile* should be Gram stained and subcultured anaerobically and aerobically on blood agar for complete identification.
5. Demonstration of the *C. difficile* toxin in feces in the presence of clinically evident pseudomembranous colitis is required for definitive diagnosis.[20]

References

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2. **Hayden, R. L.** 1923. Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med. **32**:828-849.
3. **Roseburg, T., L. J. Epps, and A. R. Clark.** 1944. A study of the isolation, cultivation and pathogenicity of *Actinomyces israeli* recovered from the human mouth and from actinomycosis in man. J. Infect. Dis. **29**:390.
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11. **Vanderzant, C., and D. F. Splittstoesser (ed).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
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20. **Onderdonk, A. B., and S. D. Allen.** 1995. *Clostridium*, p. 574 -586. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Brain Heart Infusion	100 g	0037-15
	500 g	0037-17
	2 kg	0037-07
	10 kg	0037-08
Brain Heart Infusion Agar	100 g	0418-15
	500 g	0418-17
	2 kg	0418-07
Brain Heart CC Agar	500 g	0483-17
Brain Heart Infusion w/PAB and Agar	500 g	0499-17
Brain Heart Infusion w/o Dextrose	10 kg	0502-08
Clostridium Difficile Antimicrobic Supplement CC	6 x 5 ml	3194-57*

*Store at 2-8°C

Bacto® Brain Heart Infusion, Porcine

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in distilled or deionized water. Light to medium amber, clear.
Reaction of 3.7% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare medium per label directions. Inoculate tubes with test organisms and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090*	100-1,000	fair
Streptococcus pneumoniae	6305	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Intended Use

Bacto Brain Heart Infusion, Porcine is used for cultivating a wide variety of microorganisms.

Also Known As

Brain Heart Infusion, Porcine is abbreviated as BHI, Porcine.

Summary and Explanation

Rosenow[1] devised an excellent medium for culturing streptococci by supplementing Dextrose Broth with brain tissue. Hayden,[2] revising Rosenow's procedure by adding crushed marble to the medium, reported favorable growth of organisms from dental pathogens. Brain Heart Infusion (0037) is a modification of the media described by Rosenow[1] and Hayden.[2] Infusion from calf brains has replaced the brain tissue and Disodium Phosphate has replaced the Calcium Carbonate buffer.

Brain Heart Infusion, Porcine was developed as an alternative to Brain Heart Infusion formula, and replaces calf brains and beef heart with porcine brains and heart. Brain Heart Infusion, Porcine was developed for pharmaceutical and vaccine production and can replace the traditional BHI depending on organism and production application.

BHI, Porcine was formulated with no bovine components to minimize Bovine Spongiform Encephalopathy (BSE) risk.

The nutritionally rich formula of BHI is used to grow a variety of microorganisms The original Brain Heart Infusion media are specified in standard methods for multiple applications.[3,4,5,6]

Principles of the Procedure

Infusion from pork brains, infusion from pork heart and Pork Peptone No. 2 provides nitrogen, carbon, sulfur and vitamins in Brain Heart Infusion, Porcine. Dextrose is the carbon energy source to facilitate organism growth. Sodium Chloride maintains the osmotic balance of the medium. Disodium Phosphate is the buffering agent.

Formula

Brain Heart Infusion, Porcine

Formula Per Liter	
Pork Brains, Infusion from	200 g
Pork Heart, Infusion from	250 g
Bacto Pork Peptone No. 2	10 g
Bacto Dextrose	2 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Brain Heart Infusion, Porcine

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Waterbath (optional)

Method of Preparation

1. Dissolve 37 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Dispense as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Brain Heart Infusion.

Results

Refer to appropriate references and procedures for results.

References

1. **Rosenow, E. C.** 1919. Studies on elective localization. J. Dent. Res. **1**:205-249.
2. **Hayden, R. L.** 1923. Elective localization in the eye of bacteria from infected teeth. Arch. Int. Med. **32**:828-849.
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4. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
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Packaging

Brain Heart Infusion, Porcine	500 g	0561-17

Bacto® Brewer Anaerobic Agar

Intended Use

Bacto Brewer Anaerobic Agar is used for cultivating anaerobic and microaerophilic bacteria.

Summary and Explanation

Brewer[1] described a special Petri dish cover that allowed surface growth of anaerobes and microaerophiles without anaerobic equipment. The microorganisms were grown on agar with a low oxidation-reduction potential. Brewer Anaerobic Agar was originally formulated and modified for the procedure described by Brewer.[1] This medium is suitable for standard plating procedures used in cultivating anaerobic bacteria.[2,3,4]

Anaerobic bacteria cause a variety of infections in humans, including otitis media, oral infections, endocarditis, meningitis, wound infections following bowel surgery or trauma and bacteremia.[5,6] Anaerobic bacteria are the predominant flora colonizing the skin and mucous membranes of the body.[3] Anaerobes vary in their sensitivity to oxygen and nutritional requirements.[2] Anaerobic bacteria lack cytochromes and thus are unable to use oxygen as a terminal electron acceptor.[3]

Principles of the Procedure

Tryptone, Proteose Peptone No. 3 and Yeast Extract provide the nitrogen, vitamins and amino acids in Brewer Anaerobic Agar. Dextrose

is the carbon source, and Sodium Chloride maintains osmotic equilibrium. Sodium Thioglycollate and Sodium Formaldehyde Sulfoxylate are the reducing agents. Resazurin serves as an indicator of anaerobiosis with a pink color indicating the presence of oxygen. Bacto Agar is the solidifying agent.

Formula

Brewer Anaerobic Agar

Formula Per Liter

Bacto Tryptone	5 g
Bacto Proteose Peptone No. 3	10 g
Bacto Yeast Extract	5 g
Bacto Dextrose	10 g
Sodium Chloride	5 g
Bacto Agar	20 g
Sodium Thioglycollate	2 g
Sodium Formaldehyde Sulfoxylate	1 g
Resazurin, Certified	0.002 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	5.8% solution, soluble in distilled or deionized water on boiling. Light amber, slightly opalescent while hot, turning red on aeration and cooling.
Prepared Medium:	Light pink ring at outer edge, light amber in center, slightly opalescent.
Reaction of 5.8% Solution at 25°C	pH 7.2 ± 0.2

Cultural Response

Prepare Brewer Anaerobic Agar per label directions. Inoculate the plates using the streak method. Incubate plates at 35 ± 2°C anaerobically for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacteroides fragilis	25285*	100-1,000	good
Clostridium beijerinckii	17795	100-1,000	good
Clostridium perfringens	12924	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Brewer Anaerobic Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes
Brewer Anaerobic Petri dish covers (optional)

Method of Preparation

1. Suspend 58 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense as desired.

Specimen Collection and Preparation

Anaerobic bacteria are overlooked or missed unless the specimen is properly collected and transported to the laboratory.[2] Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

Standard Petri Dishes:[2]

1. Inoculate a properly obtained specimen onto the medium, and streak to obtain isolated colonies.
2. Immediately incubate anaerobically at 35 ± 2°C.
3. Examine at 24 hours if incubating plates in an anaerobic chamber. Examine at 48 hours if incubating plates in an anaerobic jar or pouch, or if using Brewer anaerobic dish cover.
4. Extended incubation may be necessary to recover some anaerobes.

Brewer Anaerobic Agar Plates:

1. Dispense 50-60 ml of Brewer Anaerobic Agar into a standard Petri dish. For best results use porous tops to obtain a dry surface.
2. Inoculate the surface of the medium by streaking; avoid the edges of the plates.
3. Replace the standard Petri dish lid with a sterile Brewer anaerobic dish cover. The cover should not rest on the Petri dish bottom. The inner glass ridge should seal against the uninoculated periphery of the agar. It is essential that the sealing ring inside the cover is in contact with the medium. This seal must not be broken before the end of the incubation period. A small amount of air is caught over the surface of the medium, and the oxygen in this space reacts with the reducing agents to form an anaerobic environment.
4. Incubate aerobically as desired.

For a complete discussion on anaerobic and microaerophilic bacteria from clinical specimens, refer to the appropriate procedures outlined in the references.[2,3,4] For the examination of anaerobic bacteria in food refer to standard methods.[7,8,9]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Clinical specimens must be obtained properly and transported to the laboratory in a suitable anaerobic transport container.[2]
3. The microbiologist must be able to verify quality control of the medium and determine whether the environment is anaerobic.[2]
4. The microbiologist must perform aerotolerance testing on each isolate recovered to ensure the organism is an anaerobe.[2]

References

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7. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
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Packaging

Brewer Anaerobic Agar	500 g	0279-17
	10 kg	0279-08

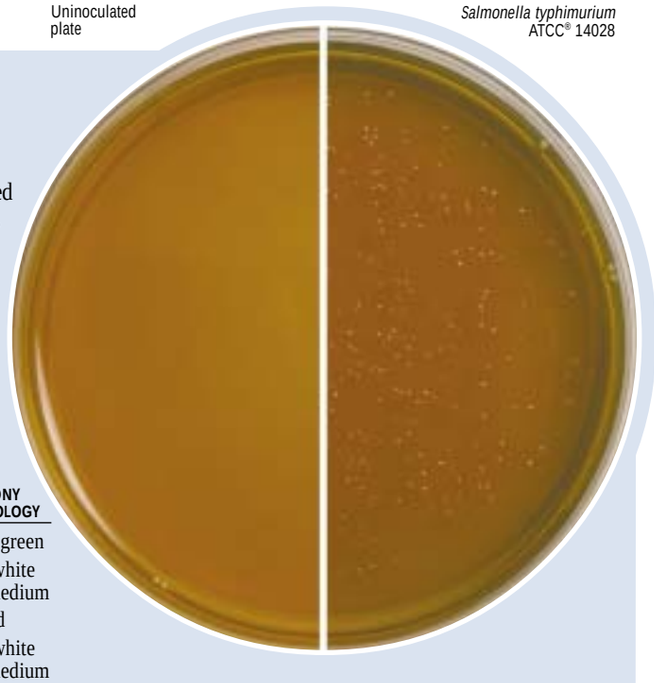
Bacto® Brilliant Green Agar

Intended Use

Bacto Brilliant Green Agar is used for isolating *Salmonella* other than *Salmonella typhi*.

Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella*



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pink, free flowing, homogeneous.
Solution:	5.8% solution, soluble in distilled or deionized water on boiling. Solution is brownish-green, clear to very slightly opalescent.
Prepared Plates:	Orangish-brown, very slightly to slightly opalescent.
Reaction of 3.6% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Prepare Brilliant Green Agar per label directions. Inoculate and incubate the plates at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY MORPHOLOGY
Escherichia coli	25922*	2,000-10,000	none to poor	yellow-green
Salmonella enteritidis	13076	100-1,000	good	pink-white w/red medium
Salmonella typhi	19430	100-1,000	none to poor	red
Salmonella typhimurium	14028*	30-300	good	pink-white w/red medium
Staphylococcus aureus	25923*	2,000-10,000	markedly inhibited	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

in domesticated animals. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness.[1] The illness results from consumption of raw, undercooked or improperly processed foods contaminated with *Salmonella*. Many of these cases of *Salmonella*-related gastroenteritis are due to improper handling of poultry products. Various poultry products are routinely monitored for *Salmonella* before their distribution for human consumption, but in many instances, contaminated food samples elude monitoring.

The use of Brilliant Green Agar as a primary plating medium for the isolation of *Salmonella* was first described by Kristensen, Lester and Jurgens[2] who reported it useful for the differentiation of "paratyphoid B" from other intestinal gram-negative bacilli. Later, Kauffmann[3] modified their formula and used Brilliant Green Agar in addition to Tetrathionate Broth for the isolation of *Salmonella* from stool specimens. Gallon and Quan[4] increased their positive *Salmonella* findings by using Tetrathionate Broth and plating on Brilliant Green Agar. Broh-Kahn[5] showed that the Kauffmann modification of Brilliant Green Agar permitted the use of heavy inocula to obtain maximum recovery of *Salmonella* from fecal specimens. Miller and Tate[6] found that the addition of 20 mg per liter of sodium novobiocin to Brilliant Green Agar reduced or completely inhibited nuisance organisms commonly seen on agar media used for isolating salmonellae. Brilliant Green Agar with Novobiocin is also recommended for use when testing food for *Salmonella*.[7]

Brilliant Green Agar is recommended for use in testing clinical specimens.[8,9] The outstanding selectivity of this medium permits the use of moderately heavy inocula, which should be evenly distributed over the surface. Brilliant Green Agar is valuable when investigating outbreaks of *Salmonella* spp., other than *S. typhi* and *S. paratyphi*.[8,9] In addition, Brilliant Green Agar is used in the microbial limits test as recommended in the United States Pharmacopeia. The microbial limits test is performed to ensure that pharmaceutical articles are free of *Salmonella* spp.[10]

Principles of the Procedure

In Brilliant Green Agar, Proteose Peptone No. 3 and Yeast Extract provide nitrogen, vitamins and minerals. Lactose and Saccharose are the carbohydrates in the medium. Phenol Red is the pH indicator that turns the medium a yellow color with the formation of acid when lactose and/or sucrose is fermented. Sodium Chloride maintains the osmotic balance in the medium. Brilliant Green inhibits gram-positive bacteria and most gram-negative bacilli other than *Salmonella* spp. Lactose/sucrose fermenters are usually inhibited.[11] Bacto Agar is the solidifying agent.

Formula

Brilliant Green Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Yeast Extract	3 g
Bacto Lactose	10 g
Bacto Saccharose	10 g
Sodium Chloride	5 g
Bacto Agar	20 g
Brilliant Green	0.0125 g
Bacto Phenol Red	0.08 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared plates at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Brilliant Green Agar

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50°C)
Petri dishes
Incubator (35°C)

Method of Preparation

1. Suspend 58 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.
4. Cool to 45-50°C in a waterbath.
5. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[7,8,9]
2. For specific information about specimen preparation and inoculation of clinical specimens, consult the appropriate references.[7,8,9]

Test Procedure

For isolation of *Salmonella* from clinical specimens, inoculate fecal specimens and rectal swabs onto a small area of one quadrant of the Brilliant Green Agar plate and streak for isolation. This will permit the development of discrete colonies. Incubate plates at 35°C. Examine plates after 18-24 hours for colonies with characteristic morphologies associated with *Salmonella* spp.

Results

The typical *Salmonella* colonies appear as pink-white opaque colonies surrounded by a brilliant red medium. The few lactose and/or sucrose fermenting organisms that grow are readily differentiated due to the formation of a yellow-green colony surrounded by an intense

yellow-green zone. Brilliant Green Agar is not suitable for the isolation of *S. typhi* or *Shigella*; however, some strains of *S. typhi* may grow forming red colonies.

Limitations of the Procedure

1. Colonies of *Salmonella* spp. vary from red-pink-white depending on length of incubation and strain.[11]
2. Medium is normally orangish-brown in color; however, on incubation, it turns bright red but returns to normal color at room temperature.[11]
3. Studies by Taylor[12] showed that slow lactose fermenters, *Proteus*, *Citrobacter*, and *Pseudomonas* may grow on Brilliant Green Agar as red colonies.
4. In routine examination of clinical specimens or other materials for the gram-negative intestinal pathogens, other primary plating media such as MacConkey Agar, and fluid enrichments such as Tetrathionate Broth and Selenite Broth, should be used with Brilliant Green Agar.
5. *S. typhi* does not grow adequately on this medium. *Shigella* spp. do not grow.[11]

References

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10. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
11. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
12. **Taylor, W. I.** 1965. Isolation of shigellae. I. Xylose lysine agars: New media for isolation of enteric pathogens. Am. J. Clin. Pathol. **44**:471.

Packaging

Brilliant Green Agar	100 g	0285-15
	500 g	0285-17

Bacto® Brilliant Green Agar Modified

Intended Use

Bacto Brilliant Green Agar Modified is used for isolating *Salmonella* from water, sewage and foodstuffs.

Summary and Explanation

Kampelmacher[1] proposed the formula for a selective medium to isolate *Salmonella* from pig feces and minced meat. Brilliant Green Agar Modified is more selective than Desoxycholate Citrate Agar and other brilliant green media, and inhibits the growth of *Pseudomonas aeruginosa* and *Proteus* sp. which may resemble *Salmonella*. *Salmonella cholerasuis* grows well on Brilliant Green Agar Modified, but poorly on Desoxycholate Citrate Agar.[2]

Brilliant Green Agar Modified is recommended for the isolation of *Salmonella*, other than *Salmonella typhi*, from water and associated materials[3] and meat and meat products.[4] It is recommended by the British Poultry Meat Society[5] for the examination of poultry and poultry products. The recommended procedures include using complementary selective culture media and techniques to increase the likelihood of isolating multiple serotypes of *Salmonella* from samples.[6]

Principles of the Procedure

Brilliant Green Agar Modified contains Beef Extract and Bacto Peptone as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Lactose and Sucrose are carbohydrate sources. In the presence of Phenol Red, a pH indicator, nonlactose and/or nonsucrose-fermenting *Salmonella* will produce red colonies. Brilliant Green inhibits gram positive organisms and many gram negative bacteria, except *Salmonella*. Bacto Agar is a solidifying agent.

Formula

Brilliant Green Agar Modified

Formula Per Liter

Bacto Beef Extract	5 g
Bacto Peptone	10 g
Bacto Yeast Extract	3 g
Disodium Hydrogen Phosphate	1 g
Sodium Dihydrogen Phosphate	0.6 g
Lactose	10 g
Sucrose	10 g
Phenol Red	0.09 g
Brilliant Green	0.0047 g
Bacto Agar	12.0 g

Final pH 6.9 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Brilliant Green Agar Modified

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (42°C)
Sterile Blender Jar
Buffered Peptone Water
Muller Kauffmann Tetrathionate Broth
Selenite Brilliant Green Medium

Method of Preparation

1. Suspend 52 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. DO NOT AUTOCLAVE.

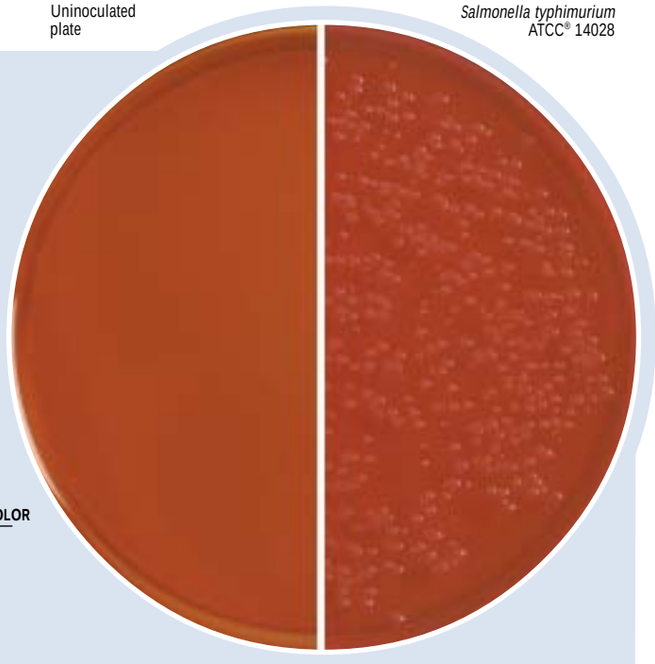
Specimen Collection and Preparation

Meat and Meat Products

1. Weigh 25 g of the sample into a sterile blender jar and add 225 ml of Buffered Peptone Water. Macerate for a sufficient time to give 15,000-20,000 revolutions.
2. Aseptically transfer the contents of the blender jar to a 500 ml flask. Incubate at 37 ± 0.1°C for 16-20 hours.
3. Transfer 10 ml samples to 100 ml Muller Kauffmann Tetrathionate Broth and to 100 ml of Selenite Brilliant Green Medium.
4. Incubate the Muller Kauffmann Tetrathionate Broth at 42-43°C and the Selenite Brilliant Green Enrichment at 37°C.

Sewage Polluted Natural Water

This procedure is applicable to the isolation of *Salmonella* spp. other than *S. typhi*.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in distilled or deionized water on boiling. Solution is orange-brown, clear to slightly opalescent.
Prepared Medium:	Orange-brown, clear to slightly opalescent.
Reaction of 5.2% Solution at 25°C:	pH 6.9 ± 0.1

Cultural Response

Prepare Brilliant Green Agar Modified per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Escherichia coli	25922*	1,000-2,000	completely to partially inhibited	green
Proteus mirabilis NCTC	11938	1,000-2,000	completely to partially inhibited	red
Salmonella typhimurium	14028*	100-1,000	good	red

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

1. Inoculate 25 ml aliquots of the sample into 25 ml of double strength Buffered Peptone Water (1810) and incubate at 37°C for 18 hours.
2. Transfer 1 ml samples into 10 ml of Muller Kauffmann Tetrathionate Broth.
3. Incubate at 43°C for 48 hours.

Test Procedure

1. Subculture the broths at 18-24 hours and at 48 hours onto Brilliant Green Agar (Modified).
2. Examine for typical colonies of *Salmonella* after overnight incubation at 37°C.

Results

Salmonella will produce red colonies.

Limitations of the Procedure

1. Due to the nutritional requirements and inhibitory characteristics of the organisms themselves, organisms other than *Salmonella* spp., such as *Morganella morgani* and some *Enterobacteriaceae* may grow on the medium.
2. Confirmatory tests, such as fermentation reactions and seroagglutination, should be carried out on all presumptive *Salmonella* spp.

References

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3. **H. M. S. O.** 1982. Methods for the isolation and identification of salmonellae (other than *Salmonella typhi*) from water and associated materials.
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5. **British Poultry Meat Society.** 1982. A manual of recommended methods for the microbiological examination of poultry and poultry products.
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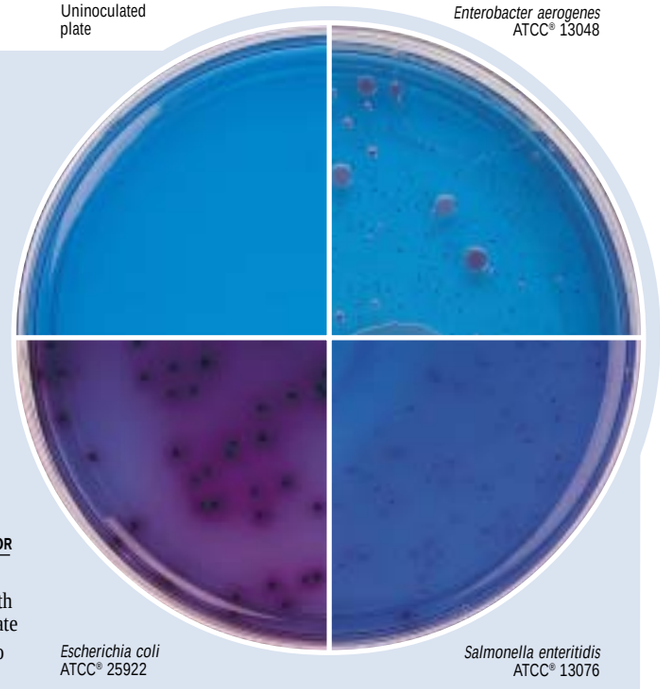
Packaging

Brilliant Green Agar Modified	500 g	1880-17

Bacto® Brilliant Green Bile Agar



User Quality Control

Identity Specifications

Dehydrated Appearance: Light purple, free-flowing, homogeneous.

Solution:	2.06% solution, soluble in distilled or deionized water on boiling. Solution is bluish purple, very slightly to slightly opalescent.
Prepared Medium:	Bluish purple, slightly opalescent.
Reaction of 2.06% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Prepare Brilliant Green Bile Agar per label directions. Inoculate using the pour plate technique and incubate the plates at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Enterobacter aerogenes	13048*	100-1,000	good	pink
Escherichia coli	25922*	100-1,000	good	deep red with bile precipitate
Salmonella enteritidis	14028	100-1,000	good	colorless to light pink
Staphylococcus aureus	25923*	1,000-2,000	marked to complete inhibition	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Intended Use

Bacto Brilliant Green Bile Agar is used for isolating, differentiating and enumerating coliform bacteria.

Also Known As

Brilliant Green Bile Agar (BGBA) is also known as Brilliant Green Agar[2] (BGA).

Summary and Explanation

Noble and Tonney[1] described Brilliant Green Bile Agar for determining the relative density of coliform bacteria in water and sewage. The medium is particularly useful in selectively isolating *Salmonella* spp. from other coliform bacteria. The American Public Health Association (APHA) specifies a qualitative procedure to isolate and identify *Salmonella* spp. from water and wastewater using concentration, enrichment and selective growth.[2]

Principles of the Procedure

Brilliant Green Bile Agar contains Bacto Peptone as a source of carbon, nitrogen, vitamins and minerals. Lactose is a fermentable carbohydrate. Oxgall (bile) and brilliant green inhibit gram-positive bacteria and most gram-negative bacteria except coliforms. Basic Fuchsin is a pH indicator. Monopotassium phosphate is a buffering agent. Agar Noble is a solidifying agent.

Differentiation of the coliforms is based on fermentation of lactose. Bacteria that ferment lactose produce acid and, in the presence of basic fuchsin, form deep red colonies with a pink halo. Bacteria that do not ferment lactose form colorless to faint pink colonies. Coliform bacteria typically ferment lactose, producing deep red colonies, while *Salmonella* spp., which do not ferment lactose, produce colorless to faint pink colonies.

Formula

Brilliant Green Bile Agar

Formula Per Liter

Bacto Peptone	8.25 g
Bacto Lactose	1.9 g
Bacto Oxgall	0.00295 g
Sodium Sulfite	0.205 g
Ferric Chloride	0.0295 g
Monopotassium Phosphate	0.0153 g
Agar Noble	10.15 g
Erioglaucine	0.0649 g
Bacto Basic Fuchsin	0.0776 g
Brilliant Green	0.0000295 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Liver, Thyroid.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

This medium is light sensitive. Protect from exposure to direct sunlight.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Brilliant Green Bile Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°)

Method of Preparation

1. Suspend 20.6 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. The medium is sensitive to light, particularly direct sunlight, which produces a decrease in the productivity of the medium and a change in color from deep blue to purple or red. The medium should be prepared just prior to use and, when necessary to store the medium, it should be kept in the dark.

References

1. **Nobel and Tonney**. 1935. J. Am. Water Works Assoc. **27**:108.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.)**. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Packaging

Brilliant Green Bile Agar	500 g	0014-17

Bacto® Brilliant Green Bile 2%

Intended Use

Bacto Brilliant Green Bile 2% is used for confirming the presence of coliform organisms in water and foods.

Also Known As

Brilliant Green Bile Broth
Brilliant Green Lactose Bile Broth, 2%
Brilliant Green Lactose Bile Broth
Brilliant Green Bile Lactose Broth

Summary and Explanation

The coliform group of bacteria includes aerobic and facultatively anaerobic gram-negative non-sporeforming bacilli that ferment lactose and form acid and gas at 35°C within 48 hours. Members of the *Enterobacteriaceae* comprise the majority of this group but organisms such as *Aeromonas* species may also be included.

Procedures to detect and confirm coliforms are used in testing water, foods, dairy products and other materials.[1,2,3,4,5] The procedures begin with a presumptive test that, when positive, is confirmed by using Brilliant Green Bile 2%.

Principles of the Procedure

Bacto Peptone is a source of carbon and nitrogen for general growth requirements. Oxgall (bile) and Brilliant Green inhibit gram-positive bacteria and many gram-negative bacteria other than coliforms. Lactose is a carbohydrate source. Bacteria that ferment lactose and produce gas are detected.

Formula

Brilliant Green Bile 2%

Formula Per Liter

Bacto Peptone	10 g
Bacto Oxgall	20 g
Bacto Lactose	10 g
Brilliant Green	0.0133 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable gloves and eye/face protection. Use only in well ventilated areas. Keep container tightly closed. TARGET ORGAN(S): Lungs.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Greenish-beige, free-flowing, homogeneous.
Solution:	4.0% solution soluble in distilled or deionized water on warming, if necessary; emerald green, clear without significant precipitate.
Prepared Medium:	Emerald green, clear.
Reaction of 4.0% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Brilliant Green Bile 2% per label directions. Inoculate medium and incubate at 35 ± 2°C for 48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	GAS PRODUCTION
Enterobacter aerogenes	13048*	100-1,000	good	+
Escherichia coli	25922*	100-1,000	good	+
Staphylococcus aureus	25923*	1,000-2,000	marked to complete inhibition	–
Enterococcus faecalis	19433	1,000-2,000	marked to complete inhibition	–

Uninoculated tube — *Escherichia coli* ATCC® 25922

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Brilliant Green Bile 2%

Materials Required but not Provided

Flask with closure
Test tubes with caps
Fermentation tubes
Distilled or deionized water
Autoclave
Incubator

Method of Preparation

1. Suspend 40 grams in 1 liter distilled or deionized water.
2. Warm slightly to dissolve completely.
3. Dispense required amount in tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Process specimens according to established procedures for the type of material being tested.[1,2,3,4,5]

Test Procedure

Consult standard references for specific instructions for the type of material being tested.[1,2,3,4,5]

1. Subculture from a positive presumptive coliform specimen in Lauryl Tryptose Broth (LST) or from typical coliform-type colonies on Violet Red Bile Agar (VRBA) to tubes of Brilliant Green Bile 2%.
2. Incubate at 35°C for 48 ± 2 hours.
3. Examine for bubbles (gas) in the fermentation tube.

Results

Positive: Bubbles (gas) in fermentation tube.

Negative: No bubbles (gas) in fermentation tube.

References

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **Christen, G. L., P. M. Davidson, J. S. McAllister, and L. A. Roth.** 1993. Coliform and other indicator bacteria, p. 247-269. *In* R. T. Marshall (ed.). Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **Hitchins, A. D., P. A. Hartman, and E. C. D. Todd.** 1992. Coliforms - *Escherichia coli* and its toxins, p. 325-369. *In* C. Vanderzant and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Hitchins, A. D., P. Feng, W. D. Watkins, S. R. Rippey, and L. A. Chandler.** 1995. *Escherichia coli* and the coliform bacteria, p. 4.01-4.29. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Andrews, W. H.** 1995. Microbial methods, p. 1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International. Arlington, VA.

Packaging

Brilliant Green Bile 2%	100 g	0007-15-6
	500 g	0007-17-4
	2 kg	0007-07-6
	10 kg	0007-08-5

Bacto® m Brilliant Green Broth

Intended Use

Bacto m Brilliant Green Broth is used for recovering and differentiating *Salmonella* from primary water samples by membrane filtration.

Summary and Explanation

m Brilliant Green Broth is primarily used as a selective-differential medium for *Salmonella* species. *Salmonella* species cause many types of infections from mild, self-limiting gastroenteritis to life-threatening typhoid fever.[4] The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than two days and diarrhea lasting less than 7 days.[4]

m Brilliant Green Broth is a modification of Kauffmann's[1] Brilliant Green Agar in which the agar has been omitted and all other ingredients are at double strength.

Kabler and Clark[2] used m Brilliant Green Broth in a membrane filtration procedure originally developed by Geldreich and Jeter.[3] In this technique, an appropriate volume of water is filtered through the membrane filter. The filter is placed on an absorbent pad saturated with m Tetrathionate Broth Base. After incubation, the membrane is transferred to another absorbent pad saturated with m Brilliant Green Broth and incubated. Following incubation, the membrane is transferred to a fresh pad saturated with urease test reagent.

Principles of the Procedure

Proteose Peptone No. 3 provides the nitrogen, minerals and amino acids in m Brilliant Green Broth. Yeast Extract is the vitamin source. Lactose and Saccharose are the carbohydrates for bacterial growth. Sodium Chloride maintains the osmotic balance of the medium and Phenol Red is the dye used as an indicator of carbohydrate fermentation. Brilliant Green is the selective agent.

Formula

m Brilliant Green Broth

Formula Per Liter

Bacto Proteose Peptone No. 3	20 g
Bacto Yeast Extract	6 g
Bacto Lactose	20 g
Bacto Saccharose	20 g
Sodium Chloride	10 g
Bacto Phenol Red	0.16 g
Brilliant Green	0.025 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Use the rehydrated medium within 24 hours.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

m Brilliant Green Broth

Materials Required But Not Provided

Glassware
Sterile absorbent pad
Membrane filtration equipment
Incubator (35°C)
Sterile Petri dishes, 50 x 9 mm
Distilled or deionized water

Method of Preparation

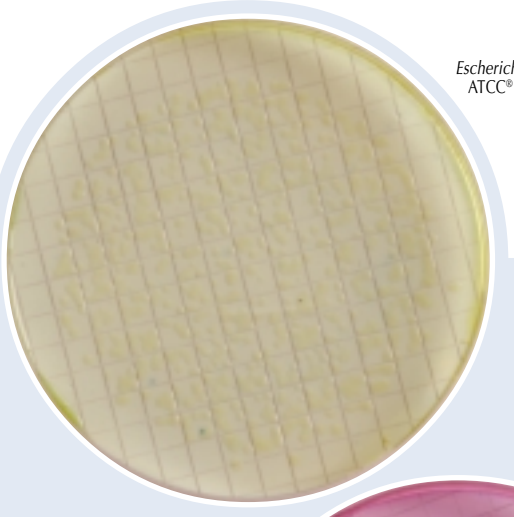
1. Suspend 7.6 grams in 100 ml of distilled or deionized water.
2. Heat to boiling to dissolve completely. Do not autoclave.
3. Cool to room temperature.
4. Dispense 2 ml amounts onto sterile absorbent pads.
5. Use the rehydrated medium within 24 hours.

Specimen Collection and Preparation

Obtain and process water samples according to the techniques and procedures established by laboratory policy.

Test Procedure

1. Inoculate a water sample using the membrane filtration procedure.
2. Place the filter on a pad saturated with m Brilliant Green Broth.
3. Incubate at 35 ± 2°C in a humid atmosphere for 18-24 hours.
4. After incubation, examine for growth and the color of the colonies.



Escherichia coli
ATCC® 25922

Salmonella typhimurium
ATCC® 14028

User Quality Control

Identity Specifications

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	7.6% solution, soluble in distilled or deionized water; greenish-red, slightly opalescent.
Prepared Medium	Greenish-red, slightly opalescent.
Reaction of 7.6% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Prepare m Brilliant Green Broth per label directions. Inoculate using the membrane filter technique and incubate at 35 ± 2°C in a humid atmosphere for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLOR OF COLONY
Escherichia coli	25922*	20-80	good	yellow
Salmonella enteritidis	13076	20-80	good	pink to red
Salmonella typhimurium	14028*	20-80	good	pink to red

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Results

Salmonella species form pink to red colonies.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Kauffmann, F.** 1935. Weitere Erfahrungen mit den kombinierten Anreicherungsverfahren fur Salmonellabacillen. Z. Hyg. Infektionskr. **117**:26.
2. **Kabler and Clark.** 1952. Am. J. Public Health **42**:390.
3. **Geldreich and Jeter.** 1952. Bacteriol. Proc. SAB., Boston, MA.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

m Brilliant Green Broth	500 g	0494-17

Bacto® Brucella Agar
Bacto Brucella Broth

Intended Use

Bacto Brucella Agar is used for isolating and cultivating *Brucella*.

Bacto Brucella Broth is used for cultivating *Brucella* and other fastidious microorganisms.

User Quality Control

Identity Specifications

Brucella Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.3% solution, soluble in distilled or deionized water with frequent agitation on boiling. Light amber, very slightly to slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent.
Reaction of 4.3% Solution at 25°C:	pH 7.0 ± 0.2

Brucella Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.8% solution, soluble in distilled or deionized water. Light amber, clear to very slightly opalescent, no precipitate.
Prepared Medium:	Light amber, clear to very slightly opalescent, no precipitate.
Reaction of 2.8% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Brucella Agar or Brucella Broth per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 24-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Brucella abortus	4315	100-1,000	good
Brucella melitensis	4309	100-1,000	good
Brucella suis	4314	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Summary and Explanation

Brucella Agar and Brucella Broth are prepared according to the APHA formula for Albimi Broth, which is used for the isolation of *Brucella* species.[1] Brucellosis is a zoonotic disease with a domestic-animal reservoir.[2] Transmission by milk, milk products, meat and direct contact with infected animals is the usual route of exposure.[2]

Brucella Agar is used as a general medium for the cultivation of fastidious microorganisms, e.g., *Streptococcus pneumoniae*, *Streptococcus viridans* and *Neisseria meningitidis*.[3] With the addition of blood, Brucella Agar can be used to determine hemolytic reactions of pathogenic bacteria.[3] Brucella Agar can be used as a base for the isolation of *Campylobacter* species.[3]

Brucella Broth is recommended for the isolation of *Brucella* species from blood cultures.[4,5] Brucella Broth is specified in the Compendium of Methods for the Microbiological Examination of Food.[6]

Principles of the Procedure

Peptamin provides nitrogen and amino acids. Tryptone provides nitrogen. Yeast Extract adds essential vitamins. Dextrose is a carbon source; Sodium Bisulfite enhances growth. Sodium Chloride maintains the osmotic balance. Bacto Agar is the solidifying agent in Brucella Agar.

Supplemental blood (5-10%) provides additional growth factors for fastidious microorganisms and is used to determine hemolytic reactions.

Formula

Brucella Agar

Formula Per Liter

Bacto Tryptone	10 g
Bacto Peptamin	10 g
Bacto Dextrose	1 g
Bacto Yeast Extract	2 g
Sodium Chloride	5 g
Sodium Bisulfite	0.1 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Brucella Broth

Formula Per Liter

Bacto Tryptone	10 g
Bacto Peptamin	10 g
Bacto Dextrose	1 g
Bacto Yeast Extract	2 g
Sodium Chloride	5 g
Sodium Bisulfite	0.1 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. *Brucella* species are classified as Biosafety Level 3 pathogens. All manipulations with live cultures and antigens must be confined to a Class II biological safety cabinet (BSC).[2]
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Brucella Agar
Brucella Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes or tubes
Sterile defibrinated blood (optional)

Method of Preparation

1. **Brucella Agar**: Suspend 43 grams in 1 liter distilled or deionized water and boil to dissolve completely.

   Brucella Broth: Dissolve 28 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes. Cool to 45-55°C.
3. OPTIONAL: To prepare Brucella Blood Agar, aseptically add 5-10% sterile defibrinated blood at 45-50°C. Mix well.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion of the isolation and identification of *Brucella*, refer to appropriate procedures outlined in the references.[2,4,5]

Results

Refer to apropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Hemolytic reactions of many microorganisms are different on horse blood from those on sheep blood agar; e.g., some Group D streptococci exhibit beta hemolysis on horse blood but not on sheep blood and are mistaken for Group A.[3]

References

1. **Hausler, W. J. (ed.).** 1976. Standard methods for the examination of dairy products, 14th ed. American Public Health Association, Washington, D.C.
2. **Moyer, N. P., and L. A. Holcomb.** 1995. *Brucella*, p. 549-555. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p.110-114. Williams & Wilkins, Baltimore, MD.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
6. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Brucella Agar	500 g	0964-17
	2 kg	0964-07
	10 kg	0964-08
Brucella Broth	500 g	0495-17
	10 kg	0495-08

Bacto® Bryant and Burkey Medium

Intended Use

Bacto Bryant and Burkey Medium is used for detecting and enumerating spores of lactate-fermenting *Clostridium* in milk and dairy products.

Summary and Explanation

Bryant and Burkey Medium is based on the lactate fermentation media described by Rosenberger[1] and Bryant and Burkey[2], as modified by Bergère et al,[3] who reported that their medium could be used for detecting and enumerating *C. tyrobutyricum* spores in milk and dairy products.[3-5]

Principles of the Procedure

Tryptone, Yeast Extract, Beef Extract Desiccated and L-Cysteine Hydrochloride provide nutrients and cofactors required for good growth of clostridia. Selectivity of this medium is achieved through the addition of Sodium Acetate, which is also the principal promoter of

germination by *C. tyrobutyricum* spores.[6] Sodium Lactate is fermented under anaerobic conditions by *C. tyrobutyricum* and other lactate-fermenting clostridia, producing hydrogen and carbon dioxide. Gas production is demonstrated by an upward movement of a paraffin plug which is overlaid on the medium. Resazurin is included in the medium to show anaerobiosis, turning from pink (aerobic) to colorless under anaerobic conditions.

During processing, the sample is heated at 75°C for 15 minutes to kill vegetative cells and activate germination of spores.

Formula

Bryant and Burkey Medium

Formula Per Liter

Bacto Tryptone	15 g
Bacto Yeast Extract	5 g
Bacto Beef Extract, Desiccated	7.5 g
Sodium Acetate	5 g
L-Cysteine Hydrochloride	0.5 g
Sodium Lactate	5 g
Resazurin	0.0025 g

Final pH 5.9 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear when hot, becoming red upon cooling.
Reaction of 3.8% Solution at 25°C:	pH 5.9 ± 0.2

Cultural Response

Prepare Bryant and Burkey Medium per label directions. Inoculate using Most Probable Number (MPN) method and incubate at 35 ± 2°C for 6 days.

ORGANISM	ATCC® OR STRAIN	GAS INOCULUM	GROWTH	PRODUCTION
Clostridium tyrobutyricum	CNRZ 500	MPN method	good	>1 cm of gas
Clostridium tyrobutyricum	CNRZ 510	MPN method	good	>1 cm of gas
Clostridium tyrobutyricum	CNRZ 608	MPN method	good	>1 cm of gas
Clostridium tyrobutyricum	25755	MPN method	good	>1 cm of gas

The cultures listed are the minimum that should be used for performance testing.

Storage

1. Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.
2. Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bryant and Burkey Medium

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Autoclave
Incubator (35 ± 2°C)

Method of Preparation

NOTE: This product contains sodium lactate; it is not necessary to add sodium lactate during preparation.

1. Dissolve 38 grams in 1 liter of distilled or deionized water.
2. Dispense 10 ml amounts into tubes.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect food samples in sterile containers and transport immediately to the laboratory following recommended guidelines.
2. Process each food sample using procedures appropriate for that sample.

Test Procedure

Three-tube Most Probable Number (MPN) Method

1. Before use, heat tubes to boiling for 10 minutes to regenerate anaerobic conditions. Note: This step is required only with tubes stored under aerobic conditions. Tubes stored under anaerobic conditions or freshly sterilized tubes do not need additional heating.
2. Prepare 10-fold dilutions of the sample and inoculate triplicate tubes of Bryant and Burkey Medium with 1 ml of each sample dilution.
3. Pour approximately 2 ml of melted paraffin (60-65°C), previously autoclaved at 121°C for 20 minutes, into each tube.
4. Heat the tubes at 75°C for 15 minutes to kill vegetative cells and activate spores; allow to cool to room temperature.
5. Incubate tubes at 35°C for 6 days.
6. Examine tubes for growth and gas production after 48 hours of incubation and daily for up to 6 days.

Results

Tubes showing both growth and production of gas (indicated by upward movement of the paraffin more than 1 cm) are considered positive for the presence of lactate-fermenting clostridial spores. Determine the spore count using the Most Probable Number (MPN) method.

References

1. **Rosenberger, K. F.** 1951. The development of methods for the study of obligate anaerobes in silage. Proc. Soc. Appl. Bacteriol. **14**:161-164.
2. **Bryant, M. P., and L. A. Burkey.** 1956. The characteristics of lactate-fermenting sporeforming anaerobes from silage. J. Bacteriol. **71**:43-46.
3. **Bergère, J. L., P. Gouet, J. Hermier, and G. Mocquot.** 1968. Les *Clostridium* du groupe butyrique dans les produits laitiers. Ann. Inst. Pasteur Lille. **19**:41-54.
4. **Cerf, O., and J. L. Bergère.** 1968. La numération des spores de *Clostridium* et son application au lait et aux produits laitiers. Numération des différents groupes de *Clostridium*. Le Lait **48**:501-519.
5. **Bergère, J. L.** 1979. Developpement de l'ensilage. Ses conséquences sur la qualité du lait et des produits laiters. Revue laitère française.
6. **Touraille, C., and J. L. Bergère.** 1974. La germination de la spore de *Clostridium tyrobutyricum*. Biochimie. **56**:404-422.

Packaging

Bryant and Burkey Medium	500 g	0645-17
	2 kg	0645-07

Bacto® Buffered Peptone Water
Bacto Buffered Peptone Water, Modified

User Quality Control

Identity Specifications

Buffered Peptone Water

Dehydrated Appearance:	Cream-white to light tan, free-flowing, homogeneous.
Solution:	2.0% solution, soluble in distilled or deionized water. Solution is light amber, clear.
Prepared Medium:	Light amber, clear.
Reaction of 2.0% Solution at 25°C:	pH 7.2 ± 0.2

Buffered Peptone Water, Modified

Dehydrated Appearance:	Light beige, free-flowing, homogenous.
Solution:	2.5% solution, soluble in distilled or deionized water. Solution is light amber, clear.
Prepared Medium:	Light amber, clear.
Reaction of 2.5% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Buffered Peptone Water or Buffered Peptone Water, Modified per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Salmonella enteritidis	13076	10-100	good
Salmonella typhi	19430	10-100	good
Salmonella typhimurium	14028*	10-100	good

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Intended Use

Bacto Buffered Peptone Water is used for preenriching damaged *Salmonella* species from food specimens to increase recovery.

Bacto Buffered Peptone Water, Modified is used for preenriching *Salmonella* species from food specimens to increase recovery.

Summary and Explanation

Edel and Kampelmacher[1] noted that food preservation techniques involving heat, desiccation, preservatives, high osmotic pressure or pH changes cause sublethal injury to salmonellae. Preenrichment in a nonselective medium allows for repair of cell damage and facilitates the recovery of salmonellae. Lactose Broth is frequently used for this purpose but it may be detrimental to recovering salmonellae.[2] Buffered Peptone Water maintains a high pH over the preenrichment period and results in repair of injured cells that may be sensitive to low pH.[3] This is particularly important for vegetable specimens which have a low buffering capacity. These media can be used for testing dry poultry feed.[4] Buffered Peptone Water is a standard methods medium.[5]

Buffered Peptone Water, Modified provides additional buffering capacity when organisms have been enriched in a pre-enrichment medium containing a high carbohydrate concentration.

Principles of the Procedure

Buffered Peptone Water and Buffered Peptone Water, Modified contain Peptone as a source of carbon, nitrogen, vitamins and minerals. Sodium Chloride maintains the osmotic balance. Phosphates buffer the medium.

Formula

Buffered Peptone Water

Formula Per Liter

Peptone	10 g
Sodium Chloride	5 g
Sodium Phosphate, Dibasic	3.5 g
Potassium Phosphate, Monobasic	1.5 g

Final pH 7.2 ± 0.2 at 25°C

Buffered Peptone Water, Modified

Formula Per Liter

Peptone	10 g
Sodium Chloride	5 g
Sodium Phosphate, Dibasic	7 g
Potassium Phosphate, Monobasic	3 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Buffered Peptone Water**

   MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   Buffered Peptone Water, Modified

   IRRITANT. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Buffered Peptone Water
Buffered Peptone Water, Modified

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Dissolve the medium in 1 liter distilled or deionized water:
   Buffered Peptone Water - 20 grams;
   Buffered Peptone Water, Modified - 25 grams.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure

Test specimens according to recommended guidelines.

Results

Growth is indicated by turbidity.

Limitations of the Procedure

1. The types and numbers of competing flora in the test sample can affect recovery and may overgrow salmonellae.

References

1. **Edel, W., and E. H. Kampelmacher.** 1973. Bull. World Hlth. Org. **48**:167-174.
2. **Angelotti, R**. 1963. Microbiological quality of foods. Academic Press, New York.
3. **Sadovski, A. Y**. 1977. J. Food Technol. **12**:85-91.
4. **Juven, B. J., N. A. Cox, J. S. Bailey, J. E. Thomson, O. W. Charles, and J. V. Schutze.** 1984. Recovery of *Salmonella* from artificially contaminated poultry feeds in non-selective and selective broth media. Jour. of Food Prot. **47**:299-302.
5. **Flowers, R. S., J-Y. D'Aoust, W. H. Andrews, and J. S. Bailey**. 1992. *Salmonella*, p. 371-422. *In* C. Vanderzant, and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Buffered Peptone Water	500 g	1810-17
	2 kg	1810-07
	10 kg	1810-08
Buffered Peptone Water, Modified	500 g	1833-17

Bacto® Bushnell-Haas Broth

Intended Use

Bacto Bushnell-Haas Broth is used for studying microbial utilization of hydrocarbons.

Also Known As

Bushnell-Haas Broth is also referred to as Bushnell-Haas marine salts broth.

Summary and Explanation

Bushnell-Haas Broth, prepared according to the formula described by Bushnell and Haas[1], is used to evaluate the ability of microorganisms to decompose hydrocarbons. It is formulated without a carbon source which allows for the addition of alternate hydrocarbons such as kerosene, light and heavy mineral oils, paraffin wax, and gasoline.

Bushnell-Haas was recommended for the microbiological examination of fuels by the Society for Industrial Microbiology (SIM) Committee on Microbiological Deterioration of Fuels.[2] The medium was used to

enumerate total heterotrophs and hydrocarbon degradation by microorganisms during bioremediation of Prince William Sound following the Exxon *Valdez* oil spill.[3,4]

Principles of the Procedure

Magnesium Sulfate, Calcium Chloride, and Ferric Chloride provide trace elements necessary for bacterial growth. Potassium Nitrate is a nitrogen source, while Monopotassium Phosphate and Ammonium Phosphate Dibasic provide buffering capability.

Formula

Bushnell-Haas Broth

Formula Per Liter	
Magnesium Sulfate	0.2 g
Calcium Chloride	0.02 g
Monopotassium Phosphate	1 g
Ammonium Phosphate Dibasic	1 g
Potassium Nitrate	1 g
Ferric Chloride	0.05 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Liver, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige with pink tint, free-flowing, homogeneous.
Solution:	0.327% solution not soluble in distilled or deionized water, white precipitate remains. Solution, after autoclaving, is colorless to very light amber, clear supernatant over yellow-orange precipitate.
Prepared Medium:	Colorless to very light amber, clear supernatant over yellow-orange precipitate.
Reaction of 0.327% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Bushnell-Haas Broth per label directions. Inoculate in duplicate with the test organisms. Add sterile mineral oil (the hydrocarbon source) to one set. Incubate at 25-30°C for up to 1 week.

ORGANISM	ATCC®	INOCULUM	PLAIN	RECOVERY w/Hydrocarbon
Pseudomonas aeruginosa	9027	100-1,000	none to poor	good
Pseudomonas aeruginosa	10145	100-1,000	none to poor	good
Pseudomonas aeruginosa	14207	100-1,000	none to poor	good
Pseudomonas aeruginosa	27853*	100-1,000	none to poor	good

The cultures listed are the minimum that should be used for performance testing.

*The cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bushnell-Haas Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (25-30°C)

Method of Preparation

1. Dissolve 3.27 grams in 1 liter distilled or deionized water.
2. Dispense as desired and autoclave at 121°C for 15 minutes.
3. Cool to 45-50ºC.

NOTE: A precipitate that is white prior to sterilization and turns yellow to orange after sterilization is normal.

Specimen Collection and Preparation

1. Collect samples in sterile containers or with sterile swabs and transport immediately to the laboratory.

Test Procedure

1. Inoculate the collected sample directly into the broth.
2. Overlay the broth with a sterile hydrocarbon source.
3. Incubate aerobically at 25-30°C.
4. Examine tubes daily for growth for up to one week.

Results

Organisms capable of degrading hydrocarbons should show growth in the Bushnell-Haas Broth supplemented with a hydrocarbon source.

Limitations of the Procedure

1. Because the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly in this medium.

References

1. **Bushnell, L. D., and H. F. Haas.** 1941. The utilization of certain hydrocarbons by microorganisms. J. Bacteriol. **41**:653-673.

2. **Allred, R. C., R. J. DeGray, R. W. Edwards, H. G. Hedrick, D. E. Klemme, M. Rogers, M. Wulf, and H. Hodge.** 1963. Proposed procedures for microbiological examination of fuels. SIM Special Publications, Number 1. Merck, Sharp & Dohme Research Laboratories, Rahway, NJ.
3. **Bragg, J. R., J. C. Roffall, and S. McMillen.** 1990. Column flow studies of bioremediation in Prince William Sound. Exxon Production Research Co., Houston, TX.
4. **Brown, E. J., and J. F. Braddock.** 1990. Sheen Screen, a miniaturized most-probable-number method for enumeration of oil-degrading microorganisms. Appl. Environ. Microbiol. **56**:3895-3896.

Packaging

Bushnell-Haas Broth	500 g	0578-17
	10 kg	0578-08

Bacto® CLED Agar

Intended Use

Bacto CLED Agar is used for cultivating, differentiating and enumerating bacteria in urine.

Also Known As

CLED Agar is an abbreviation for Cystine Lactose-Electrolyte-Deficient Agar.

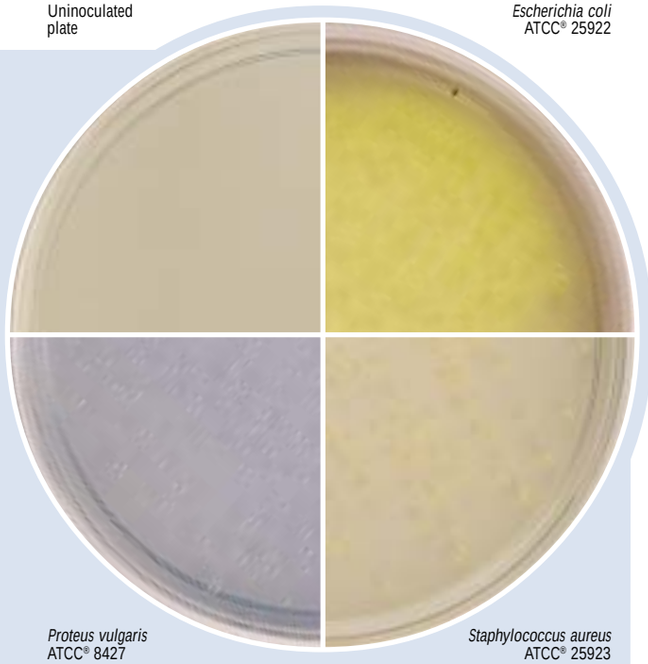
Summary and Explanation

Sandys[1] developed an electrolyte-deficient medium that prevented *Proteus* from swarming. Mackey and Sandys[2] modified the formula by substituting lactose and sucrose for mannitol, and increasing the amount of indicator and agar. While investigating this medium for a dip slide technique for urine cultures, the researchers further modified the formula. The revised formula omitted sucrose and added cysteine and was called Cystine Lactose-Electrolyte-Deficient medium.[3]

CLED Agar is recommended in the spread plate technique or as a dip slide for the detection of bacteria in urine. This medium supports the growth of urinary pathogens and provides distinct colony morphology. CLED medium lacks an electrolyte (salt) which is necessary for growth or other characteristics of certain bacteria.[4] Many European laboratories use Cystine Lactose-Electrolyte-Deficient (CLED) Agar.[5]

Principles of the Procedure

Beef Extract, Bacto Peptone and Tryptone provide the nitrogen, vitamins and amino acids in CLED Agar. L-Cystine is added as a growth supplement for cystine-dependent coliforms. Lactose is included as a carbon source. Organisms capable of fermenting lactose will lower the pH and change the color of the medium from green to yellow. Brom Thymol Blue is used as a pH indicator. Bacto Agar is used as a solidifying agent.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige with slight green tint, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water upon boiling. Solution is bluish-green, very slightly opalescent without precipitate.
Prepared Medium:	Bluish-green, very slightly opalescent without precipitate.
Reaction of 3.6% Solution:	pH 7.3 ± 0.2

Cultural Response

Prepare CLED Agar per label directions. Inoculate by spread plate technique and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Escherichia coli	25922*	100-1,000	good	yellow
Proteus vulgaris	8427	100-1,000	good, swarming inhibited	blue to blue-green
Staphylococcus aureus	25923*	100-1,000	good	yellow

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

CLED Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	4 g
Bacto Tryptone	4 g
L-Cystine	0.128 g
Bacto Lactose	10 g
Bacto Agar	15 g
Bacto Brom Thymol Blue	0.02 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

CLED Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°)
Sterile Petri dishes (optional)
Sterile dip slides (optional)

Method of Preparation

1. Suspend 36 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy. For best results, inoculate medium with specimen as soon as possible.

Test Procedure

For a complete discussion on collection and processing of urine cultures refer to appropriate references.[5,6,7]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. CLED Agar is basically non-selective. However, due to electrolyte exclusion, the growth of *Shigella* species is usually inhibited.[4]

References

1. **Sandys, G. H.** 1960. A new method of preventing swarming of *Proteus* spp. with a description of a new medium suitable for use in routine laboratory practice. J. Med. Lab. Technol. **17**:224.
2. **Mackey, J. P., and G. H. Sandys.** 1965. Laboratory diagnosis of infections of the urinary tract in general practice by means of a dip-inoculum transport medium. Br. Med. J. **2**:1286.
3. **Mackey, J. P., and G. H. Sandys.** 1966. Diagnosis of urinary tract infections. Br. Med. J. **1**:1173.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
7. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

CLED Agar	500 g	0971-17
	10 kg	0971-08

Bacto® Campylobacter Agar Base · Bacto Campylobacter Agar Kit Blaser · Bacto Campylobacter Agar Kit Skirrow

Intended Use

Bacto Campylobacter Agar Base is used with blood and Bacto Campylobacter Antimicrobic Supplement B (Blaser), Bacto Campylobacter Antimicrobic Supplement S (Skirrow) or other antibiotics in isolating and cultivating *Campylobacter.*

Also Known As

Campylobacter Agar Kit Skirrow is used to prepare Campylobacter Agar, Skirrow's or Skirrow's Campylobacter Agar.

Campylobacter Agar Kit Blaser is used to prepare Campylobacter Agar, Blaser's or Blaser's Campylobacter Agar.

Summary and Explanation

The genus *Campylobacter* was proposed in 1963 for *Vibrio fetus*, a species not exhibiting true characteristics of *Vibrionaceae*.[1] In 1977, Skirrow succeeded in isolating *C. jejuni* from fecal samples. Skirrow used a selective medium, incubated at 42°C in an atmosphere of 5% oxygen, 10% carbon dioxide and 85% nitrogen. Skirrow confirmed this organism as a major etiologic agent of human enteritis,[1] an infection acquired through ingestion of water or food contaminated with the microorganism.

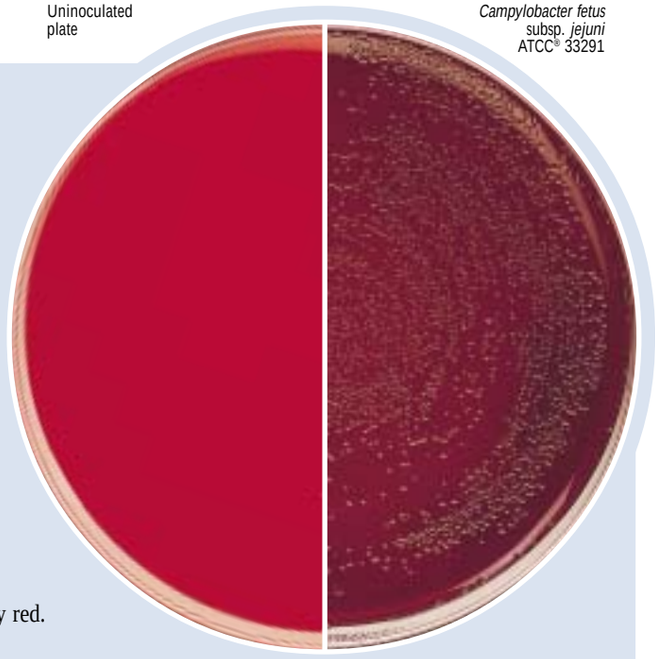
The Skirrow formulation includes blood agar supplemented with vancomycin, polymyxin B and trimethoprim for the selective isolation of *C. fetus* subsp. *jejuni*.[2] Blaser et al. further Incorporated cephalothin and amphotericin B to improve inhibition of normal enteric flora.

In 1983, spiral-shaped organisms resembling campylobacteria were isolated from the human stomach. The discovery sparked renewed interest in the etiology of human type B gastritis.[1] After genetic analysis, the genus *Helicobacter* was created and most attention focused on *H. pylori*. Specimens of gastric biopsies, brushings, or aspirates are used for the detection of *H. pylori*. Chocolate agar and brain heart infusion or brucella agar, enriched with 5 to 7% horse or rabbit blood, will support the growth of *H. pylori*.[1]

The Skirrow formulation is recommended for clinical specimens.[1] Campylobacter Agar Base is specified for food testing in Standard Methods.[3,4]

Principles of the Procedure

Campylobacter Agar Base is a nutritionally rich medium based on Blood Agar Base No. 2, rather than on Brucella Agar, to support more luxuriant *Campylobacter* growth because Trimethoprim is more active in Blood Agar Base No. 2. Supplementation of the base with antimicrobial agents as described by Skirrow[2] and Blaser et al.[5,6] provides for markedly reduced growth of normal enteric bacteria and improved recovery of *C. fetus* supsp. *jejuni* from fecal specimens. Growth of fungi is markedly to completely inhibited with Campylobacter Antimicrobic Supplement B due to the presence of amphotericin B.



User Quality Control

Identity Specifications

Campylobacter Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.95% solution, soluble in distilled or deionized water upon boiling; medium to dark amber, clear to slightly opalescent.
Prepared Medium:	Without blood: medium to dark amber, very slightly to slightly opalescent without significant precipitate.
	With 10% sheep blood: cherry red, opaque.
Reaction of 3.95% Solution at 25°C:	pH 7.4 ± 0.2

Campylobacter Antimicrobic Supplement B

Lyophilized Appearance: Bright medium yellow cake or powder.

Rehydrated Appearance: Yellow suspension.

Prepared Medium: Blaser formulation: opaque, medium cherry red.

Campylobacter Antimicrobic Supplement S

Lyophilized Appearance: White cake or powder.

Rehydrated Appearance: Colorless, clear.

Prepared Medium: Skirrow formulation: translucent, dark red.

Cultural Response

Prepare Campylobacter Agar Blaser or Skirrow per label directions. Inoculate and incubate at 42°C for 40-48 hours.

ORGANISM	ATCC®	CFU	GROWTH	APPEARANCE
Campylobacter fetus subsp. *jejuni*	33291	100-1,000	good	non-hemolytic, mucoid, gray colonies
Candida albicans	10231†	2,000-10,000	marked to complete inhibition	
Enterococcus faecalis	33186	2,000-10,000	marked to complete inhibition	
Escherichia coli	25922*	2,000-10,000	marked to complete inhibition	

†This organism is tested on Campylobacter Agar Blaser, only.

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

Campylobacter Agar Base

Formula Per Liter

Bacto Proteose Peptone No. 3	15 g
Liver Digest	2.5 g
Bacto Yeast Extract	5 g
Sodium Chloride	5 g
Bacto Agar	12 g

Campylobacter Antimicrobic Supplement B

Ingredients per vial	10 ml vial	5 ml vial
Vancomycin	10 mg	5 mg
Polymyxin B	2,500 units	1,250 units
Trimethoprim	5 mg	2.5 mg
Cephalothin	15 mg	7.5 mg
Amphotericin B	3 mg	1 mg

Campylobacter Antimicrobic Supplement S

Ingredients per vial	10 ml vial	5 ml vial
Vancomycin	10 mg	5 mg
Polymyxin B	2,500 units	1,250 units
Trimethoprim	5 mg	2.5 mg

Precautions

1. For Laboratory Use.
2. **Campylobacter Antimicrobic Supplement B**

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE SENSITIZATION BY INHALATION AND SKIN CONTACT. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. Target Organs: Blood, Kidneys, Ears, Bone Marrow.

   Campylobacter Antimicrobic Supplement S

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE SENSITIZATION BY INHALATION AND SKIN CONTACT. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. Target Organs: Kidneys, Ears, Bone Marrow.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store lyophilized and rehydrated Campylobacter Antimicrobic Supplements B and S at 2-8°C. Use the rehydrated supplement within 24 hours.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed.

Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Campylobacter Agar Base
Campylobacter Antimicrobic Supplement B
Campylobacter Antimicrobic Supplement S

Materials Required But Not Provided

Specimen collection containers or sterile rectal swabs
Microaerophilic environment system
Bunsen burner or incinerator
Sterile defibrinated blood or sterile lysed horse blood
Inoculating loops
Incubator (42°C)
Sterile Petri dishes

Method of Preparation

Campylobacter Agar Base:

1. Suspend 39.5 grams of Campylobacter Agar Base in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C. Aseptically add 5-7% sterile lysed horse blood (final concentration) or 10% sterile defibrinated sheep blood (final concentration).
5. Aseptically add 1% rehydrated Campylobacter Antimicrobic Supplement B or Campylobacter Antimicrobic Supplement S (10 ml per liter or 5 ml per 500 ml of basal medium). Mix well.
6. Dispense 20 ml amounts into 90 mm Petri dishes.

Campylobacter Antimicrobic Supplement B
Campylobacter Antimicrobic Supplement S

1. Aseptically rehydrate the lyophilized supplement with 5 or 10 ml of sterile distilled or deionized water, depending on label directions.
2. Invert the vial gently several times to dissolve the powder. Use within 24 hours of rehydration.

Specimen Collection and Preparation

Fecal specimens should be collected in sterile containers or with a sterile rectal swab and transported immediately to the laboratory for processing. If the specimen cannot be inoculated onto appropriate media within four hours after collection, the specimen should be maintained or transported in Cary-Blair Transport Medium.[1]

Test Procedure

1. Inoculate the specimen directly onto the surface of the prepared Campylobacter Agar plate and streak for isolation.
2. Incubate at 42°C under a microaerophilic atmosphere containing 5-6% oxygen and 3-10% carbon dioxide. Consult appropriate references for specific information on establishing a microaerophilic environment.[1,3,7]

Results

The colonies of *Campylobacter* species appear as non-hemolytic, flat and gray with an irregular edge or raised and round with a mucoid appearance. Some strains may appear tan or slightly pink. Swarming

or spreading may be observed on moist surfaces. Growth of normal enteric bacteria is markedly to completely inhibited. Growth of fungi is markedly to completely inhibited on Campylobacter Agar Blaser. Colonies are selected for further biochemical characterization.

Identification is based on a positive oxidase reaction and characteristic darting motility in a wet mount.[1] For further differentiation into species and biotypes, test for catalase activity, urease, hydrogen sulfide production, nitrate reduction, hippurate, indoxyl acetate, DNA hydrolysis and susceptibility to cephalothin and nalidixic acid.[1]

Limitations of the Procedure

1. Campylobacter Agar prepared with either Campylobacter Antimicrobic Supplement S or Campylobacter Antimicrobic Supplement B is selective primarily for *Campylobacter* species. Biochemical testing using a pure culture is necessary for complete identification. Consult appropriate references for further information.[1,3,7]
2. Growth of *Campylobacter fetus* subsp. *intestinalis* may be dramatically inhibited on Campylobacter Agar Blaser due to the presence of cephalothin. The use of Campylobacter Agar Skirrow and incubation at 35°C is suggested when isolating this organism from mixed populations.
3. Some strains of *C. fetus* subsp. *jejuni* may be encountered that fail to grow or grow poorly on prepared Campylobacter Agar.
4. Some strains of normal enteric organisms may be encountered that are not inhibited or only partially inhibited on Campylobacter Agar.

References

1. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Skirrow, M. D**. 1977. *Campylobacter* enteritis: A “new” disease. Br. Med. J. **2**:9-11.
3. **Vanderzant, C., and D. F. Splittstoesser (ed).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
4. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Blaser, M. J., V. Berkowitz, F. M. LaForce, J. Cravens, L. B. Reller, and W. L. Wang**. 1979. *Campylobacter* enteritis: clinical and epidemiologic features. Ann. Intern. Med. **91**:179-185.
6. **Blaser, M. J., J. Cravens, B. W. Powers, and W. L. Wang**. 1978. *Campylobacter* enteritis associated with canine infection. Lancet **(ii)**:979-980.
7. **Koneman E. W., S. D. Allen, W. M. Janda, P. C. Schreckenberger, W. C. Winn.** 1983. Color atlas and textbook of diagnostic microbiology, 5th ed. J. B. Lippencott-Raven Publishers. Washington, D.C.

Packaging

Campylobacter Agar Base	2 kg	1820-07
Campylobacter Agar Kit Blaser		3279-32
To prepare: **6 x 1 liter**		
Campylobacter Agar Base	6 x 39.5 grams	
Campylobacter Antimicrobic Supplement B	6 x 10 ml	
Campylobacter Agar Kit Blaser		3279-40
To prepare: **6 x 500 ml**		
Campylobacter Agar Base	6 x 19.75 grams	
Campylobacter Antimicrobic Supplement B	6 x 5 ml	
Campylobacter Agar Kit Skirrow		3280-32
To prepare: **6 x 1 liter**		
Campylobacter Agar Base	6 x 39.5 grams	
Campylobacter Antimicrobic Supplement S	6 x 10 ml	
Campylobacter Agar Kit Skirrow		3280-40
To prepare: **6 x 500 ml**		
Campylobacter Agar Base	6 x 19.75 grams	
Campylobacter Antimicrobic Supplement S	6 x 5 ml	

Bacto® Candida BCG Agar Base

Intended Use

Bacto Candida BCG Agar Base is used with added neomycin in isolating and differentiating *Candida* from primary specimens.

Also Known As

Candida BCG Agar Base is an abbreviation for Candida Brom Cresol Green Agar Base.

Summary and Explanation

Candida BCG Agar Base is prepared according to the formulation of Harold and Snyder.[1] Candida BCG Agar Base was developed after a study demonstrated triphenyltetrazolium chloride (TTC) employed in Pagano Levin medium retarded the growth of some *Candida* species. Harold and Snyder[1] used brom cresol green as the indicator, which is nontoxic to *Candida* species. This medium is primarily used for demonstrating morphological and biochemical reactions characterizing the different *Candida* species for clinical diagnosis.

Candidiasis is the most frequently encountered opportunistic fungal infection.[2] It is caused by a variety of species of *Candida*, with *Candida albicans* being the most frequent etiological agent, followed by *Candida tropicalis* and *Candida* (*Torulopsis*) *glabrata*.[2] *Candida* species can be present in clinical specimens as a result of environmental contamination, colonization, or actual disease process.[3]

Principle of the Procedure

Bacto Peptone provides the nitrogen and amino acids in Candida BCG Agar Base. Yeast Extract is the vitamin source. The high concentration of Dextrose provides carbon as an energy source in this formula. Bacto Agar is the solidifying agent. Brom cresol green is the pH indicator, and acid production changes the medium from blue-green to yellow. Due to pH changes, specific color patterns appear in the base and surface of colonies for differentiation of *Candida* species.

Neomycin is added to the medium in a concentration of 500 µg/ml. Neomycin and brom cresol green act as selective agents to inhibit bacteria in Candida BCG Agar Base.[4]

Formula

Candida BCG Agar Base

Formula Per Liter

Bacto Peptone	10 g
Bacto Yeast Extract	1 g
Bacto Dextrose	40 g
Bacto Agar	15 g
Brom Cresol Green	0.02 g

Final pH 6.1 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Candida BCG Agar Base

Materials Required But Not Provided

Glassware
Autoclave
Incubator (30°C)
Waterbath (45-55°C)
Neomycin (500 µg/ml)
Sterile Petri dishes

Method of Preparation

1. Suspend 66 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool the medium to 50-55°C. Add sterile neomycin (500 µg/ml). Mix well.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

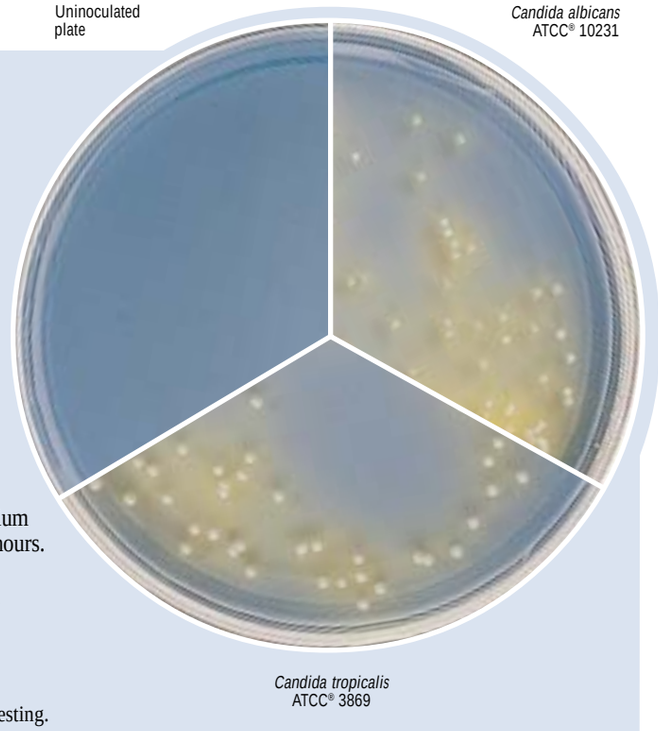
Test Procedure

Refer to the scheme for yeast identification.[3] For a complete discussion on the isolation and identification of *Candida* species refer to the procedures described in the appropriate references.[2,3,5]

Results

Identification of *Candida* species on the basis of colony morphology on Candida BCG Agar follows:

***C. albicans*:** Colonies appear as blunt cones 4.5-5.5 mm diameter with smooth edges and surfaces; coarse feathery growths may arise from the center of the colony base to penetrate the medium. The color of



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige to blue-green, free-flowing, homogeneous.
Solution:	6.6% solution, soluble in distilled or deionized water on boiling, blue-green, slightly opalescent to opalescent, may have a precipitate.
Prepared Medium:	Blue-green to greenish blue, slightly opalescent to opalescent; may have a precipitate.
Reaction of 6.6% Solution at 25°C:	pH 6.1 ± 0.1

Cultural Response

Prepare Candida BCG Agar Base per label directions. Inoculate medium using the streak plate technique, and incubate at 30 ± 2°C for 24-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLOR OF MEDIUM
Candida albicans	10231	100-1,000	good	yellow
Candida tropicalis	3869	100-1,000	good	yellow
Escherichia coli	25922*	1,000-2,000	inhibited	green

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

the base and surface of the colonies is yellowish to bluish green with the intensity diminishing from a gray green center spot to paleness at the edge, although some strains may show a distinct green outer ring.

***C. stellatoidea*:** Colonies appear convex 4.0-5.0 mm in diameter, with smooth edges and smooth to irregular surfaces; there is a fine central basal feathery growth penetrating the medium. The color of both base and surface of colonies is yellow to green, the intensity of which may or may not diminish from center to border but is usually light.

***C. tropicalis*:** Colonies appear convex or as low cones 4.5-5.0 mm in diameter with smooth to undulate edges, and smooth to granular or ridged surfaces; deeply stained feathery growth arises from several points in the base of the colony to form an effusive cloud. The color of the submerged growth is normally an intense blue green compared with that of the base which is much lighter; the surface is uniformly pale and may be yellowish green to green, reflecting a lower pH than observed of the base.

***C. pseudotropicalis*:** Colonies appear convex, 4.5-5.5 mm in diameter with undulate to smooth edges, and smooth surfaces; occasionally the surface is membranous but all colonies are shiny in appearance, and there is feathering growth emerging from several points in the base of the colony. The color of a large central area in the base of the colony is a medium green, which diminishes in intensity toward the edge; a similar distribution of color occurs on the surface, but this green is bright in hue and is never grayed as it is with *C. tropicalis*.

***C. krusei*:** Colonies appear as low cones 4.5-5.0 m in diameter with pseudohyphal edges, which may be weakly contractile or spreading, and have dull surfaces. There is abundant lightly colored growth penetrating the medium from the base of the colony. The base of the colony is a medium blue green in the center diminishing in intensity to paleness at the edge; the surface is usually a light green to yellow green without much concentration in any part.

***C. parapsilosis*:** Colonies appear as convex to low cones 3.5-4.5 mm in diameter with smooth or slightly spreading edges, but vary from smooth to granular or rough surfaces; there is no submerged growth. The color for both base and surface of the colony is blue green over much of the colony, being more intense in the base than the surface which is modified by a thin grayish film of cells; the intensity in color fades abruptly leaving a broad pale edge.

***C. guilliermondii*:** Colonies appear as low cones 4.0-5.0 mm in diameter with very smooth edges and highly glossy surfaces; there maybe a weak, fine feathered submerged growth. Both base and surface of the colony tend to have blue centers of medium intensity fading into a pale edge; however the surface may be blue green with the central third lightened with gray.

***C. glabrata*:** Colonies are smooth and convex, 4.6-5.0 mm diameter; the surface color pattern is pale green in the center which becomes medium green at the edge, and the base has the same color pattern but of less intensity.

Limitations of the Procedure

1. Since the nutritional requirements of yeast vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Harold, W., and M. Snyder.** 1968. Personal Communication.
2. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
3. **Warren, N. G., and K. C. Hazen.** 1995. *Candida*, *Cryptococcus*, and other yeasts of medical importance, p. 723-737. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 136-137. Williams & Wilkins, Baltimore, MD.
5. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

Packaging

Candida BCG Agar Base	500 g	0835-17

Bacto® Candida Isolation Agar

Intended Use

Bacto Candida Isolation Agar is used for isolating and differentiating *Candida albicans*.

Summary and Explanation

Candida Isolation Agar is a nutritionally rich medium that supports growth of many yeasts and molds and is differential for *Candida albicans*. Candida Isolation Agar was developed using a modification of YM Agar as described by Fung and Liang.[1] Goldschmidt demonstrated that YM Agar with Aniline Blue WS could be used to identify *C. albicans* in clinical samples with high accuracy and predictability.[2] Aniline Blue is metabolized by *C. albicans* to produce a fluorescent moiety that can be detected under long wave UV light.[2]

Principles of the Procedure

Yeast Extract provides nitrogen, carbon, vitamins and cofactors. Malt Extract provides carbon, protein and nutrients. Bacto Peptone provides additional carbon and nitrogen. Dextrose is an energy source. Aniline Blue is a fluorescent indicator. Bacto Agar is a solidifying agent.

Formula

Candida Isolation Agar

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Malt Extract	3 g
Bacto Peptone	5 g
Bacto Dextrose	10 g
Bacto Agar	20 g
Aniline Blue	0.1 g

Final pH 6.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Candida Isolation Agar

Materials Required but not Provided

Glassware

Autoclave

Method of Preparation

1. Suspend 41.1 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Specimens should be collected in sterile containers or with sterile swabs and transported immediately to the laboratory according to recommended guidelines.[3,4]

Test Procedure

1. Process each specimen as appropriate for that specimen and inoculate directly onto the surface of the medium. Streak for isolation.
2. Incubate plates aerobically at 30°C for 18-72 hours.
3. Examine plates for growth after 18-72 hours of incubation.

Results

Colonies of *C. albicans* fluoresce yellow-green under long wave UV light following incubation at 30°C for 18-24 hours. Non-*C. albicans* isolates do not fluoresce.

Limitations of the Procedure

1. Strains of *Candida albicans* have been reported that are false negative for fluorescence on this medium.[2]
2. Strains of *C. parapsilosis*, *C. krusei*, and *C. pulcherrima* that fluoresce on this medium may be encountered.[2] These strains may be distinguished from *C. albicans* based on germ tube formation in serum.[2,5]
3. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Fung, D. Y. C., and C. Liang.** 1988. A new fluorescent agar for the isolation of *Candida albicans*. Bull. Inf. Lab. Serv. Vet. (France) **29/30**:1-2.
2. **Goldschmidt, M. C., D. Y. C. Fung, R. Grant, J. White, and T. Brown.** 1991. New aniline blue dye medium for rapid identification and isolation of *Candida albicans*. J. Clin. Micro. **29**:1095-1099.
3. **Miller, J. M., and H. T. Holmes.** 1995. Specimen collection and handling, p. 19- 32. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken, (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Splittstoesser, D. F., and C. Vanderzant (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Murray, P. R, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for microbiology, Washington, D.C.

Packaging

Candida Isolation Agar	500 g	0507-17

User Quality Control

Identity Specifications

Dehydrated
Medium Appearance: Beige, free-flowing, homogeneous.

Solution:	4.1% solution, soluble in distilled or deionized water on boiling. Solution is medium blue, very slightly opalescent.
Prepared Plates:	Medium blue, slightly opalescent.
Reaction of 4.1% Solution at 25°C:	pH 6.2 ± 0.2

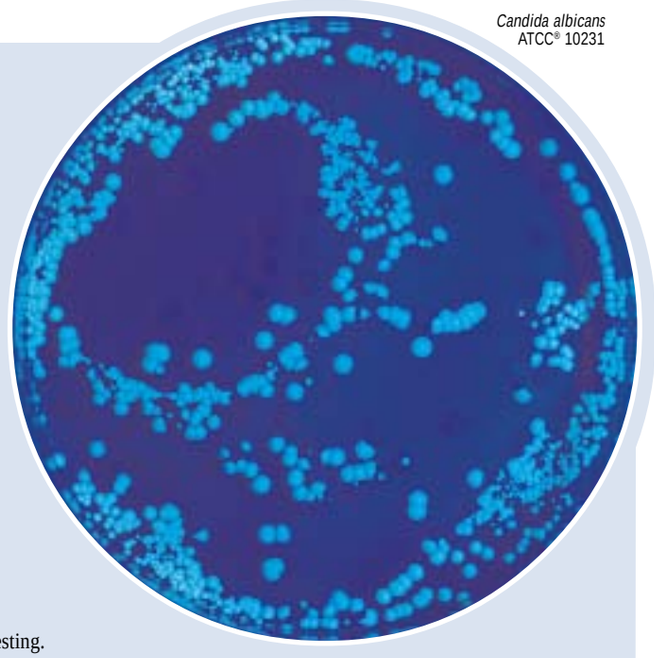
Cultural Response

Prepare Candida Isolation Agar per label instructions. Inoculate and incubate plates aerobically at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	FLUORESCENCE
Bacillus subtilis	6633	100-1,000	good	negative
Candida albicans	10231	100-1,000	good	positive
Escherichia coli	25922*	100-1,000	good	negative

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Candida albicans
ATCC® 10231

Bacto® Casamino Acids · Bacto Casamino Acids, Technical Bacto Vitamin Assay Casamino Acids

User Quality Control

Identity Specifications

Casamino Acids

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	1% solution-very light amber, clear solution.
	2% solution-Light amber, clear, soluble in distilled or deionized water upon slight heating.
Reaction of a 2% Solution at 25°C:	pH 5.8-6.65

Casamino Acids, Technical

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	1% solution, soluble in distilled or deionized water. Solution is colorless to very light amber and clear.
Reaction of 1% Solution at 25°C:	pH 5.0-7.5

Vitamin Assay Casamino Acids

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3% solution, soluble in distilled or deionized water on boiling. Very light to light amber, clear, may have a slight precipitate.
Reaction of 3% Solution at 25°C:	pH 6.5-8.5

Cultural Response

Casamino Acids and Casamino Acids, Technical

Prepare a 1% solution and adjust the pH to 7.2 ± 0.2. Inoculate tubes with the test organisms, and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	100-1,000	good
Salmonella typhi	19430	100-1,000	good

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Vitamin Assay Casamino Acids

Vitamin Assay Casamino Acids is prepared in various vitamin assay media to determine the vitamin content. It should not contain a vitamin content higher than 20% above the following values:

Vitamin B_{12}	0.2 nanograms/gram
Biotin	0.3 nanograms/gram
Folic Acid	3.3 nanograms/gram
Niacin	0.17 micrograms/gram
Pantothenate	0.04 micrograms/gram
Riboflavin	0.1 micrograms/gram
Thiamine	0.1 micrograms/gram

The cultures listed are the minimum that should be used for performance testing.

Intended Use

Bacto Casamino Acids is used in preparing microbiological culture media.

Bacto Casamino Acids, Technical is used in the preparation of microbiological culture media.

Bacto Vitamin Assay Casamino Acids is used in vitamin assay procedures.

Also Known As

Casamino Acids are also referred to as Casein Hydrolysate (Acid) or Casein Peptone, Acid Hydrolysate.

Summary and Explanation

Casamino Acids is acid hydrolyzed casein having low sodium chloride and iron concentrations. Casamino Acids is recommended for use in microbiological culture media that require a completely hydrolyzed protein as a nitrogen source. Casamino Acids is prepared according to the method described by Mueller and Miller[1] and Mueller and Johnson.[2]

Mueller[3] prepared diphtheria toxin in a medium containing a casein hydrolysate as the source of nitrogen. It was shown that the high sodium chloride content was the limiting factor in the amount of toxin that could be produced in this medium. Mueller and Miller[1] described a method to reduce the sodium chloride and iron content of the hydrolyzed casein. This hydrolyzed casein, supplemented with inorganic salts, growth factors, cystine, maltose and an optimum amount of iron, was used to prepare diphtheria toxin.[1,3] Casamino Acids duplicates this specially treated hydrolyzed casein.

In Casamino Acids, hydrolysis is carried out until all the nitrogen in the casein is converted to amino acids or other compounds of relative chemical simplicity. Casamino Acids is particularly well suited for nutritional studies, microbiological assays, and in the semi-synthetic medium for testing disinfectants.[4] Casamino Acids is also used in the preparation of tetanus toxins, and pertussis vaccines, and for sulfonamide inhibitor studies.[5]

Casamino Acids, Technical is acid hydrolyzed casein. The hydrolysis is carried out as in the preparation of Casamino Acids, but the sodium chloride and iron content of this product have not been decreased to the same extent. Casamino Acids, Technical is recommended for use in culture media where amino acid mixtures are required for a nitrogen source, and the sodium chloride content is slightly increased. It is particularly valuable in studying the growth requirements of bacteria.

Casamino Acids, Technical is prepared according to the method suggested by Mueller[1] for use in the preparation of diphtheria toxin. Mueller and Hinton[6] used Casamino Acids, Technical in a medium for primary isolation of gonococcus and meningococcus. Casamino Acids, Technical was used in agar-free media for the isolation of *Neisseria*, and in a tellurite medium for the isolation of *Corynebacterium*, described by Levin.[7] Wolf[8] used Casamino Acids, Technical in the preparation of a medium for the testing of disinfectants.

Vitamin Assay Casamino Acids is an acid digest of casein specially treated to markedly reduce or eliminate certain vitamins. It is

recommended for use in microbiological assay media and in studies of the growth requirements of microorganisms. Vitamin Assay Casamino Acids is commonly used as the amino acid source in early phases of nutrition work.[9] Sarett[10] used Vitamin Assay Casamino Acids as the acid hydrolyzed casein in his studies on *p*-aminobenzoic acid and *p*-teroylglutamic acid as growth factors for *Lactobacillus* species.

Several media containing Casamino Acids are specified in standard methods for multiple applications.[11,12,13]

Principles of the Procedure

Casamino Acids, Casamino Acids, Technical and Vitamin Assay Casamino Acids are acid hydrolyzed casein. Casein is milk protein, and a rich source of amino acid nitrogen. Casamino Acids, Casamino Acids, Technical and Vitamin Assay Casamino Acids provide nitrogen, vitamins, carbon and amino acids in microbiological culture media. Although Casamino Acids, Casamino Acids, Technical, and Vitamin Assay Casamino Acids are added to media primarily because of their organic nitrogen and growth factor components, their inorganic components also play a vital role.[14]

Formula

Casamino Acids is a dehydrated acid hydrolyzed casein in which Sodium Chloride and Iron are present in low concentrations permitting toxin production.

Casamino Acids, Technical is a dehydrated acid hydrolyzed casein. The Sodium Chloride and Iron content have not been reduced to same extent as Casamino Acids.

Vitamin Assay Casamino Acids is an acid hydrolyzed casein used to prepare media for microbiological assay of vitamins.

Typical Analysis

Physical Characteristics

Ash (%)	24.4	Loss on Drying (%)	4.5
Clarity, 1% Soln (NTU)	0.5	pH, 1% Soln	6.4
Filterability (g/cm^2)	2.9		

Nitrogen Content (%)

Total Nitrogen	10.5	AN/TN	83.8
Amino Nitrogen	8.8		

Amino Acids (%)

Alanine	3.26	Lysine	5.71
Arginine	2.20	Methionine	1.28
Aspartic Acid	4.76	Phenylalanine	2.11
Cystine	0.16	Proline	6.17
Glutamic Acid	15.30	Serine	2.19
Glycine	1.31	Threonine	2.41
Histidine	1.66	Tryptophan	<0.01
Isoleucine	3.34	Tyrosine	0.47
Leucine	5.47	Valine	4.30

Inorganics (%)

Calcium	<0.001	Phosphate	3.325
Chloride	7.400	Potassium	0.410
Cobalt	<0.001	Sodium	8.710
Copper	<0.001	Sulfate	0.045
Iron	<0.001	Sulfur	0.420
Lead	<0.001	Tin	<0.001
Magnesium	0.002	Zinc	<0.001
Manganese	<0.001		

Vitamins (µg/g)

Biotin	<0.1	PABA	<5.0
Choline (as Choline Chloride)	160.0	Pantothenic Acid	<0.1
Cyanocobalamin	<0.1	Pyridoxine	<0.1
Folic Acid	<0.1	Riboflavin	1.8
Inositol	<100.0	Thiamine	1.2
Nicotinic Acid	<20.0	Thymidine	<30.0

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	950
Salmonella	negative	Thermophile Count	25
Spore Count	390		

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated product below 30°C. The dehydrated product is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Casamino Acids
Casamino Acids, Technical
Vitamin Assay Casamino Acids

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Casamino Acids, Casamino Acids, Technical or Vitamin Assay Casamino Acids in the formula of the medium being prepared. Add Casamino Acids, Casamino Acids, Technical or Vitamin Assay Casamino Acids as required.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Casamino Acids, Casamino Acids, Technical or Vitamin Assay Casamino Acids.

Results

Refer to appropriate references and procedures for results.

References

1. **Mueller and Miller.** 1941. J. Immunol. **40**:21.
2. **Mueller and Johnson.** 1941. J. Immunol. **40**:33.
3. **Mueller**. 1939. J. Immunol. **37**:103.

4. **Klarman and Wright.** 1945. Soap and San. Chem. **21**:113.
5. **Straus, Dingle and Finland.** 1941. J. Immunol. **42**:331.
6. **Mueller and Hinton.** 1941. Proc. Soc. Exp. Biol. Med. **48**:330.
7. **Levin**. 1943. J. Bacteriol. **46**:233.
8. **Wolf**. 1945. J. Bacteriol. **49**:463.
9. **Nolan, R. A.** 1971. Amino acids and growth factors in vitamin-free casamino acids. Mycol. **63**:1231-1234.
10. **Sarett.** 1947. J. Biol. Chem. **171**:265.
11. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd. ed. American Public Health Association, Washington, D.C.
12. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
13. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
14. **Nolan, R. A., and W. G. Nolan.** 1972. Elemental analysis of vitamin-free casamino acids. Appl. Microbiol. **24**:290-291.

Packaging

Casamino Acids	100 g	0230-15
	500 g	0230-17
	2 kg	0230-07
	10 kg	0230-08
Casamino Acids, Technical	500 g	0231-17
	10 kg	0231-08
Vitamin Assay Casamino Acids	100 g	0288-15
	500 g	0288-17

Bacto® Casein Digest

Intended Use

Bacto Casein Digest is used in preparing microbiological culture media.

Also Known As

Casein Digest is similar to N-Z-Amine A.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	1%, 2%, and 10% solutions, soluble in distilled or deionized water:
	1%-Light amber, clear;
	2%-Medium amber, clear;
	10%-Dark amber, clear, no significant precipitate.
Reaction of 1% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare NZM Broth per formula. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacillus subtilis†	6633	100-1,000	good
Escherichia coli (HB101)	33694	100-1,000	good
Escherichia coli (JM107)	47014	100-1,000	good
Escherichia coli (DH5)	53868	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good
Streptomyces avermitilis	31267	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

†*Bacillus subtilis* is available as Subtilis Spore Suspension.

Summary and Explanation

Casein Digest, an enzymatic digest of casein, was developed for use in molecular genetics media. This product is digested under conditions different from other enzymatic digests of casein, including Tryptone and Casitone.

Casein Digest is contained in the formulas of NZ media (NZCYM Broth, NZYM Broth and NZM Broth), which are used for cultivating recombinant strains of *Escherichia coli*. *E. coli* grows rapidly in these rich media because they provide amino acids, nucleotide precursors, vitamins and other metabolites that the cells would otherwise have to synthesize.[1] Consult appropriate references for recommended test procedures using NZ media.[1,2]

Principles of the Procedure

Casein Digest is a nitrogen and amino acid source for microbiological culture media. Casein is raw milk protein, a rich source of amino acid nitrogen.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store Casein Digest below 30°C. The product is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Casein Digest

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Casein Digest in the formula of the medium being prepared. Add Casein Digest as required.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Casein Digest.

Results

Refer to appropriate references and procedures for results.

References

1. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1994. Current protocols in molecular biology, vol.1. Current Protocols, New York, NY.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Packaging

Casein Digest	500 g	0116-17

Bacto® Casitone

Intended Use

Bacto Casitone is used in preparing microbiological culture media.

User Quality Control

Identity Specifications

Dehydrated Appearance: Tan, free-flowing granules.

Solution:
- 1%, 2% and 10% solutions are soluble in distilled or deionized water.
- 1%-Light amber, clear, no precipitate.
- 2%-Light to medium amber, clear, may have a slight precipitate.
- 10%-Medium to dark amber, clear to very slightly opalescent, may have a precipitate.

Reaction of 1% Solution at 25°C: pH 6.8 - 7.4

Cultural Response

All solutions are prepared with the pH adjusted to 7.2 - 7.4.

TEST	SOLUTION	ORGANISM	ATCC®	RESULT	INOCULUM
Fermentable Carbohydrates	2%	*Escherichia coli*	25922*	negative	–
Indole Production	0.1%	*Escherichia coli*	25922*	positive	–
Acetylmethylcarbinol Production	0.1%	*Enterobacter aerogenes*	13048*	positive	–
Hydrogen Sulfide Production	1%	*Salmonella typhi*	6539	positive	–
Toxicity	2%w/0.5% NaCl & 1.5% Agar	*Escherichia* coli	25922*	good growth	100-1,000
Toxicity	2%w/0.5% NaCl & 1.5% Agar	*Staphylococcus aureus*	25923*	good growth	100-1,000

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Summary and Explanation

Casitone, is recommended for preparing media where an enzymatic hydrolyzed casein is desired. Casitone is used to support the growth of fastidious microorganisms. The high tryptophan content of Casitone makes it valuable for use in detecting indole production.

Dubos Broth and Dubos Oleic Agar media that support the growth of *Mycobacterium tuberculosis* contain Casitone. Media used for the enumeration of coliforms in water, m Endo Agar and m Endo Broth MF®, use Casitone as a nitrogen source. Several Thioglycollate media used for detecting microorganisms in normally sterile materials, include Casitone as a nitrogen and amino acid source.

Casitone is recommended for preparing media for sterility testing according to US Pharmacopeia XXIII (USP).[1] Several media containing Casitone are specified in standard methods[2,3,4,5] for multiple applications.

Principles of the Procedure

Casitone is a pancreatic digest of casein. Casein is the main protein of milk, and a rich source of amino acid nitrogen.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated product below 30°C. The dehydrated product is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Typical Analysis

Physical Characteristics

Ash (%)	7.0	Loss on Drying (%)	3.7
Clarity, 1% Soln (NTU)	0.6	pH, 1% Soln	7.2
Filterability (g/cm^2)	1.7		

Carbohydrate (%)

Total	0.2

Nitrogen Content (%)

Total Nitrogen	13.3	AN/TN	35.3
Amino Nitrogen	4.7		

Amino Acids (%)

Alanine	3.01	Lysine	13.62
Arginine	3.76	Methionine	1.71
Aspartic Acid	6.61	Phenylalanine	4.02
Cystine	0.02	Proline	8.57
Glutamic Acid	20.03	Serine	4.82
Glycine	1.97	Threonine	3.74
Histidine	2.17	Tryptophan	0.14
Isoleucine	4.16	Tyrosine	2.09
Leucine	8.74	Valine	4.06

Inorganics (%)

Calcium	0.010	Phosphate	2.604
Chloride	0.110	Potassium	0.162
Cobalt	<0.001	Sodium	3.073
Copper	<0.001	Sulfate	0.339
Iron	0.003	Sulfur	0.676
Lead	<0.001	Tin	<0.001
Magnesium	0.019	Zinc	0.004
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.2	PABA	15.9
Choline (as Choline Chloride)	550.0	Pantothenic Acid	7.7
Cyanocobalamin	<0.1	Pyridoxine	1.3
Folic Acid	0.8	Riboflavin	0.4
Inositol	980.0	Thiamine	<0.1
Nicotinic Acid	20.3	Thymidine	342.9

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	1850
Salmonella	negative	Thermophile Count	100
Spore Count	300		

Procedure

Materials Provided

Casitone

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Casitone in the formula of the medium being prepared. Add Casitone as required.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Casitone.

Results

Refer to appropriate references and procedures for results.

References

1. **The United States Pharmacopeial Convention.** 1995. The United States Pharmacopeia, 23rd ed. Sterility test, p. 1686-1690. The United States Pharmacopeial Convention Inc., Rockville, MD.
2. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
3. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed., American Public Health Association, Washington, D.C.

Packaging

Casitone	100 g	0259-15
	500 g	0259-17
	10 kg	0259-08

Bacto® Casman Medium Base

Intended Use

Bacto Casman Medium Base is used with blood in isolating fastidious microorganisms under reduced oxygen tension.

Summary and Explanation

In 1947, Casman[1,2,3] described an infusion-free medium enriched with 5% blood for fastidious microorganisms incubated anaerobically. This medium replaced labor intensive formulas containing fresh meat infusion and unheated and heated blood.[1] Casman adjusted the medium after experiments revealed that nicotinamide disrupted the action of a blood enzyme that inactivates V factor (NAD).[2] Using unheated human blood in the formula, *Haemophilus influenzae* grew well and *Neisseria* was inhibited. The concentration of nicotinamide was lowered to support growth of *Neisseria* species.[2,3]

Casman Agar Base with rabbit blood can be used for the cultivation and maintenance of *Gardnerella vaginalis*.[4]

Principles of the Procedure

Proteose Peptone No.3, Tryptose and Beef Extract provide nitrogen, vitamins and amino acids. Nicotinamide enhances growth of *N. gonorrhoeae* and *H. influenzae* by impeding the removal of coenzyme (V factor) by nucleotidase from the enriched blood. The small amount of Dextrose is added to enhance growth of pathogenic cocci. Sodium chloride maintains the osmotic balance of the medium. Para-aminobenzoic acid is a preservative. Corn starch is added to ensure that any toxic metabolites produced are absorbed, to neutralize glucose inhibition of beta-hemolysis[4] and to enhance *growth of Neisseria* species. Agar Noble is a solidifying agent.

Formula

Casman Medium Base

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Tryptose	10 g
Bacto Beef Extract	3 g
Nicotinamide	0.05 g
p-Aminobenzoic Acid	0.05 g
Bacto Dextrose	0.5 g
Corn Starch	1 g
Sodium Chloride	5 g
Agar Noble	14 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Casman Medium Base

Materials Required But Not Provided

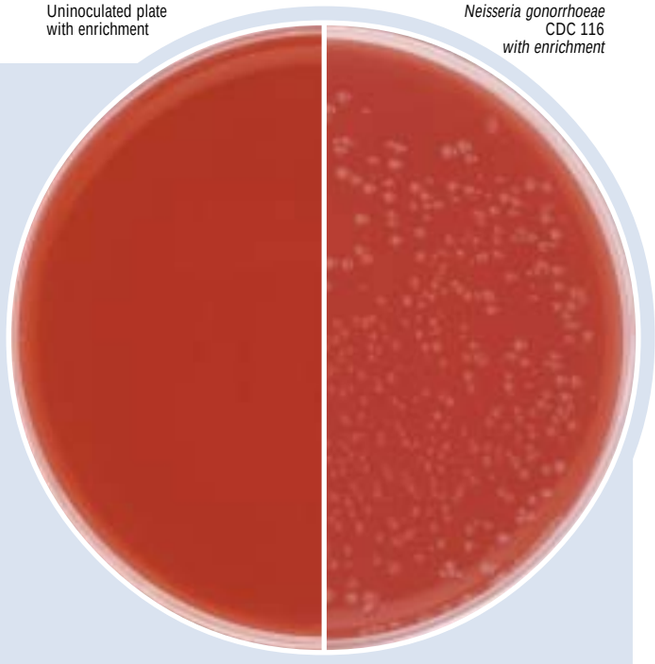
Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile defibrinated blood
Sterile water-lysed blood

Method of Preparation

1. Suspend 43 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50°C.
4. Add 5% sterile blood and 0.15% sterile water-lysed blood solution (one part blood to three parts water). Omit water-lysed blood if sterile blood is partially lysed.
5. Dispense into sterile Petri dishes or as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.3% solution, soluble in distilled or deionized water with frequent agitation on boiling. Light to medium amber with a ground glass appearance.
Prepared Medium:	Without blood, light to medium amber with a ground glass appearance. With 5% blood, cherry red opaque.
Reaction of 4.3% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Casman Medium Base per label directions, enrich with 5% sterile blood and 0.15% sterile water-lysed blood solution. Inoculateprepared medium and incubate at 35 ± 2°C under increased CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH w/BLOOD
Haemophilus influenzae	10211	100-1,000	good
Neisseria gonorrhoeae	CDC116	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Test Procedure

For a complete discussion on the isolation and identification of fastidious microorganisms, refer to the procedures described in appropriate references.[5,6]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Nicotinamide in concentrations greater than 0.005% inhibits growth of some *N. gonorrhoeae* strains; however, only slight stimulation of growth of *H. influenzae* occurs with this amount.[1]
3. Hemolytic reactions of some strains have been shown to be affected by differences in animal blood.[6]
4. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[6]
5. Casman Medium Base is intended for use with supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.[5,6]
6. Improper specimen collection, environment, temperature, CO_2 level, moisture and pH can adversely affect the growth and viability of the organism.

References

1. **Casman, E. P.** 1947. A noninfusion blood agar base for *Neisseriae*, *Pneumococci* and *Streptococci*. Am. J. Clin. Pathol. **27**:281.
2. **Casman, E. P.** 1942. J. Bacteriol. **43**:33.
3. **Casman, E. P.** 1947. J. Bacteriol. **53**:561.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1, p. 141-143. Williams & Wilkins, Baltimore, MD.
5. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology. Washington, D.C.

Packaging

Casman Medium Base	500 g	0290-17

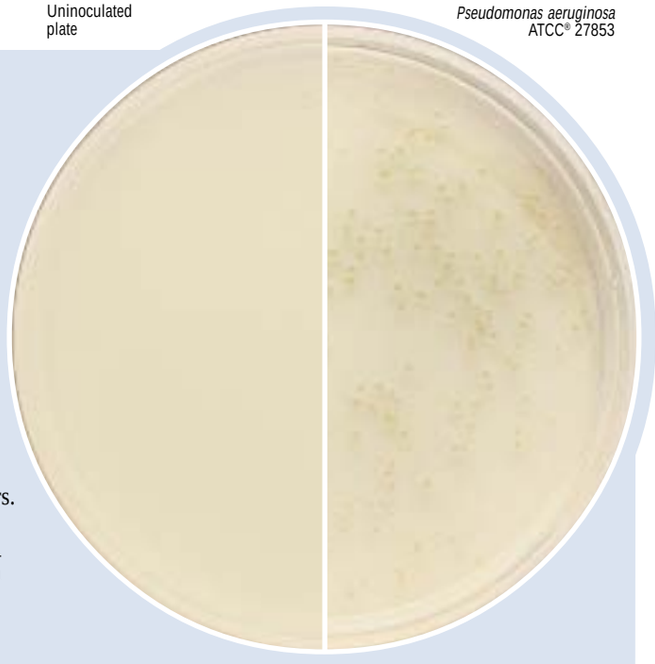
Bacto® Cetrimide Agar Base

Intended Use

Bacto Cetrimide Agar Base is used for isolating and cultivating *Pseudomonas aeruginosa*.

Also Known As

Cetrimide Agar Base is also referred to as Pseudosel™ Agar, Pseudomonas Selective Agar Base or Pseudomonas Selective Medium.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.53% solution with 1% glycerol, soluble on boiling in distilled or deionized water. Light amber, opalescent, with precipitate.
Prepared Medium:	Light amber, opalescent, with precipitate.
Reaction of 4.53% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Cetrimide Agar Base per label directions with 1% glycerol. Inoculate prepared medium and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU(approx.)	COLONY GROWTH	COLOR
Pseudomonas aeruginosa	27853*	1,000	good	yellow-green to blue
Escherichia coli	25922*	1,000-2,000	inhibited	–
Staphylococcus aureus	25923*	1,000-2,000	inhibited	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Summary and Explanation

Pseudomonas aeruginosa has one of the broadest ranges of infectivity among pathogens, and is the most frequently isolated nonfermentative bacillus in clinical specimens.[3] It is a significant cause of burn and nosocomial infections.[4] The ability of *P. aeruginosa* strains to destroy tissue may be related to the production of various extracellular enzymes.[3] In addition, virulent strains produce an exotoxin A, which inhibits protein synthesis.[3]

Pseudomonas aeruginosa produces a number of water-soluble pigments, including the yellow-green or yellow-brown fluorescent pigment pyoverdin.[4] When pyoverdin combines with the blue water-soluble pigment pyocyanin, the bright green color characteristic of *P. aeruginosa* is created.[4] Fluorescent pigment-producing strains fluoresce under short-wave ultraviolet light, and are observed at 254 nm using a standard Wood's lamp.[3] Agar containing cetrimide has been used successfully to isolate *P. aeruginosa* from contaminated specimens.[5]

King, Ward and Raney[1] developed Medium A (Tech Agar) to enhance the production of pyocyanin in *Pseudomonas* species. Cetrimide Agar Base is prepared according to this formula with the addition of cetrimide.[1] Brown and Lowbury[2] used cetrimide in the Medium B formulation of King, Ward and Raney[1] to demonstrate the production of fluorescein in *P. aeruginosa*.

Cetrimide Agar Base is recommended in the examination of food[6] and in United States Pharmacopeia (USP XXIII) for use in Microbial Limit Tests.[7]

Principles of the Procedure

Bacto Peptone provides the nitrogen, vitamins and amino acids in Cetrimide Agar Base. Magnesium Chloride and Potassium Sulfate enhance the production of pyocyanin and fluorescein.[8] Cetrimide (cetyltrimethylammonium bromide) is the selective agent. Cetrimide acts as a quaternary ammonium cationic detergent causing nitrogen and phosphorous to be released from bacterial cells other than *P. aeruginosa*. Bacto Agar is the solidifying agent. Cetrimide Agar Base is supplemented with 1% Glycerol as a source of carbon.

Formula

Cetrimide Agar Base

Formula Per Liter

Bacto Peptone	20 g
Magnesium Chloride	1.4 g
Potassium Sulfate	10 g
Cetrimide (Cetyltrimethylammonium Bromide)	0.3 g
Bacto Agar	13.6 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Procedure

Materials Provided

Cetrimide Agar Base

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Glycerol
Sterile Petri dishes

Method of Preparation

1. Suspend 45.3 grams in 1 liter distilled or deionized water containing 10 ml of glycerol.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Dispense into sterile Petri dishes, or as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For the isolation of *P. aeruginosa* plates of Cetrimide Agar Base may be inoculated by the streak method from nonselective medium or directly from the specimen. When plating directly from the specimen the inoculum level should be sufficiently high.

Results

Examine plates or tubes for the presence of characteristic blue, blue-green, yellow-green pigment. *Pseudomonas aeruginosa* typically produce both pyocyanin and fluorescein.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. The type of peptone used in base may affect pigment production.[1,9]
3. No single medium can be depended upon to exhibit all pigment producing *P. aeruginosa* strains.
4. Occasionally some enterics will exhibit a slight yellowing of the medium; however, this coloration is easily distinguished from fluorescein production since this yellowing does not fluoresce.[1]
5. Some nonfermenters and some aerobic spore formers may exhibit a water-soluble tan to brown pigmentation on this medium. *Serratia* strains may exhibit a pink pigmentation.[1]
6. Studies of Lowbury and Collins[10] showed *Ps. aeruginosa* may lose its fluorescence under UV if the cultures are left at room temperature for a short time. Fluorescence reappears when plates are reincubated.
7. Further tests are necessary for definitive identification of *P. aeruginosa*.

References

1. **King, E. O., M. K. Ward, and E. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44**:301.

2. **Brown, V.I., and E. J. L. Lowbury.** 1965. Use of an improved Cetrimide Agar Medium and of culture methods for Pseudomonas aeruginosa. J. Clin. Pathol. **18**:752.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Nonfermentative gram-negative bacilli and coccobacilli, p. 386-405. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
4. **Gilligan, P. H.** 1995. *Pseudomonas* and *Burkholderia*, p. 509-519. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.)., Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Robin, T., and J. M. Janda.** 1984. Enhanced recovery of *Pseudomonas aeruginosa* from diverse clinical specimens on a new selective agar. Diag. Microbiol. Infect. Dis. **2**:207.
6. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
7. **The United States Pharmacopeia**. 1995. Microbiological Limits Tests, The United States pharmacopeia, 23rd ed. United States Pharmacopeial Convention, Rockville, MD.
8. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 146-149. Williams & Wilkins, Baltimore, MD.
9. **Goto, S., and S. Enomoto.** 1970. Nalidixic acid cetrimide agar: A new selective plating medium for the selective isolation of *Pseudomonas aeruginosa*. Jpn. J. Microbiol. **14**:65.
10. **Lowbury, E. J. L., and A. G. Collins.** 1955. The use of a new cetrimide product in a selective medium for *Pseudomonas aeruginosa*. J. Clin. Pathol. **8**:47.

Packaging

Cetrimide Agar Base	100 g	0854-15
	500 g	0854-17
Glycerol	100 g	0282-15
	500 g	0282-17

Bacto® Chapman Stone Medium

Intended Use

Bacto Chapman Stone Medium is used for isolating and differentiating staphylococci based on mannitol fermentation and gelatinase activity.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous with a tendency to cake.
Solution:	20.2% solution, soluble in distilled or deionized water on boiling. Solution is light amber, opalescent with precipitation.
Prepared medium:	Light to medium amber, opalescent with a precipitate.
Reaction of 20.2% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Chapman Stone Medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours. Add Brom Cresol Purple indicator to determine mannitol fermentation (yellow = positive).

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HALO (Gelatinase)	MANNITOL FERMENTATION
Escherichia coli	25922*	100-1,000	inhibited	–	–
Staphylococcus aureus	25923*	100-1,000	good	+	+
Staphylococcus epidermidis	12228*	100-1,000	good	+	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Also Known As

Chapman Stone Medium conforms with Chapman Stone Agar.

Summary and Explanation

Chapman Stone Medium is prepared according to the formula described by Chapman.[1] It is similar to Staphylococcus Medium 110, previously described by Chapman,[2] except that the sodium chloride concentration is reduced to 5.5% and ammonium sulfate is included in the formulation. The inclusion of ammonium sulfate in the medium negates the need to add a reagent after growth has been obtained in order to detect gelatinase activity by Stone's method. Chapman Stone Medium is especially recommended for suspected food poisoning studies involving *Staphylococcus*.[3] It is selective, due to the relatively high salt content, and is differential due to pigmentation, mannitol fermentation, and the presence or absence of gelatin liquefaction.

Principles of the Procedure

Yeast Extract and Tryptone provide nitrogen, carbon, sulfur, vitamins, and trace nutrients essential for growth. Gelatin serves as a substrate for gelatinase activity. Ammonium Sulfate allows detection of gelatin hydrolysis. D-Mannitol is the fermentable carbohydrate. Sodium Chloride acts as a selective agent because most bacterial species are inhibited by the high salt content. Dipotassium Phosphate provides buffering capability. Bacto Agar is the solidifying agent.

Formula

Chapman Stone Medium

Formula Per Liter

Bacto Yeast Extract	2.5 g
Bacto Tryptone	10 g
Bacto Gelatin	30 g
Bacto D-Mannitol	10 g
Sodium Chloride	55 g
Ammonium Sulfate	75 g
Dipotassium Phosphate	5 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** HARMFUL IF SWALLOWED. (EC) IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Lungs, Intestines.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Chapman Stone Medium

Materials Required But Not Provided

Glassware
Autoclave
Incubator (30°C)
Sterile Petri dishes
Brom Cresol Purple

Method of Preparation

1. Suspend 20.2 grams in 100 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 10 minutes. Omit sterilization if prepared medium is to be used within 12 hours.
4. Dispense as desired.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Streak a sample of the specimen onto the surface of the agar. Make several stabs into the medium along the streak.
2. Incubate, aerobically, at 30 ± 2°C for up to 48 hours.
3. Examine for growth and the presence or absence of clear zones around colonies.
4. To determine mannitol fermentation, add a few drops of Brom Cresol Purple to areas on the medium from which colonies have been removed. Any change in color of the indicator, compared with that of the uninoculated medium, indicates fermentation of mannitol.

Results

Mannitol fermentation: Positive = change in color of the indicator to yellow.

Gelatinase activity: Positive Stone reaction = formation of clear zones around the colonies.

Any mannitol-positive, yellow or orange colonies surrounded by a clear zone are presumptively identified as *Staphylococcus aureus*. White or nonpigmented colonies, with or without a clear zone, are probably *S. epidermidis*.

Limitations of the Procedure

1. Confirm the presumptive identification of pathogenic staphylococci with additional tests, such as coagulase activity.
2. Enterococci and/or Group D streptococci may exhibit growth on the medium and show slight mannitol fermentation. The colonies, however, are tiny and can easily be differentiated from staphylococci by Gram stain and the catalase test.[3]

References

1. **Chapman, G. H.** 1948. An improved Stone medium for the isolation and testing of food-poisoning staphylococci. Food Res. **13**:100-105.
2. **Chapman, G. H.** 1946. A single culture medium for selective isolation of plasma-coagulating staphylococci and for improved testing of chromogenesis, plasma coagulation, mannitol fermentation, and the Stone reaction. J. Bacteriol. **51**:409-410.
3. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Packaging

Chapman Stone Medium	500 g	0313-17
	10 kg	0313-08

Bacto® Charcoal Agar

Intended Use

Bacto Charcoal Agar is used for cultivating fastidious organisms, especially *Bordetella pertussis*, for vaccine production and stock culture maintenance.

Summary and Explanation

Charcoal Agar is prepared according to the method of Mishulow, Sharpe and Cohen.[1] The authors found this medium to be an efficient substitute for Bordet-Gengou Agar in the production of *B. pertussis* vaccines.

The genus *Bordetella* consists of four species: *Bordetella pertussis*, *B. parapertussis*, *B. bronchiseptica* and *B. avium*.[2] All *Bordetella* are

respiratory pathogens, residing on the mucous membranes of the respiratory tract. *B. pertussis* is the major cause of whooping cough or pertussis. *B. parapertussis* is associated with a milder form of the disease.[3] *B. bronchiseptica* is an opportunistic human pathogen associated with both respiratory and non-respiratory infections, often occurring in patients having close contact with animals.[2] *B. bronchiseptica* has not been reported to cause pertussis. There have been no reports of recovery of *B. avium* from humans.[2]

Charcoal Agar supplemented with Horse Blood is used for the cultivation and isolation of *Haemophilus influenzae*.[4]

Principles of the Procedure

Infusion from Beef Heart and Bacto Peptone provide the nitrogen, carbon and amino acids in Charcoal Agar. Yeast Extract is a vitamin source. Sodium Chloride maintains osmotic balance. Bacto Agar is a solidifying agent. Soluble Starch absorbs toxic metabolites. Norit SG, charcoal, provides growth requirements and selective properties.

Formula

Charcoal Agar

Formula Per Liter

Beef Heart, Infusion from	500 g
Bacto Peptone	10 g
Sodium Chloride	5 g
Bacto Soluble Starch	10 g
Bacto Yeast Extract	3.5 g
Norit SG	4 g
Bacto Agar	18 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Charcoal Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Sterile Petri dishes

Method of Preparation

1. Suspend 62.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Mix thoroughly during dispensing to uniformly distribute the charcoal.

User Quality Control

Identity Specifications

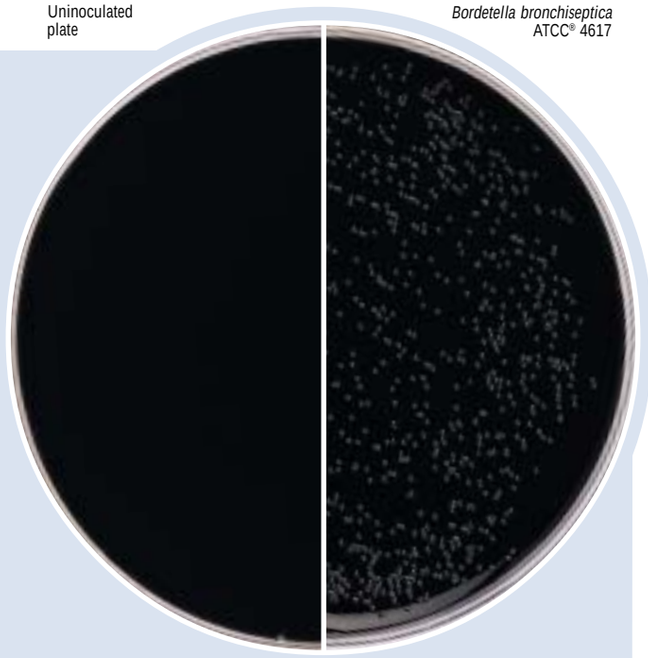
Dehydrated Appearance:	Gray, free-flowing, homogeneous.
Solution:	6.25% solution, soluble in distilled or deionized water on boiling; black, opaque with a precipitate.
Prepared Medium:	Black, opaque.
Reaction of 6.25% Solution at 25°C	pH 7.3 ± 0.2

Cultural Response

Prepare Charcoal Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bordetella bronchiseptica	4617	100-1,000	good
Bordetella parapertussis	15237	100-1,000	good
Bordetella pertussis	8467	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.



Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion on the isolation and maintenance of fastidious microorganisms refer to the procedures described in appropriate references.[2,4,5]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Charcoal has a tendency to settle out of the medium. Swirl the flask gently when dispensing to obtain a uniform charcoal suspension.[4]

References

1. **Mishulow, L., L. S. Sharpe, and L. L. Cohen.** 1953. Beef-heart charcoal agar for the preparation of pertussis vaccines. Am. J. Public Health, **43**:1466.
2. **Marcon, M. J.** 1995. *Bordetella*, p. 566-573. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.)., Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Linneman, C. C., and E. B. Pery.** 1977. *Bordetella parapertussis*; recent experience and a review of the literature. Am. J. Dis. Child. **131**:560-563. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.)., Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol 1, p. 154-159. Williams & Wilkins, Baltimore, MD.
5. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Packaging

Charcoal Agar	500 g	0894-17

Bacto® Choline Assay Medium

Intended Use

Bacto Choline Assay Medium is used for determining choline concentration by the microbiological assay technique.

Summary and Explanation

Vitamin Assay Media are used in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test.

Choline Assay Medium is a slight modification of the medium described by Horowitz and Beadle.[1] *Neurospora crassa* ATCC® 9277 is the test organism used in this microbiological assay.

User Quality Control

Identity Specifications

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	2.85% (single strength) and 5.7% (double strength) solution, soluble in distilled or deionized water upon boiling. Solution is colorless, clear, may have a slight precipitate.
Prepared Medium:	Colorless, clear, may have a slight precipitate.
Reaction of 2.85% Solution at 25°C:	pH 5.5 ± 0.2

Cultural Response

Prepare Choline Assay Medium per label directions. Prepare a standard curve using choline at levels from 0 to 25 µg per 10 ml. The medium supports the growth of *Neurospora crassa* ATCC® 9277 when supplemented with choline chloride.

Principles of the Procedure

Choline Assay Medium is a choline-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *N. crassa* ATCC® 9277. The addition of choline standard in specified increasing concentrations gives a growth response by this organism that can be measured gravimetrically.

Formula

Choline Assay Medium

Formula Per Liter

Bacto Sucrose	40 g
Ammonium Nitrate	2 g
Biotin	10 µg
Potassium Sodium Tartrate	11.4 g
Monopotassium Phosphate	2 g
Magnesium Sulfate	1 g
Sodium Chloride	0.2 g
Calcium Chloride	0.2 g
Sodium Borate	700 µg
Ammonium Molybdate	500 µg
Ferrous Sulfate	1.1 mg
Cuprous Chloride	300 µg
Manganese Sulfate	110 µg
Zinc Sulfate	17.6 mg

Final pH 5.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
4. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Choline Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Neurospora crassa* ATCC® 9277
Sterile 0.85% saline
Distilled or deionized water
Inoculating loop
Neurospora Culture Agar
Wire needle or glass rod
Paper towels
Vacuum oven
Porcelain spot plate
Scale

Method of Preparation

1. Suspend 5.7 grams in 100 ml of distilled or deionized water.
2. Boil 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into flasks, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust flask volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assay, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Remove 1 loop of spores from a 48-hour culture of *N. crassa* ATCC® 9277 grown on Neurospora Culture Agar and suspend it in 100 ml sterile saline. Add 1 drop of this spore suspension to each flask of medium. Incubate at 25-30°C for 3 days. At the end of the incubation period, steam the flask at 100°C for 5 minutes. Remove all the mycelium from the flask using a stiff wire needle or glass rod, press dry between paper towels, and roll into a small pellet. Dry the pellet at 100°C in a vacuum oven for 2 hours. (A glazed porcelain spot plate is convenient for handling the mycelium during drying and weighing.) Weigh to the nearest 0.5 mg. A standard curve is then constructed from the weights obtained, and the unknown determined by interpolation. In the assay for choline, 50 ml Erlenmeyer flasks containing a total volume of 10 ml each are used.

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. The standard curve is obtained by using choline at levels of 0.0, 2.5, 5, 10, 15, 20 and 25 µg per assay flask (10 ml). The most effective assay range using Choline Assay Medium is between 2.5 and 30 µg choline.

The concentration of choline required for the preparation of the standard curve may be prepared by dissolving 0.5 grams choline chloride in 1,000 ml distilled water. This is the stock solution (500 µg per ml). Dilute the stock solution by adding 1 ml to 99 ml distilled water. Use 0.0, 0.5, 1, 2, 3, 4 and 5 ml of this diluted solution per flask. Prepare the stock solution fresh daily.

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average. Use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results to these procedures, all conditions of the assay must be followed precisely.

References

1. **Horowitz and Beadle.** 1943. J. Biol. Chem. **150**:325.

Packaging

Choline Assay Medium	100 g	0460-15

Bacto® Columbia Blood Agar Base · Bacto Columbia Blood Agar Base EH · Bacto Columbia Blood Agar Base No. 2

Intended Use

Bacto Columbia Blood Agar Base is used for cultivating fastidious microorganisms with or without the addition of blood.

Bacto Columbia Blood Agar Base EH is used with blood in isolating and cultivating fastidious microorganisms.

Bacto Columbia Blood Agar Base No. 2 is used with blood in isolating and cultivating fastidious microorganisms.

Also Known As

Blood Agar Base may be abbreviated as BAB.

Summary and Explanation

Columbia blood agar base media are typically supplemented with 5-10% sheep, rabbit or horse blood for use in isolating, cultivating and determining the hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, Columbia Blood Agar Base can be used as a general purpose medium.

Columbia Blood Agar Base was patterned after the Columbia Agar formulation described by Ellner *et al.* of Columbia University.[1] Columbia Blood Agar Base No. 2 and Columbia Blood Agar Base EH (Enhanced Hemolysis) are modifications of Columbia Blood Agar Base. Columbia Blood Agar Base No. 2 provides clearer hemolytic reactions with *Streptococcus* group A while Columbia Blood Agar Base EH provides dramatic, enhanced hemolysis.

Columbia Blood Agar Base is specified in the Compendium of Methods for the Microbiological Examination of Foods.[2]

Principles of the Procedure

Columbia Blood Agar Base uses specially selected raw materials to support good growth of fastidious microorganisms. Two peptones, Pantone (a casein hydrolysate) and Bitone (an infusion peptone), provide nitrogen, carbon, amino acids and vitamins. Tryptic Digest of Beef Heart provides additional nitrogen and amino acids. Corn Starch, originally proposed by the authors of this medium, increases growth of *Neisseria* and enhances the hemolytic reactions of some streptococci.[1] Agar is a solidifying agent. Sodium Chloride maintains the osmotic balance of the medium.

Columbia Blood Agar Base No. 2 and Columbia Blood Agar Base EH are similar in composition to Columbia Blood Agar Base. However, different peptones are used to improve and enhance hemolysin production while minimizing antagonism or loss in activity of streptococcal hemolysins. Columbia Blood Agar Base No. 2 contains Bitone H while Columbia Blood Agar Base EH contains Bitone H Plus. Both formulations contain Pantone, Enzymatic Digest of Animal Tissue, Starch, Sodium Chloride and Agar.

Blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of ß-hemolytic streptococci.[3] Supplementation with blood (5-10%) provides additional growth factors for fastidious microorganisms and aids in determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood and the type of basal medium used.[4]

User Quality Control

Identity Specifications

Columbia Blood Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.4% solution, soluble in distilled or deionized water on boiling, light to medium amber, opalescent with a fine precipitate.
Prepared Medium:	Plain - light to medium amber, slightly opalescent to opalescent with a fine precipitate.
	With 5% sheep blood - cherry red, opaque.
Reaction of 4.4% Solution at 25°C:	pH 7.3 ± 0.2

Columbia Blood Agar Base No. 2

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.9% solution, soluble in distilled or deionized water on boiling, light to medium amber, opalescent.
Prepared Medium:	With 5% sheep blood - cherry red, opaque.
Reaction of 3.9% Solution at 25°C:	pH 7.3 ± 0.2

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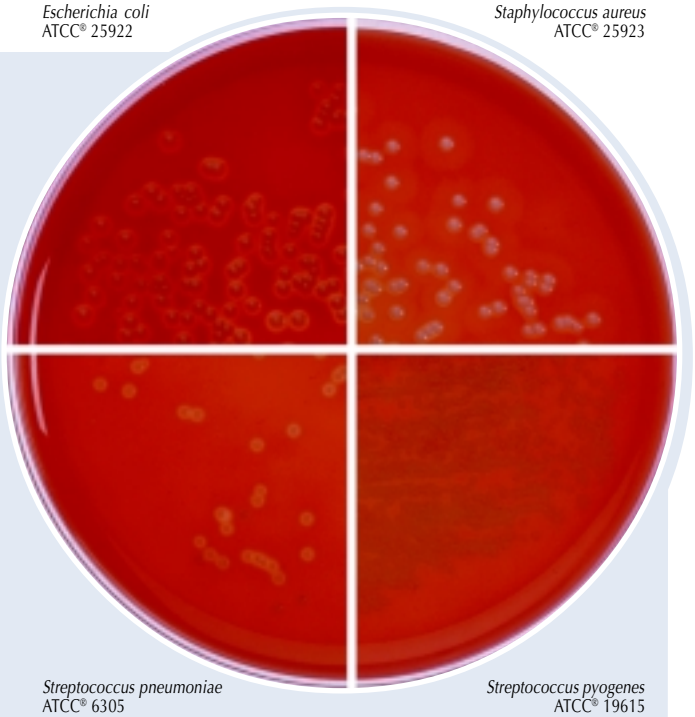


On Columbia Blood Agar Base

Formula

Columbia Blood Agar Base

Formula Per Liter

Bacto Pantone	10 g
Bacto Bitone	10 g
Tryptic Digest of Beef Heart	3 g
Corn Starch	1 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Columbia Blood Agar Base EH

Formula Per Liter

Bacto Pantone	12 g
Bacto Bitone H Plus	6 g
Enzymatic Digest of Animal Tissue	3 g
Starch	1 g
Sodium Chloride	5 g
Agar	12 g

Final pH 7.3 ± 0.2 at 25°C

Columbia Blood Agar Base No. 2

Formula Per Liter

Bacto Pantone	12 g
Bacto Bitone H	6 g
Enzymatic Digest of Animal Tissue	3 g
Starch	1 g
Sodium Chloride	5 g
Agar	12 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Columbia Blood Agar Base
Columbia Blood Agar Base EH
Columbia Blood Agar Base No. 2

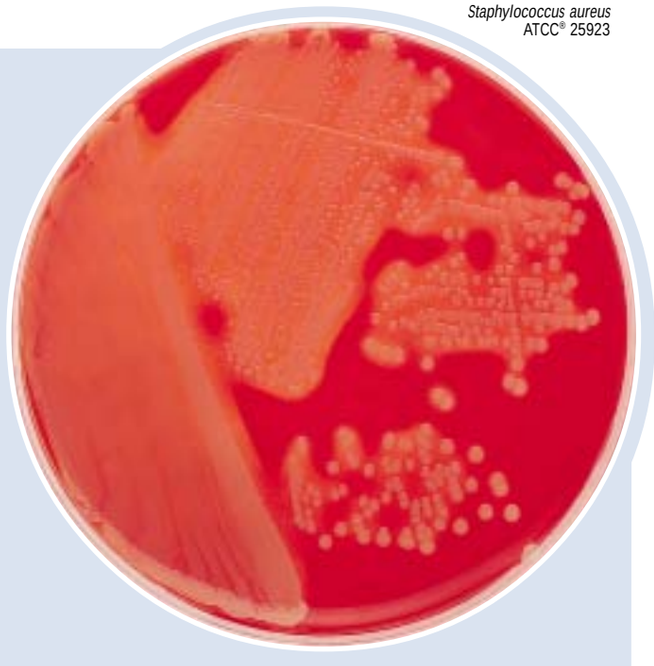
Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile defibrinated blood
Sterile Petri dishes

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water:
   Columbia Blood Agar Base - 44 grams;
   Columbia Blood Agar Base EH - 39 grams;
   Columbia Blood Agar Base No. 2 - 39 grams.

Staphylococcus aureus
ATCC® 25923



Columbia Blood Agar Base EH

User Quality Control cont.

Columbia Blood Agar Base EH

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.9% solution, soluble in distilled or deionized water on boiling, light to medium amber, clear to slightly opalescent.
Prepared Medium:	With 5% sheep blood - medium to bright cherry red, opaque.
Reaction of 3.9% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare the medium with and without 5% sterile defibrinated sheep blood per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HEMOLYSIS
Escherichia coli	25922*	100-1,000	good	N/A
Staphylococcus aureus	25923*	100-1,000	good	beta
Streptococcus pneumoniae	6305	100-1,000	good	alpha
Streptococcus pyogenes	19615*	100-1,000	good	beta

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory in accordance with recommended guidelines outlined in the references.

Test Procedure

1. Process each specimen as appropriate and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit ß-hemolytic streptococci beneath the agar surface. Subsurface growth will demonstrate the most reliable hemolytic reactions due to the activity of both oxygen-stable and oxygen-labile streptolysins.[4]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.

Results

1. Examine plates for growth and hemolytic reactions after 18-24 and 48 hours of incubation. Four types of hemolysis on blood agar media can be described:[5]
   a. Alpha-hemolysis (α) is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony, causing a greenish discolorization of the medium.
   b. Beta-hemolysis (ß) is the lysis of red blood cells, producing a clear zone surrounding the colony.
   c. Gamma-hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
   d. Alpha-prime-hemolysis (ά) is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Limitations of the Procedure

1. Blood agar base media are intended for use with blood supplementation. Although certain diagnostic tests may be performed directly on these media, biochemical and, if indicated, immunological testing using pure cultures is recommended for complete identification. Consult appropriate references for further information.
2. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
3. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are ß-hemolytic on horse, human and rabbit blood agar and α-hemolytic on sheep blood agar.[4]
4. Colonies of *Haemophilus haemolyticus* are ß-hemolytic on horse and rabbit blood agar and must be distinguished from colonies of ß-hemolytic streptococci using other criteria. The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of *H. haemolyticus*.[6]
5. Atmosphere of incubation has been shown to influence hemolytic reactions of ß-hemolytic streptococci.[4] For optimal performance, incubate blood agar media under increased CO_2 or anaerobic conditions.

References

1. **Ellner, P. D., C. J. Stoessel, E. Drakeford, and F. Vasi.** 1966. A new culture medium for medical bacteriology. Am. J. Clin. Pathol. **45:**502-504.
2. **Vanderzant, C. and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin Pathol. **17**:281-289.
4. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Isenberg, H. D. (ed).** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.
6. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.

Packaging

Columbia Blood Agar Base	500 g	0792-17
	2 kg	0792-07
	10 kg	0792-08
Columbia Blood Agar Base EH	500 g	0790-17
	2 kg	0790-07
	10 kg	0790-08
Columbia Blood Agar Base No. 2	500 g	0793-17
	2 kg	0793-07
	10 kg	0793-08

Bacto® Columbia Broth

Intended Use

Bacto Columbia Broth is used for cultivating fastidious microorganisms.

Summary and Explanation

Columbia Broth is prepared according to the formulation described by Morello and Ellner.[1] In their study Columbia Broth, a medium developed for blood cultures, was superior to a commonly used general purpose broth for faster growth of *Staphylococcus aureus*, *E. coli* and streptococci (viridans and enterococcus groups). Columbia Broth, in the presence of CO_2 and supplemented with SPS, is an excellent blood culture medium.[2] In the study by Morello and Ellner,[1] the addition of sodium polyanetholsulfonate (SPS) in Columbia Broth was emphasized. SPS is an anticoagulant that inhibits serum bactericidal activity against many bacteria, inhibits phagocytosis, inactivates complement, and neutralizes lysozymes and the aminoglycoside class of antibiotics.[2]

Principles of the Procedure

Columbia Broth was formulated from Pantone and Bitone. Dextrose is added to the formula as a carbon energy source. The medium is

buffered with Tris. Corn Starch is omitted to reduce opalescence.[1] Cysteine is the reducing agent. Magnesium and Iron are added to facilitate organism growth.

Formula

Columbia Broth

Formula Per Liter

Bacto Pantone	10 g
Bacto Bitone	10 g
Tryptic Digest of Beef Heart	3 g
L-Cysteine Hydrochloride	0.1 g
Bacto Dextrose	2.5 g
Sodium Chloride	5 g
Magnesium Sulfate Anhydrous	0.1 g
Ferrous Sulfate	0.02 g
Sodium Carbonate	0.6 g
Tris (Hydroxymethyl) Aminomethane	0.83 g
Tris (Hydroxymethyl) Aminomethane HCl	2.86 g

Final pH 7.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water on warming. Solution is light amber, clear to very slightly opalescent, may have a slight amount of fine precipitate.
Prepared Medium:	Light amber, clear to very slightly opalescent, may have a slight amount of fine precipitate.
Reaction of 3.5% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

Prepare Columbia Broth per label directions. Inoculate and incubate at 35 ± 2°C under appropriate conditions for 18-48 hours. Incubate *Bacteroides fragilis* anaerobically.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090	100-1,000	good
Staphylococcus aureus	25923*	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good
Bacteroides fragilis	25285	100-1,000	good
Pseudomonas aeruginosa	27853	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Columbia Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Sterile tubes
Sodium polyanetholesulfonate (SPS)

Method of Preparation

1. Dissolve 35 grams in 1 liter distilled or deionized water.
2. Warm slightly if necessary to dissolve completely.
3. OPTIONAL: Sodium polyanetholesulfonate (SPS) may be added at this time with agitation to ensure a uniform solution. The culture medium should contain 0.025 to 0.05% SPS.
4. Distribute in suitable containers. Autoclave at 121°C for 15 minutes.
5. Allow to cool to room temperature before using.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

Process clinical specimens from different body sites as described in Clinical Microbiology Procedures Handbook,[2] Manual of Clinical Microbiology[3] or according to laboratory procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. *Neisseria* spp. may be inhibited by SPS in Columbia Broth. The addition of 1.2% gelatin may counteract the inhibitory effect, but SPS may also inhibit other organisms.[2]
3. Opalescence in Columbia Broth cannot always be relied upon as evidence of bacterial growth in the bottle.
4. It is possible for significant numbers of viable bacteria to be present in an inoculated and incubated blood culture bottle without the usual signs of bacterial growth.

References

1. **Morello, J. A., and P. D. Ellner**. 1969. New medium for blood cultures. Appl. Microbiol. **17**:68-70.
2. **Isenberg, H. D. (ed).** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.

3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Columbia Broth	500 g	0944-17
	2 kg	0944-07

Bacto® Columbia CNA Agar

Intended Use

Bacto Columbia CNA Agar is used with added blood in isolating gram-positive cocci.

Also Known As

Columbia CNA Agar is also referred to as Colistin Nalidixic Acid Agar.

Summary and Explanation

Ellner et al.[1] described Columbia CNA Agar, a variation of Columbia Blood Agar Base that is selective for gram-positive cocci. The antimicrobics colistin and nalidixic acid select for gram-positive organisms and fungi by suppressing gram-negative bacteria.[2] Columbia CNA Agar is recommended as a primary plating medium when culturing urine specimens.[3]

Principles of the Procedure

Columbia CNA Agar is Columbia Blood Agar Base supplemented with colistin (10 µg/ml) and nalidixic acid (15 µg/ml). The antimicrobial agents suppress growth of *Enterobacteriaceae* and *Pseudomonas* species while allowing yeasts, staphylococci, streptococci and enterococci to grow.[4] Certain gram-negative organisms, such as *Gardnerella vaginalis* and some *Bacteriodes* species, can grow very well on Columbia CNA Agar with blood.[4] Colistin disrupts the cell membrane of gram-negative organisms; it is particularly effective against *Pseudomonas* species.[2] Nalidixic acid blocks DNA replication in susceptible bacteria and acts against many gram-negative bacteria.[2]

Formula

Columbia CNA Agar

Formula Per Liter

Bacto Pantone	10 g
Bacto Bitone	10 g
Tryptic Digest of Beef Heart	3 g
Corn Starch	1 g
Sodium Chloride	5 g
Colistin Sulfate	10 mg
Nalidixic Acid	15 mg
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

User Quality Control

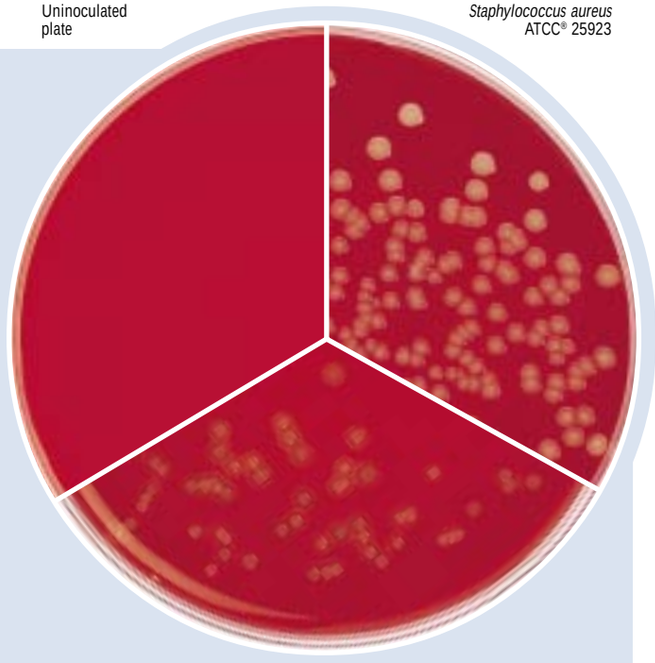
Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.4% solution, soluble in distilled or deionized water upon boiling, light to medium amber, slightly opalescent to opalescent with a fine precipitate.
Prepared Medium:	Without blood: light to medium amber, slightly opalescent to opalescent with a fine precipitate. With 5% sheep blood: cherry red, opaque.
Reaction of 4.4% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Columbia CNA Agar with and without 5% sheep blood per label directions. Inoculate both media and incubate at 35 ± 2°C for 18-24 hours under 10% CO_2.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH w/o BLOOD	GROWTH w/BLOOD	HEMOLYSIS
Proteus mirabilis	12453	1,000-2,000	markedly inhibited	markedly inhibited	–
Staphylococcus aureus	25923*	100-1,000	good	good	beta
Streptococcus pneumoniae	6305	100-1,000	good	good	alpha
Streptococcus pyogenes	19615*	100-1,000	good	good	beta



The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container whenstored as directed. Do not use a product if it fails to meet specificationfor identity and performance.

Procedure

Materials Provided

Columbia CNA Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath
Sterile Petri dishes

Method of Preparation

1. Suspend 44 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121° C for 15 minutes. Avoid overheating.
4. Cool to 45-50°C.
5. Aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
6. Dispense into sterile Petri dishes or tubes as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

1. Inoculate specimens directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions due to the activity of both oxygen-stable and oxygen-labile streptolysins.[5]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.

Results

Examine plates for growth and hemolytic reactions after 18-24 and 48 hours incubation. Four different types of hemolysis on blood agar media can be described:[3]

a. Alpha (α)-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This causes a greenish discolorization of the medium.
b. Beta (β)-hemolysis is the lysis of red blood cells, resulting in a clear zone surrounding the colony.
c. Gamma (γ)-hemolysis indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
d. Alpha-prime (ὰ)-hemolysis is a small zone of complete hemolysis that is surrounded by an area of partial lysis.

Limitations of the Procedure

1. Because the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-hemolytic on horse, human and rabbit blood agar and alpha-hemolytic on sheep blood agar.[5]
3. Colonies of *Haemophilus haemolyticus* are beta-hemolytic on horse and rabbit blood agar and must be distinguished from colonies on beta-hemolytic streptococci using other criteria. The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of *H. haemolyticus*.[4]
4. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[5] For optimal performance, incubate blood agar base media under increased CO_2 or anaerobic conditions.
5. *Proteus* species occasionally grow on CNA Agar and may initially be confused with streptococci because of the small size of the colonies.[2]

References

1. **Ellner, P. D., C. J. Stoessel, E. Drakeford, and F. Vasi.** 1966. A new culture medium for medical bacteriology. Am. J. Clin. Pathol. **45**:502-504.
2. **Estevez, E. G.** 1984. Bacteriologic plate media: review of mechanisms of action. Lab Med. **15**:258-262.
3. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.
4. **Baron, E. J., L. R. Peterson, and S.M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
5. **Ruoff, K. L.** 1995. Streptococcus, p. 299-305. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Columbia CNA Agar	500 g	0867-17
	2 kg	0867-07

Bacto® Cooke Rose Bengal Agar Bacto Antimicrobic Vial A

Intended Use

Bacto Cooke Rose Bengal Agar is used with or without Bacto Antimicrobic Vial A in isolating fungi from environmental and food specimens.

Bacto Antimicrobic Vial A is used in preparing microbiological culture media.

Summary and Explanation

Cooke Rose Bengal Agar is a selective medium for the isolation of fungi prepared according to the formula of Cooke.[1] Selectivity of the medium may be increased by the addition of antibiotics.

A variety of materials and methods have been used to inhibit bacteria in an attempt to isolate fungi from mixed flora. Fungi are extremely successful organisms, as evidenced by their ubiquity in nature.[2] Waksman[3] described an acid medium consisting of peptone, dextrose, inorganic salts and agar for the isolation of fungi from soil. Cooke[1] used the Waksman[3] medium without adjustment to investigate the isolation of fungi from sewage. It was discovered that Soytone was particularly suitable for use in this medium and that the combination of chlortetracycline, or oxytetracycline, with rose bengal increased the selectivity of the medium.

Antimicrobic Vial A contains sterile, desiccated chlortetracycline (Aureomycin®). It was originally used in preparing DTM Agar described by Taplin, Azias, Rebell and Blank[4] for the isolation of dermatophytes. Antimicrobic Vial A is applicable for use in various media requiring this antibiotic. Cooke[1] preferred chlortetracycline in Cooke Rose Bengal Agar due to the increased stability of the antibiotic.

Principles of the Procedure

Soytone provides nitrogen, carbon and vitamins in Cooke Rose Bengal Agar. Dextrose is an energy source. Rose Bengal and chlortetracycline selectively inhibit bacterial growth and restrict the size and height of colonies of more rapidly growing molds. Monopotassium Phosphate provides buffering capability. Magnesium Sulfate is a source of divalent cations. Bacto Agar is a solidifying agent.

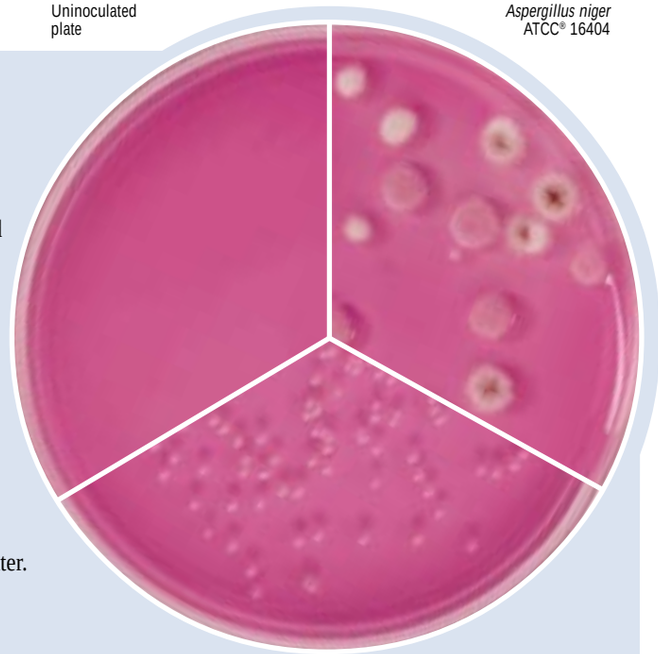
Formula

Cooke Rose Bengal Agar

Formula Per Liter

Bacto Soytone	5 g
Bacto Dextrose	10 g
Monopotassium Phosphate	1 g
Magnesium Sulfate	0.5 g
Bacto Agar	20 g
Rose Bengal	0.035 g

Final pH 6.0 ± 0.2 at 25°C



User Quality Control

Identity Specifications

Cooke Rose Bengal Agar

Dehydrated Appearance:	Pinkish tan, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water on boiling. Solution is pinkish red, very slightly to slightly opalescent without a significant precipitate.
Prepared Medium:	Deep pink, slightly opalescent without a precipitate.
Reaction of 3.6% Solution at 25°C:	pH 6.0 ± 0.2

Antimicrobic Vial A

Lyophilized Appearance:	Yellow cake or powder.
Rehydrated Appearance:	Yellow, clear solution.
Solution:	Soluble in 10 ml distilled or deionized water.
Microbial Limits Test:	Negative.
Potency (Cup-Plate Assay):	90-140% of labeled potency.

Cultural Response

Prepare Cooke Rose Bengal Agar with 35 µg per ml chlortetracycline (Antimicrobic Vial A) per label directions. Inoculate and incubate at 25-30°C for up to 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	30-300	good
Candida albicans	26790	30-300	good
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition
Saccharomyces cerevisae	9763	30-300	good

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Antimicrobic Vial A

Antimicrobic Vial A contains 25 mg sterile desiccated chlortetracycline (Aureomycin®) per 10 ml vial.

Precautions

1. For Laboratory Use.
2. **Antimicrobic Vial A**

   HARMFUL. MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) POSSIBLE RISK OF HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. Target Organs: Teeth, Bones.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Antimicrobic Vial A at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Cooke Rose Bengal Agar
Antimicrobic Vial A

Materials Required but not Provided

Glassware
Autoclave
Sterile Petri dishes
Incubator
Waterbath (optional)

Method of Preparation

Antimicrobic Vial A

1. Aseptically add 10 ml sterile distilled or deionized water to Antimicrobic Vial A.
2. Agitate gently to dissolve completely.
3. The resulting concentration of the rehydrated solution is 2.5 mg chlortetrycycline per ml.

Cooke Rose Bengal Agar

1. Suspend 36 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45°C.
5. OPTIONAL: To increase selectivity, aseptically add 14 ml of rehydrated Antimicrobic Vial A to achieve a final concentration of 35 µg of chlortetracycline per ml of medium or an appropriate amount of another antibiotic.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

Refer to appropriate references for specific procedures on the isolation and cultivation of fungi.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Cooke.** 1922. J. Bact. **7:**339.
2. **Dixon, D. M., and R. A. Fromtling.** 1995. Morphology, taxonomy, and classification of the fungi, p. 699-708. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Waksman.** 1954. Antibiotics and Chemotherapy **4:**657.
4. **Taplin, Azias, Rebell, and Blank.** 1969. Arch. Dermatol. **99**:203.

Packaging

Cooke Rose Bengal Agar	500 g	0703-17
Antimicrobic Vial A	6 x 10 ml	3333-60

Bacto® Cooked Meat Medium

Intended Use

Bacto Cooked Meat Medium is used for cultivating anaerobic microorganisms and for maintaining stock cultures.

Also Known As

Cooked Meat Medium (CMM) is also called Chopped Meat Medium.

Summary and Explanation

In 1890, Theobald Smith[1] made use of fresh unheated animal tissue for cultivating anaerobic organisms. Tarozzi[2] confirmed Smith's[1] findings and discovered the meat-broth could be heated to 104-105°C for 15 minutes without destroying medium nutrients. A steam sterilized emulsion of brain tissue in water was employed by von Hibler[3,4] for cultivating anaerobic microorganisms. Von Hibler[3,4] found organisms in cooked brain broth were less susceptible to harmful effects of toxic metabolic products than in

carbohydrate serum media. Robertson[5] substituted beef heart for brain tissue and found successful results. Cooked Meat Medium is prepared according to the formulation of Robertson.[5]

The capacity of Cooked Meat Medium to detoxify metabolic products of microorganisms makes it an excellent maintenance and growth medium. A study of various formulations used to grow and maintain clinical isolates of anaerobic bacteria found Chopped Meat Broth superior.[6]

Cooked Meat Medium's ability to initiate growth in a small inoculum makes it valuable for the primary culture of clinical specimens. Cooked Meat Medium can be supplemented with vitamin K_1 (1% alcohol solution) and hemin (1% solution) for clinical isolates.[7] This modification is used as a general enrichment for anaerobes, and as a backup for anaerobic jar or chamber failure.[7]

Chopped Meat Carbohydrate Medium and Chopped Meat Glucose Medium is used for cultivation and maintenance of anaerobic bacteria.[7,8,9] Cooked Meat Medium is recommended in the Bacteriological Analytical Manual[10] for use in the examination of *Clostridium botulinum* from food and in the Compendium of Methods for the Microbiological Examination of Foods.[11]

Principles of the Procedure

Beef Heart and Proteose Peptone provide the nitrogen, vitamins and amino acids in Cooked Meat Medium. Sodium Chloride maintains the osmotic balance of the medium. The low concentration of Dextrose is sufficient as the energy source, but not high enough to accumulate toxic metabolites. This formulation provides an effective maintenance medium.

Solid meat particles provide favorable growth conditions for anaerobes due to the reducing action of -SH (sulfhydryl) groups of muscle protein.[2,3,4] Sulfhydryl groups are more accessible in denatured proteins, therefore the use of cooked meat particles is preferred.[9]

Formula

Cooked Meat Medium

Formula Per Liter

Beef Heart	454 g
Bacto Proteose Peptone	20 g
Bacto Dextrose	2 g
Sodium Chloride	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Cooked Meat Medium

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Distilled or deionized water
Sterile tubes with closures

User Quality Control

Identity Specifications

Dehydrated Appearance:	Brown pellets.
Prepared Medium:	Medium amber, clear supernatant over insoluble pellets.
Reaction of 12.5% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Cooked Meat Medium per label directions. Inoculate and incubate medium at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacteroides vulgatus	8482	100-1,000	good
Clostridium novyi	7659	100-1,000	good
Clostridium perfringens	12924	100-1,000	good
Clostridium sporogenes	11437	100-1,000	good
Staphylococcus aureus	25923*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube

Clostridium sporogenes ATCC® 11437

Method of Preparation

1. Suspend 12.5 grams in 100 ml distilled or deionized water (1.25 g per 10 ml).
2. Let stand until all particles are thoroughly wetted and form an even suspension.
3. Autoclave at 121°C for 15 minutes. Reduce pressure slowly.
4. Cool without agitation.
5. If not used within 24 hours, reheat (100°C) prior to use to drive off dissolved oxygen.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

1. Inoculate specimen well into the meat particles (bottom of the tube). Tissue specimens should be ground prior to inoculation.
2. Growth is indicated by turbidity and/or the presence of gas bubbles.
3. For a complete discussion on the isolation and identification of aerobic and anaerobic bacteria, refer to appropriate procedures outlined in the references.

Results

Refer to appropriate references and procedures for results.

Limitations

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Smith, T.** 1890. Centr. Bakteriol. **7**:509.
2. **Tarozzi, G.** 1905. Uber ein leicht in aerober Weise ausfuhrbares Kulturmittel von einigen bis jetzt fuu strenge Anaeroben gehlatenen Keimen. Zentralb. Bakteriol. **38**:619.
3. **von Hibler, E.** 1899. Beitrage zur Kenntnis der durch anaerobe Spaltpilze erzeugen Infektions-krankheiten der Tiere und des Menschen etc. Centr. Bakteriol. **25**: 513,594,631.
4. **von Hibler, E.** 1908. Untersuchungen uber die pathogenen Anaerobier, Jena: Verlag Fischer.
5. **Robertson, M.** 1916. Notes upon certain anaerobes isolated from wounds. J. Pathol. Bacteriol. **20**:327.
6. **Claros, M. C., D. M. Citron, and E. J. C. Goldstein.** 1995. Survival of anaerobic bacteria in various thioglycollate and chopped meat broth formulations. J. Clin. Microbiol. **33**:2505-2507.
7. **Isenberg, H. E. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
8. **Atlas, R. M.** 1993. Handbook of microbiological media, p. 224-226. CRC Press, Boca Raton, FL.
9. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol. 1, p. 240-246. Williams & Wilkins, Baltimore, MD.
10. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
11. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Cooked Meat Medium	100 g	0267-15
	500 g	0267-17
	10 kg	0267-15r

Bacto® Corn Meal Agar

Intended Use

Bacto Corn Meal Agar is used for stimulating the production of chlamydospores by most strains of *Candida albicans* and for cultivating phytopathological fungi.

Summary and Explanation

Numerous culture media formulations have been described for the detection, isolation, and identification of *Candida albicans*, the etiological agent in candidiasis. The various media were designed to bring out morphological or physiological characteristics in this organism which would differentiate it from other members of the genus as well as from other genera.

One of the most important differential characteristics of *C. albicans* in its ability to form chlamydospores on certain media. This property is perhaps the best criterion for identification. Corn Meal Agar is valuable for morphologic differentiation of many yeast-like organisms. It suppresses vegetative growth of many fungi while stimulating sporulation.[1]

Corn Meal Agar has been used with varying degrees of success for showing chlamydospore formation in *C. albicans*. Chlamydospore production is the best diagnostic criterion for identification of the pathogenic yeast *C. albicans*.[2] Kelly and Funigeillo[3] reported that the addition of 1% Tween 80 enhanced chlamydospore formation by *C. albicans*. With this improvement, Corn Meal Agar may be the most accurate routine tool available for identification of *C. albicans*.[4]

Principles of the Procedure

Infusion from corn meal is a source of carbon, protein and nutrients. Bacto Agar is a solidifying agent.

Formula

Corn Meal Agar

Formula Per Liter

Corn Meal, Infusion from	50 g
Bacto Agar	15 g

Final pH 6.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.

2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Corn Meal Agar

Materials Required but not Provided

Glassware
Autoclave
Sterile Inoculating Needle
Cover Glass

Method of Preparation

1. Suspend 17 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Specimens should be collected in sterile containers or with sterile swabs and transported immediately to the laboratory according to recommended guidelines.[5]

Test Procedure[6]

1. Using a sterile inoculating needle, lightly touch the yeast colony, then make two streaks approximately 1.5 cm long each and 1.0 cm apart.
2. Flame the needle, and allow it to cool. Lightly make an S-shaped streak back and forth across the two streak lines.
3. Flame sterilize a cover glass. Allow it to cool, then place it over the streak marks.
4. Incubate at 22-26°C for 72 hours.

Results

1. Examine plates for the presence of chlamydospores.

User Quality Control

Identity Specifications

Dehydrated Medium	
Appearance:	Yellow, free-flowing, homogeneous.
Solution:	1.7% solution, soluble in distilled or deionized water on boiling. Solution is light amber, slightly opalescent to opalescent, may have a slight, fine precipitate.
Reaction of 1.7% Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

Prepare Corn Meal Agar per label directions. Inoculate using the spread plate method. Prepare a heavy suspension of *C. albicans*, dip a sterile inoculating loop into the suspension, and cut a 2 cm "X" through the medium. Place a cover slip over the "X". Incubate at 20-25°C for 40-48 hours and up to four days, if required. Examine plates for chlamydospores which, when produced by some *Candida* species, appear as double walled spheres on cover slip plates.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	CHLAMYDOSPORES
Aspergillus niger	16404	100-1,000	good	–
Candida albicans	10231*	100-1,000	good	+
Saccharomyces cerevisiae	9763	100-1,000	good	–

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Limitations of the Procedure

1. Corn Meal Agar with the addition of 1% Tween 80 should not be the only medium used for identification of *C. albicans* since *C. stellatoidea* and *C. tropicalis* also produce chlamydospores on this medium.[7]
2. Repeated subculture of some *Candida* strains will result in the reduced ability to form chlamydospores.

References

1. **Baron, E. J., and S. M. Finegold.** 1990. Formulas and preparation of culture media and reagents, p. A-10. Bailey & Scott's Diagnostic Microbiology, 8th ed. The C. V. Mosby Company, St. Louis, MO.
2. **Duncan, J., and J. Floeder.** 1963. A comparison of media for the production of chlamydospores by Candida albicans. Am. J. Med. Tech. **29:**199-206.
3. **Kelly, J. P., and F. Funigiello.** 1959. Candida albicans: A study of media designed to promote chlamydospore production. J. Lab. & Clin. Med. **53**:807- 809.
4. **Gordon, M. A., and G. N. Little**.1963. Effective dehydrated media with surfactants for identification of Candida albicans. J. of Int. Soc. for Human and Animal Mycol. **2**:171-175.
5. **Miller, J. M., and H. T. Holmes.** 1995. Specimen collection and handling, p. 19- 32. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken, (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
7. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 247-250. Williams & Wilkins, Baltimore, MD.

Packaging

Corn Meal Agar	500 g	0386-17

Bacto® Cystine Heart Agar

Intended Use

Bacto Cystine Heart Agar is used with Bacto Hemoglobin for cultivating *Francisella tularensis* and without enrichment for cultivating gram-negative cocci and other microorganisms.

Also Known As

Cystine Heart Agar with added hemoglobin is also referred to as Cystine Glucose Blood Agar.

Summary and Explanation

Francisella tularensis was first described in humans in 1907.[1] Several media formulations were employed to isolate this microorganism. Initial formulations contained egg or serum and were difficult to prepare. Edward Francis,[2] who dedicated his career to the study of this organism, reported that blood dextrose cystine agar was a satisfactory medium for cultivating this fastidious pathogen. Shaw[3] added 0.05% cystine and 1% dextrose to Heart Infusion Agar for the cultivation of *F. tularensis*.

While experimenting with Francis' blood dextrose cystine agar, Rhamy[4] added hemoglobin to Cystine Heart Agar to develop a satisfactory medium for growth of *F. tularensis*.

Cystine Heart Agar is the medium of choice for isolating *F. tularensis*.[1,5]

Principles of the Procedure

Infusions from Beef Heart, Proteose Peptone and L-Cystine provide nitrogen, vitamins and amino acids in Cystine Heart Agar. Dextrose is a carbon source. Sodium chloride maintains the osmotic balance and Bacto Agar is a solidifying agent.

Enrichment with 2% hemoglobin provides additional growth factors. Without enrichment, Cystine Heart Agar supports excellent growth of gram-negative cocci and other pathogenic microorganisms.[6] Rabbit blood and antimicrobial agents can be added to this medium.[5]

Formula

Cystine Heart Agar

Formula Per Liter

Beef Heart, Infusion from	500 g
Bacto Proteose Peptone	10 g
Bacto Dextrose	10 g
Sodium Chloride	5 g
L-Cystine	1 g
Bacto Agar	15 g

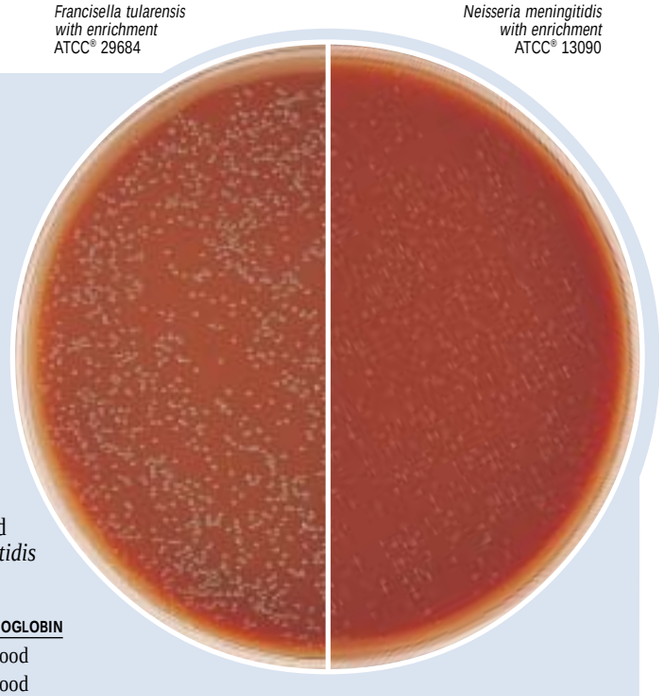
Final pH 6.8 ± 0.2 at 25%C

Precautions

1. For Laboratory Use.
2. *Francisella tularensis* is a Biosafety Level 2 pathogen that can be transmitted by aerosols or by penetration of unbroken skin.[5] Wearing of gowns, gloves and masks is advocated for laboratory staff handling suspected infectious material.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.1% solution, soluble in distilled or deionized water upon boiling; light to medium amber, very slightly to slightly opalescent, may have fine precipitate.
Prepared Medium:	Plain - Light to medium amber, slightly opalescent, may have fine precipitate.
	With Hemoglobin - Chocolate, opaque.
Reaction of 5.1% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Cystine Heart Agar per label directions. Incubate inoculated medium at 35 ± 2°C aerobically for 18-48 hours. *Neisseria meningitidis* should be incubated under 5- 10% CO_2.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH w/o HEMOGLOBIN	GROWTH w/HEMOGLOBIN
Francisella tularensis	29684	100-1,000	fair	good
Neisseria meningitidis	13090*	100-1,000	good	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Cystine Heart Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Hemoglobin Solution 2% or Hemoglobin (optional)
Sterile Petri dishes or tubes

Method of Preparation

Enriched Medium:

1. Suspend 10.2 grams in 100 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50-60°C.
4. Add 100 ml sterile 2% hemoglobin solution and mix well. Use:
   - Hemoglobin Solution 2%; or,
   - Prepare a 2% hemoglobin solution as follows: Place 2 grams of Hemoglobin in a dry flask. Add 100 ml of cold distilled or deionized water while agitating vigorously. Continue intermittent agitation for 10-15 minutes until solution is complete. Autoclave at 121°C for 15 minutes. Cool to 50-60°C
5. Dispense into sterile Petri dishes or tubes.

Unenriched Medium:

1. Suspend 51 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Collect specimens in sterile containers or with sterile swabs. Transport immediately to the laboratory in accordance with recommended guidelines outlined in the references.

Test Procedure

1. Inoculate and streak specimens as soon as possible. For a complete discussion on the inoculation and identification of *Francisella*, consult appropriate references.
2. Overgrowth by contaminating organisms can be reduced by incorporating 100- 500 units penicillin per ml into the medium.[6]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Stewart, S. J**. 1995. *In* P. R. Murray., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Francis, E.** 1928. Symptoms, diagnosis and pathology of tularemia. J. Am. Med. Assoc. **91**:1155-1160.
3. **Shaw, F. W.** 1930. Culture medium for *Bacterium tularense*. Zentr. Bakt. I. Abt. Orig. **118**:216-217.
4. **Rhamy, B. W.** 1933. A new and simplified medium for *Pasteurella tularensis* and other delicate organisms. Am. J. Clin. Pathol. **3**:121-124.
5. **Isenberg, H. D.** (ed.). 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.
6. **Stewart, S. J.** 1995. *Francisella*, p. 545-548. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Cystine Heart Agar	500 g	0047-17
Hemoglobin	100 g	0136-15
	500 g	0136-17
	2 kg	0136-07
	10 kg	0136-08
Hemoglobin Solution 2%	6 x 100 ml	3248-73

Bacto® Cystine Tryptic Agar

Intended Use

Bacto Cystine Tryptic Agar is used with added carbohydrates in differentiating microorganisms based on fermentation reactions and motility.

Also Known As

Cystine Tryptic Agar is abbreviated as CTA, and referred to as CT Medium.

Summary and Explanation

Cystine Tryptic Agar is a semi-solid basal medium prepared according to the formula of Vera.[1] Many tests used to differentiate among members of the *Enterobacteriaceae* determine the organism's ability to utilize a carbohydrate with the production of acid metabolic end products.[2] CTA is free of fermentable carbohydrates, and the carbohydrate content can be adjusted for specific reactions. The carbohydrate concentration used most frequently in fermentation reactions is 0.5 or 1%.

Some researchers prefer 1% to insure against reversion of the reaction due to depletion of the carbohydrate by the microorganism.

The low agar content of Cystine Tryptic Agar provides a suitable environment for motility studies. Motility determination aids in the identification of bacteria. CTA can also be used as a maintenance medium for stock cultures.[3,4] This formula will support the growth of fastidious organisms, e.g., *Streptococcus pneumonia* and *Corynebacterium* species.[4]

Principles of the Procedure

Tryptose provides the nitrogen, vitamins and amino acids in Cystine Tryptic Agar. L-Cystine and Sodium Sulfite are added to this formula to stimulate growth. Sodium Chloride maintains the osmotic balance of the medium. Phenol Red is the pH indicator. Bacto Agar maintains an Eh potential which facilitates anaerobic growth, and aids in dispersion of reducing substances and CO_2 formed in the environment.[5] The agar is also used for the determination of motility.

Formula

Cystine Tryptic Agar

Formula Per Liter

Bacto Tryptose	20 g
L-Cystine	0.5 g
Sodium Chloride	5 g
Sodium Sulfite	0.5 g
Bacto Agar	2.5 g
Bacto Phenol Red	0.017 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Cystine Tryptic Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (50-55°C) (optional)
Sterile 5-10% carbohydrate solution
Sterile tubes

Method of Preparation

1. Suspend 28.5 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. To prepare fermentation medium, use one of the following methods:
   A. Add 5-10 grams carbohydrate before sterilization.
   or B. Dissolve 28.5 grams medium in 900 ml water, sterilize and aseptically add 100 ml sterile of carbohydrate solution.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	2.85% solution, soluble in distilled or deionized water upon boiling. Red, very slightly opalescent without significant precipitate.
Prepared Medium:	Red, very slightly opalescent without precipitate.
Reaction of 2.85% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Cystine Tryptic Agar per label directions with and without 0.5% dextrose. Inoculate tubes by straight stab and incubate at 35°C for 18-48 hours.

ORGANISM	ATCC®	MOTILITY	ACID PRODUCTION w/ DEXTROSE
Corynebacterium diphtheriae subsp. *mitis*	8024	–	+
Escherichia coli	25922*	+	+
Neisseria gonorrhoeae (CDC 98)	43070*	–	+

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube | *Corynebacterium diptheriae* ATCC® 8024 with dextrose | *Escherichia coli* ATCC® 25922 with dextrose

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion on motility and carbohydrate fermentation studies refer to procedures described in appropriate references.[2,6,7]

Results

1. Fermentation of the test carbohydrate is observed when acid is formed and the medium turns from red to yellow.
2. Motility of an organism is evident as a haze of growth extending into the agar from the stab line.[2]

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. CTA requires a heavy inoculum.[5]
3. Prolonged incubation may lead to changes in pH indicator or abnormal lactose/sucrose reactions with Neisseria pathogens.[8,9]
4. *Neisseria* species usually produce acid only in the area of stabs (upper third). If there is a strong acid (yellow color) throughout the medium, a contaminating organism may be present. If in doubt about a tube containing a *Neisseria* species, a Gram stain and oxidase test should be performed on the growth.[5]

References

1. **Vera, H. D.** 1948. A simple medium for identification and maintenance of the gonococcus and other bacteria. J. Bacteriol. **55**:531.
2. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's Diagnostic Microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
3. **Myers, R. M., and G. Koshy.** 1961. Beta-hemolytic streptococci in survey throat cultures in an Indian population. Am. J. Public Health **51**:1872.
4. **Alford, J. A., G. E. Wiese, and J. J. Gunter.** 1955. Heat resistance in *Corynebacterium* and the relationship of the genus to *Microbacterium*. J. Bacteriol. **69**:516.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 254-259, 802-804. Williams & Wilkins, Baltimore, MD.
6. **Isenberg, H. D. (ed.).** 1995. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.
7. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
8. **Faur, Y. C., M. H. Weisburd, and M. E. Wilson.** 1975. Carbohydrate fermentation plate medium for confirmation of *Neisseria* species. J. Clin. Microbiol. **1**:294.
9. **Applebaum, P. C., and R. B. Lawrence.** 1979. Comparison of three methods for identification of pathogenic *Neisseria* species. J. Clin. Microbiol. **9**:598.

Packaging

Cystine Tryptic Agar	500 g	0523-17

Bacto® Czapek-Dox Broth Bacto Czapek Solution Agar

Intended Use

Bacto Czapek-Dox Broth and Czapek Solution Agar are used for cultivating fungi and bacteria capable of using inorganic nitrogen.

Summary and Explanation

Czapek-Dox Broth and Czapek Solution Agar are a modification of the Czapek[1] and Dox[2] formula prepared according to Thom and Raper.[3] The media are prepared with only inorganic sources of nitrogen and chemically defined compounds sources of carbon. Czapek-Dox media are useful in a variety of microbiological procedures, including soil microbiology and fungi and mildew resistance tests. Thom and Raper[3] reported Czapek-Dox Broth and Czapek Solution Agar will produce moderately vigorous growth of most saprophytic aspergilli and yield characteristic mycelia and conidia.

Czapek Solution Agar is recommended in Standard Methods for the Examination of Water and Wastewater[5] for the isolation of *Aspergillus*, *Penicillium* and related fungi.

Principles of the Procedure

Saccharose is the sole carbon source, and Sodium Nitrate is the sole nitrogen source in Czapek-Dox Broth and Czapek Solution Agar. Dipotassium Phosphate is the buffering agent, and Potassium Chloride contains essential ions. Magnesium Sulfate and Ferrous Sulfate sources of cations. Bacto Agar is the solidifying agent in Czapek Solution Agar.

Formula

Czapek-Dox Broth

Formula Per Liter	
Bacto Saccharose	30 g
Sodium Nitrate	3 g
Dipotassium Phosphate	1 g
Magnesium Sulfate	0.5 g
Potassium Chloride	0.5 g
Ferrous Sulfate	0.01 g

Final pH 7.3 ± 0.2 at 25°C

Czapek Solution Agar

Formula Per Liter	
Bacto Saccharose	30 g
Sodium Nitrate	2 g
Dipotassium Phosphate	1 g
Magnesium Sulfate	0.5 g
Potassium Chloride	0.5 g
Ferrous Sulfate	0.01 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

Czapek-Dox Broth

1. For Laboratory Use.

Czapek Solution Agar

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

User Quality Control

Identity Specifications

Czapek-Dox Broth

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water. Solution is colorless, clear to very slightly opalescent and may have a slight precipitate.
Prepared Medium:	Colorless, clear to very slightly opalescent, may have slight precipitate.
Reaction of 3.5% Solution at 25°C:	pH 7.3 ± 0.2

Czapek Solution Agar

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	4.9% solution, soluble in distilled or deionized water on boiling; light amber, opalescent with a uniform flocculent precipitate.
Prepared Medium:	Light amber, slightly opalescent; may have slight precipitate.
Reaction of 4.9% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Czapek-Dox Broth

Prepare Czapek-Dox Broth per label directions. Inoculate tubes with the test organisms. Incubate inoculated medium at 30 ± 2°C for 48-72 hours.

Czapek Solution Agar

Prepare Czapek Solution Agar per label directions. Inoculate prepared medium with the test organisms. Incubate at 30 ± 2°C for 18-48 hours, or up to 72 hours if necessary.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	9642	100-1,000	good
Candida albicans	10231	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Procedure

Materials Provided

Czapek-Dox Broth
Czapek Solution Agar

Materials Required but not Provided

Glassware
Autoclave
Incubator
Waterbath (optional)

Method of Preparation

Czapek-Dox Broth

1. Dissolve 35 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Dispense as desired.

Czapek Solution Agar

1. Suspend 49 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Dispense as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

Refer to appropriate references for specific procedures for the cultivation of fungi and bacteria capable of utilizing inorganic nitrogen.

Results

Refer to appropriate references and procedures for results.

References

1. **Czapek, F.** 1902-1903. Untersuchungen uber die stickstoffgewinnung und EiweiBbildung der Pflanze. Beitr. Chem. Physiol. Pathol. **1**:540.
2. **Dox, A. W.** 1910. The intracellular enzymes of *Penicillium* and *Aspergillus* with special references to those of *P. camenberti*. U.S. Dept. Agr. Bur. Anim. Ind. Bull. **120**:70.
3. **Thom, C., and K. B. Raper.** 1945. Manual of the aspergilli, vol. 39.
4. **Thom, C., and M. B. Church.** 1926. The aspergilli. Williams and Wilkins Co., Baltimore, MD.
5. **Eaton, A. D., L. S. Cleseri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Packaging

Czapek-Dox Broth	500 g	0338-17
Czapek Solution Agar	500 g	0339-17

Bacto® DCLS Agar

Intended Use

Bacto DCLS Agar is used for isolating gram-negative enteric bacilli.

Also Known As

DCLS is an abbreviation for Desoxycholate Citrate Lactose Saccharose Agar.

Summary and Explanation

DCLS Agar is a modification of SS Agar and the Desoxycholate Citrate Agar described by Leifson[1]. Coliform organisms capable of fermenting lactose or sucrose are generally inhibited. Gram positive bacteria are suppressed.

While studying enteric pathogens on Endo medium, Holt-Harris and Teague[2] used lactose and sucrose in the development of a nutrient agar containing methylene blue and eosin. Some coliforms ferment sucrose more readily than lactose. The addition of sucrose (saccharose) allows nonpathogenic sucrose-fermenting organisms to produce red colonies. The red colonies are easily recognized, reducing the number of false positive reactions.

Principles of the Procedure

Beef Extract and Proteose Peptone No. 3 provide nitrogen, vitamins and amino acids. Lactose and Saccharose (sucrose) provide fermentable carbohydrates. Sodium Citrate, Sodium Thiosulfate and Sodium Desoxycholate are selective agents. Bacto Agar is the solidifying agent. Neutral Red is the indicator.

Formula

DCLS Agar

Formula Per Liter

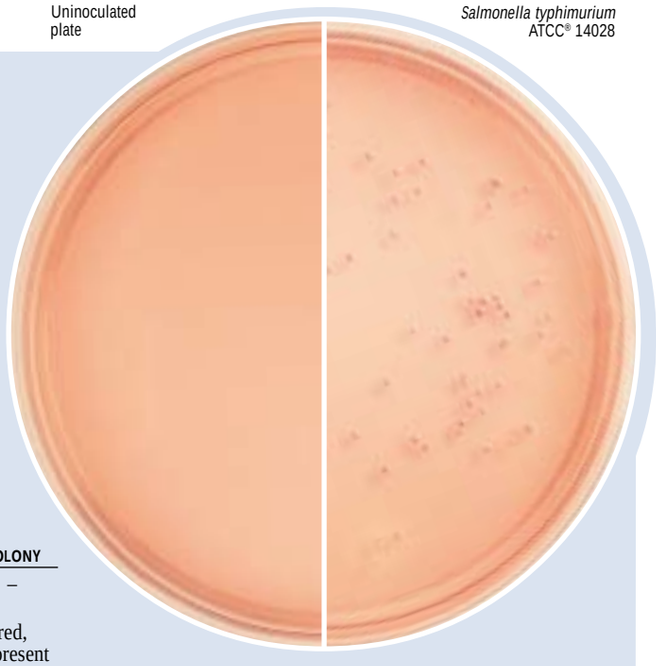
Bacto Beef Extract	3 g
Bacto Proteose Peptone No.3	7 g
Bacto Lactose	5 g
Bacto Saccharose (sucrose)	5 g
Sodium Citrate	10 g
Sodium Thiosulfate	5 g
Sodium Desoxycholate	2.5 g
Bacto Agar	12 g
Neutral Red	0.03 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige to light pink, free-flowing, homogeneous.
Solution:	4.95% solution, soluble in distilled or deionized water upon boiling. After boiling, orange-red, clear to very slightly opalescent, without significant precipitation.
Prepared Medium:	Orange-red, slightly opalescent.
Reaction of 4.95% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare DCLS Agar per label directions. Inoculate prepared medium and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	COLOR OF GROWTH	COLONY
Enterococcus faecalis	29212*	1,000-2,000	marked to complete inhibition	–
Escherichia coli	25922*	100-1,000	marked to complete inhibition	red, if present
Salmonella typhimurium	14028*	100-1,000	good	colorless to pink
Shigella flexneri	12022*	100-1,000	fair to good	colorless to pink

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

DCLS Agar

Materials Required But Not Provided

Glassware
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes

Method of Preparation

1. Suspend 49.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. DO NOT AUTOCLAVE.
3. Cool to 50-55°C.
4. Dispense into sterile Petri dishes, or as desired.
5. Allow prepared medium to dry for about 2 hours with the covers partially removed.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion on the isolation and identification of enteric pathogens from clinical specimens, refer to the procedures described in appropriate references.[3,4]

Results

Typical coliforms that rapidly ferment sucrose and/or lactose will form red, opaque colonies. *Shigella* and *Salmonella* species will produce colorless to slightly pink, transparent colonies.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. DO NOT AUTOCLAVE MEDIUM. DO NOT OVERHEAT.
3. DCLS Agar is intended for selective use and should be inoculated in parallel with nonselective media.
4. Colonies suspected of being enteric pathogens must be confirmed biochemically and, if required, serologically.

References

1. **Leifson, E.** 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol. **40**:581-599.
2. **Holt-Harris, J. E., and O. Teague.** 1916. A new culture medium for the isolation of Bacillus typhosus from stools. J. Infect. Dis. **18**:596-601.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.

Packaging

DCLS Agar	500 g	0759-17

Bacto® D/E Neutralizing Agar
Bacto D/E Neutralizing Broth

Intended Use

Bacto D/E Neutralizing Agar is used for neutralizing and determining the bactericidal activity of antiseptics and disinfectants.

Bacto D/E Neutralizing Broth is used for determining the bactericidal activity of antiseptics and disinfectants based on neutralizing the chemical and detecting organisms remaining after treatment.

Also Known as

D/E Neutralizing Agar and Broth are also known as Dey-Engley Neutralizing Agar and Broth.

Summary and Explanation

D/E Neutralizing media, developed by Dey and Engley,[1] neutralize a broad spectrum of disinfectants and preservative antimicrobial chemicals. D/E Neutralizing media neutralize higher concentrations of residual antimicrobials when compared with other standard neutralizing formulations such as Letheen media, Thioglycollate media, and Neutralizing Buffer.[2,3]

Complete neutralization of disinfectants is important because disinfectant carryover can cause a false no-growth test result. D/E Neutralizing media effectively neutralize the inhibitory effects of disinfectant carryover,[4,5] allowing differentiation between bacteriostasis and the true bactericidal action of disinfectant chemicals. This is a critical characteristic to consider when evaluating a disinfectant. D/E Neutralizing media are recommended for use in disinfectant evaluation, environmental sampling (swab and contact plate methods) and the testing of water-miscible cosmetics in accordance with Cosmetic, Toiletry and Fragrance Association (CTFA) guidelines.[6]

Principles of the Procedure

D/E Neutralizing Agar and Broth contain Tryptone which provides the carbon and nitrogen sources required for growth of a wide variety of

organisms. Yeast Extract provides vitamins and cofactors required for growth and additional nitrogen and carbon. Dextrose is a source of fermentable carbohydrate. Sodium Thioglycollate neutralizes mercurials. Sodium Thiosulfate neutralizes iodine and chlorine. Sodium Bisulfite neutralizes formaldehyde and gluteraldehyde. Lecithin neutralizes quaternary ammonium compounds and Polysorbate 80 neutralizes phenols, hexachlorophene, formalin and, with lecithin, ethanol.[11] Brom Cresol Purple is used as a colorimetric indicator to demonstrate the production of acid from the fermentation of dextrose.

D/E Neutralizing Agar uses Bacto Agar as a solidifying agent.

Formula

D/E Neutralizing Agar

Formula Per Liter

Bacto Tryptone	5 g
Bacto Yeast Extract	2.5 g
Bacto Dextrose	10 g
Sodium Thioglycollate	1 g
Sodium Thiosulfate	6 g
Sodium Bisulfite	2.5 g
Polysorbate 80	5 g
Lecithin (Soy Bean)	7 g
Bacto Agar	15 g
Bacto Brom Cresol Purple	0.02 g

Final pH 7.6 ± 0.2 at 25° C

D/E Neutralizing Broth

Formula Per Liter

Bacto Tryptone	5 g
Bacto Yeast Extract	2.5 g
Bacto Dextrose	10 g
Sodium Thioglycollate	1 g
Sodium Thiosulfate	6 g
Sodium Bisulfite	2.5 g
Polysorbate 80	5 g
Lecithin (Soy Bean)	7 g
Bacto Brom Cresol Purple	0.02 g

Final pH 7.6 ± 0.2 at 25° C

Precautions

1. For Laboratory Use.
2. **D/E Neutralizing Agar**
   D/E Neutralizing Broth

   HARMFUL. MAY CAUSE SENSITIZATION BY INHALATION. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh

User Quality Control

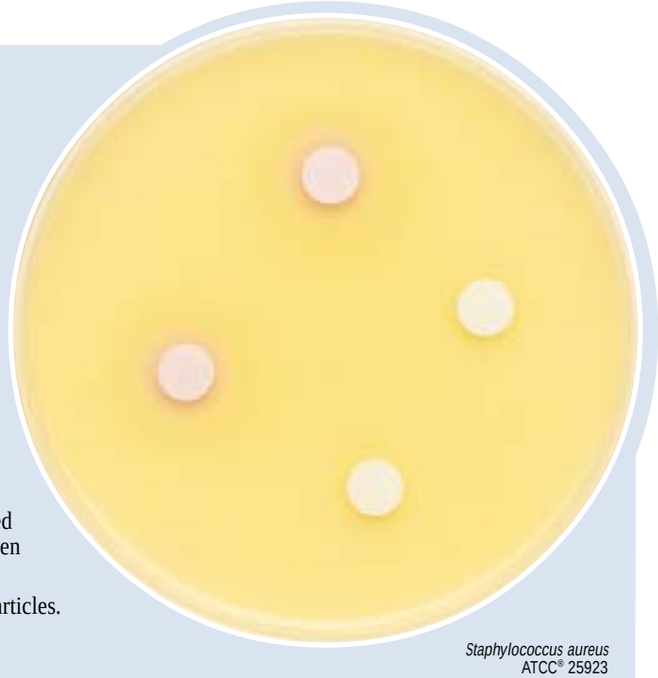
Identity Specifications

D/E Neutralizing Agar

Dehydrated Medium:	Bluish-grey, homogeneous, appears moist and lumpy.
Solution:	5.4% solution, soluble in distilled or deionized water on boiling. Lavender, opaque with an even suspension of fine particles.
Prepared Medium:	Lavender, opaque with a fine precipitate.
Reaction of 5.4% Solution at 25°C:	7.6 ± 0.2

D/E Neutralizing Broth

Dehydrated Medium:	Bluish-grey, homogeneous, appears moist and lumpy.
Solution:	3.9% solution, soluble in distilled or deionized water on warming. Purple, opaque with an even suspension of fine particles.
Prepared Medium:	Purple, opaque with an even suspension of particles.
Reaction 3.9% Solution at 25°C:	7.6 ± 0.2



Staphylococcus aureus
ATCC® 25923

Cultural Response

D/E Neutralizing Agar: Neutralization Test

Prepare medium per label directions. Inoculate 50 ml of D/E Neutralizing Agar with 0.1 ml of a heavy suspension of each test organism and dispense into 150 x 15 mm Petri dishes of D/E Neutralizing Agar and Plate Count Agar. Place 1/2 inch sterile blank disks on each plate. Dispense 0.1 ml of each disinfectant solution onto two disks per medium. Incubate at 35 ± 2°C for 40-48 hours. D/E Neutralizing Agar should exhibit no zones of inhibition or zones significantly smaller than those found on Plate Count Agar.

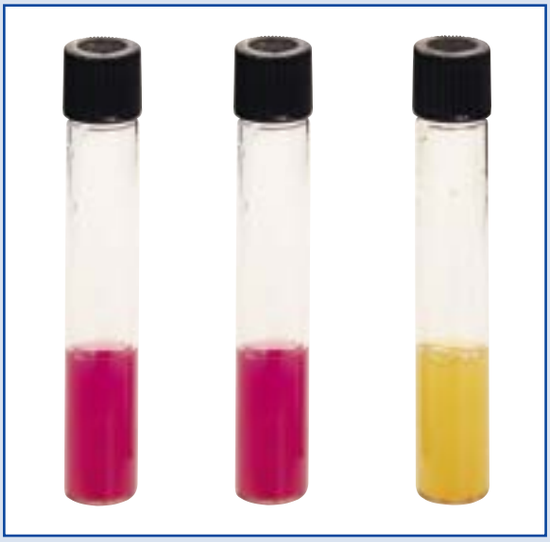
continued on following page

User Quality Control cont.

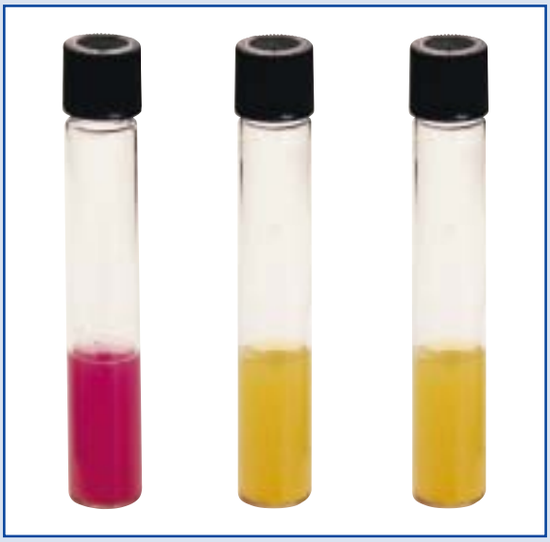
Cultural Response

D/E Neutralizing Broth: Toxicity Test
Prepare medium per label directions with and without added disinfectants. Inoculate with 100-1,000 CFU of test organism. Incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacillus subtilis	6633	100-1,000	good
Escherichia coli	25922	100-1,000	good
Pseudomonas aeruginosa	27853	100-1,000	good
Salmonella typhimurium	14028	100-1,000	good
Staphylococcus aureus	25923	100-1,000	good



Uninoculated tube | *Bacillus subtilis* ATCC® 6633 | *Escherichia coli* ATCC® 25922



Pseudomonas aeruginosa ATCC® 27853 | *Salmonella typhimurium* ATCC® 14028 | *Staphylococcus aureus* ATCC® 25923

air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

D/E Neutralizing Agar
D/E Neutralizing Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave

Method of Preparation

D/E Neutralizing Agar

1. Suspend 54 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve.
3. Autoclave at 121°C for 15 minutes.

D/E Neutralizing Broth

1. Suspend 39 grams in 1 liter of distilled of deionized water.
2. Heat to dissolve.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

D/E Neutralizing Agar and D/E Neutralizing Broth are used in a variety of procedures. Consult appropriate references for further information.[6]

Results

Refer to appropriate references and procedures for results.

References

1. **Engley, F. B., Jr., and B. P. Dey.** 1970. A universal neutralizing medium for antimicrobial chemicals. Presented at the Chemical Specialties Manufacturing Association (CSMA) Proceedings, 56th Mid-Year Meeting.
2. **Dey, B. P., and F. B. Engley, Jr.** 1983. Methodology for recovery of chemically treated *Staphylococcus aureus* with neutralizing medium. Appl. Environ. Microbiol. **45**:1533-1537.

3. **Dey, B. P., and F. B. Engley, Jr.** 1978. Environmental sampling devices for neutralization of disinfectants. Presented at the 4th International Symposium on Contamination Control.
4. **Dey, B. P., and F. B. Engley, Jr.** 1994. Neutralization of antimicrobial chemicals by recovery media. J. Microbiol. Methods **19**:51-58.
5. **Dey, B. P., and F. B. Engley, Jr.** 1995. Comparison of Dey and Engley (D/E) Neutralizing medium to Letheen Medium and Standard Methods Medium for recovery of *Staphylococcus aureus* from sanitized surfaces. J. Ind. Microbiol. **14**:21-25.
6. **Curry, A. S., J. G. Graf, and G. N. McEwen, Jr. (ed.).** 1993. CTFA Microbiology Guidelines. The Cosmetic, Toiletry and Fragrance Association, Washington, D.C.

Packaging

D/E Neutralizing Agar	500 g	0686-17
	10 kg	0686-08
D/E Neutralizing Broth	500 g	0819-17

Bacto® DNase Test Agar
Bacto DNase Test Agar w/Methyl Green

Intended Use

Bacto DNase Test Agar and Bacto DNase Test Agar w/Methyl Green are used for differentiating microorganisms based on deoxyribonuclease activity.

Summary and Explanation

In 1956, Weckman and Catlin[1] showed a correlation between increased DNase activity of *Staphylococcus aureus* and positive coagulase activity. They suggested that DNase activity could be used to identify potentially pathogenic staphylococci. DiSalvo[2] confirmed their results by obtaining excellent correlation between the coagulase and DNase activity of staphylococci isolated from clinical specimens. Jeffries, Holtman and Guse[3] incorporated DNA in an agar medium to study DNase production by bacteria and fungi. Polymerized DNA precipitates in the presence of 1N HCl, making the medium opaque. Organisms that degrade DNA produce a clear zone around an inoculum streak. Fusillo and Weiss[4] studied the calcium requirements of staphylococci for DNase production and concluded that additional calcium was unnecessary when a complete nutritive medium was used.

Kurnick[5] showed that methyl green combines with highly polymerized DNA at pH 7.5. When combination does not take place, the color fades,

User Quality Control

Identity Specifications

DNase Test Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.2% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly to slightly opalescent, may have a slight precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent, may have a slight precipitate.
Reaction of 4.2% Solution at 25°C:	pH 7.3 ± 0.2

DNase Test Agar w/Methyl Green

Dehydrated Appearance:	Light beige with a slight green tint, free-flowing, homogeneous.
Solution:	4.2% solution, soluble in distilled or deionized water on boiling. Solution is green, very slightly to slightly opalescent, may have a slight precipitate.
Prepared Medium:	Green, slightly opalescent, may have a slight precipitate.
Reaction of 4.2% Solution at 25°C:	pH 7.3 ± 0.2

continued on following page

Staphylococcus aureus
ATCC® 25923
DNase Test Agar

creating a clear zone around the growth. Applying this principle, Smith, Hancock and Rhoden[6] modified DNase Test Agar with added methyl green to detect staphylococci, streptococci and *Serratia*. When using DNase Test Agar w/Methyl Green, acid does not have to be added to the plate.

Mannitol fermentation can be determined simultaneously with DNase production by adding 10 grams of mannitol and 0.025 grams of phenol red to the DNase Test Agar prior to sterilization.[7]

Principles of the Procedure

Tryptose is a source of nitrogen, amino acids and carbon. Deoxyribonucleic Acid enables the detection of DNase that depolymerizes DNA. Sodium Chloride provides essential ions while maintaining osmotic balance. Methyl Green is a colorimetric indicator. Bacto Agar is a solidifying agent.

Formula

DNase Test Agar

Formula Per Liter

Bacto Tryptose	20 g
Deoxyribonucleic Acid	2 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

DNase Test Agar w/Methyl Green

Formula Per Liter

Bacto Tryptose	20 g
Deoxyribonucleic Acid	2 g
Sodium Chloride	5 g
Bacto Agar	15 g
Methyl Green	0.05 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

DNase Test Agar
DNase Test Agar w/Methyl Green

Materials Required but not Provided

Glassware
Autoclave
1N Hydrochloric acid (DNase Test Agar)

Method of Preparation

1. Suspend 42 grams of either DNase Test Agar or DNase Test Agar w/Methyl Green in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

User Control Quality cont.

Cultural Response

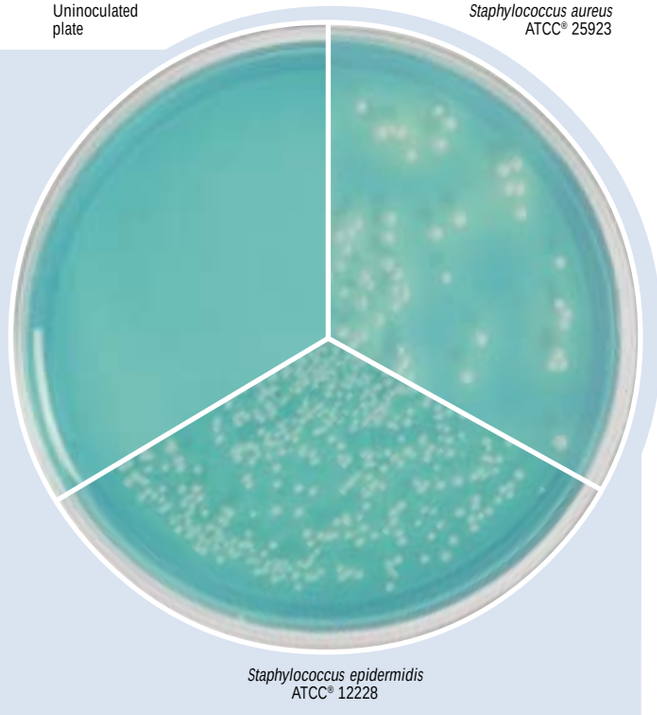
DNase Test Agar
DNase Test Agar w/Methyl Green

Prepare DNase Test Agar or DNase Test Agar w/Methyl Green per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 and up to 48 hours. Flood DNase Test Agar (only) with 1N hydrochloric acid prior to observing DNase activity.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	DNASE TEST
Serratia marcescens	8100*	100-1,000	good	+
Staphylococcus aureus	25923*	100-1,000	good	+
Staphylococcus epidermidis	12228*	100-1,000	good	–
Streptococcus pyogenes	19615*	100-1,000	good	+

The cultures listed are the minimum that should be used for performance testing.

*****These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate plates by spotting or streaking with a heavy inoculum of the test organism. Use a spot approximately 5 mm in diameter or a 1-2 cm streak approximately 5 mm wide.
2. Incubate plates at 35 ± 2°C for 18-24 hours and up to 48 hours.
3. DNase Test Agar: Flood the plates with 1N hydrochloric acid.

   DNase Test Agar w/Methyl Green: Do NOT flood with 1N hydrochloric acid.
4. Observe for clearing around the spot or streak. Record results.

Results

DNase Test Agar:	A zone of clearing around the spot or streak indicates DNase activity.
DNase Test Agar w/Methyl Green:	A decolorized zone (halo) around the spot or streak indicates DNase activity.

Limitations of the Procedure

1. The composition of the culture medium, the degree of aeration, pH, temperature and incubation period are important factors influencing DNase activity in the culturing and testing the micrococci.[7]

References

1. **Weckman, B. G., and B. W. Catlin.** 1957. Deoxyribonuclease activity of micrococci from clinical sources. J. Bacteriol. **73**:747-753.
2. **DiSalvo, J. W.** 1958. Deoxyribonuclease and coagulase activity of micrococci. Med. Tech. Bull. U. S. Armed Forces Med. J. **9**:191.
3. **Jeffries, C. D., D. F. Holtman, and D. G. Guse.** 1957. Rapid method for determining the activity of microorganisms on nucleic acid. J. Bacteriol. **73**:590- 591.
4. **Fusillo, M. H., and D. L. Weiss.** 1959. Qualitative estimation of staphylococcal deoxyribonuclease. J. Bacteriol. **78**:520.
5. **Kurnick, N. B.** 1950. The determination of deoxyribonuclease activity by methyl green: application to serum. Arch. Biochem. **29:**41.
6. **Smith, P. B., G. A. Hancock, and D. L. Rhoden.** 1969. Improved medium for detecting deoxyribonuclease-producing bacteria. Appl. Microbiol. **18**:991.
7. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 275-284. Williams & Wilkins, Baltimore, MD.

Packaging

DNase Test Agar	100 g	0632-15
	500 g	0632-17
DNase Test Agar w/Methyl Green	100 g	0220-15
	500 g	0220-17

Bacto® DRBC Agar

User Quality Control

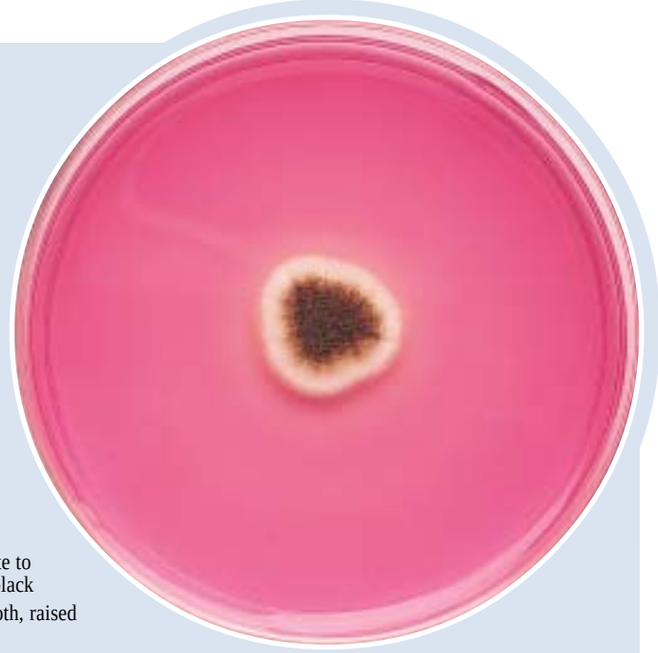
Identity Specifications

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	3.16% solution, soluble in distilled or deionized water upon boiling. Solution is reddish pink, very slightly to slightly opalescent.
Prepared Medium:	Bright pink, very slightly to slightly opalescent.
Reaction of 3.16% Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response

Prepare DRBC Agar per label directions. Inoculate and incubate plates at 25°C for 5 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	1015	Stab	good-colonies white to salt and pepper to black
Candida albicans	10231	100-1,000	good-colonies pink,smooth, raised
Escherichia coli	25922*	1,000-2,000	none to poor
Micrococcus luteus	10240	1,000-2,000	none to poor



Aspergillus niger
ATCC® 1015

The cultures listed are the minimum that should be used for performance testing.
*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Intended Use

Bacto DRBC Agar is used for the enumeration of yeasts and molds.

Also Known As

Dichloran Rose Bengal Chloramphenicol Agar

Summary and Explanation

DRBC Agar is based on the Dichloran Rose Bengal Chlortetracycline (DRBC) Agar formula described by King, Hocking and Pitt.[1] DRBC Agar conforms with APHA guidelines for the mycological examination of foods, containing chloramphenicol rather than chlortetracycline as proposed by King, Hocking and Pitt.[2] DRBC Agar is a selective medium that supports good growth of yeasts and molds.

Principles of the Procedure

Proteose Peptone No. 3 provides nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Phosphate is a buffering agent. Magnesium Sulfate is a source of divalent cations and sulfate. The antifungal agent, Dichloran, is added to the medium to reduce colony diameters of spreading fungi. The pH of the medium is reduced from 7.2 to 5.6 for improved inhibition of the spreading fungi.[1] The presence of Rose Bengal in the medium suppresses the growth of bacteria and restricts the size and height of colonies of the more rapidly growing molds. The concentration of Rose Bengal is reduced from 50 µg/ml to 25 µg/ml as found in Rose Bengal Chloramphenicol Agar for optimal performance with Dichloran. Chloramphenicol is included in this medium to inhibit the growth of bacteria present in environmental and food samples. Inhibition of growth of bacteria and restriction of spreading of more rapidly growing molds aids in the isolation of slow-growing fungi by preventing their overgrowth by more rapidly growing species. In addition, Rose Bengal is taken up by yeast and mold colonies, which allows these colonies to be easily recognized and enumerated. Reduced recovery of yeasts may be encountered due to increased activity of Rose Bengal at pH 5.6.[1] Bacto Agar is a solidifying agent.

Formula

DRBC Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Dextrose	10 g
Potassium Phosphate Monobasic	1 g
Magnesium Sulfate	0.5 g
Dichloran	0.002 g
Rose Bengal	0.025 g
Chloramphenicol	0.1 g
Bacto Agar	15 g

Final pH 5.6 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **TOXIC.** MAY CAUSE CANCER. POSSIBLE RISK OF HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Nerves, Lymph Glands, Eyes.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

1. Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.
2. Protect medium from light.
3. Store prepared medium in the dark at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure[2,3]

Materials Provided

DRBC Agar

Materials Required But Not Provided

Peptone Water
Flasks with closures
Distilled or deionized water
Autoclave
Incubator (25°C)

Method of Preparation

1. Suspend 31.6 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Prepare sample for surface inoculation following recommended guidelines.[2,3] The use of 0.1% Peptone Water as the diluent is recommended.

Test Procedure

1. Inoculate 0.1 ml of appropriate decimal dilutions of the sample in duplicate onto the surface of DRBC Agar plates. The plates should be dried overnight at room temperature. Spread the inoculum over the entire surface of the plate using a sterile, bent-glass rod.
2. Incubate plates upright at 22-25°C. Examine for growth of yeasts and molds after 3, 4 and 5 days incubation.

Results

Colonies of molds and yeasts should be apparent within 5 days of incubation. Colonies of yeast appear pink due to the uptake of Rose Bengal. Report the results as colony forming units per gram or milliliter of sample.

References

1. **King, A. D., A. D. Hocking, and J. I. Pitt.** 1979. Dichloran-rose bengal medium for the enumeration and isolation of molds from foods. Appl. and Environ. Microbiol. **37:**959-964.

2. **Mislivec, P. B., L. R. Beuchat, and M. A. Cousin.** 1992. Yeasts and molds, p. 239-249. *In* C. Vanderzant, and D. F. Splittstoesser, (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association. Washington, D.C.

3. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association. Washington, D.C.

Packaging

DRBC Agar	500 g	0587-17

Decarboxylase Differential Media

Bacto® Decarboxylase Base Moeller · Bacto Decarboxylase Medium Base · Bacto Lysine Decarboxylase Broth

Intended Use

Bacto Decarboxylase Base Moeller is a basal medium which, with added lysine, arginine, ornithine, or another amino acid, is used for differentiating bacteria based on their ability to decarboxylate amino acids.

Bacto Decarboxylase Medium Base, with added lysine, arginine, or ornithine, is used for differentiating bacteria based on amino acid decarboxylation.

Bacto Lysine Decarboxylase Broth is used for differentiating microorganisms based on lysine decarboxylation.

Also Known As

Decarboxylase Base Moeller is also referred to as Moeller Decarboxylase Broth Base. Decarboxylase Medium Base is also known as Decarboxylase Medium Base, Falkow or Decarboxylase Basal Medium.

Summary and Explanation

Moeller [1,2,3] described the amino acid decarboxylase test for distinguishing between various microorganisms. He determined the usefulness of using this enzyme system in the differentiation of the *Enterobacteriaceae.*[4,5] The production of lysine, arginine, ornithine, and glutamic acid decarboxylase by various members of this family provided a useful adjunct to other biochemical tests used for the speciation and identification of the *Enterobacteriaceae.*

Carlquist[6] developed a medium using the lysine decarboxylase reaction to differentiate *Salmonella arizonae (Arizona)* from *Citrobacter* (Bethesda-Ballerup biotype). Falkow[7] obtained valid and reliable results with a lysine decarboxylase medium he developed to differentiate and identify *Salmonella* and *Shigella.* Although his modification of the Moeller formula was originally described as a lysine medium only, further study by Falkow and then by Ewing, Davis and Edwards,[8] substantiated the use of the medium for ornithine and arginine decarboxylase reactions as well.

Ewing, Davis and Edwards[8] compared the Falkow decarboxylase medium base to the Moeller medium and reported that, although the two methods compared favorably in most cases, the Moeller medium was found to be more reliable for cultures of *Klebsiella* and *Enterobacter.* They concluded that the Moeller method should be regarded as the standard or reference method, although the Falkow formula is suitable for determining decarboxylase reactions for most members of the *Enterobacteriaceae* except for *Klebsiella* and *Enterobacter.* The Moeller medium is also particularly useful in the identification of *Aeromonas*, *Plesiomonas*, *Vibrio* spp., and nonfermentative gram- negative bacilli.[9]

Decarboxylase Base Moeller conforms with the Moeller formulation while Decarboxylase Medium Base is prepared according to the formula described by Falkow. Lysine Decarboxylase Broth is the Falkow medium with L-Lysine added in 0.5% concentration.

Decarboxylase tests are important in the differentiation and identification of a wide variety of microorganisms and are outlined in numerous standard methods.[10-13]

Principles of the Procedure

Decarboxylase Base Moeller, Decarboxylase Medium Base and Lysine Decarboxylase Broth consist of Bacto Peptone and Beef Extract which supply carbon and nitrogen. Dextrose is a fermentable carbohydrate. Yeast Extract provides vitamins and cofactors required for growth as well as additional sources of nitrogen and carbon. As applicable, Brom Cresol Purple and Cresol Red are pH indicators. The Pyridoxal is an enzyme cofactor for the amino acid decarboxylase. The amino acids lysine, ornithine, and arginine are added to the basal media to detect the production of the enzymes specific for these substrates.

When the media are inoculated with bacteria that are able to ferment the dextrose, acids are produced that lower the pH and change the indicator from purple to yellow. If the bacteria produce the appropriate decarboxylase, the production of amines raises the pH of the medium causing the indicator to change from yellow to a light or deep purple. Decarboxylation of lysine yields cadaverine, while decarboxylation of ornithine yields putrescine. Arginine is first hydrolyzed to ornithine and then decarboxylated to putrescine. If decarboxylation does not occur the medium remains acidic (yellow). Control tubes of basal media, that do not contain an amino acid, should be inoculated to verify reactions.

To obtain proper reactions, inoculated tubes must be protected from the air. This is done to avoid false alkalinization at the surface of the medium, which could cause a decarboxylase negative bacteria to appear to be positive. This can be done by overlaying a medium with sterile mineral oil as suggested by Ewing, Davis and Edwards.[8]

Formula

Decarboxylase Base Moeller

Formula Per Liter

Bacto Peptone	5 g
Bacto Beef Extract	5 g
Bacto Dextrose	0.5 g
Bacto Brom Cresol Purple	0.01 g
Cresol Red	0.005 g
Pyridoxal	0.005 g

Final pH 6.0 ± 0.2 at 25°C

Decarboxylase Medium Base

Formula Per Liter

Bacto Peptone	5 g
Bacto Yeast Extract	3 g
Bacto Dextrose	1 g
Bacto Brom Cresol Purple	0.02 g

Final pH 6.8 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Decarboxylase Base Moeller

Dehydrated Appearance:	Light to medium tan, homogeneous, free-flowing.
Solution:	1.05% solution, soluble in distilled or deionized water on warming.
Prepared Tubes:	Yellowish-red, slightly opalescent.
pH at 25°C:	6.0 ± 0.2

Decarboxylase Medium Base

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	0.9% solution, soluble in distilled or deionized water on warming.
Prepared Tubes:	Purple, clear.
pH at 25°C:	6.8 ± 0.2

Lysine Decarboxylase Base

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	1.4% solution, soluble in distilled or deionized water on warming.
Prepared Tubes:	Purple, clear w/o significant precipitate.
pH at 25°C:	6.8 ± 0.2

Cultural Response

Prepare media per label directions. Where necessary add appropriate amounts of amino acids to be tested. Inoculate with approx. 1,000 CFUs of test organisms and overlay test tubes with sterile mineral oil. Incubate at 35 ± 2°C for 18-48 hours. Purple color indicates a positive decarboxylase reaction. Yellow color indicates a negative decarboxylase reaction.

Decarboxylase Base Moeller

ORGANISM	ATCC®	GROWTH	REACTION w/o LYSINE	REACTION w/ LYSINE
Escherichia coli	25922*	good	yellow (–)	purple (+)
Shigella flexneri	12022*	good	yellow (–)	yellow (–)

Decarboxylase Medium Base

ORGANISM	ATCC®	GROWTH	REACTION LYSINE	REACTION ORNITHINE	REACTION ARGININE
Salmonella typhimurium	14028*	good	purple (+)	purple (+)	purple (+)
Proteus vulgaris	13315*		yellow (–)	yellow (–)	yellow (–)

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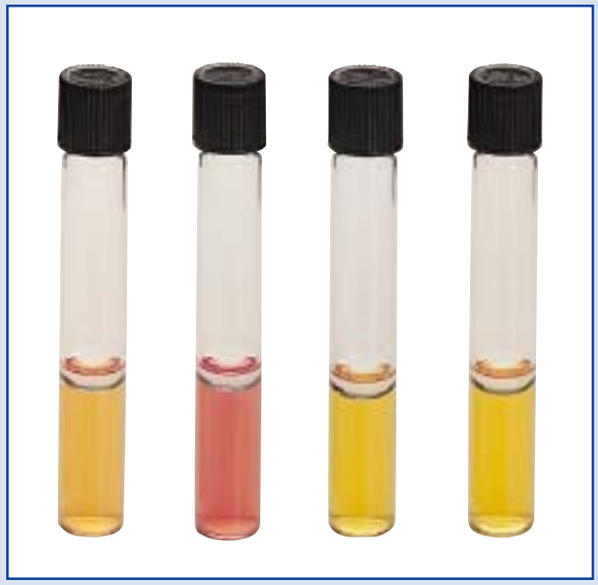


Escherichia coli ATCC® 25922 | *Escherichia coli* ATCC® 25922 w/ Lysine | *Shigella flexneri* ATCC® 12022 | *Shigella flexneri* ATCC® 12022 w/ Lysine

Decarboxylase Base Moeller

Uninoculated tube | *Salmonella typhimurium* ATCC® 14028 | *Proteus vulgaris* ATCC® 13315

Decarboxylase Medium Base

Lysine Decarboxylase Broth

Formula Per Liter

Bacto Peptone	5 g
Bacto Yeast Extract	3 g
Bacto Dextrose	1 g
L-Lysine	5 g
Bacto Brom Cresol Purple	0.02 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated media below 30°C. The dehydrated media is very hygroscopic. Keep containers tightly closed.

Expiration Date

The expiration date applies to the products in their intact containers when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

User Quality Control cont.

Lysine Decarboxylase Base

ORGANISM	ATCC®	GROWTH	REACTION
Escherichia coli	25922*	good	purple (+)
Proteus vulgaris	13315*	good	yellow (–)

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Escherichia coli ATCC® 25922 *Proteus vulgaris* ATCC® 13315

Lysine Decarboxylase Broth

Procedure

Materials Provided

Decarboxylase Base Moeller
Decarboxylase Medium Base
Lysine Decarboxylase Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (37°C)
Wire loops (bacteriological)
L-lysine, L-arginine, L-ornithine, or other L-amino acids (to be added to either Decarboxylase Base Moeller or Decarboxylase Medium Base)
Sterile mineral oil
1N NaOH

Methods of Preparation

Decarboxylase Base Moeller

1. Suspend 10.5 grams in 1 liter distilled or deionized water and heat to dissolve completely.
2. Add 10 grams L-amino acid (or 20 grams DL-amino acid) and agitate to dissolve completely. When adding ornithine which is highly acidic, adjust the pH with NaOH (approximately 4.6 ml 1 N NaOH per liter) prior to sterilizing.
3. Dispense 5 ml amounts into screw capped test tubes.
4. Autoclave at 121°C for 10 minutes.

Decarboxylase Medium Base

1. Suspend 9 grams in 1 liter distilled or deionized water and warm to dissolve completely.
2. Add 5 grams L-amino acid (or 10 grams DL-amino acid) and warm to dissolve completely. Adjust the pH with NaOH (if necessary) prior to sterilizing.
3. Dispense 5 ml amounts into screw capped test tubes.
4. Autoclave at 121°C for 15 minutes.

Lysine Decarboxylase Broth

1. Suspend 14 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Dispense 5 ml amounts into screw capped test tubes.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Only pure cultures of enteric bacteria taken from purification plates or agar slants are to be used for biochemical tests. A presumptive identification of the bacteria under investigation should be made on the basis of morphological and cultural characteristics prior to biochemical testing.

Test Procedure

1. Inoculate the prepared tubes with a 24 hour pure culture using a bacteriological loop. A control tube should also be inoculated.
2. Aseptically overlay all inoculated tubes, including the control tube, with 4-5 mm sterile mineral oil.
3. Incubate tubes at 35 ± 2°C for up to 4 days. Tubes must be read daily after 24 hours incubation. Observe for a change in color from purple to yellow to purple.

Results

See appropriate reference for the expected decarboxylase reactions of the *Enterobacteriaceae* and other organisms.[14]

Limitations of the Procedure

1. Biochemical characteristics of the *Enterobacteriaceae* serve to confirm presumptive identification based on cultural, morphological, and/or serological findings. Therefore, biochemical testing should be attempted on pure culture isolates only and subsequent to differential determinations.
2. The decarboxylase reactions are part of a total biochemical profile for members of the *Enterobacteriaceae* and related organisms. Results obtained from these reactions, therefore, can be considered indicative of a given genus or species. However, conclusive and final identification of these organisms cannot be made solely on the basis of the decarboxylase reactions.
3. If layers of yellow and purple appear after incubation, shake the test tube gently before attempting to interpret results.
4. If a reaction is difficult to interpret, compare the tube in question to an uninoculated control tube. Any trace of purple after 24 hours of incubation is a positive test.
5. A gray color may indicate reduction of the indicator. Additional indicator may be added before the results are interpreted.[12]
6. *Salmonella gallinarum* gives a delayed positive ornithine decarboxylase reaction, requiring 5-6 days incubation.[3] Many strains of *E. coli*, including those that ferment adonitol, may exhibit a delayed reaction.[3]
7. Decarboxylase Medium Base is not satisfactory for the determination of lysine decarboxylase activity with the two genera *Klebsiella* and *Enterobacter.*
8. The lysine decarboxylase activity in *Salmonella* is used to differentiate this group from *Citrobacter freundii*. *Salmonella paratyphi A,* however, gives an atypical negative reaction (yellow color of medium) in 24 hours when Decarboxylase Medium Base is used.[15]

References

1. **Moeller, V.** 1954. Activity determination of amino acid decarboxylases in *Enterobacteriaceae*. Acta Pathol. Microbiol. Scand. **34**: 102-111.
2. **Moeller, V.** 1954. Distribution of amino acid decarboxylases in *Enterobacteriaceae*. Acta Pathol. Microbiol. Scand. **34**: 259-277.
3. **Moeller, V.** 1955. Simplified tests of some amino acid decarboxylases for arginine dihydrolase system. Acta Pathol. Microbiol. Scand. **36**: 158-172.
4. **Gale, E. F.** 1940. The production of amines by bacteria. Biochem. J. **34**: 392, 583, 846.
5. **Gale, E. F.** 1941. Production of amines by bacteria. 4. The decarboxylation of amino-acids by organisms of the groups *Clostridium* and *Proteus*. Biochem. J. **35**: 66-79.
6. **Carlquist, P. R.** 1956. A biochemical test for separating paracolon groups. J. Bacteriol. **71**: 339-341.
7. **Falkow, S.** 1958. Activity of lysine decarboxylase as an aid in the identification of *Salmonella* and *Shigella*. Am. J. Clin. Pathol. **29**: 598.
8. **Ewing, W. H., B. R. Davis, and P. R. Edwards.** 1960. The decarboxylase reaction of *Enterobacteriaceae* and their value in taxonomy. Publ. Health Lab. **18**: 77-83.
9. **Baron, E J., L R. Peterson, and S. M. Finegold (eds.).** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
10. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
11. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
12. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
13. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
14. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken.** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
15. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Decarboxylase Base Moeller	100 g	0890-15
	500 g	0890-17
Decarboxylase Medium Base	500 g	0872-17
	10 kg	0872-08
Lysine Decarboxylase Broth	100 g	0215-15
	500 g	0215-17
	10 kg	0215-08

Bacto® Demi-Fraser Broth Base
Bacto Fraser Broth Supplement

Intended Use

Bacto Demi-Fraser Broth Base is used with Bacto Fraser Broth Supplement in selectively and differentially enriching *Listeria* from foods.

Summary and Explanation

Fraser Broth Base and Fraser Broth Supplement are based on the Fraser Broth formulation of Fraser and Sperber.[1] The medium is used in the rapid detection of *Listeria* from food and environmental samples. Demi-Fraser Broth Base is a modification of Fraser Broth Base in which the nalidixic acid and acriflavine concentrations have been reduced to 10 mg/l and 12.5 mg/l respectively, in accordance with AFNOR guidelines.[2]

Principles of the Procedure

Tryptose, Beef Extract and Yeast Extract provide carbon and nitrogen sources and the cofactors required for good growth of *Listeria*. Sodium Phosphate and Potassium Phosphate buffer the medium. Selectivity is provided by Lithium Chloride, Nalidixic Acid and Acriflavine. The high Sodium Chloride concentration of the medium inhibits growth of enterococci.

All *Listeria* species hydrolyze esculin, as evidenced by a blackening of the medium. This blackening results from the formation of 6,7-dihydroxycoumarin, which reacts with ferric ions.[1] Ferric ions are added to the final medium as Ferric Ammonium Citrate in Fraser Broth Supplement.

Formula

Demi-Fraser Broth Base

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	5 g
Bacto Yeast Extract	5 g
Sodium Chloride	20 g
Sodium Phosphate, Dibasic	9.6 g
Potassium Phosphate, Monobasic	1.35 g
Esculin	1 g
Nalidixic Acid	0.01 g
Acriflavine HCl	0.0125 g
Lithium Chloride	3 g

Final pH 7.2 ± 0.2 at 25°C

Fraser Broth Supplement

Ingredients per 10 ml vial

Ferric Ammonium Citrate	0.5 g

One vial is added to one liter of basal medium

Precautions

1. For Laboratory Use.
2. **Demi-Fraser Broth Base: HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Kidneys, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   Fraser Broth Supplement: **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

User Quality Control

Identity Specifications

Demi-Fraser Broth Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.5% solution, soluble in distilled or deionized water. Solution is medium amber, clear to slightly opalescent, may have a fine precipitate.
Prepared Medium:	Medium amber, very slightly to slightly opalescent, may have a slight precipitate.
Reaction of 5.5% Solution at 25°C:	pH 7.2 ± 0.2

Fraser Broth Supplement

Solution Appearance: Dark brown solution.

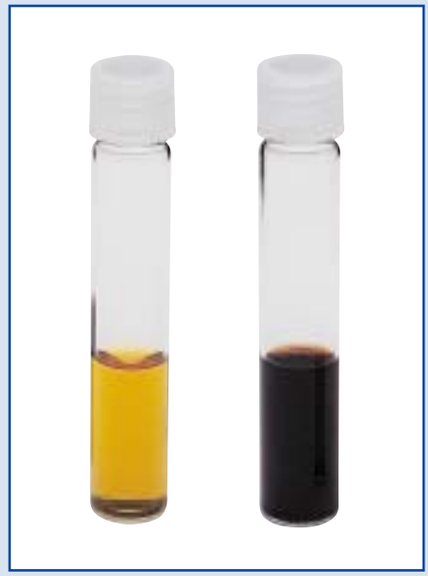
Cultural Response

Prepare Demi-Fraser Broth per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH/APPEARANCE
Escherichia coli	25922*	1,000-2,000	inhibited
Listeria monocytogenes	19114	100-1,000	good growth/blackening of the medium
Enterococcus faecalis	29212*	1,000-2,000	markedly to completely inhibited

The cultures listed above are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Uninoculated tube — *Listeria monocytogenes* ATCC® 19114

3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Fraser Broth Supplement at 2-8°C.

Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Demi-Fraser Broth Base
Fraser Broth Supplement

Materials Required But Not Provided

Glassware
Autoclave
Fraser Broth
Oxford Medium
PALCAM Medium
Sterile tubes with closures

Method of Preparation

1. Dissolve 55 grams of Demi-Fraser Broth Base in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes. Cool to room temperature.
3. Aseptically add 10 ml Fraser Broth Supplement. Mix well.

Sample Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure[2]

1. Pre-enrich the sample in Demi-Fraser Broth. Incubate for 18-24 hours at 35 ± 2°C. Subculture onto Oxford Medium or PALCAM Medium.
2. Transfer 0.1 ml of the pre-enrichment culture into 10 ml of Fraser Broth and incubate for 48 hours at 37°C. Subculture onto Oxford Medium or PALCAM Medium after 18-24 hours and again after 42-48 hours of incubation.
3. Examine Oxford Medium or PALCAM Medium plates for the appearance of presumptive *Listeria* colonies.
4. Confirm the identity of all presumptive *Listeria* by biochemical and/or serological testing.

Results

The presence of *Listeria* is presumptively indicated by the blackening of Demi-Fraser Broth after incubation for 24-48 hours at 35°C. Confirmation of the presence of *Listeria* is made following subculture onto appropriate media and biochemical/serological identification.

References

1. **Fraser, J., and W. Sperber.** 1988. Rapid detection of *Listeria* in food and environmental samples by esculin hydrolysis. Journal of Food Protection **51**:762- 765.
2. **L'association française de normalisation (AFNOR).** 1993. Food Microbiology- Detection of *Listeria monocytogenes*-Routine Method, V 08-055. AFNOR, Paris, France.

Packaging

Demi-Fraser Broth Base	500 g	0653-17-0
	10 kg	0653-07-0
Fraser Broth Supplement	6 x 10 ml	0211-60-2

Bacto® Desoxycholate Agar

Intended Use

Bacto Desoxycholate Agar is used for isolating and differentiating gram-negative enteric bacilli.

Also Known As

Deoxycholate Agar (Sodium Deoxycholate Agar)

NOTE: Alternate spelling[1] - Deoxy-.

Summary and Explanation

Desoxycholate Agar as formulated by Leifson[2] demonstrated improved recovery of intestinal pathogens from specimens containing normal intestinal flora. The medium was an improvement over other media of the time because the chemicals, citrates and sodium desoxycholate, in specified amounts, worked well as inhibitors. This medium has been used to screen for *Salmonella* sp. and *Shigella* sp. from clinical specimens.[3]

Principles of the Procedure

Bacto Peptone provides nitrogen and carbon for general growth requirements. Lactose is the fermentable carbohydrate. Sodium chloride and dipotassium phosphate maintain the osmotic balance of the medium. Sodium desoxycholate, ferric citrate and sodium citrate inhibit growth of gram-positive bacteria. Neutral red is a pH indicator. Bacto® Agar is a solidifying agent.

Differentiation of enteric bacilli is based on fermentation of lactose. Bacteria that ferment lactose produce acid and, in the presence of neutral red, form red colonies. Bacteria that do not ferment lactose form colorless colonies. The majority of normal intestinal bacteria ferment lactose (red colonies) while *Salmonella* and *Shigella* species do not ferment lactose (colorless colonies).

Formula

Desoxycholate Agar

Formula Per Liter

Bacto Peptone	10 g
Bacto Lactose	10 g

Sodium Desoxycholate	1 g
Sodium Chloride	5 g
Dipotassium Phosphate	2 g
Ferric Citrate	1 g
Sodium Citrate	1 g
Bacto Agar	15 g
Neutral Red	0.03 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Desoxycholate Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Bunsen burner or heating plate
Incubator (35°C)
Petri dishes

Method of Preparation

1. Suspend 45 grams in 1 liter distilled or deionized water.
2. Boil 1 minute with frequent, careful agitation to dissolve completely. Avoid overheating.
3. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

For a complete discussion on the isolation of enteric bacilli, refer to appropriate procedures outlined in the references.

Results

Refer to appropriate references and procedures for results.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 269-275, vol 1. Williams & Wilkins, Baltimore, MD.
2. **Leifson, E.** 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol. **40**:581-599.

User Quality Control

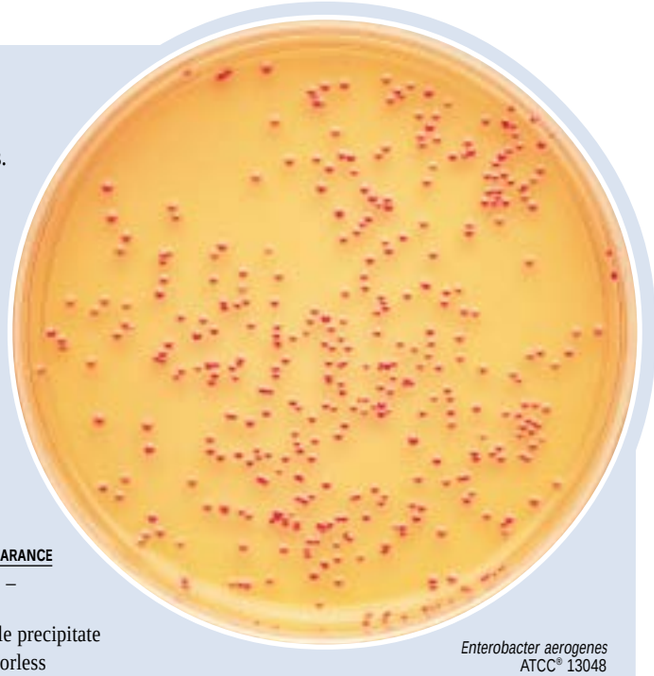
Identity Specifications

Dehydrated Appearance:	Pinkish beige, free-flowing, homogeneous.
Solution:	4.5% solution, soluble in distilled or deionized water on boiling. Solution is reddish orange, very slightly to slightly opalescent with no significant precipitate.
Prepared Medium:	Orange, slightly opalescent.
Reaction of 4.5% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Desoxycholate Agar per label directions. Inoculate using the pour plate method and incubate plates at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	1,000-2,000	marked to complete inhibition	–
Escherichia coli	25922*	30-300	good	pink w/bile precipitate
Salmonella typhimurium	14028*	30-300	good	colorless



Enterobacter aerogenes
ATCC® 13048

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

3. **Balows, A., W. J. Mausler, K. L. Herrmann, H. D. Isenberg, and H. J. Shadony (ed.)** 1991. Manual of clinical microbiology. 5th ed. American Society for Microbiology, Washington, D.C.

Packaging

Desoxycholate Agar	500 g	0273-17

Bacto® Desoxycholate Citrate Agar

Intended Use

Bacto Desoxycholate Citrate Agar is used for isolating enteric bacilli, particularly *Salmonella* and many *Shigella* species.

Also Known As

NOTE: Deoxy-; alternate spelling.[1]

Summary and Explanation

Desoxycholate Citrate Agar is a modification of Desoxycholate Agar formulated by Leifson.[2] His original medium demonstrated improved recovery of intestinal pathogens from specimens containing normal intestinal flora by using citrates and sodium desoxycholate in specified amounts as inhibitors to gram positive bacteria.

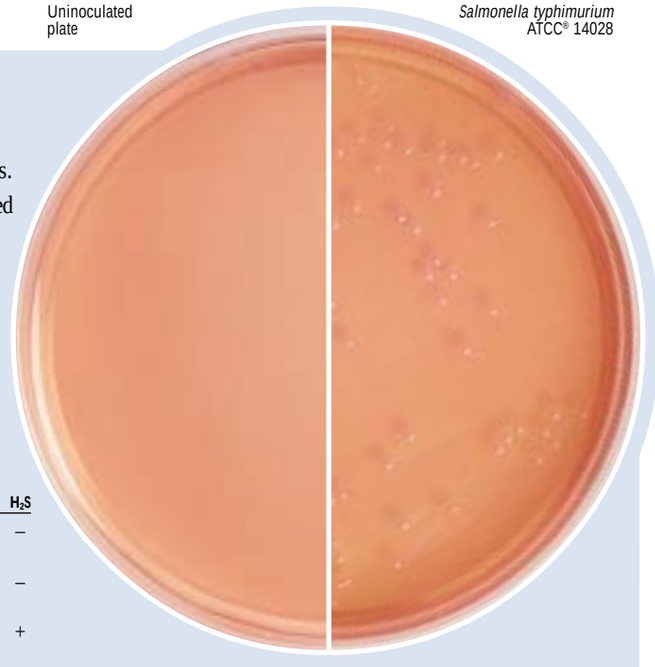
Leifson modified his original medium by increasing the concentration of sodium citrate and sodium desoxycholate and found Desoxycholate Citrate Agar reliable for isolating many *Salmonella* and *Shigella* species.[2]

Desoxycholate Citrate Agar effectively isolates intestinal pathogens (*Salmonella* and *Shigella* species) by inhibiting coliforms and many *Proteus* species.[1] This medium is widely used by clinical laboratories.[3]

Principles of the Procedure

Infusion from Meat is a source of carbon and nitrogen. This ingredient is used because the inhibition of coliforms produced is greater than when an extract or simple peptone is used.[2] Desoxycholate Citrate Agar contains Proteose Peptone No. 3 as a source of carbon, nitrogen, vitamins and minerals. Lactose is a carbohydrate. Sodium Citrate and Sodium Desoxycholate inhibit gram positive bacteria, coliforms and *Proteus* species. Ferric Ammonium Citrate aids in the detection of H_2S producing bacteria. Neutral Red is a pH indicator. Bacto Agar is a solidifying agent.

In the presence of neutral red, bacteria that ferment lactose produce acid and form red colonies. Bacteria that do not ferment lactose form colorless colonies. If the bacteria produce H_2S, the colonies will have black centers. The majority of normal intestinal bacteria ferment lactose and do not produce H_2S (red colonies without black centers). *Salmonella* and *Shigella* spp. do not ferment lactose but *Salmonella* may produce H_2S (colorless colonies with or without black centers). Lactose-fermenting colonies may have a zone of precipitation around them caused by the precipitation of desoxycholate in the presence of acid.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pinkish-beige, free-flowing, homogeneous.
Solution:	7.0% solution, soluble in distilled or deionized water on boiling. Solution is orange-red, very slightly to slightly opalescent.
Prepared Medium:	Orange-red, slightly opalescent.
Reaction of 7.0% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

Prepare Desoxycholate Citrate Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	H_2S
Enterococcus faecalis	29212*	1,000-2,000	marked to complete inhibition	–	–
Escherichia coli	25922*	100-1,000	partial inhibition	pink with bile precipitate	–
Salmonella typhimurium	14028*	100-1,000	fair to good	colorless	+

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

Desoxycholate Citrate Agar

Formula Per Liter

Meat, Infusion from	330 g
Bacto Proteose Peptone No. 3	10 g
Bacto Lactose	10 g
Sodium Citrate	20 g
Ferric Ammonium Citrate	2 g
Sodium Desoxycholate	5 g
Bacto Agar	13.5 g
Neutral Red	0.02 g

Final pH 7.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Desoxycholate Citrate Agar

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Incubator (35°C)

Method of Preparation

1. Suspend 70 grams in 1 liter distilled or deionized water.
2. Heat and boil briefly with frequent, careful agitation to dissolve completely. Avoid overheating.
3. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure

1. Inoculate specimen directly onto surface of medium.
2. Incubate plates at 35 ± 2°C for 18-24 hours. Plates can be incubated for an additional 24 hours if no lactose fermenters are observed.

Results

Non-lactose fermenters produce transparent, colorless to light pink or tan colored colonies with or without black centers. Lactose fermenters produce a red colony with or without a bile precipitate.

Limitations of the Procedure

1. Coliform strains may be encountered that will grow on this medium, making it difficult to detect pathogens.
2. Heavy inocula should be distributed over the entire surface of the medium prevent complete masking of pathogens by coliform organisms.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 269-275. Williams & Wilkins, Baltimore, MD.
2. **Leifson, E.** 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol. **40**: 581-599.
3. **Farmer III, J. J., and M. T. Kelly.** 1991. *Enterobacteriaceae.* p. 360-383. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

Packaging

Desoxycholate Citrate Agar	500 g	0274-17

Bacto® Desoxycholate Lactose Agar

Intended Use

Bacto Desoxycholate Lactose Agar is used for isolating and differentiating gram-negative enteric bacilli and for enumerating coliforms from water, wastewater, milk and dairy products.

Also Known As

NOTE: Deoxy-; alternate spelling.[1]

Summary and Explanation

Desoxycholate Lactose Agar is a modification of Desoxycholate Agar formulated by Leifson.[2] His original medium demonstrated improved recovery of intestinal pathogens from specimens containing normal intestinal flora by using citrates and sodium desoxycholate in specified amounts as inhibitors to gram-positive bacteria.

Standard Methods manuals for dairy[3] and water[4] specified a modification of Desoxycholate Agar to contain less sodium desoxycholate and, accordingly, be less inhibitory to gram-positive bacteria. This formulation, known as Desoxycholate Lactose Agar, was used in pour plate procedures for isolation and enumeration of coliforms in milk, water and other specimens.

Principles of the Procedure

Bacto Peptone provides nitrogen and carbon for general growth requirements. Lactose is a fermentable carbohydrate. Sodium

Chloride maintains the osmotic balance of the medium. Sodium Desoxycholate and Sodium Citrate inhibit growth of gram-positive bacteria. Neutral Red is a pH indicator. Bacto Agar is a solidifying agent.

Differentiation of enteric bacilli is based on fermentation of lactose. Bacteria that ferment lactose produce acid and, in the presence of neutral red, form red colonies. Bacteria that do not ferment lactose form colorless colonies. The majority of normal intestinal bacteria ferment lactose (red colonies) while *Salmonella* and *Shigella* species do not ferment lactose (colorless colonies).

Formula

Bacto Desoxycholate Lactose Agar

Formula Per Liter

Bacto Proteose Peptone	10 g
Bacto Lactose	10 g
Sodium Desoxycholate	0.5 g
Sodium Chloride	5 g
Sodium Citrate	2 g
Bacto Agar	15 g
Neutral Red	0.03 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bacto Desoxycholate Lactose Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Bunsen burner or heating plate
Incubator(35°C)
Petri dishes

Method of Preparation

1. Suspend 42.5 grams in 1 liter distilled or deionized water.
2. Boil 1 minute with frequent, careful agitation to dissolve completely. Avoid overheating.
3. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

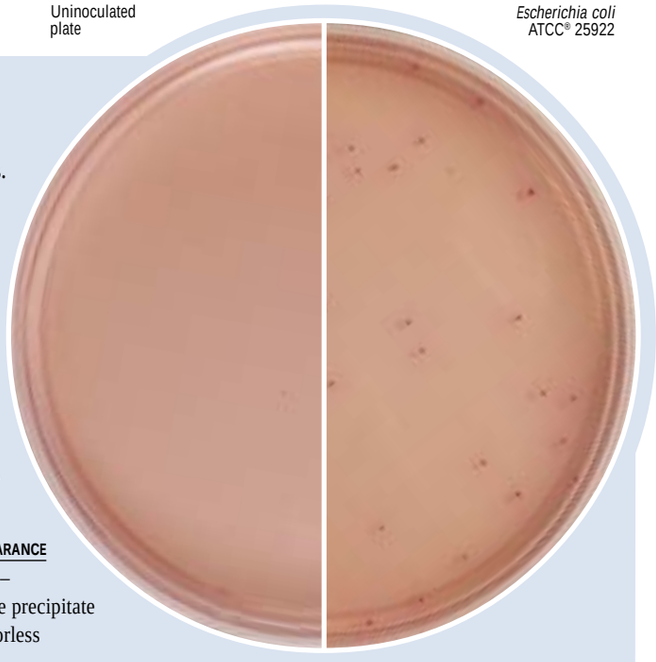
Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pinkish beige, free-flowing, homogeneous.
Solution:	4.25% solution, soluble in distilled or deionized water on boiling. Solution is pinkish-red, very slightly to slightly opalescent.
Prepared Medium:	Pinkish-red, very slightly to slightly opalescent.
Reaction of 4.25% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Prepare Desoxycholate Lactose Agar per label directions. Inoculate the medium and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	1000-2,000	markedly inhibited	–
Escherichia coli	25922*	30-300	good	pink w/bile precipitate
Salmonella typhimurium	14028*	30-300	good	colorless

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 269-275, vol 1. Williams & Wilkins, Baltimore, MD.
2. **Leifson, E.** 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol. **40**:581-599.
3. **American Public Health Association.** 1960. Standard methods for the examination of dairy products microbiological and chemical, 11th ed. American Public Health Association, Washington, D.C.
4. **American Public Health Association.** 1960. Standard methods for the examination of water and wastewater, 11th ed. American Public Health Association, Washington, D.C.

Packaging

Desoxycholate Lactose Agar	500 g	0420-17

Bacto® Dextrose Agar
Bacto Dextrose Broth

Intended Use

Bacto Dextrose Agar is used for cultivating a wide variety of microorganisms with or without added blood.

Bacto Dextrose Broth is used for cultivating fastidious microorganisms and for detecting gas from enteric bacilli.

Summary and Explanation

In 1932, Norton[1] recommended a basal medium containing 0.5-1% dextrose with approximately 5% defibrinated blood for the isolation of many fastidious bacteria, including *Haemophilus* and *Neisseria*. Dextrose is an energy source used by many organisms. The high concentration of this ingredient makes Dextrose Agar a suitable medium for the production of early, abundant organism growth and shortening the lag periods of older cultures. Because of the increased dextrose content, Dextrose Agar is not suitable for observation of hemolysis when supplemented with 5% sheep, rabbit or horse blood.

Dextrose Broth is a highly nutritious broth suitable for the isolation of fastidious organisms and specimens containing a low inoculum. The addition of 0.1-0.2% agar to Dextrose Broth facilitates anaerobic growth and aids in dispersion of reducing substances and CO_2 formed in the environment.[2] The low agar concentration provides suitable conditions for both aerobic growth in the clear upper zone and for microaerophilic and anaerobic growth in the lower, flocculent agar zones.

Dextrose Agar and Dextrose Broth are specified in the Compendium of Methods for the Microbiological Examination of Foods.[3]

Principles of the Procedure

Beef Extract and Tryptose provide nitrogen, amino acids and vitamins. Dextrose is a carbon source, and the increased concentration is a distinguishing characteristic of this medium from other formulations used

User Quality Control

Identity Specifications

Dextrose Agar

Dehydrated Appearance:	Medium beige, homogeneous, free-flowing.
Solution:	4.3% solution, soluble in distilled or deionized water on boiling; medium amber, very slightly to slightly opalescent.
Prepared Medium:	Plain - Medium amber, slightly opalescent without significant precipitate. With blood - Cherry-red, opaque.
Reaction of 4.3% Solution at 25°C	pH 7.3 ± 0.2

Dextrose Broth

Dehydrated Appearance:	Light tan, homogeneous, free-flowing.
Solution:	2.3% solution, soluble in distilled or deionized water; light to medium amber, clear without significant precipitate.
Prepared Medium:	Light to medium amber.
Reaction of 2.3% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Dextrose Agar

Prepare Dextrose Agar per label directions with and without sterile defibrinated sheep blood. Inoculate and incubate at 35 ± 2°C under proper atmospheric conditions for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH w/o BLOOD	GROWTH w/5% SHEEP BLOOD
Neisseria meningitidis	13090*	100-1,000	poor	good
Staphylococcus aureus	25923*	100-1,000	good	good
Streptococcus pyogenes	19615*	100-1,000	good	good

Dextrose Broth

Prepare Dextrose Broth per label directions with and without 0.1% Bacto Agar; dispense into tubes containing fermentation vials. Inoculate and incubate at 35 ± 2°C under proper atmospheric conditions. Read growth and gas production at 15-24 and 40-48 hours.

ORGANISM	ATCC®	GROWTH	CFU	GAS PRODUCTION	GROWTH w/1% AGAR
Escherichia coli	25922*	good	100-1,000	+	good
Neisseria meningitidis	13090*	good	100-1,000	–	good
Streptococcus pyogenes	19615*	good	100-1,000	–	good
Staphylococcus aureus	25923*	good	100-1,000	–	good

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

as blood agar bases. Bacto Agar is a solidifying agent.

Supplementation with 5% blood provides additional growth factors for fastidious microorganisms.

Formula

Dextrose Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Tryptose	10 g
Bacto Dextrose	10 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Dextrose Broth

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Tryptose	10 g
Bacto Dextrose	5 g
Sodium Chloride	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Dextrose Agar
Dextrose Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile defibrinated blood (optional)
Sterile Petri dishes

Method of Preparation

Dextrose Agar

1. Suspend 43 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. OPTIONAL: To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. Dispense into sterile Petri dishes or as desired.

Dextrose Broth

1. Suspend 23 grams in 1 liter distilled or deionized water.
2. Dissolve in distilled or deionized water.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense into tubes.

Specimen Collection and Preparation

Specimens are obtained and processed according to the techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion on microorganism isolation and identification, refer to appropriate references.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Because the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Norton.** 1932. Bacteriology of pus. J. Lab. Clin. Med. **17**:558-565.
2. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol. 1, p. 802-804. Williams & Wilkins, Baltimore, MD.
3. **Vanderzant, C. and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Dextrose Agar	500 g	0067-17
Dextrose Broth	500 g	0063-17

Bacto® Dextrose Starch Agar

Intended Use

Bacto Dextrose Starch Agar is used for cultivating pure cultures of *Neisseria gonorrhoeae* and other fastidious microorganisms.

Summary and Explanation

Dextrose Starch Agar is recommended as a complete solid medium for the propagation of pure cultures of *Neisseria gonorrhoeae*. This highly nutritious medium without additives will also support excellent growth of *N. meningitidis*, *Streptococcus pneumoniae* and *S. pyogenes*. Dextrose Starch Agar, in half concentration, is recommended as a Stock Culture Agar for the maintenance of *N. gonorrhoeae*, *N. meningitidis* and other organisms not capable

of hydrolyzing starch. This medium cannot be used to maintain stock cultures of organisms capable of splitting starch; acid production from starch will create an unsatisfactory environment.

Dextrose Starch Agar was used by Wilkins, Lewis and Barbiers[1] in an agar dilution procedure to test the activity of antibiotics against *Neisseria* species.

Principles of the Procedure

Proteose Peptone No. 3 and Gelatin provide the nitrogen, vitamins and amino acids in Dextrose Starch Agar. Soluble Starch improves growth response. Dextrose is a carbon source. Sodium chloride maintains the osmotic balance of the medium, and disodium phosphate is a buffering agent. Bacto Agar is the solidifying agent.

Formula

Dextrose Starch Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	15 g
Bacto Dextrose	2 g
Bacto Soluble Starch	10 g
Sodium Chloride	5 g
Disodium Phosphate	3 g
Bacto Gelatin	20 g
Bacto Agar	10 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	6.5 % solution, soluble in distilled or deionized water on boiling; light amber, opalescent with a precipitate.
Prepared Medium:	Light amber, opalescent with a precipitate.
Reaction of 6.5% Solution at 25°C	pH 7.3 ± 0.2

Cultural Response

Prepared Dextrose Starch Agar per label directions. Incubate inoculated medium at 35 ± 2°C for 18-48 hours under 5-10% CO_2.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria gonorrhoeae	CDC 98*	100-1,000	good
Neisseria meningitidis	13090 98*	100-1,000	good
Streptococcus pyogenes	19615 98*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Material Provided

Dextrose Starch Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes

Method of Preparation

1. Suspend 65 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion of the isolation and identification of *N. gonorrhoeae* and other fastidious pathogens, refer to the procedures described in Clinical Microbiology Procedures Handbook[2] and Manual of Clinical Microbiology.[3]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. This medium is not recommended for isolation of gonococci from mixed cultures.

References

1. **Wilkins, Lewis, and Barbiers.** 1956. Antibiot. Chemother. **6**:149.
2. **Isenberg, H. D. (ed.)** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Dextrose Starch Agar	500 g	0066-17
	10 kg	0066-08

Bacto® Dextrose Tryptone Agar

Intended Use

Bacto Dextrose Tryptone Agar is used for cultivating thermophilic "flat-sour" microorganisms associated with food spoilage.

Summary and Explanation

In the 1930's, the National Canners Association specified the use of Dextrose Tryptone Agar for isolating "flat sour" organisms from food products.[1] "Flat sour" spoilage of canned foods is caused by *Bacillus coagulans* (*Bacillus thermoacidurans*). Bacterial growth results in a 0.3-0.5 drop in pH, while the ends of the can remain flat. *B. coagulans* is a soil microorganism that can be found in canned tomato products and dairy products. Conditions favorable for multiplication of the bacterium can result in spoilage of the food product.[2]

Dextrose Tryptone Agar can also be used to isolate other food spoilage bacteria: mesophilic aerobic spore formers in the genera *Bacillus* and *Sporolactobacillus* and thermophilic flat sour spore formers such as *B. stearothermophilus*.[2]

Principles of the Procedure

Dextrose Tryptone Agar contains Tryptone to provide carbon and nitrogen sources for general growth requirements. Dextrose is the carbohydrate source. Brom Cresol Purple is the pH indicator. Bacto Agar is the solidifying agent.

Formula

Bacto Dextrose Tryptone Agar

Formula Per Liter

Bacto Tryptone	10 g
Bacto Dextrose	5 g
Bacto Agar	15 g
Bacto Brom Cresol Purple	0.04 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bacto Dextrose Tryptone Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Petri dishes
Incubator

User Quality Control

Identity Specifications

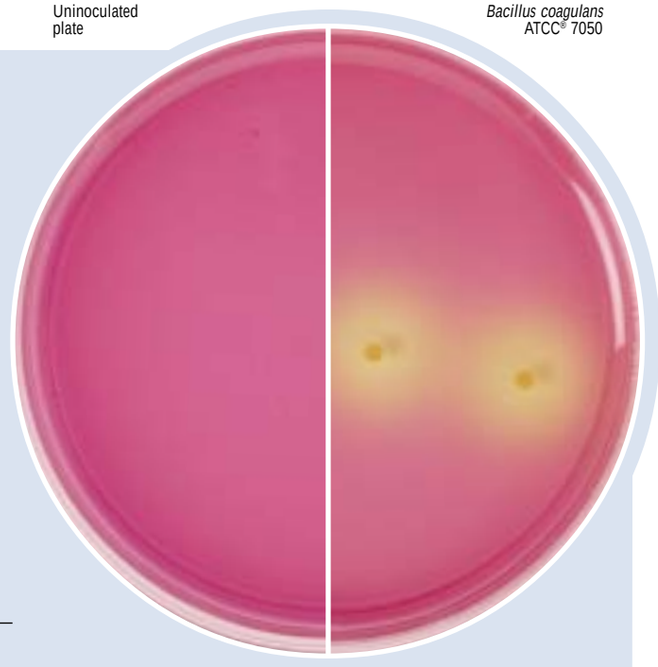
Dehydrated Appearance:	Light, greenish-beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in distilled or deionized water on boiling; purple, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Purple, slightly opalescent without significant precipitate.
Reaction of 3.0% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare Dextrose Tryptone Agar per label directions. Inoculate plates and incubate at 55°C for 36-48 hours. Examine cultures for growth. A change in the color of the medium from purple to yellow indicates dextrose fermentation.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Bacillus coagulans	7050	100-1,000	good	yellow
Bacillus stearothermophilus	7953	100-1,000	good	yellow

The cultures listed are the minimum that should be used for performance testing.



Method of Preparation

1. Suspend 30 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to room temperature.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **National Canners Association**. 1933. Bacterial standards for sugar.
2. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Dextrose Tryptone Agar	500 g	0080-17

Bacto® Differential Reinforced Clostridial Agar

Intended Use

Bacto Differential Reinforced Clostridial Agar is used for enumerating and cultivating sulfite-reducing clostridia.

Also Known As

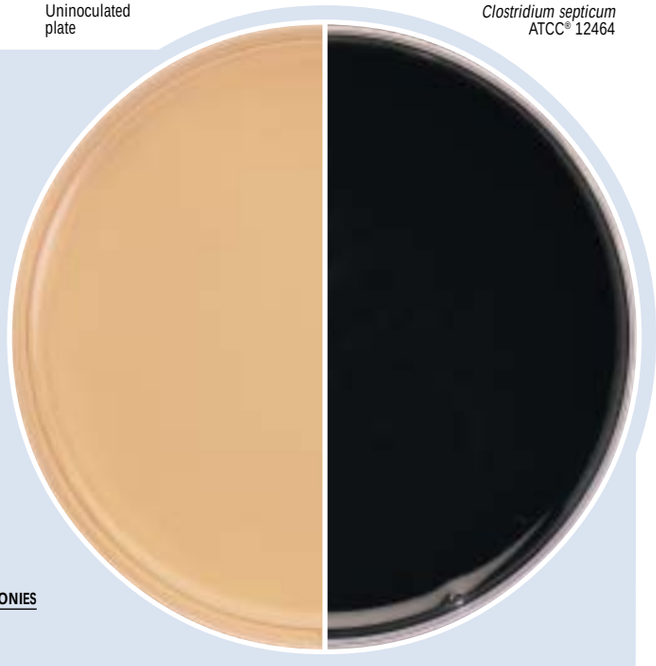
Differential Reinforced Clostridial Agar is also known as DRCA.

Summary and Explanation

Differential Reinforced Clostridial Medium was developed by Gibbs and Freame in 1965[1]. The medium could be used to enumerate clostridia in foods using the Most Probable Number (MPN) method.

Differential Reinforced Clostridial Agar (DRCA) is based on Differential Reinforced Clostridial Medium, but with the addition of agar.

The assay is performed using unheated and heat shocked tubes of DRCA containing replicate dilutions of the test sample. Blackening of the medium is presumptive evidence for the presence of sulfite-reducing clostridia. In this method, heat shocked tubes showing blackening are confirmatory for clostridia. Non-heat shocked tubes showing blackening



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.25% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, clear to slightly opalescent while hot; upon cooling, solution becomes light red.
Prepared Medium:	Light pink, very slightly to slightly opalescent.
Reaction of 4.25% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Prepare Differential Reinforced Clostridial Agar per label directions. Inoculate and incubate at 35°C in an anaerobic environment for 72 hours.

ORGANISM	ATCC®	APPROXIMATE INOCULUM CFU	RECOVERY	BLACK COLONIES
Clostridium bifermentans	638	100	Good	+
Clostridium perfringens	12924	100	Good	+
Clostridium septicum	12464	100	Good	+

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

must be heat shocked to kill off vegetative cells and subcultured into DRCA to confirm the presence of sulfite-reducing clostridia.

Principles of the Procedure

Tryptone, Bacto Peptone, Beef Extract, Yeast Extract, Starch, and L-Cysteine provide nutrients and co-factors required for good growth of clostridia. Dextrose is included in the medium as an energy source. Partial selectivity of the medium is achieved through the addition of Sodium Acetate. Bacto Agar has been incorporated into this medium as a solidifying agent. Anaerobiosis in the medium is detected by the redox indicator Resazurin. The addition of Ferric Ammonium Citrate to the medium is used to detect sulfite reduction. Blackening of the medium is due to the formation of iron sulfide.

Formula

Differential Reinforced Clostridial Agar

Formula Per Liter

Bacto Tryptone	5 g
Bacto Peptone	5 g
Bacto Beef Extract, Desiccated	8 g
Bacto Yeast Extract	1 g
L-Cysteine HCl	0.5 g
Starch	1 g
Dextrose	1 g
Sodium Acetate	5 g
Sodium Bisulfite	0.5 g
Ferric Ammonium Citrate	0.5 g
Resazurin	0.002 g
Bacto Agar	15 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Differential Reinforced Clostridial Agar

Material Required But Not Provided

Anaerobic Jar Complete
Flasks with closures
Distilled or deionized water
Autoclave
Incubator (35°C)
Ringer's solution or 0.1% peptone water

Method of Preparation

1. Suspend 42.5 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense 10 ml portions into tubes.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect samples in sterile containers and transport immediately to the laboratory following recommended guidelines.[4,5]
2. Process each sample using procedures appropriate for that sample.[4,5]

Test Procedure

1. Prepare serial 10-fold dilutions of the sample in 1/4 strength Ringer's solution or 0.1% peptone water.
2. Depending on the amount of the initial sample, transfer 1 ml or 0.1 ml of the appropriate dilution, prepared in step 1, to the bottom of a molten (45-50°C) DRCA tube. Prepare a duplicate tube using the same procedure.
3. Tighten the caps on the tubes.
4. Heat one of the duplicate DRCA tubes prepared in step 2 to 80 ± 1°C for 10 minutes to kill vegetative cells.
5. Incubate both tubes, heat-shocked and non-heat-shocked, at 35 ± 1°C for 5 days; examine for sulfite reduction.

Results

The presence of clostridia is presumptively indicated by blackening in the medium. Heat-shocked tubes showing blackening should be considered confirmatory for the presence of sulfite-reducing clostridia.

Limitations of the Procedure

1. Non-heat-shocked cultures showing blackening must be heat shocked and subcultured to DRCA for confirmation.

References

1. **Gibbs, B. M., and B. Freame.** 1965. Methods for the recovery of clostridia from foods. J. Appl. Microbiol. **28:**95-143.
2. **Miller, N. J., O. W. Gerrett, and T. S. Prickett.** 1939. Anaerobic technique, a modified deep agar shake. Food Research **4:**447-51.
3. Mikrobiologische Untersuchungsverfahren gemäß Anlage 3 (zu § 4 Abs. 3) der Mineral-und Tafelwasserverordnung vom 1.8. 1984, Untersuchung auf sulfitreduzierende, sporenbildende Anaerobier.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Differential Reinforced Clostridial Agar	500 g	0641-17

Bacto® Dubos Albumin Broth · Bacto Dubos Broth Base Bacto Dubos Medium Albumin · Bacto Dubos Oleic Agar Base Bacto Dubos Oleic Albumin Complex

Intended Use

Bacto Dubos Albumin Broth is used for rapidly cultivating *Mycobacterium tuberculosis*.

Bacto Dubos Broth Base is used with Bacto Dubos Medium Albumin for rapidly cultivating pure cultures of *Mycobacterium tuberculosis*.

Bacto Dubos Oleic Agar Base is used with Bacto Dubos Oleic Albumin Complex and penicillin for isolating and determining the susceptibility of *Mycobacterium tuberculosis*.

Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.[1] During the mid 1980s, the number of tuberculosis (TB) cases in the U.S. began increasing. Prior to this time, the number of cases in the U.S. had been decreasing, reaching a low in 1984.[2] Non-tuberculous mycobacteria infections have also increased since the mid 1980s.[3]

Dubos Broth is prepared according to the Dubos, Fenner and Pierce[4] modification of the medium originally described by Dubos and Davis[5] and Dubos and Middlebrook.[6]

Dubos and Middlebrook[6] described Dubos Oleic Medium Albumin as suitable for primary isolation and cultivation of the tubercle bacillus and for studying colony morphology. In comparative studies, Dubos Oleic Albumin Agar Medium was superior to other media studied for primary isolation.[7,8]

There are two types of solid culture media for the primary isolation of mycobacteria, those that have coagulated egg as a base and those that have agar. Lowenstein formulations are examples of media that contain egg; Middlebrook and Dubos formulations contain agar.

Agar based media are not liquified by contaminating proteolytic organisms but overgrowth may occur. These media are recommended for specimens from nonsterile sites.[9] The medium is clear so colonies of mycobacteria can be viewed through a stereo microscope even if contaminating organisms are present. Colonies can be observed in 10 to 12 days.

Drugs may be added to Dubos media in exact concentrations because the medium is solidified with agar rather than by inspissation. Also, there is less drug inactivation when egg ingredients are not present.

Mycobacteria grow more rapidly in broth media. Primary culture of all specimens in broth media is recommended.[10] Tween 80 in the medium acts as a surfactant, dispersing the bacilli, which increases growth.

User Quality Control

Identity Specifications

Dubos Albumin Broth

Appearance:	Almost colorless, clear to very slightly opalescent.
Reaction of Solution at 25°C:	pH 7.0 ± 0.2

Dubos Broth Base

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	0.65% solution, soluble in distilled or deionized water. Solution is very light to light amber, clear, may have a slight precipitate.
Reaction of 0.65% Solution at 25°C:	pH 6.6 ± 0.2

Dubos Medium Albumin

Appearance:	Very light amber, clear liquid.
Reaction of Solution at 25°C:	pH 6.6 ± 0.2

Dubos Oleic Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2% solution, soluble in distilled or deionized water upon boiling. Solution is light amber, slightly opalescent to opalescent with fine precipitate.
Reaction of 2% Solution at 25°C:	pH 6.6 ± 0.2

Dubos Oleic Albumin Complex

Appearance:	Light amber, clear liquid without precipitate.
Reaction of Solution at 25°C:	pH 6.8 ± 0.2

continued on following page

Principles of the Procedure

Casitone and Asparagine are sources of nitrogen. Disodium Phosphate and Monopotassium Phosphate are sources of phosphates and, along with Calcium Chloride, help maintain the pH of the medium. Magnesium Sulfate, Ferric Ammonium Sulfate, Zinc Sulfate and Copper Sulfate are sources of trace metals and sulfates. Bacto Agar is the solidifying agent.

Formula

Dubos Albumin Broth

Formula Per Liter		
Bacto Dubos Broth Base	6.5	g
Distilled or Deionized Water	900	ml
Bacto Dubos Medium Albumin	100	ml

Dubos Broth Base

Formula Per Liter

Bacto Casitone	0.5 g
Bacto Asparagine	2 g
Tween® 80	0.2 g
Monopotassium Phosphate	1 g
Disodium Phosphate (Anhyd.)	2.5 g
Ferric Ammonium Citrate	50 mg
Magnesium Sulfate	10 mg
Calcium Chloride	0.5 mg
Zinc Sulfate	0.1 mg
Copper Sulfate	0.1 mg

Dubos Medium Albumin

A 5% solution of albumin fraction V from bovine plasma and 7.5% dextrose in normal saline.

Dubos Oleic Agar Base

Formula Per Liter

Bacto Casitone	0.5 g
Bacto Asparagine	1 g
Monopotassium Phosphate	1 g
Disodium Phosphate (Anhyd.)	2.5 g
Ferric Ammonium Citrate	50 mg
Magnesium Sulfate	10 mg
Calcium Chloride	0.5 mg
Zinc Sulfate	0.1 mg
Copper Sulfate	0.1 mg
Bacto Agar	15 g

User Quality Control

Cultural Response

Dubos Albumin Broth

Prepare medium from Dubos Broth Base and Dubos Medium Albumin per label directions or use prepared Dubos Albumin Broth. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for up to 21 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Mycobacterium fortuitum	6841	300-1,000	good
Mycobacterium intracellulare	13950	300-1,000	good
Mycobacterium kansasii	12478	300-1,000	good
Mycobacterium scrofulaceum	19981	300-1,000	good
Mycobacterium tuberculosis H37 Ra	25177	300-1,000	good

Dubos Oleic Agar

Prepare medium from Dubos Oleic Agar Base and Dubos Oleic Albumin Complex per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for up to 21 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	1,000-2,000	partial inhibition
Mycobacterium fortuitum	6841	300-1,000	good
Mycobacterium intracellulare	13950	300-1,000	good
Mycobacterium kansasii	12478	300-1,000	good
Mycobacterium scrofulaceum	19981	300-1,000	good
Mycobacterium tuberculosis H37 Ra	25177	300-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Dubos Oleic Albumin Complex

A 0.05% solution of alkalinized oleic acid in a 5% solution of albumin fraction V in normal saline (0.85%).

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.
3. **Dubos Broth Base**

   IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   Dubos Oleic Agar Base

   MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

Storage

Store **Dubos Broth Base** and **Dubos Oleic Agar Base** dehydrated below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store **Dubos Albumin Broth**, **Dubos Medium Albumin** and **Dubos Oleic Albumin Complex** at 2-8°C.

Store prepared media at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Dubos Albumin Broth
Dubos Broth Base
Dubos Medium Albumin
Dubos Oleic Agar Base
Dubos Oleic Albumin Complex

Materials Required but not Provided

Glycerol
Penicillin (for preparing Dubos Oleic Agar Base)
Glassware
Distilled or deionized water
Autoclave
Incubator (CO_2, 35°C)

Method of Preparation

Dubos Broth

1. Dissolve 1.3 grams **Dubos Broth Base** in 180 ml distilled or deionized water (or 170 ml water and 10 ml Glycerol).

2. Autoclave at 121°C for 15 minutes.
3. Cool below 50°C.
4. Aseptically add 20 ml **Dubos Medium Albumin** and mix thoroughly.
5. Dispense into tubes.

Dubos Oleic Agar

1. Suspend 4 grams **Dubos Oleic Agar Base** in 180 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50-55°C.
5. Aseptically add 20 ml **Dubos Oleic Albumin Complex** and 5,000 -10,000 units penicillin (25-50 units per ml medium).
6. Mix thoroughly.
7. Dispense into sterile tubes or plates.

Specimen Collection and Preparation[7]

1. Collect specimens in sterile containers and transport immediately to the laboratory following recommended guidelines.
2. Process each specimen as appropriate for that specimen.

Test Procedure

1. Inoculate the specimen onto/into the medium and incubate tubes for up to eight weeks.
2. Examine tubes for growth.

Results

Mycobacteria grow on the medium or in the broth.

Limitations of the Procedure

1. Negative culture results do not rule out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:
   - The specimen was not representative of the infectious material, i.e., saliva instead of sputum.
   - The mycobacteria were destroyed during digestion and decontamination of the specimen.
   - Gross contamination interfered with the growth of the mycobacteria.
   - Proper aerobic conditions and increased CO_2 tension were not provided during incubation.
2. Mycobacteria are strict aerobes and growth is stimulated by increased levels of CO_2. Screw caps on tubes or bottles should remain loose for a free exchange of CO_2.

References

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4. Am. Rev. Tuberculosis, 1950, **61**:66.
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9. **Isenberg, H. D. (ed.).** 1994. Clinical microbiology procedures handbook, suppl. 1. American Society for Microbiology, Washington, D.C.
10. **Tenover, F. C., J. T. Crawford, R. E. Huebner, L. J. Geiter, C. R. Horsburgh, Jr., and R. C. Good**. 1993. The resurgence of tuberculosis: is your laboratory ready? J. Clin. Microbiol. **31**:767-770.

Packaging

Dubos Albumin Broth	20	tubes	1022-39
Dubos Broth Base	500	g	0385-17
Dubos Medium Albumin	12 x 20	ml	0309-64
Dubos Oleic Agar Base	500	g	0373-17
Dubos Oleic Albumin Complex	12 x 20	ml	0375-64

Bacto® m E Agar
Bacto Esculin Iron Agar

Intended Use

Bacto m E Agar is used with nalidixic acid and triphenyl tetrazolium chloride in isolating and differentiating enterococci from water by membrane filtration and in an *in situ* esculin test on Bacto Esculin Iron Agar.

Bacto Esculin Iron Agar is used for enumerating enterococci from water by membrane filtration based on esculin hydrolysis.

Also Known As

Esculin Iron Agar is abbreviated as EIA.

Summary and Explanation

Enterococcus species are a subgroup of fecal streptococci that includes *E. faecalis*, *E. faecium*, *E. gallinarum*, and *E. avium*.[1] Enterococci are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6, and at 10°C and 45°C.[1] The enterococci portion of the fecal streptococcus group is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters.[1]

Slanetz and Bartley[2] first reported quantitating enterococci by the membrane filter method in 1957. A wide range of levels of enterococci in water can be enumerated and detected because small or large volumes of water can be analyzed by the membrane filter technique.[3] In 1961, Kenner et al.[4] described the KF method for detecting and quantitating fecal streptococci. In 1966, Isenberg et al.[5] reported a plating procedure with differentiation based on esculin hydrolysis. Levin, Fischer and Cabelli[6] compared the KF method with Isenberg's

plating method, and found the latter method resulted in better recovery of fecal streptococci. They developed m E Agar as a primary isolation medium for enterococci, and Esculin Iron Agar as an *in situ* substrate test medium for identifying organisms capable of hydrolyzing esculin.[6]

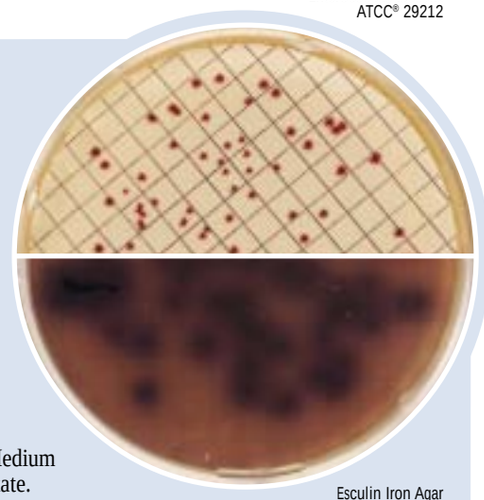
Two research projects by the Environmental Protection Agency (EPA) evaluated the relationships between swimming-associated illness and the ambient densities of indicator bacteria.[7,8] The studies demonstrated that enterococci have a better correlation with swimming-associated illness for both marine and fresh waters than fecal coliforms. *Escherichia coli* has a correlation in fresh water equal to enterococci but does not correlate as well in marine waters.[7,8] This suggests that enterococci may be better indicator organisms for some recreational waters.[7,8]

m E Agar and Esculin Iron Agar are prepared according to the formulas specified in Standard Methods.1 These media are used in the membrane filter technique for the isolation of fecal streptococcus and enterococcus groups.[1] This procedure can be used to test marine and fresh water sources.

m E Agar with the addition of 0.075% indoxyl B-D glucoside (m EI Agar) is recommended by the U.S. EPA as a one step procedure for the isolation and identification of enterococci in recreational water.[9] This method is used in the EPA Beaches Environmental Assessment Closure and Health (BEACH) Program. m EI Agar eliminates the necessity of transferring the incubated membrane to Esculin Iron Agar.

Principles of the Procedure

m E Agar is a highly selective and differential primary isolation medium that supports good growth of enterococci. Bacto Peptone and Yeast Extract provides carbon, nitrogen, minerals, vitamins and other growth factors for organism growth. Sodium Chloride maintains the osmotic balance of the medium. Nalidixic Acid and Sodium Azide act as selective agents to inhibit gram negative bacteria. Actidione® inhibits fungi. At the concentration in the formula, 2,3,5 triphenyl tetrazolium chloride (TTC) dyes enterococci colonies. TTC slightly inhibits growth of other microorganisms. In addition, the elevated incubation temperature of 41°C inhibits some indigenous microbial flora. Esculin is hydrolyzed by enterococci to form esculetin and dextrose. The esculetin reacts with the iron salt (ferric ammonium citrate) contained in the medium to produce a black to reddish brown complex that appears in the medium surrounding the colonies. The production of black to reddish brown complex verifies the colonies as enteroccci and facilitates their enumeration. Bacto Agar is the solidifying agent in the medium.



m E Agar
Enterococcus faecalis
ATCC® 29212

Esculin Iron Agar
Enterococcus faecalis
ATCC® 29212

User Quality Control

Identity Specifications

m E Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	7.12% solution, soluble in distilled or deionized water upon boiling. Light to medium amber with bluish cast, very slightly opalescent.
Prepared Medium:	Light to medium amber with blue cast, slightly opalescent.
Reaction of 7.12% Solution at 25°C:	pH 7.1 ± 0.2

Esculin Iron Agar

Dehydrated Appearance:	Tan to dark tan, free-flowing, homogeneous.
Solution: amber with	1.65%, soluble in distilled or deionized water upon boiling. Medium blue cast, very slightly opalescent without significant precipitate.
Prepared Medium:	Medium amber with blue cast, slightly opalescent without precipitate.
Reaction of 1.65% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Prepare m E Agar per label directions and pour into 9 x 50 mm plates. Dilute the test organisms and filter through membrane filters. Place the filters on m E Agar plates and incubate the plates in an upright position for 48 hours at 41 ± 0.5°C. Remove the filters and place over prepared Esculin Iron Agar plates. After 20 minutes incubation at 41 ± 0.5°C, count colonies giving positive esculin reaction (formation of black or reddish brown precipitate).

ORGANISM	ATCC®	INOCULUM CFU/10 ml	GROWTH ON m E AGAR	REACTION ON ESCULIN IRON AGAR
Enterococcus faecalis	29212*	20-60	good/pink to red colonies	black or red/brown ppt
Enterococcus faecalis	33186	20-60	good/pink to red colonies	black or red/brown ppt
Escherichia coli	25922*	20-60	marked to complete inhibition	inhibited

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

m E Agar

Formula Per Liter

Bacto Yeast Extract	30 g
Bacto Peptone	10 g
Sodium Chloride	15 g
Esculin	1 g
Actidione®	0.05 g
Sodium Azide	0.15 g
Bacto Agar	15 g

Final pH 7.1 ± 0.2 at 25°C

Esculin Iron Agar

Formula Per Liter

Esculin	1 g
Ferric Ammonium Citrate	0.5 g
Bacto Agar	15 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **m E Agar**

   HARMFUL BY INHALATION AND IF SWALLOWED. (US) IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Cardiovascular, Lungs, Nerves

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

m E Agar
Esculin Iron Agar

Materials Required But Not Provided

Bacto TTC Solution 1%
Nalidixic acid
Indoxyl β-D glucoside (optional)
Sterile Petri dishes, 50 x 9 mm
Membrane filter equipment
Sterile pipettes
Sterile 47 mm, 0.45 µm, gridded membrane filters
Autoclave
Glassware
Dilution blanks
41°C incubator or waterbath
Fluorescent lamp
Magnifying lens

Method of Preparation

m E Agar

1. Suspend 7.12 grams in 100 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45°C.
4. Add 0.024 grams of nalidixic acid and 1.5 ml TTC Solution 1% (0.015 grams triphenyl tetrazolium chloride).
5. Adjust to pH 7.1 if necessary.
6. Dispense 4-5 ml into 9 x 50 mm Petri dishes.

Note: Nalidixic acid is soluble in water with an alkaline pH.

Esculin Iron Agar

1. Suspend 1.65 grams in 100 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Dispense 4-5 ml into 9 x 50 mm Petri dishes.

Specimen Collection and Preparation

Collect water samples as described in Standard Methods for the Examination of Water and Wastewater.[1]

Test Procedure

1. Follow the membrane filter procedure described in Standard Methods for the Examination of Water and Wastewater.[1]
2. Choose a sample size so that 20-60 colonies will result.
3. Place the filter on an m E Agar plate and incubate for 48 hours at 41 ± 0.5°C.
4. After incubation, remove the filter from m E Agar and place it on Esculin Iron Agar plate. Retain at room temperature for approximately 20-30 minutes.
5. Incubate Esculin Iron Agar at 41 ± 0.5°C for 20 minutes.

Results

Pink to red enterococci develop a black or reddish-brown precipitate on the underside of the filter.[1] Count colonies using a fluorescent lamp and a magnifying lens.[1] Report results as estimated number or organisms per 100 ml of water.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. m E Agar and Esculin Iron Agar should be used in sequence.

3. Incubation at 41°C is recommended.
4. Approximately 10% false-positive esculin reactions may be expected. When used as m EI Agar, U.S. EPA reports a 6.0% false positive and 6.5% false negative rate with mE Agar.

References

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2. **Slanetz, L. W., and C. H. Bartley.** 1957. Numbers of enterococci in water, sewage, and feces determined by the membrane filter technique with an improved medium. J. Bacteriol. **74**:591-595.
3. **ASTM.** 1996. Annual book of ASTM standards. Section 11, Water and environmental technology. PCN: 01-110296-16. ASTM, West Conshohocken, PA.
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9. **U.S. Environmental Protection Agency.** 1997. EPA method 1600: Membrane filter test method for enterococci in water. U.S. Environmental Protection Agency. EPA-821-R-97-004. Washington, D.C.

Packaging

m E Agar	100 g	0333-15
	500 g	0333-17
Esculin Iron Agar	100 g	0488-15

Bacto® EC Medium

Intended Use

Bacto EC Medium is used for differentiating and enumerating coliforms in water, wastewater, shellfish and foods.

Also Known As

EC Medium is also referred to as EC Broth. EC is an abbreviation for *Escherichia coli.*

Summary and Explanation

EC Medium was developed by Hajna and Perry1 in an effort to improve the methods for the detection of the coliform group and *E. coli*. This medium consists of a buffered lactose broth with the addition of 0.15% Bile Salts No. 3. Growth of spore forming bacteria and fecal streptococci is inhibited by the bile salts, while growth of *E.coli* is enhanced by its presence. The medium can be

User Quality Control

Identity Specifications

Dehydrated Appearance	Light beige, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in distilled or deionized water. Light amber, clear.
Prepared Medium:	Light amber, clear.
Reaction of 3.7% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Prepare EC Medium per label directions. Inoculate tubes with the test organisms, and incubate at 44.5 ± 0.2°C for 24 ± 2 hours. Read tubes for growth and gas production.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	GAS PRODUCTION
Enterococcus faecalis	19433	1,000	inhibited	–
Escherichia coli	25922*	1,000	good	+
Escherichia coli	8739	1,000	good	+

Uninoculated tube — *Escherichia coli* ATCC® 25922

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disk and should be used as directed in Bactrol Disk Technical Information.

used at 37°C for the detection of coliform organisms or at 45.5°C for the isolation of *E. coli*.

In a further evaluation of EC Medium and Lauryl Tryptose Broth, Perry and Hajna[2] reported the results obtained from eleven different laboratories examining a variety of waters, milk and shellfish. The results indicate that the media are highly specific for coliform bacteria. Fishbein and Surkiewicz[3] used the EC confirmation test for recovery of *E. coli* from frozen foods and nut meats. This study[3] showed that the test is optimal when conducted at 45.5°C, with incubation limited to 24 hours.

EC Medium is employed in elevated-temperature tests for distinguishing organisms of the total coliform group that also belong to the fecal coliform group.[4] The fecal coliform test, using EC Medium, is applicable to investigations of drinking water, stream pollution, raw water sources, wastewater treatment systems, bathing waters, seawaters and general water-quality monitoring. Prior enrichment in presumptive media is required for optimum recovery of fecal coliforms when using EC Medium.

EC Medium is used in standard methods for food and water testing.[4,5,6]

Principles of the Procedure

Tryptose provides the nitrogen, vitamins and amino acids in EC Medium. Lactose is the carbon source. Bile Salts No. 3 is the selective agent against gram positive bacteria, particularly bacilli and fecal streptococci. Dipotassium Phosphate and Monopotassium Phosphate are the buffering agents. Sodium Chloride maintains the osmotic balance of the medium.

Formula

EC Medium

Formula Per Liter

Bacto Tryptose	20 g
Bacto Lactose	5 g
Bacto Bile Salts No. 3	1.5 g
Dipotassium Phosphate	4 g
Monopotassium Phosphate	1.5 g
Sodium Chloride	5 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet the specifications for identity and performance.

Procedure

Materials Provided

EC Medium

Materials Required But Not Provided

Glassware
Fermentation vials
Autoclave
Incubator or waterbath

Method of Preparation

1. Suspend 37 grams in 1 liter distilled or deionized water.
2. Warm slightly to dissolve completely.
3. Dispense into tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Obtain and process specimens according to the procedures established by laboratory policy or standard methods.[4,5,6]

Test Procedure

Follow the methods and procedures as stated in standard methods.[4,5,6]

Results

Gas production with growth in EC Medium within 24 hours or less is considered a positive fecal coliform reaction. Failure to produce gas with little or no growth, is a negative reaction.[4]

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. False-negative reactions in recovering coliforms from water supplies can occur due to low pH, refrigeration and use of bactericidal or bacteriostatic agents.[7]

References

1. **Hajna and Perry.** 1943. Am. J. Public Health **33**:550.
2. **Hajna and Perry.** 1944. Am. J. Public Health **34**:735.
3. **Fishbein and Surkiewicz.** 1964. Appl. Microbiol. **12**:127.
4. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
7. **Ray, B.** 1986. Impact of bacterial injury and repair in food microbiology: Its past, present and future. J. Food Prot. **49**:651.

Packaging

EC Medium	100 g	0314-15
	500 g	0314-17
	10 kg	0314-08

Bacto® EC Medium with MUG

Intended Use

Bacto EC Medium with MUG is used for detecting *Escherichia coli* in water, food and milk.

Also Known As

EC is an abbreviation for *Escherichia coli*.

Summary and Explanation

EC Medium was developed by Hajna and Perry[1] to improve the methods for the detection of coliforms and *E. coli*. This medium consists of a buffered lactose broth with the addition of 0.15% Bile Salts No. 3. Growth of spores formers and fecal streptococci were inhibited by the bile salts, while growth of *E. coli* is enhanced. EC Medium with MUG is the same formula as EC Medium with the addition of 4-methylumbelliferyl-ß-D-glucuronide.

Feng and Hartman[2] developed a rapid assay for *E. coli* by incorporating 4-methylumbelliferyl-ß-D-glucuronide (MUG) into Lauryl Tryptose Broth at a final concentration of 100 µg/ml. Robison[3] compared the fluorogenic assay with present methodology and found that total agreement between the two methods was 94.8%. Moburg[4] determined the amount of MUG could be reduced to a final concentration of 50 µg/ml without adversely affecting results. Koburger and Miller[5] recommended the incorporation of MUG into EC Broth for use in testing shellfish.

EC Medium with MUG is prepared according to the formula specified by US EPA[6] and standard methods for water and food testing.[7,8]

Principles of the Procedure

Tryptose provides the nitrogen, vitamins and amino acids in EC Medium with MUG. Lactose is the carbon source in this medium. Bile Salts No. 3 is the selective agent against gram-positive bacteria, particularly bacilli and fecal streptococci. Dipotassium Phosphate and Monopotassium Phosphate are buffering agents. Sodium Chloride maintains the osmotic balance of the medium.

E. coli produces the enzyme glucuronidase that hydrolyzes MUG to yield a fluorogenic product that is detectable under long-wave (366 nm) UV light. The addition of MUG to EC Medium provides another criterion, in addition to growth response and gas production, to determine the presence of *E. coli* in food and environmental samples.

Formula

EC Medium with MUG

Formula Per Liter

Bacto Tryptose	20 g
Bacto Lactose	5 g
Bacto Bile Salts No. 3	1.5 g
Dipotassium Phosphate	4 g
Monopotassium Phosphate	1.5 g
Sodium Chloride	5 g
MUG (4-methylumbelliferyl-ß-D-glucuronide)	0.05 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.71% solution, soluble in distilled or deionized water; light amber, clear.
Prepared Medium:	Light amber, clear.
Reaction of 3.71% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Prepare EC Medium with MUG per label directions. Inoculate tubes in duplicate. Incubate the first set at 35 ± 2°C for 24 hours and the second set at 44.5 ± 0.2°C. Read fluorescence under a long-wave UV light.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH AT 35°C/GAS	GROWTH AT 44.5°C/GAS	FLUORESCENCE
Enterobacter aerogenes	13048*	100-1,000	good/+	inhibited/–	–
Escherichia coli	25922*	100-1,000	good/+	good/+	+
Enterococcus faecalis	19433*	100-1,000	inhibited/–	inhibited/–	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Escherichia coli
ATCC® 25922

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

EC Medium with MUG

Materials Required But Not Provided

Test tubes
Fermentation vials
Sterile pipettes
Incubator 35°C, 44.5°C
Long-wave UV lamp
Autoclave

Method of Preparation

1. Suspend 37.1 grams in 1 liter distilled or deionized water.
2. Warm slightly to dissolve completely.
3. Dispense into test tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 15 minutes.
5. Before opening the autoclave, allow the temperature to drop below 75°C to avoid entrapping air bubbles in the fermentation vials.

Specimen Collection and Preparation

Collect food, water or other environmental samples in accordance with recommended procedures.[6,7,8]

Test Procedure

Follow the methods and procedures as stated in appropriate references.[6,7,8]

Results

Following incubation, observe tubes for growth, production of gas and fluorescence. Positive gas production is demonstrated by displacement of the medium from the fermentation vial. Positive MUG reactions exhibit a bluish fluorescence under long-wave (approximately 366 nm) UV light. Typical strains of *E. coli* are positive for both gas production and fluorescence. Non-*E. coli* coliforms that grow may exhibit fluorescence but will not produce gas.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Strains of *E. coli* that fail to grow in EC Medium with MUG, fail to produce gas, or fail to produce glucuronidase may infrequently be encountered.
3. Strains of *Salmonella*, *Shigella* and *Yersinia* that produce glucuronidase may be encountered. These strains must be distinguished from *E. coli* on the basis of other parameters, i.e., gas production, growth at 44.5°C.
4. The presence of endogenous glucuronidase in shellfish samples may cause false positive fluorescent reactions at the presumptive stage. To prevent this problem, the use of EC Medium with MUG in the confirmatory stage has been recommended.[5]

References

1. **Hajna and Perry.** 1943. Am. J. Public Health **33**:550.
2. Feng, P. C. S., and P. A. Hartman. 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. 43:1320-1329.
3. **Robison, B. J.** 1984. Evaluation of a fluorogenic assay for detection of *Escherichia coli* in foods. App. Environ. Microbiol. **48**:285-288.
4. **Moberg, L. J.** 1985. Fluorogenic assay for rapid detection of *Escherichia coli* in food. Appl. Environ. Microbiol. **50**:1383-1387.
5. **Koburger, J. A., and M. L. Miller.** 1985. Evaluation of a fluorogenic MPN procedure for determining *Escherichia coli* in oysters. J. Food Prot. **48**:244-245.
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7. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
8. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

EC Medium with MUG	100 g	0022-15
	500 g	0022-17

Bacto® EE Broth Mossel

Intended Use

Bacto EE Broth Mossel is used for selectively enriching and detecting *Enterobacteriaceae*, particularly from foods.

Summary and Explanation

EE Broth Mossel is prepared according to the formula of Mossel, Visser and Cornelissen.[1] The formula contains dextrose to facilitate growth of most *Enterobacteriaceae*, thus insuring the detection of *Salmonella* and other lactose- negative organisms. EE Broth Mossel should be used as an enrichment broth, followed by a selective medium, e.g., Violet Red Bile Agar.

The enumeration of *Enterobacteriaceae* is of great concern in monitoring the sanitary condition of food. *Enterobacteriaceae* can be injured in food-processing procedures, which include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives or sanitizers.[2] Recovery relies on proper resuscitation of damaged cells.

Principles of the Procedure

Tryptose provides nitrogen, vitamins and amino acids. Dextrose is a carbon source. Disodium Phosphate and Monopotassium

Phosphate are buffering agents. Brilliant Green and Oxgall are selective agents.

Formula

EE Broth Mossel

Formula Per Liter

Bacto Tryptose	10 g
Bacto Dextrose	5 g
Disodium Phosphate	8 g
Monopotassium Phosphate	2 g
Brilliant Green	0.0135 g
Bacto Oxgall	20 g

Final pH 7.2± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

EE Broth Mossel

Materials Required But Not Provided

Glassware
Incubator (35°C)
Waterbath

Method of Preparation

1. Dissolve 45 grams in 1 liter distilled or deionized water.
2. Dispense 120 ml amounts into 250 ml flasks.
3. Heat at 100°C (waterbath or flowing steam) for 30 minutes. **Do Not Autoclave.**

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

1. Inoculate flasks of EE Broth Mossel with approximately 10 grams of homogenized food or other material to be tested.
2. Shake the inoculated medium thoroughly for a few seconds to mix well.
3. Incubate for a total of 20-24 hours at 35-37°C. Shake the flasks after the first 3 hours of incubation.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light green, homogeneous, free-flowing.
Solution:	4.5% solution, soluble in distilled or deionized water; emerald green and clear.
Prepared Medium:	Emerald green, clear.
Reaction of 4.5% Solution at 25°C	pH 7.2 ± 0.2

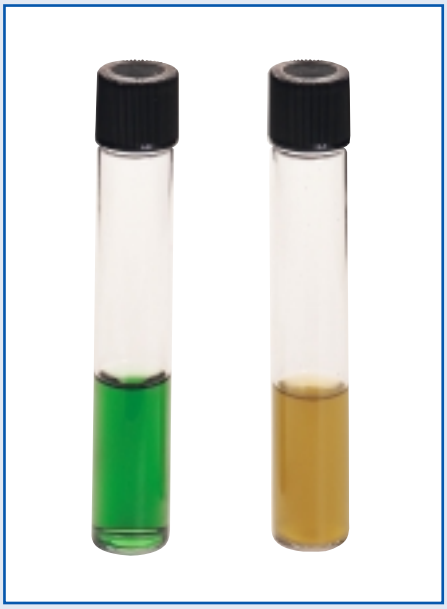
Cultural Response

Prepare EE Broth Mossel per label directions. Inoculate the medium and incubate at 35 ± 2°C for 18-24 hours and up to 48 hours if necessary.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID PRODUCTION
Escherichia coli	25922*	100-1,000	good	+ (yellow)
Staphylococcus aureus	25923*	100-1,000	inhibited	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Uninoculated tube | *Escherichia coli* ATCC® 25922

4. Prepare plates such as Violet Red Bile Agar for streaking. To ensure recovery of dextrose fermenters, add 1% dextrose before boiling.
5. Streak a loopful of the enrichment culture onto the prepared plates.
6. Incubate the plates for 18-24 hours at 35-37°C. Examine for the presence of coliforms which appear pink to purplish-red on Violet Red Bile Agar. The color of coliform colonies may vary if a different medium is used.

For a complete discussion on *Enterobacteriaceae* in food testing, refer to procedures in Standard Methods.[3,4]

Results

Acid production causes the color of EE Broth Mossel to become yellow. A negative reaction results in no color change and the medium remains green.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Mossel, D. A. A., M. Vissar, and A. M. R. Cornellisen.** 1963. The examination of foods for *Enterobacteriaceae* using a test of the type generally adopted for the detection of salmonellae. J. Appl. Bacteriol. **26**:444-452.
2. **Hartman, P. A., and S. A. Minnich.** 1981. Automation for rapid identification of *Salmonella* in foods. J. Food Prot. **44**:385-386.
3. **Association of Official Analytical Chemists**. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

EE Broth Mossel	500 g	0566-17
	10 kg	0566-08

Bacto® EMB Agar

Intended Use

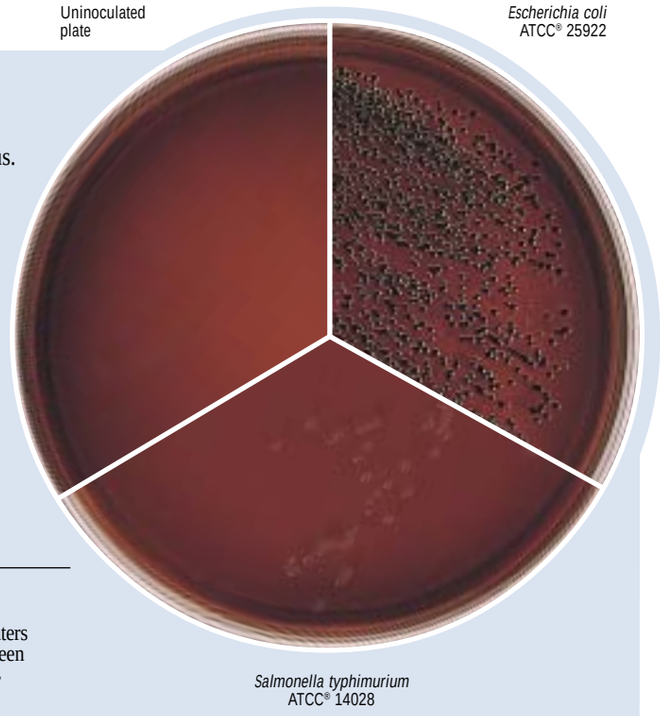
Bacto EMB Agar is used for isolating and differentiating gram-negative enteric bacilli.

Also Known As

EMB Agar is also known as Eosin Methylene Blue Agar

Summary and Explanation

The original Eosin Methylene Blue Agar was the formulation of Holt-Harris and Teague.[1] The use of eosin and methylene blue as indicators gave sharp and distinct differentiation between colonies of lactose fermenting and nonfermenting organisms. Sucrose was included in the medium to detect members of the coliform group that fermented sucrose more readily than lactose. Lactose-positive colonies were black



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pinkish purple, free flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water on boiling. Solution is green with orange cast, opalescent with a uniform flocculent precipitate.
Prepared Plates:	Purple with a greenish-orange cast, opalescent, may have a fine precipitate.
Reaction of 3.6% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare EMB Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	1,000	partial inhibition	colorless
Escherichia coli	25922*	100-1,000	good	blue-black w/dark centers and green metallic sheen
Salmonella typhimurium	14028*	100-1,000	good	colorless to amber

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

or possessed dark centers with transparent, colorless peripheries. Lactose- or sucrose-negative colonies were colorless. The Eosin Methylene Blue Agar of Holt-Harris and Teague had definite advantages over the Fuchsin Sulfite Agar of Endo. The EMB Agar formulation was more sensitive, more accurate, more stable, and gave an earlier differentiation between the lactose fermenters and lactose and sucrose nonfermenters.

Two years after Holt-Harris and Teague had introduced their new medium, Levine[2] described an Eosin Methylene Blue Agar for differentiating fecal and nonfecal coliforms. Levine's medium differentiated salmonellae and other lactose nonfermenters from the coliform organisms.

EMB Agar is a combination of the Levine and the Holt-Harris and Teague formulae. EMB Agar is selective due to the presence of inhibitors and differential based on the ability of some organisms to ferment carbohydrates with the absorption of eosin and methylene blue.

EMB Agar is recommended for use in examining clinical specimens for enteric pathogens.[3,4,5] The medium enables the isolation and differentiation of gram-negative enteric bacilli.

Principles of the Procedure

Peptone is a source of nitrogen and other nutrients in the formulation. Eosin and methylene blue are dyes which combine to form a precipitate at an acid pH. The dyes act both as pH indicators and inhibitors. Gram-positive bacteria are partially inhibited on the medium. Lactose and Sucrose are fermentable carbohydrates. Phosphate acts as a buffer. Bacto Agar is a solidifying agent.

Formula

EMB Agar

Formula Per Liter

Bacto Peptone	10 g
Bacto Lactose	5 g
Bacto Sucrose	5 g
Dipotassium Phosphate	2 g
Bacto Agar	13.5 g
Eosin Y	0.4 g
Methylene Blue	0.065 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared plates at 2-8°C.

Expiration Date

Expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

EMB Agar

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50)°C
Petri dishes
Incubator (35°C)

Method of Preparation

1. Suspend 36 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.
4. Cool to 45-50°C in a waterbath.
5. Dispense into sterile Petri dishes. Evenly disperse the precipitate when dispensing.

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[3,4,5]
2. For specific information about specimen preparation and inoculation of clinical specimens, consult appropriate references.[3,4,5]

Test Procedure

For isolation of enteric pathogens from clinical specimens, inoculate fecal specimens and rectal swabs onto a small area of one quadrant of the EMB Agar plate and streak for isolation. This will permit development of discrete colonies. Incubate plates at 35°C. Examine plates at 24 hours and again at 48 hours for colonies with characteristic morphologies associated with potential pathogens.

Results

Salmonella and *Shigella* colonies are translucent and amber colored or colorless. Coliforms that use lactose and/or sucrose produce blue-black colonies with dark centers and greenish metallic sheen. Other coliforms such as *Enterobacter* form mucoid, pink colonies. Strains of *Enterococcus faecalis* are partially inhibited on this medium and appear as colorless colonies.

Limitations of the Procedure

1. EMB Agar is only moderately inhibitory. Some staphylococci, streptococci and yeast may grow. They will appear as small, pinpoint colonies. Gram-negative nonfermenting bacilli may grow and appear as non-lactose fermenters. Biochemical tests are necessary for further identification to genus or species.[6]
2. Some strains of *Salmonella* and *Shigella* may not grow on EMB Agar.[6] It is recommended that a nonselective, differential medium (MacConkey Agar or Hektoen Enteric Agar) and a selective medium (Bismuth Sulfite Agar, SS Agar or Desoxycholate Citrate Agar) be run in parallel with EMB Agar.
3. Sterilization reduces the methylene blue, leaving the medium orange in color. The normal purple color of the medium may be restored by gentle mixing. If the reduced medium is not shaken to oxidize the methylene blue, a dark zone beginning at the top and extending downward through the medium will gradually appear. The sterilized medium normally contains a flocculent precipitate which should not be removed. By cooling to 50°C and gently

mixing the medium before pouring it into plates, the flocculation will be finely dispersed.

4. Greenish metallic sheen is **not** always present. The presence of the greenish metallic sheen is **not** diagnostic for *E. coli*.[6]
5. Store and incubate EMB Agar plates in the dark. Visible light can alter the ability of the medium to support microbial growth, especially of *Proteus* spp.[7]

References

1. **Holt-Harris, J. E., and O. Teague.** 1916. A new culture medium for the isolation of *Bacillus typhosa* from stools. J. Infect. Dis. **18:**596-600.
2. **Levine, M. M.** 1918. Differentiation of *E. coli* and *B. aerogenes* on a simplified Eosin-Methylene Blue Agar. J. Infect. Dis. **23:**43.
3. **Gray, L. D.** 1995. *Escherichia, Salmonella, Shigella and Yersinia*, p. 450-456. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Pezzlo, M. (ed.).** 1992. Aerobic bacteriology, p. 1.0.1-1.20.47. *In* H. D. Isenberg, (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
7. **Girolami, R. L., and J. M. Stamm.** 1976. Inhibitory effect of light on growth-supporting properties of Eosin Methylene Blue Agar. Appl. Environ. Microbiol. **31:**141.

Packaging

EMB Agar	100 g	0076-15
	500 g	0076-17
	2 kg	0076-07
	10 kg	0076-08

Bacto® EVA Broth

Intended Use

Bacto EVA Broth is used for detecting and confirming enterococci in water and other specimens as an indication of fecal contamination.

Also Known As

EVA Broth is also known as Ethyl Violet Azide Broth.

Summary and Explanation

The presence of enterococci in water and other specimens indicates fecal contamination. Mallmann and Seligmann[1] compared various enrichment media for detecting fecal streptococci and found that Azide Dextrose Broth presumptively identified the streptococci. However, because gram-positive bacteria other than enterococci grow in the medium, confirmation is necessary. Litsky et al.[2] studied various dyes and selective agents and formulated a medium using ethyl violet and sodium azide as selective agents. The medium known as Ethyl Violet Azide (EVA) Broth is specific for enterococci. In conjunction with Azide Dextrose Broth, EVA Broth is used to confirm the presence of enterococci.

Principles of the Procedure

EVA Broth contains Tryptose as a source of carbon, nitrogen, vitamins and minerals. Dextrose is the carbohydrate. Sodium Azide and

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.58% solution, soluble in distilled or deionized water. Solution is light amber, clear to very slightly opalescent.
Reaction of 3.58% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare EVA Broth per label directions. Inoculate and incubate the tubes at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus faecalis	19433*	100-1,000	good
Enterococcus faecalis	29212*	100-1,000	good
Escherichia coli	25922*	1,000-2,000	inhibited

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube — *Enterococcus faecalis* ATCC® 29212

Ethyl Violet inhibit gram-positive bacilli and gram-positive cocci other than enterococci. Monopotassium and Dipotassium Phosphates buffer the medium. Sodium Chloride provides osmotic balance.

Formula

EVA Broth

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	5 g
Dipotassium Phosphate	2.7 g
Monopotassium Phosphate	2.7 g
Sodium Chloride	5 g
Sodium Azide	0.4 g
Ethyl Violet	0.00083 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Nerves, Lungs, Cardiovascular.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

EVA Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 35.8 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Growth of enterococci.

References

1. **Mallmann and Seligmann**. 1950. Am. J. Pub. Health **40**:286.
2. **Litsky, Mallmann, and Fifield**. 1953. Am. J. Pub. Health **43**:873.

Packaging

EVA Broth	500 g	0606-17

Bacto® Egg Meat Medium

Intended Use

Bacto Egg Meat Medium is recommended for cultivating *Clostridium* cultures used in detecting the sporicidal activity of disinfectants.

Summary and Explanation

Egg Meat Medium is a dehydrated medium containing particles of meat, egg white and calcium carbonate.

The use of a combination meat and egg white culture medium was reported by Rettger[1] in his studies on *Escherichia coli* and *Enterobacter*

User Quality Control

Identity Specifications

Dehydrated Appearance:	Brown, free-flowing, homogeneous pellets.
Solution:	15% solution, insoluble in distilled or deionized water. Solution is a light to medium amber, clear to very slightly opalescent supernatant over insoluble pellets.
Reaction of 15% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Egg Meat Medium per label directions. Inoculate tubes and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacillus subtilis	19659*	100-1,000	good
Clostridium sporogenes	3584*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

aerogenes. Later, he described the use of this medium in studies of intestinal putrefaction.[2] In 1923, Reddish and Rettger[3] used the medium in their detailed study of *Clostridium putrificum* and, the following year, in a study of other spore-forming anaerobes.[4]

AOAC[5] recommends Egg Meat Medium to propagate and maintain cultures of *Clostridium* for use in testing sporicidal activity of liquid and gaseous chemicals.

Principles of the Procedure

Beef muscle provides carbon, nitrogen, inorganic salts, vitamins, and a variety of other nutrients to support bacterial growth. Egg whites are a source of protein. Calcium carbonate helps neutralize acids and displaces oxygen in the media.

Formula

Egg Meat Medium

Formula per liter

Beef Muscle, from	454 g
Egg White, from	6 eggs
Calcium Carbonate	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Egg Meat Medium

Materials Required but not Provided

Glassware
Garden soil extract (AOAC, 1995, 6.3.05, A.[a.][1.])[5]
Autoclave

Method of Preparation

1. Suspend 1.5 grams in 15 ml garden soil extract.[5] Let stand at least 15 minutes to form a thoroughly wetted, even suspension.
2. Autoclave at 121°C for 15 minutes. Allow to cool.
3. Avoid a rapid release of pressure after sterilization to prevent expelling the medium from the test tubes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Refer to AOAC[5] for detailed procedures to determine presence or absence of sporicidal activity of disinfectants against specified spore-forming bacteria.

Results

Refer to appropriate references and procedures for results.

References

1. **Rettger, L. F.** 1903. An experimental study of the chemical products of Bacillus coli communis and Bacillus lactis aerogenes. Am. J. Physiol. **8:**284.
2. **Rettger, L.** 1906. Studies on putrefaction. J. Biol. Chem. **2:**71.
3. **Reddish, G. F., and L. F. Rettger.** 1923. Clostridium putrificum. II. Morphological, cultural and biochemical study. J. Bact. **8:**375.
4. **Reddish, G. F., and L. F. Rettger.** 1924. A morphological, cultural and biochemical study of representative spore-forming anaerobic bacteria. J. Bact. **9:**13.
5. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Egg Meat Medium	500 g	0042-17

Bacto® Elliker Broth

Intended Use

Bacto Elliker Broth is used for cultivating streptococci and lactobacilli, particularly in dairy procedures.

Also Known As

Elliker Broth is also called Lactobacilli Broth.

Summary and Explanation

Testing for lactic acid bacteria in dairy products may be useful for various reasons.[3] These include determining the cause of acid defects in dairy products, evaluating lactic starter cultures, and controlling the quality of cured cheese, cultured milks, and uncultured products.[3] Lactic acid bacteria found in dairy products are primarily *Streptococcus*, *Lactococcus*, *Leuconostoc* and *Lactobacillus*.[3]

Elliker Broth is prepared according to the formulation of Elliker, Anderson and Hannesson,[1] and modified by McLaughlin.[2] This slightly acidic medium contains nutrients to support the growth of streptococci and lactobacilli.

A modification of Elliker Broth, Lactic (Elliker) Agar is recommended for general purpose enumeration of lactic acid bacteria.[3]

Principles of the Procedure

Tryptone and Gelatin provide the nitrogen and amino acids in Elliker Broth. Yeast Extract is the vitamin source in this formula. Dextrose, Lactose and Saccharose are the fermentable carbohydrates. Sodium

Chloride maintains the osmotic balance of the medium, and ascorbic acid is added to create a proper environment for organism growth. Sodium Acetate is a selective agent against gram negative bacteria.

Formula

Elliker Broth

Formula Per Liter

Bacto Tryptone	20 g
Bacto Yeast Extract	5 g
Bacto Gelatin	2.5 g
Bacto Dextrose	5 g
Bacto Lactose	5 g
Bacto Saccharose	5 g
Sodium Chloride	4 g
Sodium Acetate	1.5 g
Ascorbic Acid	0.5 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet the specifications for identity and performance.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.85% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, clear without significant precipitate.
Prepared Medium:	Light to medium amber, clear without significant precipitate.
Reaction of 4.85% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Elliker Broth per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours except *Streptococcus cremoris* which is incubated at 30 ± 2°C.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus casei	7469	100-1,000	good
Lactobacillus delbrueckii subsp. *lactis*	8000	100-1,000	good
Streptococcus cremoris	9596	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Procedure

Materials Provided

Elliker Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Sterile tubes

Method of Preparation

1. Suspend 48.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Dispense as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion on the isolation and identification of streptococci and lactobacilli, refer to standard methods in food testing.[3,4,5]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Elliker, P. R., A. W. Anderson, and G. Hannesson.** 1956. An agar culture medium for lactic acid streptococci and lactobacilli. J. Dairy Sci. **39**:1611.
2. **McLaughlin, C. B.** 1946. Readily prepared medium for cultivation of lactobacilli. J. Bacteriol. **51**:560.
3. **Frank, J. F., G. L. Christen, and L. B. Bullerman.** 1993. Test for groups of microorganisms, p. 271-286. *In* R. T. Marshall (ed.), Standard methods for the examination of dairy products. 16th ed. American Public Health Association, Washington, D.C.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological association of food, 3rd. ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

Elliker Broth	500 g	0974-17

Bacto® Emerson YpSs Agar

Intended Use

Bacto Emerson YpSs Agar is used for cultivating *Allomyces* and other fungi.

Summary and Explanation

Emerson YpSs Agar is prepared according to the formula given by Emerson.[1] Emerson and Wilson[2] used the medium at half strength for streaking zygotes or zoospores to obtain single germlings.

Fungi are extremely successful organisms, as evidenced by their ubiquity in nature.[3] Of the estimated 250,000 species, fewer than 150 are known primary human pathogens.[3] Opportunistic fungal pathogens are increasing at an impressive rate relating directly to the expanding size of the immunocompromised to patient population.[3]

Principles of the Procedure

Yeast Extract provides a source of trace elements, vitamins and amino acids. Soluble Starch provides starch for hydrolysis, detoxification of metabolic byproducts and as a carbon source. Dipotassium Phosphate is a buffer. Magnesium Sulfate is a source of divalent cations and sulfate. Bacto Agar is the solidifying agent.

Formula

Emerson YpSs Agar

Formula Per Liter

Bacto Yeast Extract	4 g
Bacto Soluble Starch	15 g
Dipotassium Phosphate	1 g
Magnesium Sulfate	0.5 g
Bacto Agar	20 g

Final pH 7.0 + 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.05% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, slightly opalescent, may have a slight flocculent precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent, may have a slight flocculent precipitate.
Reaction of 4.05% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Emerson YpSs Agar per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Allomyces macrogynus	38327	100-300	good
Allomyces reticulatus	42465	100-300	good
Aspergillus niger	16404	100-300	good
Saccharomyces cerevisiae	9763	100-300	good

The cultures listed are the minimum that should be used for performance testing.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Emerson YpSs Agar

Materials Required But Not Provided

Glassware
Autoclave
Sterile Petri dishes
Waterbath (optional)

Method of Preparation

1. Suspend 40.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

Refer to appropriate references for specific procedures on the isolation and cultivation of fungi.

Results

Refer to appropriate references and procedures for results.

References

1. **Lloydia.** 1941. **4**:77.
2. **Mycologia.** 1954. **46**:393.
3. **Dixon, D. M., and R. A. Fromtling.** 1995. Morphology, taxonomy, and classification of the fungi, p. 699-708. *In* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Emerson YpSs Agar	500 g	0739-17

Bacto® Endo Agar

Intended Use

Bacto Endo Agar is used for confirming the presence of coliform organisms.

Summary and Explanation

Endo[1] developed a medium using a fuchsin sulfite indicator to differentiate lactose fermenting and lactose non-fermenting organisms. Coliform organisms that ferment lactose produce red colonies and color the surrounding medium. Typical reactions of this medium are not caused by acid production but by the intermediate product acetaldehyde, which reacts with sodium sulfite.[2-3]

Endo Agar was formerly a standard methods medium for the microbiological examination of water[4] and dairy products.[5]

Principles of the Procedure

Lactose-fermenting bacteria produce acetaldehyde. The aldehyde is fixed by the sodium sulfite and in the presence of fuchsin forms red colonies. A sheen is produced by rapid lactose fermenting organisms. Lactose non-fermenting bacteria form clear, colorless colonies.

Endo Agar contains Bacto Peptone as a source of carbon, nitrogen, vitamins and minerals. Lactose is the carbohydrate source. Basic Fuchsin in the presence of Sodium Sulfite produces the red colonies. Bacto Agar is the solidifying agent.

Formula

Endo Agar

Formula Per Liter

Bacto Peptone	10 g
Bacto Lactose	10 g
Dipotassium Phosphate	3.5 g
Bacto Agar	15 g
Bacto Basic Fuchsin	0.5 g
Sodium Sulfite	2.5 g

Final pH 7.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Liver, Thyroid.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Medium purple, free-flowing, homogeneous.
Solution:	4.15% solution, soluble in distilled or deionized water on boiling. Solution is pink, slightly opalescent, may have a slight precipitate.
Prepared Medium:	Pink, slightly opalescent, may have a slight precipitate.
Reaction of 4.15% Solution at 25°C:	pH 7.5 ± 0.2

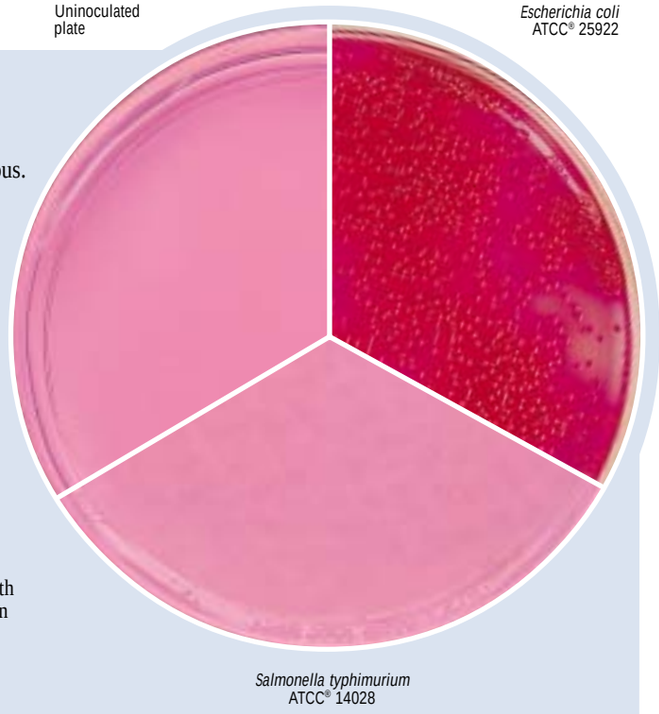
Cultural Response

Prepare Endo Agar per label directions. Inoculate plates and incubate at 35 ± 2°C for 24 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Escherichia coli	25922*	100-1,000	good	pink to red with metallic sheen
Salmonella typhimurium	14028*	100-1,000	good	colorless to light pink
Staphylococcus aureus	25923	1,000-2,000	marked to complete inhibition	

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Endo Agar

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 41.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Evenly disperse the precipitate when dispensing.
5. Use immediately.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure

See appropriate references for specific procedures.

Results

Rapid lactose fermenting organisms will produce red colonies that have a metallic sheen. Slow lactose fermenting organisms will produce red colonies. Lactose non-fermenting organisms will produce colorless colonies.

Limitations of the Procedure

If the medium is to be used the same day it is rehydrated, it does not need to be autoclaved. Boil to dissolve completely before dispensing into plates.

References

1. **Endo, S.** 1904. Uber ein Verfahren zum Nachweis der Typhusbacillen. Centr. Bakt., Abt 1, Orig. **35**:109-110.
2. **Margolena, L. A., and P. A. Hansen.** 1933. The nature of the reaction of the colon organism on Endo's medium. Stain Tech. **8**:131-139.
3. **Neuberg, C., and F. F. Nord.** 1919. Anwendungen der abfangmethode auf die bakteriengarungen. Biochem. Zeit. **96**:133-174.
4. **American Public Health Association**. 1975. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D.C.
5. **American Public Health Association**. 1972. Standard methods for the examination of dairy products, 13th ed. American Public Health Association, Washington, D.C.

Packaging

Endo Agar	500 g	0006-17

Bacto® m Endo Agar LES

Intended Use

Bacto m Endo Agar LES is used for enumerating coliforms in water by membrane filtration.

Also Known As

LES (Lawrence Experimental Station) Endo Agar

Summary and Explanation

McCarthy, Delaney and Grasso[1] formulated Endo Agar LES (Lawrence Experimental Station) for testing water for coliform bacteria by a two-step membrane filter procedure using Lauryl Tryptose Broth as a preliminary enrichment. They recovered higher numbers of coliforms by this method compared with the one step technique using m Endo Broth.

The American Public Health Association specifies using m Endo Agar LES in the standard total coliform membrane filtration procedure for testing drinking water[2] and bottled water.[3] It is also specified for use in the completed phase of the standard total coliform fermentation technique.[2] The coliform bacteria are bacteria that produce a red colony with a metallic (golden) sheen within 24 hours incubation at 35°C on an Endo-type medium.

The U. S. Environmental Protection Agency specifies using m Endo Agar LES in the total coliform methods for testing water using single-step, two-step and delayed incubation membrane filtration methods.[4,5]

Principles of the Procedure

m Endo Agar LES contains tryptose, casitone and thiopeptone as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins, which stimulate bacterial growth. Lactose is the carbohydrate. Phosphates are buffering agents. Sodium Chloride maintains the osmotic balance of the medium. Sodium Desoxycholate and Sodium Lauryl Sulfate are added as inhibitors. Basic Fuchsin is a pH indicator. Sodium Sulfite is added to decolorize the Basic Fuchsin solution. Bacto Agar is the solidifying agent.

Lactose-fermenting bacteria produce acetaldehyde that reacts with the sodium sulfite and fuchsin to form red colonies. The development of a metallic sheen occurs when the organism produces aldehydes with the rapid fermentation of lactose. If the inoculum is too heavy, the sheen will be suppressed. Lactose non-fermenting bacteria form clear, colorless colonies.

Formula

m Endo Agar LES

Formula Per Liter

Bacto Yeast Extract	1.2 g
Bacto Casitone	3.7 g
Thiopeptone	3.7 g
Bacto Tryptose	7.5 g
Bacto Lactose	9.4 g
Potassium Phosphate Dibasic	3.3 g
Potassium Phosphate Monobasic	1 g
Sodium Chloride	3.7 g
Sodium Desoxycholate	0.1 g
Sodium Lauryl Sulfate	0.05 g
Sodium Sulfite	1.6 g
Bacto Basic Fuchsin	0.8 g
Bacto Agar	15 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Liver, Thyroid.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Purple, free-flowing, homogeneous.
Solution:	5.1% solution, soluble in distilled or deionized water containing 2% ethanol on boiling. Solution is pinkish-red, slightly opalescent to opalescent with precipitate.
Prepared Medium:	Rose colored, slightly opalescent, with precipitate.
Reaction of 5.1% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare m Endo Agar LES per label directions. Use the membrane filter technique to inoculate filters and preincubate on pads saturated with Lauryl Tryptose Broth (0241) at 35 ± 2°C for 1 1/2-2 hours. Transfer filters to plates of m Endo Agar LES and incubate at 35 ± 2°C for 22 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Escherichia coli	25922*	20-80	good	red with sheen
Salmonella typhimurium	14028	20-80	good	pink
Staphylococcus aureus	25923*	1,000-2,000	marked to complete inhibition	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

m Endo Agar LES
Lauryl Tryptose Broth
Materials Required but not Provided
95% Ethanol (Not denatured)
Glassware
Distilled or deionized water
Membrane filter apparatus
Membrane filter absorbent pads
Petri dishes, 60 mm
Incubator (35°C)

Method of Preparation

m Endo Agar LES

1. Suspend 51 grams in 1 liter distilled or deionized water containing 20 ml ethanol (95% not denatured).
2. Boil to dissolve completely. **Do Not Autoclave**.
3. Dispense in 5-7 ml quantities into 60 mm sterile petri dishes.

Lauryl Tryptose Broth

1. Suspend 35.6 g in 1 liter of distilled or deionized water.
2. Warm slightly to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Collect samples and process according to recommended guidelines for enumerating coliforms in water.[2,4,5]

Test Procedure

1. Place a membrane filter absorbent pad inside the cover.
2. Add 1.8-2.0 ml Lauryl Tryptose Broth to each pad.
3. Run the water sample through a membrane filter.
4. Place the filter, top side up, onto the pad containing Lauryl Tryptose Broth. Use a rolling motion to avoid entrapping air bubbles.
5. Incubate at 35 ± 2°C for 1½-2½ hours. Transfer the membrane from the pad to the surface of the m Endo Agar LES medium in the petri dish bottom, keeping the side on which the bacteria have been collected facing upward.

6. Leave the filter pad in the lid and incubate the plates in the inverted position at 35 ± 2°C for 22 ± 2 hours.
7. Observe and count all colonies that are red and have a metallic sheen.

Results

All colonies that are red and have the characteristic metallic sheen are considered coliforms. The sheen may cover the entire colony, may only be in the center or may appear only around the edges.

Limitations of the Procedure

1. Occasionally, noncoliform organisms may produce typical sheen colonies. Coliform organisms may also occasionally produce atypical colonies (dark red or nucleated colonies without sheen). It is advisable to verify both colony types.[2]

References

1. **McCarthy, J. A., J. E. Delaney, and R. J. Grasso**. 1961. Measuring coliforms in water. Water Sewage Works. **108**:238.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg** (ed.), 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Cowman, S., and R. Kelsey.** 1992. Bottled water, p. 1031-1036. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Bordner, R., and J. Winter (ed.)**, 1978. Microbiological methods for monitoring the environment, water and wastes. EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Cincinnati, OH.
5. **Environmental Protection Agency**. 1992. Manual for the certification of laboratories analyzing drinking water. EPA-814B-92-002. Office of Ground Water and Technical Support Division, U. S. Environmental Protection Agency, Cincinnati, OH.

Packaging

m Endo Agar LES	100 g	0736-15
	500 g	0736-17
Lauryl Tryptose Broth	100 g	0241-15
	500 g	0241-17
	2 kg	0241-07
	10 kg	0241-08

Bacto® m Endo Broth MF®

Intended Use

Bacto m Endo Broth MF® is used for enumerating coliform organisms in water by membrane filtration.

Also Known As

m Endo Medium. MF is a registered trademark of Millipore Filter.

Summary and Explanation

Bacto m Endo Broth MF is prepared according to the formulation of the Millipore Filter Corporation.[1] for selectively isolating coliform bacteria from water and other specimens using the membrane filtration technique. The medium is a combination of the former m HD Endo Medium and Lauryl Tryptose Broth.

The American Public Health Association specifies using m Endo Broth MF in the standard total coliform membrane filtration procedure for testing water[2] and bottled water.[3] APHA also specifies using m Endo Broth MF in the delayed-incubation total coliform procedure by adding sodium benzoate to make m Endo preservative medium.[2] The coliform bacteria are defined as bacteria that produce a red colony with a metallic sheen within 24 hours incubation at 35°C on an Endo-type medium.

The U. S. Environmental Protection Agency specifies using m Endo Broth MF in the total coliform methods for testing water using single-step, two-step and delayed incubation membrane filtration methods.[4,5]

Principles of the Procedure

m Endo Broth MF contains Tryptose, Casitone and Thiopeptone as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins, which stimulate bacterial growth. Lactose is the carbohydrate. Phosphates are buffering agents. Sodium Chloride maintains the osmotic balance of the medium. Sodium Desoxycholate and Sodium Lauryl Sulfate are added as inhibitors. Basic Fuchsin is a pH indicator. Sodium Sulfite is added to decolorize the Basic Fuchsin solution. Bacto Agar is the solidifying agent.

Lactose-fermenting bacteria produce acetaldehyde that reacts with the sodium sulfite and fuchsin to form red colonies. The development of a metallic sheen occurs when the organism produces aldehydes with the rapid fermentation of lactose. If the inoculum is too heavy, the sheen will be suppressed. Lactose non-fermenting bacteria form clear, colorless colonies.

Formula

m Endo Broth MF

Formula Per Liter

Bacto Yeast Extract	1.5 g
Bacto Casitone	5 g
Thiopeptone	5 g
Bacto Tryptose	10 g
Bacto Lactose	12.5 g
Sodium Desoxycholate	0.1 g
Dipotassium Phosphate	4.375 g
Monopotassium Phosphate	1.375 g
Sodium Chloride	5 g
Sodium Lauryl Sulfate	0.05 g
Sodium Sulfite	2.1 g
Bacto Basic Fuchsin	1.05 g

Final pH 7.2 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM

AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Liver, Thyroid

FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

m Endo Broth MF

Materials Required but not Provided

95% Ethanol (Not denatured)
Glassware
Distilled or deionized water
Membrane filter apparatus
Membrane filters
Membrane filter absorbent pads
Petri dishes, 60 mm
Incubator (35°C)

Method of Preparation

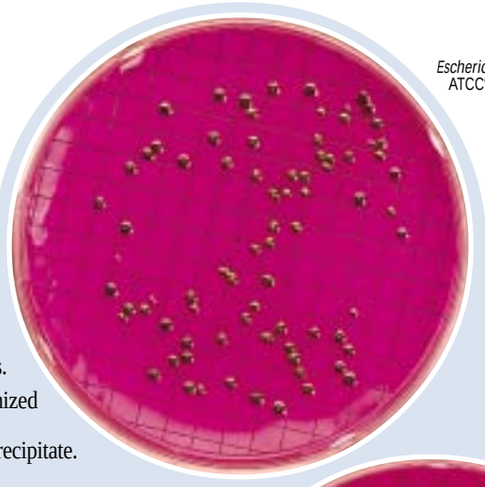
1. Suspend 48 grams in 1 liter distilled or deionized water containing 20 ml ethanol (95%, not denatured).
2. Boil to dissolve completely. **Do Not Autoclave**.

Specimen Collection and Preparation

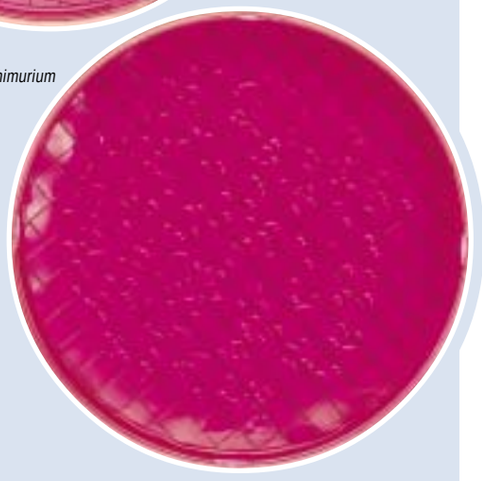
Collect samples and process according to recommended guidelines for enumerating coliforms in water.[2,3,4,5]

Test Procedure

1. Place a membrane filter absorbent pad inside a sterile 60 mm petri dish.
2. Add 1.8-2.0 ml m Endo Broth MF to each pad.
3. Filter the water sample through a membrane filter.
4. Place filter top side up on the pad using a rolling motion to avoid entrapping air bubbles.
5. Incubate at 35°C for 24 ± 2 hours.
6. Observe and count all colonies that are red and have a metallic sheen.



Escherichia coli
ATCC® 25922



Salmonella typhimurium
ATCC® 14028

User Quality Control

Identity Specifications

Dehydrated Appearance:	Pinkish purple, free-flowing, homogeneous.
Solution:	4.8% solution, soluble in distilled or deionized water containing 2% ethanol on boiling. Solution is pinkish-red, opalescent with precipitate.
Reaction of 4.8% Solution at 25°C:	pH 7.2 ± 0.1

Cultural Response

Prepare m Endo Broth MF per label directions. Use the membrane filter technique to inoculate filters. Incubate on pads saturated with m Endo Broth MF at 35 ± 2°C for 24 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Escherichia coli	25922*	20-80	good	red with green metallic sheen
Salmonella typhimurium	14028	20-80	good	colorless to pink
Staphylococcus aureus	25923*	1,000-2,000	marked to complete inhibition	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Results

All colonies that are red and have the characteristic metallic sheen are considered coliforms. The sheen may cover the entire colony, may only be in the center or may appear only around the edges.

Limitations of the Procedure

1. Occasionally, noncoliform organisms may produce typical sheen colonies. Coliform organisms may also occasionally produce atypical colonies (dark red or nucleated colonies without sheen). It is advisable to verify both colony types.[2]

References

1. **Fifield, C. W., and C. P. Schaufus.** 1958. Improved membrane filter medium for the detection of coliform organisms. J. Amer. Water Works Assoc. **50:**193.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Cowman, S., and R. Kelsey.** 1992. Bottled water, p. 1031-1036. *In* C. Vanderzant, and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Bordner, R., and J. Winter (ed).** 1978. Microbiological methods for monitoring the environment, water and wastes. EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Cincinnati, OH.
5. **Environmental Protection Agency**. 1992. Manual for the certification of laboratories analyzing drinking water. EPA-814B-92-002. Office of Ground Water and Technical Support Division, U. S. Environmental Protection Agency, Cincinnati, OH.

Packaging

m Endo Broth MF	100 g	0749-15
	500 g	0749-17
	25 x 2 ml	0749-36

Bacto® Enteric Fermentation Base

Intended Use

Bacto Enteric Fermentation Base is used with added carbohydrate and indicator for differentiating microorganisms based on fermentation reactions.

Summary and Explanation

The fermentative properties of bacteria are valuable criteria in their identification.[1,2,3,4] A basal medium for determining the fermentation reactions of microorganisms must be capable of supporting growth of

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	1.8% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Medium plain + Andrede's Indicator:	Light pinkish amber, clear without precipitate.
Reaction of 1.8% Solution at 25°C:	pH 7.2 ± 0.1

Cultural Response

Prepare Enteric Fermentation Base per label directions, with addition of 1% Andrade's Indicator, with and without 1% dextrose. Inoculate each tube with one drop from an undiluted suspension of the test organism. Incubate at 35 ± 2°C for 18-24 hours. Acid production is indicated by a change in color from light amber to dark pink or red. Check for gas production in at least 3% of the volume of the fermentation vial.

ORGANISM	ATCC®	GROWTH	PLAIN ACID/GAS	w/ DEXTROSE ACID/GAS
Escherichia coli	25922*	good	–/–	+/+
Salmonella typhimurium	14028*	good	–/–	+/+
Shigella flexneri	12022*	good	–/–	+/–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube | *Escherichia coli* ATCC® 25922 with Dextrose | *Escherichia coli* ATCC® 25922 plain

test organisms and be free from fermentable carbohydrates. Enteric Fermentation Base is prepared according to the formula described by Edwards and Ewing.[5,6]

Principles of the Procedure

Beef Extract and Peptone provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Sodium Chloride maintains the osmotic balance of the medium. The microorganisms tested are differentiated by their ability to ferment a particular carbohydrate that has been added to the Enteric Fermentation Base. The fermentation and resultant acid production are indicated by a change in color of the pH indicator (Andrade's indicator) which is also added to the Enteric Fermentation Base.

Formula

Enteric Fermentation Base

Formula Per Liter

Beef Extract	3 g
Peptone	10 g
Sodium Chloride	5 g

Final pH 7.2 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Enteric Fermentation Base

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Choice of filter-sterilized carbohydrate(s)
Andrade's Indicator (0.5 g Acid Fuchsin in 100 ml water plus 16 ml of 1.0 N Sodium Hydroxide)

Method of Preparation

1. Suspend 18 grams in 1 liter distilled or deionized water.
2. Add 10 ml Andrade's Indicator.
3. Heat to boiling to dissolve completely.
4. Autoclave at 121°C for 15 minutes.
5. Cool to 45 to 50°C in a waterbath.
6. Add appropriate amounts of sterile carbohydrates as indicated in the table below.

CARBOHYDRATE	FINAL CONCENTRATION	ADD BEFORE AUTOCLAVING	ADD AFTER AUTOCLAVING
Adonitol	0.5%	X	–
Arabinose	0.5%	–	X
Cellobiose	0.5%	–	X
Dextrose (Glucose)	1%	X	–
Dulcitol	0.5%	X	–
Glycerol*	0.5%	X	–
Inositol	0.5%	X	–
Lactose	1%	–	X
Mannitol	1%	X	–
Salicin	0.5%	X	–
Sucrose	1%	–	X
Xylose	0.5%	–	X

*Medium containing glycerol should be autoclaved for 10 minutes at 15 lbs pressure (121°C).

7. Dispense 3 ml amounts into test tubes containing inverted fermentation vials (Durham tubes).

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[7,8,9]
2. Process each specimen, using procedures appropriate for that specimen or sample.[7,8,9]

Test Procedure

For a complete discussion on identification of *Enterobacteriaceae*, refer to the appropriate procedures outlined in the references.[5,6,10]

Results

A positive result for gas includes production in at least 3% of the volume of the fermentation tube. A positive reaction for gas production is a change in color from light amber to dark pink or red.

Limitations of the Procedure

1. Negative tubes remain colorless and should be observed regularly for a total of 30 days.

References

1. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th edition. Mosby-Year Book, Inc., St. Louis, MO.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken. (ed.)**. 1995. Manual of clinical microbiology, 6th edition. ASM Press, Washington, D.C.
3. **Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams.** 1994. Bergey's manual of determinative bacteriology, 9th edition. Williams & Wilkins, Baltimore, MD.
4. **Ewing, W. H.** 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th edition. Elsevier Science Publishing Co., Inc., New York, NY
5. **Edwards, P. R., and W. H. Ewing**. 1972. Identification of *Enterobacteriaceae*, 3rd ed. Burgess Publishing Co., Minneapolis.
6. **Balows, A., and W. J. Hausler**. 1981. Diagnostic Procedures for Bacteria, Mycotic and Parasitic Infections, 6th ed. American Public Health Association, Washington, D.C.

7. **Baron, E. J., and S. M. Finegold.** 1990. Bailey & Scott's Diagnostic Microbiology, 8th ed. C.V. Mosby Company, St. Louis, MO.
8. **Gilligan, P. H.** 1995. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
9. **Pezzlo, M. (ed.).** 1994. Aerobic bacteriology, p. 1.0.0-1.20.47. *In* H. D. Isenberg (ed.), Clinical microbiology procedures handbook, Vol. 1. American Society for Microbiology, Washington, D.C.
10. **Isenberg, H. D. (ed.).** 1992. Conventional tests (18 to 24 hours) - Carbohydrate Fermentation Test, p. 1.19.28. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology. Washington, D.C.

Packaging

Enteric Fermentation Base	500 g	1828-17

Bacto® m Enterococcus Agar

Intended Use

m Enterococcus Agar is used for isolating and enumerating enterococci in water and other materials by membrane filtration or pour plate technique.

Also Known As

m Enterococcus Agar is also referred to as m Azide Agar

Summary and Explanation

The enterococcus group is a subgroup of the fecal streptococci that include *E. faecalis*, *E. faecium*, *E. gallinarum*, and *E. avium*.[1] Enterococci are differentiated from other streptococci by their ability to grow in 6.5% sodium chloride, at pH 9.6, and at 10°C and 45°C.[1] The enterococci portion of the fecal streptococcus group is a valuable bacterial indicator for determining the extent of fecal contamination of recreational surface waters.[1] m Enterococcus Agar is used in standard methods for the detection of fecal streptococcus and enterococcus groups using the membrane filtration technique.[1]

m Enterococcus Agar was developed by Slanetz et al.[2] for the enumeration of enterococci by the membrane filtration technique. A modification of m Enterococcus Agar, adding triphenyltetrazolium chloride (TTC), was described by Slanetz and Bartley[3]. This modified medium proved to be a superior membrane filtration medium for the enumeration of enterococci. Increased recovery and larger colonies were obtained by incubating the inoculated membranes on the agar surface instead of on pads saturated with liquid medium. The membrane filtration method has the advantages of being simpler to perform, not requiring confirmation and permitting a direct count of enterococci in 48 hours. Burkwell and Hartman[4] added 0.2% sodium carbonate and 0.05% Tween® 80 to m Enterococcus Agar to increase the sensitivity for the direct plating method.

Principles of the Procedure

Tryptose provides the nitrogen, minerals and amino acids in m Enterococcus Agar. Yeast Extract is the vitamin source and Dextrose supplies carbon. Dipotassium Phosphate acts as a buffer for the

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.2% solution, soluble in distilled or deionized water upon boiling. Solution is light amber, very slightly to slightly opalescent, without significant precipitate.
Prepared Medium:	Light amber, slightly opalescent, without precipitate.
Reaction of 4.2% Solution at 25°C:	pH 7.2 ± 0.2

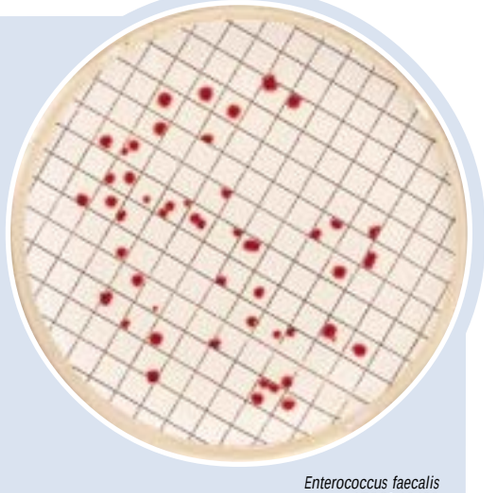
Cultural Response

Prepare m Enterococcus Agar per label directions. Inoculate medium using the membrane filter technique. Incubate in humid atmosphere inoculated medium at 35 ± 0.5°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY OF COLONY
Enterococcus faecalis	19433	20-60	good	pink to red
Enterococcus faecalis	29212*	20-60	good	pink to red
Escherichia coli	25922*	1,000	marked to complete inhibition	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol™ Disks Technical Information.



Enterococcus faecalis ATCC® 19433

medium. Sodium Azide is the selective agent to suppress the growth of gram negative organisms. Bacto Agar is the solidifying agent. Triphenyltetrazolium Chloride (TTC) is the dye used as an indicator of bacterial growth. TTC is reduced to the insoluble formazan inside the bacterial cell, resulting in the production of red colonies.

Formula

m Enterococcus Agar

Formula Per Liter

Bacto Tryptose	20 g
Bacto Yeast Extract	5 g
Bacto Dextrose	2 g
Dipotassium Phosphate	4 g
Sodium Azide	0.4 g
Bacto Agar	10 g
2,3,5-Triphenyl Tetrazolium Chloride	0.1 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Cardiovascular, Lungs, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

m Enterococcus Agar

Materials Required But Not Provided

Glassware
Sterile Petri dishes, 50 x 9 mm
Membrane filtration equipment
Incubator (35°C)
Fluorescent lamp
Magnifying lens
Distilled or deionized water

Method of Preparation

1. Suspend 42 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. DO NOT AUTOCLAVE.
4. Dispense into 9 x 50 mm Petri dishes to a depth of 4-5 mm (approximately 4-6 ml).

Specimen Collection and Preparation

Collect water samples as described in Standard Methods for the Examination of Water and Wastewater, Section 9230[1] or by laboratory policy.

Test Procedure

Membrane filtration procedure

1. Follow the membrane filtration procedure as described in Standard Methods for the Examination of Water and Wastewater, Section 9230C.[1]
2. Choose a sample size so that 20-60 colonies will result.
3. Transfer the filter to agar medium in a Petri dish, avoiding air bubbles beneath the membrane.
4. Let plates stand for 30 minutes.
5. Invert plates and incubate at 35 ± 0.5°C for 48 hours.

Direct plating procedure

1. Inoculate medium with a specimen using the streak plate method.
2. Incubate plates at 35 ± 2°C for 24-48 hours.

Results[1]

Count all light and dark red colonies as enterococci. Count colonies using a fluorescent lamp and a magnifying lens.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **Slanetz, Bent, and Bartley.** 1955. Public Health Rep. **70**:67.
3. **Slanetz, and Bartley.** 1957. J. Bacteriol. **74**:591.
4. **Burkwell, and Hartman.** 1964. Appl. Microbiol. **12**:18.

Packaging

m Enterococcus Agar	100 g	0746-15
	500 g	0746-17

Bacto® Eugon Agar
Bacto Eugon Broth

Intended Use

Bacto Eugon Agar and Eugon Broth are used for cultivating a wide variety of microorganisms, particularly in mass cultivation procedures.

Also Known As

Eugon media are also referred to as Eugonic Agar and Eugonic Broth.

User Quality Control

Identity Specifications

Eugon Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.54% solution, soluble in distilled or deionized water upon boiling. Light amber, slightly opalescent, precipitate may be visible.
Prepared Medium:	Light amber, slightly opalescent, precipitate may be visible.
Reaction of 4.54% Solution at 25°C:	pH 7.0 ± 0.2

Eugon Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.04% solution, soluble in distilled or deionized water upon boiling. Light amber, clear, may have a slight precipitate.
Prepared Medium:	Light amber, clear, may have a slight precipitate.
Reaction of 3.04% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepared Eugon Agar and Eugon Broth per label directions. Inoculate prepared medium and incubate for 18-48 hours (up to 72 hours if necessary). *Candida albicans* and *Aspergillus niger* should be incubated at 30 ± 2°C; all other cultures should be incubated at 35 ± 2°C.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	fair to good
Brucella abortus	4315	100-1,000	good
Candida albicans	26790	100-1,000	good
Lactobacillus fermentum	9338	100-1,000	good
Shigella flexneri	12022*	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Summary and Explanation

Eugon Agar and Eugon Broth are prepared according to the formula described by Vera.[1] Eugon media were developed to obtain eugonic (luxuriant) growth of fastidious microorganisms.[2] These formulations can be used with or without enrichment. Enriched with blood, Eugon media support the growth of pathogenic fungi including *Nocardia*, *Histoplasma* and *Blastomyces*. With the addition of Supplement B, excellent growth of *Neisseria*, *Francisella* and *Brucella* is achieved. The unenriched media support rapid growth of lactobacilli associated with cured meat products, dairy products and other food.

Niven[3] reported the use of Eugon Agar for the detection of lactic acid in cured meats, and recommended it for investigating spoilage in meats. Harrison and Hansen[4] employed the medium for plate counts of the intestinal flora of turkeys. Frank[5] showed its usefulness in germinating anaerobic spores pasteurized at 104°C.

Eugon Agar is specified in the Compendium of Methods for the Microbiological Examination of Food.[6]

Principles of the Procedure

Tryptose and Soytone provides the nitrogen, vitamins and amino acids in Eugon Agar and Eugon Broth. The high concentration of Dextrose is the energy source for rapid growth of bacteria. L-Cystine and Sodium Sulfite are added to stimulate growth. Sodium Chloride maintains the osmotic balance of the media. The high carbohydrate content along with high sulfur (cystine) content improves growth with chromogenicity.[2] Bacto Agar is the solidifying agent in Eugon Agar.

Formula

Eugon Agar

Formula Per Liter

Bacto Tryptose	15 g
Bacto Soytone	5 g
Bacto Dextrose	5.5 g
L-Cystine	0.7 g
Sodium Chloride	4 g
Sodium Sulfite	0.2 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Eugon Broth

Formula Per Liter

Bacto Tryptose	15 g
Bacto Soytone	5 g
Bacto Dextrose	5.5 g
L-Cystine	0.7 g
Sodium Chloride	4 g
Sodium Sulfite	0.2 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Eugon Agar
Eugon Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-55°C) (optional)
Sterile Petri dishes or Sterile tubes
5% sterile defibrinated blood (optional)
Bacto Supplement B (optional)

Method of Preparation

1. Suspend the appropriate amount of medium 1 liter distilled or deionized water:

Eugon Agar	45.4 g/l
Eugon Broth	30.4 g/l

2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. OPTIONAL: When an enriched medium is being prepared, cool to 50-55°C prior to adding the desired enrichment. After the enrichment is added, mix well.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion on bacteria and fungi from clinical specimens, refer to the appropriate procedures outlined in the references.[7,8] For the examination of bacteria and fungi in food refer to standard methods.[6,9]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Eugon Agar is not recommended as a blood agar base for hemolytic reactions because of its high sugar content.
3. It is suggested that Eugon Agar be prepared as required. Do not melt and resolidify media containing enrichments.

References

1. **Vera, H. D.** 1947. The ability of peptones to support surface growth of lactobacilli. J. Bacteriol. **54**:14.
2. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, p.301-303. vol. 1. Williams & Wilkins, Baltimore, MD.
3. **Niven**. 1949. J. Bacteriol. **58**:633.
4. **Harrison, A. P., Jr., and P. A. Hansen.** 1950. The bacterial flora of the cecal feces of health turkeys. J. Bacteriol. **59**:197.
5. **Frank, H. A.** 1955. The influence of various media on spore count determinations of a putrefactive anaerobe. J. Bacteriol. **70**:269.
6. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
7. **Isenberg, H. D. (ed.),** 1992. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.
8. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.),** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
9. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

Eugon Agar	500 g	0589-17
Eugon Broth	500 g	0590-17

Bacto® m FC Agar
Bacto m FC Broth Base
Bacto Rosolic Acid

Intended Use

Bacto m FC Agar and Bacto m FC Broth Base are used with Bacto Rosolic Acid in cultivating and enumerating fecal coliforms by the membrane filter technique at elevated temperatures.

Also Known As

M-FC Medium

Summary and Explanation

Geldreich et al.[1] formulated a medium to enumerate fecal coliforms (MFC) using the membrane filter (MF) technique without prior enrichment. Fecal coliforms, i.e., those found in the feces of warm-blooded animals, are differentiated from coliforms from environmental sources by their ability to grow at 44.5 ± 0.5°C.[2]

Many Standard Methods membrane filtration procedures specify M-FC medium for testing water. The American Public Health Association (APHA) specifies M-FC medium and incubation at 44.5 ± 0.5°C in the fecal coliform membrane filter procedure, the delayed-incubation fecal coliform procedure, the two-layer agar method for recovering injured fecal coliforms,[2] and in the membrane filter method for fecal coliforms in bottled water.[3] The Association of Official Analytical Chemists (AOAC) specifies m-FC Agar for detecting total coliforms and fecal coliforms in foods.[4]

The U. S. Environmental Protection Agency specifies using M-FC medium in fecal coliform methods for testing water by the direct MF method or the delayed-incubation MF method.[5,6]

Principles of the Procedure

m FC Agar and m FC Broth Base contain Tryptose and Proteose Peptone No. 3 as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins that stimulate bacterial growth. Lactose is a carbohydrate. Bile Salts No. 3 inhibits growth of gram-positive bacteria. m FC Agar contains Bacto Agar as the solidifying agent. The differential indicator system combines Aniline Blue and Rosolic Acid.

Colonies of fecal coliforms are blue; non-fecal coliforms and other organisms are gray to cream-colored.

Formula

m FC Agar

Formula Per Liter

Bacto Tryptose	10 g
Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Bacto Lactose	12.5 g
Bacto Bile Salts No. 3	1.5 g
Sodium Chloride	5 g
Bacto Agar	15 g
Aniline Blue	0.1 g

Final pH 7.4 ± 0.2 at 25°C

m FC Broth Base

Formula Per Liter

Bacto Tryptose	10 g
Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Sodium Chloride	5 g
Bacto Lactose	12.5 g
Bacto Bile Salts No. 3	1.5 g
Aniline Blue (Water Blue)	0.1 g

Final pH 7.4 ± 0.2 at 25°C

Rosolic Acid

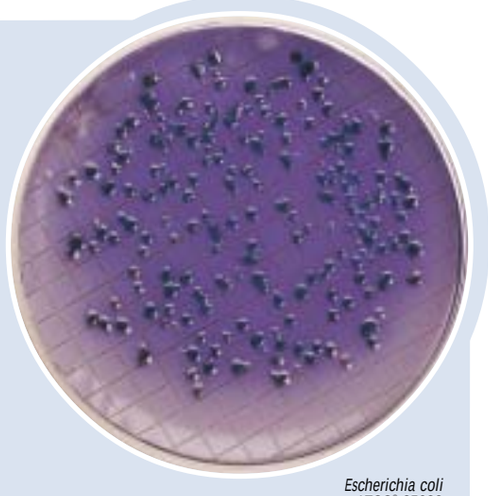
Rosolic Acid	1 g/vial

User Quality Control

Identity Specifications

m FC Agar

Dehydrated Appearance:	Beige with slight blue tint, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in distilled or deionized water on boiling. Without 1% rosolic acid: blue, very slightly to slightly opalescent, may have a slight precipitate. With 1% rosolic acid: cranberry red, slightly opalescent, may have a slight precipitate.
Prepared Medium:	Without 1% rosolic acid: blue, slightly opalescent. With 1% rosolic acid: cranberry red, slightly opalescent.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2 (without 1% rosolic acid)



Escherichia coli ATCC® 25922

m FC Broth Base

Dehydrated Appearance:	Beige with slight blue tint, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in distilled or deionized water on boiling. Without 1% rosolic acid: blue, slightly opalescent, may have a very fine precipitate. With 1% rosolic acid: cranberry red, slightly opalescent, may have a very fine precipitate.
Reaction of 3.7% Solution at 25°C:	pH 7.4 ± 0.2 (without 1% rosolic acid)

Rosolic Acid

Dehydrated Appearance:	Dark reddish-brown with metallic green particles, free-flowing, fine crystalline powder.
Solution:	1.0% solution, soluble in 0.2 N NaOH. Solution is deep red, clear to very slightly opalescent.

Cultural Response

m FC Agar and m FC Broth Base

Prepare mFC Agar and mFC Broth Base per label directions with 1% Rosolic Acid. Using the membrane filter technique, inoculate and incubate plates at 44.5 ± 0.5°C for 24 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	19433*	1,000-2,000	markedly to completely inhibited	–
Escherichia coli	25922*	20-80	good	blue

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Precautions

1. For Laboratory Use.
2. Rosolic Acid: **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Rosolic Acid at 15-30°C. Rehydrated Rosolic Acid (1% solution) is stable for 2 weeks if stored at 2-8°C in the dark.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

m FC Agar
m FC Broth Base
Rosolic Acid

Materials Required but not Provided

0.2 N sodium hydroxide
1 N hydrochloric acid
Glassware
Distilled or deionized water
Waterproof plastic bags
Waterbath (35°C)
Waterbath (44.5 ± 0.5°C)

Method of Preparation

Rosolic Acid

1. Prepare a 1% solution, dissolving 1 gram in 100 ml 0.2 N NaOH.

m FC Agar

1. Suspend 52 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Add 10 ml of a 1% solution of Rosolic Acid in 0.2 N NaOH.
4. Continue heating for 1 minute. Do Not Autoclave.
5. If necessary, adjust to pH 7.4 with 1 N HCl.

m FC Broth Base

1. Suspend 3.7 grams in 100 ml distilled or deionized water.
2. Add 1 ml of a 1% solution of Rosolic Acid in 0.2 N NaOH.
3. If necessary, adjust to pH 7.4 with 1 N HCl.
4. Heat to boiling.
5. Cool before dispensing.

Specimen Collection and Preparation

Collect samples and process according to recommended guidelines.[2-6]

Test Procedure

m FC Agar

1. Filter duplicate samples through separate membrane filters.
2. Transfer the filters to the surface of separate mFC Agar plates.
3. Place each plate in a separate waterproof plastic bags Submerge in different waterbaths, one set at 35 ± 2°C and one set at 44.5 ± 0.5°C; incubate for 24 ± 2 hours.
4. Incubate one set of plates at 35°C and one set at 44.5 ± 0.5°C for 24 ± 2 hours.

Results

Colonies of fecal coliforms will be various shades of blue. Non-fecal coliforms are gray to cream-colored.

Limitations of the Procedure

1. A few nonfecal coliform colonies may be observed on m FC media due to the selective action of the elevated temperature and the addition of the Rosolic Acid. It may be useful to elevate the temperature to 45 ± 0.2°C to eliminate *Klebsiella* strains from the fecal coliform group.[2]

References

1. **Geldreich, E. E., H. F. Clark, C. B. Huff, and L. C. Best**. 1965. Fecal-coliform-organism medium for the membrane filter technique. J. Am. Water Works Assoc. **57**:208-214.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Cowman, S., and R. Kelsey.** 1992. Bottled water, p. 1031-1036. *In* C. Vanderzant, and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Andrews, W.** 1995. Microbial methods, p. 17.1-17-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International. Arlington, VA.
5. **Bordner, R., and J. Winter (ed).** 1978. Microbiological methods for monitoring the environment. EPA-600/8-78-017. Environmental Monitoring and Support Laboratory, Office of Research and Development, U. S. Environmental Protection Agency, Cincinnati, OH.
6. **Environmental Protection Agency**. 1992. Manual for the certification of laboratories analyzing drinking water. EPA-814B-92-002. Office of Ground Water and Technical Support Division, U. S. Environmental Protection Agency, Cincinnati, OH.

Packaging

m FC Agar	100 g	0677-15
	500 g	0677-17
m FC Broth Base	100 g	0883-15
	500 g	0833-17
Rosolic Acid	6 x 1 g	3228-09

Bacto® m FC Basal Medium

Intended Use

Bacto m FC Basal Medium is used with MUG or BCIG for cultivating and enumerating fecal coliforms by the membrane filter technique at elevated temperatures.

Summary and Explanation

Ciebin et al.[1] described a modification of m FC Medium called FC Basal Medium, in which the chromogenic substrate 5-bromo-6-chloro-3-indolyl-β-D-glucuronide (BCIG) is added for quantitative recovery of *Escherichia coli* from untreated water samples to show fecal contamination using membrane filter methods.

Standard method procedures use media with the fluorogenic substrate, 4-methylumbelliferyl β-D-glucuronide (MUG) to enumerate *E. coli* by membrane filter methods.[2] Disadvantages of using MUG include the requirement of ultra violet light, possible diffusion of fluorescence from the colony to the surrounding medium and background fluorescence of membrane filters.[3] Using BCIG in place of MUG to detect β-glucuronidase activity, gives visible blue colonies and an indigo-blue complex that remains within the colony. Ciebin et al.[1] found FC-BCIG Medium comparable to standard MUG-based media for detection of ß-glucuronidase activity of *E. coli*.

In another study, Ciebin et al.[4] formulated DC Medium using FC Basal Medium supplemented with lactose, BCIG and cefsulodin. It is a differential coliform medium for the enumeration of coliforms and *E. coli* in potable water using membrane filtration. Ciebin et al. compared DC Medium to LES Endo Medium and FC-BCIG Medium. They found DC Medium superior to LES Endo Medium in recovering coliforms and equivalent to FC-BCIG Medium in recovering *E. coli*.

Principles of the Procedure

m FC Basal Medium contains Tryptose and Proteose Peptone No. 3 as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins that stimulate bacterial growth. Bile Salts No. 3 inhibits the growth of gram-positive microorganisms. Bacto Agar is the solidifying agent.

Formula

m FC Basal Medium

Formula Per Liter

Bacto Tryptose	10 g
Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Bacto Bile Salts No. 3	1.5 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.95% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent, may have slight precipitate.
Prepared Medium:	Light amber, slightly opalescent.
Reaction of 3.95% Solution at 25°C:	pH 7.4 ± 0.2

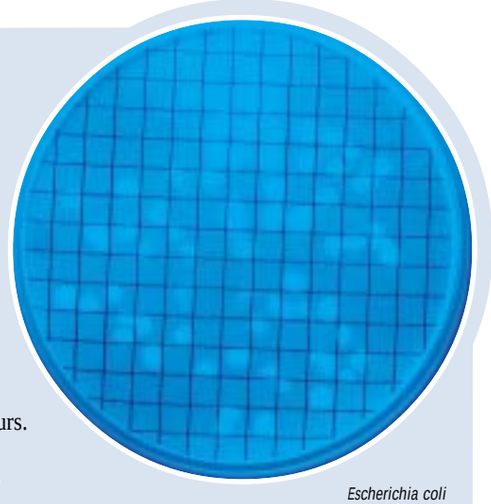
Cultural Response

Prepare m FC Basal Medium per label directions, with the addition of 0.01% MUG. Using membrane filter technique, inoculate and incubate at 44.5 ± 0.5°C for 24 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	19433*	300-1,000	markedly to completely inhibited	–
Escherichia coli	25922*	30-200	good	blue-white fluorescence

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Escherichia coli ATCC® 25922

Procedure

Materials Provided

m FC Basal Medium

Materials Required but not Provided

MUG (4-methylumbelliferyl b-D-glucuronide)
BCIG (5-bromo-6-chloro-3-indolyl b-D-glucuronide)
1 N HCl
Glassware
Membrane filtration apparatus
Petri dishes, 60 mm
Distilled or deionized water
Waterproof plastic bags
Waterbath (35°C)
Waterbath (44.5°C)

Method of Preparation

1. Suspend 39.5 grams in 1 liter distilled or deionized water.
2. Add 100 mg MUG or BCIG, as desired, and boil to dissolve completely. **Do Not Autoclave.**
3. If necessary, adjust to pH 7.4 with 1 N HCl.
4. Dispense into 60 mm sterile petri dishes.

Specimen Collection and Preparation

Collect samples and process according to recommended guidelines for water testing.[2]

Test Procedure

1. Filter duplicate water samples through membrane filtration apparatus.
2. Transfer each membrane to the surface of the m FC Basal Medium plate. Place cultures in waterproof plastic bags. Submerge petri dishes in waterbath. Incubate one set of plates at 35 ± 2°C and one set at 44.5 ± 0.5°C for 24 ± 2 hours.
3. Count number of colonies and record observations. m FC-MUG plates are read under long-wavelength (366 nm) ultraviolet light.

Results

m FC-MUG

β-glucuronidase-positive organisms produce a blue-white fluorescence.

β-glucuronidase-positive organisms do not fluoresce.

m FC-BCIG

β-glucuronidase-positive organisms produce a visible blue colony.

β-glucuronidase-positive organisms produce a non-blue colony.

References

1. **Ciebin, B. W., M. H. Brodsky, R. Eddington, G. Horsnell, A. Choney, G. Palmateer, A. Ley, R. Joshi, and G. Shears.** 1995. Comparative evaluation of modified m-FC and m-TEC medium for membrane filter enumeration of *Escherichia coli* in water. Appl. Environ. Microbiol. **61**:3940-3942.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Farber, J. M.** 1986. Potential use of membrane filters and a fluorogenic reagent-based solid medium for the enumeration of Escherichia coli in foods. Can. Inst of Food Sci. and Tech. J. **19**:34-37.
4. **Ciebin, B. W., R. N. Schop, and M. H. Brodsky.** 1997. DC medium: a differential coliform medium for simultaneous enumeration of coliforms and *Escherichia coli* in water by membrane filtration. Q-152. Abstr. 97th Annu. Meet. Am. Soc. for Microbiol. 1997. American Society for Microbiology, Washington, D.C.

Packaging

m FC Basal Medium	500 g	0698-17

Bacto® Fildes Enrichment

User Quality Control

Identity Specifications

Solution Appearance: Dark brown solution.

Cultural Response

Prepare Heart Infusion Agar per label directions. Aseptically add 5% Fildes Enrichment to the cooled medium at 50-55°C and pour plates. Inoculate and incubate at 35 ± 2°C under increased CO_2 for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Haemophilus influenzae	19418	100-1,000	good
Haemophilus parainfluenzae	7901	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Intended Use

Bacto Fildes Enrichment is used to enrich media for cultivating *Haemophilus influenzae*.

Also Known As

Fildes Enrichment is also referred to as Fildes Broth.

Summary and Explanation

Fildes Enrichment, a sterile digest of sheep blood, is prepared according to Fildes.[1,2] This enrichment is recommended for use in liquid and solid media for culturing *Haemophilus influenzae* and other fastidious microorganisms requiring blood derivatives for optimal growth.

Body fluid specimens likely to harbor *Haemophilus* species should be inoculated on 5% sheep blood agar, chocolate agar and a suitable enrichment broth, such as Fildes Broth.[3] Fildes-enriched gonococcal medium, composed of gonococcal agar base containing 5% Fildes Enrichment, 5% horse blood and 3 µg of vancomycin per ml, is used for lymph node aspirates and lesion scrapings from patients with suspected chancroid.[3]

Principles of the Procedure

Fildes Enrichment is a rich source of factors that will promote growth of fastidious microorganisms. The growth factors include hemin (X factor) and nicotinamide adenine dinucleotide (NAD or V factor) required by *H. influenzae* and other *Haemophilus* species.

Formula

Fildes Enrichment

A sterile digest of sheep blood.

Precautions

1. For In Vitro Diagnostic Use.
2. Follow proper, established laboratory procedure in handling and disposing of infectious materials.

Storage

Store Fildes Enrichment at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Fildes Enrichment

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Fildes Enrichment is a ready-to-use solution. Many factors in Fildes Enrichment are heat labile. This enrichment cannot be heated and must be added aseptically in the proper amounts to media that have been sterilized in the autoclave and cooled to 50-55°C.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy or appropriate references.[3,4]

Test Procedure

Fildes Enrichment is usually employed in prepared media at a final concentration of 5% for optimal results. Some formulas may require higher or lower concentrations. Add Fildes Enrichment as required.

Body fluids and other clinical specimens inoculated in Fildes Enrichment should be incubated for 7 days at 35-37°C.[3]

Results

Carefully examine clinical specimens incubated in Fildes Enrichment for evidence of growth.[3]

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly with this enrichment.
2. *Haemophilus* species cannot be depended on to impart obvious turbidity to broths even when they are present at densities exceeding 10^9 CFU/ml.[3]

References

1. **Fildes.** 1920. Br. J. Exp. Pathol. **1**:129-130.
2. **Fildes.** 1921. Br. J. Exp. Pathol. **2**:16-25.
3. **Campos, J. M.** 1995. *Haemophilus*, p. 556-565. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D.** (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Packaging

Fildes Enrichment	6 x 5 ml	0349-57
	100 ml	0349-72
	6 x 100 ml	0349-73

Bacto® Fish Peptone No. 1

Intended Use

Bacto Fish Peptone No. 1 is used in preparing microbiological culture media.

Summary and Explanation

Fish Peptone No. 1 is a non-bovine origin peptone. Fish Peptone No. 1 was developed by Difco Laboratories for pharmaceutical and vaccine production to reduce Bovine Spongiform Encephalopathy (BSE) risk. Fish Peptone No. 1 may substitute for other peptones, depending on organism and production application.

Principles of the Procedure

Fish Peptone No. 1 is a non-mammalian/animal peptone used as a nitrogen source in microbiological culture media.

Typical Analysis

Physical Characteristics

Ash (%)	34.8	Loss on Drying (%)	3.4
Clarity, 1% Soln (NTU)	0.9	pH, 1% Soln	6.9
Filterability (g/cm^2)	3.4		

Carbohydrate (%)

Total	<0.1

Nitrogen Content (%)

Total Nitrogen	10.6	AN/TN	30.2
Amino Nitrogen	3.2		

Amino Acids (%)

Alanine	3.48	Lysine	2.51
Arginine	2.19	Methionine	0.83
Aspartic Acid	3.21	Phenylalanine	0.95
Cystine	0.24	Proline	2.19
Glutamic Acid	5.27	Serine	1.27
Glycine	5.29	Threonine	1.17
Histidine	1.54	Tryptophan	0.15
Isoleucine	0.92	Tyrosine	0.45
Leucine	2.16	Valine	1.43

Inorganics (%)

Calcium	0.020	Phosphate	3.848
Chloride	9.326	Potassium	4.183
Cobalt	<0.001	Sodium	9.351
Copper	0.001	Sulfate	1.004
Iron	0.003	Sulfur	1.629
Lead	<0.001	Tin	<0.001
Magnesium	0.017	Zinc	0.002
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.3	PABA	95.0
Choline (as Choline Chloride)	4170.7	Pantothenic Acid	63.2
Cyanocobalamin	0.3	Pyridoxine	7.2
Folic Acid	1.5	Riboflavin	26.8
Inositol	2820.0	Thiamine	NA
Nicotinic Acid	603.0	Thymidine	55.0

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	18
Salmonella	negative	Thermophile Count	379

User Quality Control

Identity Specifications

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 1% solution is light to medium amber, clear to very slightly opalescent, may have a slight precipitate.

Reaction of 1% Solution at 25°C: pH 6.7 ± 0.2

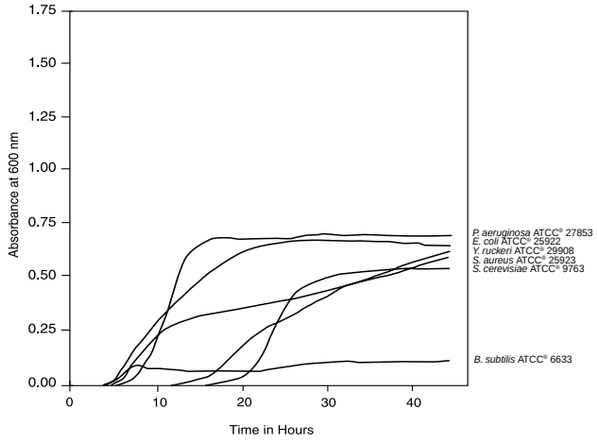
Cultural Response

Prepare a 1% concentration of Fish Peptone No. 1 with the addition of 0.5% sodium chloride. Inoculate test organisms and incubate for 18-48 hours at 35 ± 2°C. Incubate *Vibrio tubiashii* for 18-48 hours at 25 ± 2°C. *Saccharomyces cerevisiae* is tested with the addition of 0.5% dextrose. *Vibrio tubiashii* is tested with the addition of 1.5% sodium chloride.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacillus subtilis†	6633	100-1,000	fair to good
Escherichia coli	25922*	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good
Staphylococcus aureus	25923*	100-1,000	good
Vibrio tubiashii	19105	100-1,000	good

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

†*Bacillus subtilis* is available as Bacto Subtilis Spore Suspension.



Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated product below 30°C. The dehydrated product is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Fish Peptone No. 1

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of peptone in the formula of the medium being prepared. Add Fish Peptone No. 1 as required.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures on the medium being prepared or the sample being analyzed.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on prepared medium.

Packaging

Fish Peptone No. 1	500 g	0551-17
	10 kg	0551-08

Bacto® Fletcher Medium Base

Intended Use

Bacto Fletcher Medium Base is used with sterile normal rabbit serum for isolating and cultivating *Leptospira*.

Summary and Explanation

In 1816, Adolf Weil described the first recognized infections of Leptospirosis in humans.[5] These cases were caused by *Leptospira interogans* serovar *icterohaemorrhagiae* and the disease was subsequently named Weil's Disease.[5] Leptospirosis is a zoonotic disease, having its reservoir in wild, domestic, and peridomestic animals.[6] Infection usually results from direct or indirect exposure to the urine of leptospiruric animals.[6] Indirect exposure through contaminated water and soil accounts for most sporadic cases.[3] Direct exposure occurs in pet owners, veterinarians and persons working with livestock.[3]

Leptospirosis is typically a biphasic illness.[3,8] The infection is acute in onset, with a flu- like syndrome persisting for 4 to 7 days.[4] Onset of a second "immune" phase, in which meningitis, skin rash, and hepatic and renal involvement may be present, occurs within a few days.[4]

Fletcher Medium Base is prepared according to the formulation of Fletcher.[1] Myers et al.[2] reported Fletcher Medium, with a basal agar layer containing charcoal, to be superior to the standard medium for the maintenance of leptospiral cultures. Fletcher Medium Base prepared with sterile normal rabbit serum is specified for the isolation of *Leptospira*.[3,4,7]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.5 g in 920 ml distilled or deionized water, soluble upon boiling. Solution is very light amber, clear to very slightly opalescent without significant precipitate.
Prepared Medium:	Very light amber, very slightly to slightly opalescent without significant precipitate.
Reaction of 2.5 g in 920 ml distilled water:	pH 7.9 ± 0.1 at 25°C

Cultural Response

Prepare Fletcher Medium Base per label directions. Enrich with sterile normal rabbit serum. Inoculate and incubate at 30 ± 2°C for up to 5 days.

ORGANISM	ATCC®	INOCULUM	GROWTH
Leptospira interrogans serovar *australis*	23605	2-3 drops	good
Leptospira interrogans serovar *canicola*	23470	2-3 drops	good
Leptospira kirschneri serovar *grippotyphosa*	23604	2-3 drops	good

The cultures listed are the minimum that should be used for performance testing.

Principles of the Procedure

Bacto Peptone and Beef Extract provide the nitrogen, vitamins, carbon and amino acids in Fletcher Medium Base. Sodium chloride maintains the osmotic balance of the medium. Bacto Agar is the solidifying agent.

Sterile normal rabbit serum is added to the formula to stimulate growth of *Leptospira*.

Formula

Fletcher Medium Base

Formula Per Liter

Bacto Peptone	0.3 g
Bacto Beef Extract	0.2 g
Sodium Chloride	0.5 g
Bacto Agar	1.5 g

Final pH 7.9 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. *Leptospira* species are BioSafety Level 2 pathogens. Handling clinical specimen material potentially infected with *Leptospira* species should be performed in a Class II biological safety cabinet (BSC).[7]

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Fletcher Medium Base

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (56°C)
Sterile normal rabbit serum

Method of Preparation

1. Suspend 2.5 grams in 920 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 56°C.
4. Aseptically add 80 ml sterile normal rabbit serum at 56°C. Mix well.
5. Determine pH. If necessary, aseptically adjust to pH 7.9 ± 0.1 with 1 N HCl or 1 N NaOH.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy. Blood, cerebrospinal fluid (CSF) and

urine are the specimens of choice for the recovery of leptospires from patients with leptospirosis.[3]

Test Procedure[7]

1. Aseptically dispense into sterile screw-cap tubes in 5-7 ml amounts. Store at room temperature overnight.
2. Inactivate the whole medium the day following its preparation by placing the tubes in a water bath at 56°C for 1 hour.
3. Allow the medium to cool before inoculation.
4. Growth is first seen in approximately 10 days at 35°C (2 to 4 weeks at 25°C) as a cloud of minute granules that develop into microcolonies just below the surface.
5. Gram stain is not satisfactory. The microcolonies can be fixed with methanol and stained with Giemsa stain to show rod forms at the edges.[9]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Fletcher, W.** 1928. Recent work on leptospirosis, tsutsugamushi disease and tropical typhus in the federated Malay States. Trans. Roy. Soc. Trop. Med. Hyg. **21**: 265-287.
2. **Myers, D. M., V. M. Varela-Diaz, and A. A. Siniuk.** 1973. Long-term survival of Leptospira in a biphasic culture medium containing charcoal. Am. Soc. Microbiol. **25**:514-516.
3. **Kaufmann, A. F., and R. S. Weyant**. 1995. *Leptospiraceae*, p. 621-625. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Koneman, E. W., S. D. Allen, V. R. Dowell, Jr., W. M. Janda, H. M. Sommers, and W. C. Winn, Jr.** 1988. Color atlas and textbook of diagnostic microbiology, 3rd ed. J. B. Lippincott Company, Philadelphia, PA.
5. **Elliott, S. H.** 1980. Discussion and clinical diagnosis of Leptospirosis. J. Am. Med. Tech. **42**:37-44.
6. **Faine, S. (ed.).** 1982. Guidelines for the control of Leptospirosis. W. H. O. Offset publication no. 67. World Health Organization, Geneva.
7. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
8. **Kelley, P. W.** 1992. Leptospirosis, p. 1295-1301. *In* S. L. Gorbach, J. G. Bartlett, and N. R. Blacklow (ed.), Infectious diseases. The W. B. Saunders Co., Philadelphia, PA.
9. **Weinman, D.** 1981. Bartonellosis and anemias associated with bartonella-like structures, p. 235-248. *In* A. Balows, and W. J. Hausler, Jr. (ed.), Diagnostic procedures for bacterial, mycotic and parasitic infections, 6th ed. American Public Health Association, Washington, D.C.

Packaging

Fletcher Medium Base	500 g	0987-17

Bacto® Folic AOAC Medium

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	5.50% solution (single strength) and 11.0% solution (double strength), soluble in distilled or deionized water on boiling.
	Solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	Very light amber, clear.
Reaction of 5.50% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Prepare Folic AOAC Medium per label directions. This medium should support the growth of *E. hirae* ATCC® 8043 when prepared in single strength and supplemented with folic acid.

Intended Use

Bacto Folic AOAC Medium is used for determining folic acid concentration by the microbiological assay technique.

Also Known As

AOAC is an abbreviation for Association of Official Analytical Chemists.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose.
2. Inoculum Media: To condition the test culture for immediate use.
3. Assay Media: to permit quantitation of the vitamin under test.

Folic AOAC Medium is prepared for use in the microbiological assay of folic acid according to the procedures of the Folic Acid Assay in AOAC.[1] *Enterococcus hirae* ATCC® 8043 (*Streptococcus faecium*) is the test organism in this assay.

Principles of the Procedure

Folic Acid AOAC Medium is a folic acid-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *E. hirae* ATCC® 8043. The addition of folic acid in specified

increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Folic AOAC Medium

Formula Per Liter		
Bacto Vitamin Assay Casamino Acids	10	g
L-Asparagine	0.6	g
L-Tryptophane	0.2	g
L-Cysteine Hydrochloride	0.76	g
Bacto Dextrose	40	g
Adenine Sulfate	10	mg
Guanine Hydrochloride	10	mg
Uracil	10	mg
Xanthine	20	mg
p-Aminobenzoic Acid	1	mg
Pyridoxine Hydrochloride	4	mg
Thiamine Hydrochloride	400	µg
Calcium Pantothenate	800	µg
Nicotinic Acid	800	µg
Biotin	20	µg
Riboflavin	1	mg
Glutathione	5.2	mg
Sorbitan Monooleate Complex	0.1	g
Sodium Citrate	52	g
Dipotassium Phosphate	6.4	g
Magnesium Sulfate	0.4	g
Manganese Sulfate	20	mg
Sodium Chloride	20	mg
Ferrous Sulfate	20	mg

Final pH 6.7 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware is heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
4. Take precautions to keep sterilizing and cooling conditions uniform throughout the assay.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Folic AOAC Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Enterococcus hirae* ATCC® 8043
Sterile tubes
Distilled or deionized water
Folic Acid
0.01 N NaOH
Dilute HCl
Spectrophotometer or Nephelometer

Method of Preparation

1. Suspend 11 grams in 100 ml distilled or deionized water.
2. Heat to boiling for 2-3 minutes.
3. Distribute 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 5 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assays, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Follow assay procedures as outlined in AOAC.[1] It is essential that a standard curve be set up for each separate assay. Autoclaving and incubation conditions that can influence the standard curve readings cannot always be duplicated. The standard curve is obtained by using folic acid at levels of 0.0, 1, 2, 4, 6, 8 and 10 ng per assay tube (10 ml). Folic AOAC Medium may be used for both turbidimetric and titrimetric analysis. Turbidimetric readings should be taken after incubation at 35-37°C for 16-18 hours. Titrimetric determinations are best made following incubation at 35-37°C for 72 hours.

The folic acid required for the preparation of the standard curve may be prepared as follows:

A. Dissolve 50 mg dried folic acid in about 30 ml 0.01N NaOH and 300 ml distilled water.

B. Adjust the pH reaction to 7.5 ± 0.5 with diluted HCl solution. Dilute to 500 ml with distilled water.

C. Add 2 ml of the solution to 50 ml distilled water. Adjust the pH reaction to 7.5 ± 0.5. Dilute to 100 ml with distilled water. This yields a stock solution containing 2 mcg folic acid per ml.

D. Prepare the stock solution fresh daily.

The standard solution for the assay is made by diluting 1 ml of this stock solution to 1 liter with distilled water. This solution contains 2 ng folic acid per ml. Use 0.0, 0.5, 1, 2, 3, 4, and 5 ml per assay tube.

Some laboratories may wish to alter the concentration of folic acid recommended above for the standard curve. This is permissible if the concentration used is within the limits specified by AOAC.[1]

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.

2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average. Use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC international, 16th ed. AOAC International, Arlington, VA.

Packaging

Folic AOAC Medium	100 g	0967-15*

*Store at 2-8°C

Bacto® Folic Acid Assay Medium

Intended Use

Bacto Folic Acid Assay Medium is used for determining folic acid concentration by the microbiological assay technique.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of medium are used for this purpose:

1. Maintenance Medium: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose.
2. Inoculum Medium: To condition the test culture for immediate use.
3. Assay Medium: To permit quantitation of the vitamin under test.

Folic Acid Assay Medium is used in the microbiological assay of folic acid with *Enterococcus hirae* ATCC® 8043 as the test organism. Folic Acid Assay Medium is prepared according to the formula described by Capps, Hobbs and Fox,[1] modified with sodium citrate instead of sodium acetate.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off white to very light beige, free-flowing, homogeneous.
Solution:	3.75% (single strength) or 7.5% (double strength) solution, soluble in distilled or deionized water upon boiling for 2-3 minutes. Solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	Very light amber, clear, may have a very slight precipitate.
Reaction of 3.75% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare single-strength Folic Acid Assay Medium per label directions. The medium should support the growth of *E. hirae* ATCC® 8043. The most effective range is 2-10 ng folic acid per 10 ml tube.

Principles of the Procedure

Folic Acid Assay Medium is a folic acid-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *E. hirae* ATCC® 8043. The addition of folic acid in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

Formula

Folic Acid Assay Medium

Formula Per Liter	
Bacto Vitamin Assay Casamino Acids	12 g
Bacto Dextrose	40 g
Sodium Citrate	20 g
L-Cystine	0.2 g
DL-Tryptophane	0.2 g
Adenine Sulfate	20 mg
Guanine Hydrochloride	20 mg
Uracil	20 mg
Thiamine Hydrochloride	2 mg
Pyridoxine Hydrochloride	4 mg
Riboflavin	2 mg
Niacin	2 mg
p-Aminobenzoic Acid	200 µg
Biotin	0.8 µg
Calcium Pantothenate	400 µg
Dipotassium Phosphate	1 g
Monopotassium Phosphate	1 g
Magnesium Sulfate	0.4 g
Sodium Chloride	20 mg
Ferrous Sulfate	20 mg
Manganese Sulfate	20 mg

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small

amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.

4. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Folic Acid Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Enterococcus hirae* ATCC® 8043
Sterile tubes
Sterile 0.85% saline
Distilled or deionized water
0.01 N NaOH
Dilute HCl
Folic Acid USP
Lactobacilli Agar AOAC
Lactobacilli Broth AOAC
Centrifuge
Spectrophotometer

Method of Preparation

1. Suspend 7.5 grams in 100 ml distilled or deionized water.
2. Boil 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

Prepare assay samples according to references given in the specific assay procedures. Dilute the samples to approximately the same concentration as the standard solution.

Test Procedure

Prepare stock cultures of *E. hirae* ATCC® 8043 by stab inoculation of Lactobacilli Agar AOAC. Incubate at 35-37°C for 24-48 hours. Store tubes in the refrigerator. Make transfers at monthly intervals. Prepare the inoculum for assay by subculturing a stock culture of *E. hirae* ATCC® 8043 into a tube containing 10 ml of Lactobacilli Broth AOAC. After incubation at 35-37°C for 18-24 hours, centrifuge the cells under aseptic conditions and decant the supernatant. Wash the cells three times with 10 ml of sterile 0.85% saline. After the third wash, dilute the cell suspension 1:100 with sterile 0.85% saline. Use one drop of this latter suspension to inoculate each of the assay tubes.

It is essential that a standard curve be set up for each separate assay. Autoclaving and incubation conditions that influence the standard curve readings cannot always be duplicated. The standard curve is obtained by using folic acid at levels of 0.0, 2, 4, 6, 8 and 10 ng per 10 ml assay tube. Turbidimetric readings should be made after incubation at 35-37°C for 18-24 hours. Refrigerate tubes for 15-30 minutes to stop growth before reading.

Prepare the folic acid stock solution required for the standard curve as follows:

1. Dissolve 50 mg dried Folic Acid USP Reference Standard or equivalent in about 30 ml of 0.01 N NaOH and 300 ml distilled water.
2. Adjust to pH 7.5 ± 0.5 with diluted HCl solution. Add distilled water to give a volume of 500 ml.
3. Add 2 ml of the solution from step 2 to 50 ml distilled water. Adjust the pH to 7.5 ± 0.5 with HCl solution. Dilute to 100 ml with distilled water to give a stock solution containing 2 mcg folic acid per ml. Prepare the stock solution fresh daily.

Prepare the standard solution for the assay by diluting 1 ml of this stock solution in 1 liter with distilled water. This solution contains 2 ng folic acid per ml. Use 0.0, 0.5, 1, 2, 3, 4 and 5 ml per assay tube.

Following incubation, place the tubes in the refrigerator for 15-30 minutes to stop growth. The growth can be measured by a turbidimetric method and the curve constructed from the values obtained. The most effective assay range is between the levels of 2 and 10 ng folic acid per 10 ml tube.

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average. Use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. **Capps, Hobbs, and Fox.** 1948. J. Bacteriol. **55**:869.

Packaging

Folic Acid Assay Medium	100 g	0318-15

Bacto® Folic Acid Casei Medium
Bacto Folic Buffer A, Dried

Intended Use

Bacto Folic Acid Casei Medium is used for determining folic acid concentration by the microbiological assay technique.

Bacto Folic Buffer A, Dried is prepared for use with Folic Acid Casei Medium in the microbiological assay of serum folic acid.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test.

Folic Acid Casei Medium is prepared for the microbiological assay of folic acid, particularly folic acid in serum. *Lactobacillus casei* subsp. *rhamnosus* ATCC® 7469 is used as the test organism in this assay. Folic Acid Casei Medium is prepared according to the formulation described by Flynn, Williams, O'Dell and Hogan[1] and modified by Baker et al.[2] and Waters and Mollin.[3]

Total serum folic acid activity can vary depending on the disease state. It has been reported that normal subjects have a mean serum folic acid level of 9.9 ng per ml. Patients with uncomplicated pernicious anemia have a mean serum folic acid level of 16.6 ng per ml while patients with megaloblastic anemia have levels less than 4.0 ng per ml.

Folic Buffer A, Dried is used for preparing both the standard and the serum specimen in the microbiological assay of folic acid.

User Quality Control

Identity Specifications

Folic Acid Casei Medium

Dehydrated Appearance:	Off-white, homogeneous, with a tendency to clump.
Solution:	4.7% (single strength) and 9.4% (double strength) solution, soluble in distilled or deionized water upon boiling 1-2 minutes. Single-strength solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	Single-strength solution is very light amber, clear, may have a very slight precipitate.
Reaction of 4.7% Solution at 25°C:	6.7 ± 0.1

Folic Buffer A, Dried

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous.
Solution:	1.54% solution, soluble in distilled or deionized water.
Solution Appearance:	Colorless to very light amber, clear.
Reaction of 1.54% Solution at 25°C:	pH 6.1 ± 0.05

Cultural Response

Prepare Folic Acid Casei Medium per label directions. The medium is tested by creating a standard curve using Folic Acid at concentrations of 0 to 1.0 ng per 10 ml. This medium should support the growth of *L. casei* subsp. *rhamnosus* ATCC® 7469 when prepared in single strength and supplemented with ascorbic acid and Folic Acid.

Principles of the Procedure

Folic Acid Casei Medium is a folic acid-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *L. casei* subsp. *rhamnosus* ATCC® 7469. The addition of folic acid in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

Formula

Folic Acid Casei Medium

Formula Per Liter

Charcoal Treated Casitone	10 g
Bacto Dextrose	40 g
Sodium Acetate	40 g
Dipotassium Phosphate	1 g
Monopotassium Phosphate	1 g
DL-Tryptophane	0.2 g
L-Asparagine	0.6 g
L-Cysteine Hydrochloride	0.5 g
Adenine Sulfate	10 mg
Guanine Hydrochloride	10 mg
Uracil	10 mg
Xanthine	20 mg
Sorbitan Monooleate Complex	0.1 g
Glutathione (reduced)	5 mg
Magnesium Sulfate, Anhydrous	0.2 g
Sodium Chloride	20 mg
Ferrous Sulfate	20 mg
Manganese Sulfate	15 mg
Riboflavin	1 mg
p-Aminobenzoic Acid	2 mg
Pyridoxine Hydrochloride	4 mg
Thiamine Hydrochloride	400 µg
Calcium Pantothenate	800 µg
Nicotinic Acid	800 µg
Biotin	20 µg

Final pH 6.7 ± 0.1

Folic Buffer A, Dried

Formula Per Liter

Monopotassium Phosphate	10.656 g
Dipotassium Phosphate	3.744 g
Ascorbic Acid	1 g

Final pH 6.1 ± 0.05

Precautions

1. For Laboratory Use.
2. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
3. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.
4. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store Folic Acid Casei Medium and Folic Buffer A, Dried at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Folic Acid Casei Medium
Folic Buffer A, Dried

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus casei* subsp. *rhamnosus* ATCC® 7469
Ascorbic acid (25 mg)
Sterile 0.85% saline
Distilled and deionized water
0.01 N NaOH
0.05 N HCl
Lactobacilli Agar AOAC
Folic Acid
Incubator (35-37°C)
Micro Inoculum Broth
Centrifuge
Spectrophotometer

Method of Preparation

Folic Acid Casei Medium

1. Suspend 9.4 grams in 100 ml distilled or deionized water.
2. Add 50 mg ascorbic acid if standard and test samples are not prepared in Folic Buffer A.
3. Boil for 1-2 minutes.
4. Dispense 5 ml amounts into tubes, evenly dispersing any precipitate.
5. Add standard or test samples.
6. Adjust tube volume to 10 ml with distilled or deionized water.
7. Autoclave at 121°C for 5 minutes.

Folic Buffer A Dried

1. Dissolve the contents of one vial (15.4 grams) in 1 liter distilled or deionized water.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assays, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Preparation of Stock Cultures and Inoculum

Prepare stock cultures of the test organism, *L. casei* subsp. *rhamnosus* ATCC® 7469, by stab inoculation into prepared tubes of Lactobacilli Agar AOAC. Incubate the cultures at 35-37°C for 18-24 hours. Store cultures in the refrigerator at 2-8°C. Stock transfers are made at monthly intervals.

Prepare the inoculum for assay by subculturing from a stock culture of *L. casei* subsp. *rhamnosus* into a tube containing 10 ml prepared Micro Inoculum Broth. Incubate at 35-37°C for 16-18 hours. Under aseptic conditions, centrifuge the tubes to sediment the cells and decant the supernatant. Wash the cells in 10 ml sterile single-strength Folic Acid Casei Medium. Resediment the cells by centrifuging aseptically and decant the supernatant. Repeat washing two more times. After the third washing, resuspend the cells in 10 ml sterile single-strength medium and dilute 1 ml with 99 ml of the same medium. One drop of this suspension is used to inoculate each of the assay tubes. Read the growth response of the assay tubes turbidimetrically after 18-24 hours incubation at 35-37°C. (Some laboratories use 0.85% saline instead of the single-strength basal medium to wash and dilute the inoculum.)

Preparation of the Standard

It is essential that a standard curve be constructed for each separate assay. Autoclave and incubation conditions can influence the standard curve readings and cannot always be duplicated. The standard curve may be obtained by using folic acid at levels of 0.0, 0.1, 0.2, 0.4, 0.6, 0.8 and 1 ng per assay tube (10 ml).

The folic acid required for preparation of the standard curve may be prepared as follows:

Dissolve 50 mg dried folic acid in about 30 ml 0.01 N NaOH and 300 ml distilled water. Adjust to pH 7-8 with 0.05 N HCl and dilute to 500 ml with distilled water. Dilute 10 ml of this solution with 500 ml distilled water. Further dilute 1 ml in 1 liter distilled water to make a stock solution containing 2 ng per ml folic acid. Prepare the standard solution containing 0.2 ng per ml folic acid by diluting 10 ml of stock solution with 90 ml of Folic Buffer A, Dried solution. Use 0.0, 0.5, 1, 2, 3, 4 and 5 ml per assay tube.

Prepare the stock solution fresh daily.

Preservation of Serum Specimens

1. Allow the blood specimen to clot and the serum to separate from the clot.
2. Aspirate the serum into a clean dry tube and centrifuge to remove any cells that may be present. Avoid hemolysis. Dispense 5 ml of each serum sample into clean dry test tubes and add 25 mg ascorbic acid to each tube.
3. If the test is not begun immediately, place tubes in a freezer and hold below -20°C.

Preparation of Serum Specimen

1. Thaw the serum containing ascorbic acid.
2. Add 5 ml of the uniform sample to 45 ml rehydrated Folic Buffer A, Dried.
3. Incubate the serum-buffer solution at 37°C for 90 minutes. Autoclave the incubated mixture at 121°C for 2.5 minutes.
4. Remove the coagulated protein by centrifuging and transfer the clear supernatant to a clean dry tube. The clear solution is the sample to use in the folic acid assay.

Procedure for Total Folic Acid

1. Use 0.5, 1.0, 1.5 ml or other volumes of the prepared serum extracts as described above.
2. Fill each assay tube with 5 ml of rehydrated Folic Acid Casei Medium and sufficient distilled or deionized water to give a total volume of 10 ml per tube.
3. Autoclave tubes at 121°C for 5 minutes.
4. Add 1 drop of inoculum described under **Preparation of Stock Culture and Inoculum** to each assay.
5. Incubate at 35-37°C for 18-24 hours. Tubes are refrigerated for 15-30 minutes to stop growth before reading turbidimetrically.

Results

The amount of folic acid in the test samples can be determined by interpolating the results with the values obtained on the standard curve, taking into consideration the dilutions of the samples.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. **Flynn, Williams, O'Dell, and Hogan.** 1951. Anal. Chem. **23**:180.
2. **Baker, Herbert, Frank, Pasher, Hunter, Wasserman, and Sobotka.** 1959. Clin. Chem. **5**:275.
3. **Waters and Molin.** 1961. J. Clin. Pathol. **14**:335.

Packaging

Folic Acid Casei Medium	100 g	0822-15
Folic Buffer A, Dried	6 x 15.4 g	3246-33

Fraser Broth

Bacto® Fraser Broth Base · Fraser Broth Supplement

Intended Use

Bacto Fraser Broth Base is used with Bacto Fraser Broth Supplement in selectively enriching and detecting *Listeria*.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[1] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant women.[2] The first reported food-borne outbreak of listeriosis was in 1985,[3] and since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has indicated that the principle route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[4]

Implicated vehicles of transmission include turkey frankfurters,[5] coleslaw, pasteurized milk, Mexican-style cheese, paté, and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants, and is ubiquitous in nature, being present in a wide range of unprocessed foods as well as in soil, sewage, silage and river water.[6]

Bacto Fraser Broth Base and Bacto Fraser Broth Supplement are based on the formulation of Fraser and Sperber.[7] The medium is use in the rapid detection of *Listeria* from food[8] and environmental samples. Many common food contaminants such as streptococci, enterococci, *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* interfere with the isolation of *Listeria monocytogenes*.[9]

Listeria species grow over a pH range of 5.0-9.6, and survive in food products with pH levels outside these parameters.[10] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Principles of the Procedure

Bacto Tryptose, Bacto Beef Extract and Bacto Yeast Extract provide nitrogen, vitamins and minerals. Sodium phosphate and potassium phosphate are buffering agents. Differentiation is aided by including ferric ammonium citrate in the final medium. Since all *Listeria* species hydrolyze esculin, the addition of ferric ions to the medium will detect the reaction. A blackening of the medium by cultures containing esculin-hydrolyzing bacteria is the result of the formation of 6,7-dihydroxycoumarin that reacts with the ferric ions.[7]

Selectivity is provided by the presence of lithium chloride, nalidixic acid and acriflavine in the formula. The high salt tolerance of *Listeria* is used as a means to inhibit growth of enterococci.

Formula

Fraser Broth Base

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	5 g
Bacto Yeast Extract	5 g
Sodium Chloride	20 g
Sodium Phosphate, Dibasic	9.6 g
Potassium Phosphate, Monobasic	1.35 g
Esculin	1 g
Nalidixic Acid	0.02 g
Acriflavine HCl	0.024 g
Lithium Chloride	3 g

Final pH 7.2 ± 0.2 at 25°C

Fraser Broth Supplement

Ingredients per 10 ml vial

Ferric Ammonium Citrate	0.5 g

Precautions

1. For Laboratory Use.
2. **Fraser Broth Base**:

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Kidneys, Nerves

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   Fraser Broth Supplement:

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Bacto Fraser Broth Supplement at 2-8°C.

Store the prepared medium at 2-8°C.

User Quality Control

Identity Specifications

Fraser Broth Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	5.5% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, clear to slightly opalescent with a fine precipitate.
Prepared Tubes:	Medium amber, clear to slightly opalescent with a fine precipitate.
Reaction of 5.5% Solution at 25°C:	pH 7.2 ± 0.2

Bacto Fraser Broth Supplement

Solution Appearance:	Dark brown solution.

Cultural Response

Prepare Fraser Broth Base per label directions. Add Fraser Broth Supplement. Inoculate and incubate at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ESCULINE REACTION
Escherichia faecalis	29212*	1,000-2,000	marked to complete inhibition	–
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition	–
Listeria monocytogenes	19114	100-1,000	good	+

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube — *Listeria monocytogenes* ATCC® 19114

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Fraser Broth Base
Fraser Broth Supplement

Materials Required But Not Provided

Flasks with closure
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50°C)
Test tubes with closures
Incubator (30°C)
Incubator (35°C)

Method of Preparation

1. Suspend 55 grams of Fraser Broth Base in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Aseptically add 10 ml Fraser Broth Supplement. Mix well.
5. Dispense into tubes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

To isolate *Listeria monocytogenes* from processed meats and poultry, the following procedure is recommended by the U.S.D.A.[8]

1. Add 25 grams of test material to 225 ml of UVM Modified Listeria Enrichment Broth and mix or blend thoroughly.
2. Incubate for 20-24 hours at 30°C.
3. Transfer 0.1 ml of the incubated broth to Fraser Broth. Incubate at 35°C for 26 ± 2 hours.
4. At 24 and 48 hours, streak the Fraser Broth culture to Modified Oxford Agar.
5. Incubate the Modified Oxford plates at 35°C for 24-48 hours.

Results

1. Examine agar plates for suspect colonies. For further identification and confirmation of *Listeria* spp., consult appropriate references.[8,10,11,12]
2. Rapid slide and macroscopic tube tests can be used for definitive serological identification.

Limitations of the Procedure

1. Since *Listeria* species other than *L. monocytogenes* can grow on these media, an identification of *Listeria monocytogenes* must be confirmed by biochemical and serological testing.[11,12]
2. Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.

References

1. **Murray, E. G. D., R. A. Webb, and M. B. R. Swann.** 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n. sp.). J. Path. Bact. **29:**407- 439.
2. **Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low- and high-fat, frozen and refrigerated ground beef. J. Food Prot. **57**:969-974.
3. **Wehr, H. M.** 1987. *Listeria monocytogenes* - a current dilemma special report. J. Assoc. Off. Anal. Chem. **70**:769-772.
4. **Bremer, P. J., and C. M. Osborne.** 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels (*Perna canaliculus*) prepared for hot smoking. J. Food Prot. **58:**604-608.
5. **Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
6. **Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett**. 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
7. **Fraser, J., and W. Sperber**. 1988. Rapid detection of *Listeria* in food and environmental samples by esculin hydrolysis. J. Food Prot. **51:**762-765.
8. **Lee, W. H., and D. McClain**. 1994. Laboratory Communication No. 57 (revised February 8, 1994), U.S.D.A., F.S.I.S. Microbiology Division, Bethesda, MD.
9. **Kramer, P. A., and D. Jones**. 1969. Media selective for *Listeria monocytogenes*. J. Appl. Bacteriol. **32:**381-394.
10. **Donnelly, C. W., R. E. Bracket, D. Doores, W. H. Lee, and J. Lovett.** 1992. *Listeria*, p. 637-663. *In* C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
11. **Swaminathan, B., J. Rocourt, and J. Bille.** 1995. *Listeria*, p. 342-343. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
12. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products. *In* R. T. Marshall (ed.), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Packaging

Fraser Broth Base	500 g	0219-17
	2 kg	0219-07
Fraser Broth Supplement	6 x 10 ml	0211-60*

*Store at 2-8°C

Bacto® GC Medium Base · Bacto Supplement B Bacto Supplement VX · Bacto Hemoglobin Bacto Antimicrobic Vial CNV · Bacto Antimicrobic Vial CNVT

Intended Use

Bacto GC Medium Base is used with various additives in isolating and cultivating *Neisseria gonorrhoeae* and other fastidious microorganisms.

Bacto Supplement B with Bacto Reconstituting Fluid B is used for supplementing media to culture fastidious organisms, particularly *Neisseria gonorrhoeae* and *Haemophilus influenzae*.

User Quality Control

Identity Specifications

GC Medium Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water upon boiling; light to medium amber, opalescent, may have slight precipitate, "ground glass" appearance.
Prepared Medium:	With Hemoglobin and Supplement: chocolate brown, opaque.
Reaction of 3.6% Solution at 25°C:	pH 7.2 ± 0.2

Hemoglobin

Dehydrated Appearance:	Dark brown, fine, free-flowing.
Solution:	2% solution, insoluble in distilled or deionized water; chocolate brown, opaque with a dispersed precipitate.
Reaction of 2% Solution at 25°C:	pH 8.2 ± 0.2

Supplement B

Lyophilized Appearance:	Tan to reddish brown lyophilized powder or cake.
Rehydrated Appearance:	Medium to dark amber may have a reddish tint, clear to slightly opalescent solution.
Reconstituting Fluid:	Colorless, clear solution.
Sterility Test:	Satisfactory.
Reaction of Solution at 25°C:	pH 6.5-7.2

Supplement VX

Lyophilized Appearance:	Pink, lyophilized powder.
Rehydrated Appearance:	Pink, clear solution without precipitate.
Reconstituting Fluid:	Colorless, clear solution.
Sterility Test:	Satisfactory.
Reaction of Solution at 25°C:	pH 0.75-2.5

continued on following page

Bacto Supplement VX with Bacto Reconstituting Fluid VX is used to culture fastidious microorganisms, particularly *Neisseria gonorrhoeae*.

Bacto Hemoglobin is used in preparing microbiological culture media.

Bacto Antimicrobic Vial CNV and Bacto Antimicrobic Vial CNVT are sterile lyophilized preparations containing inhibitory agents to be used in selective media for culturing *Neisseria gonorrhoeae* and *Neisseria meningitidis*.

Also Known As

GC Medium is also referred to as Chocolate Agar Base, Chocolate Agar, Enriched and GC Agar.

Summary and Explanation

In 1945, Johnston[1] described a medium that could successfully produce colonies of *N. gonorrhoeae* in 24 rather than 48 hours. The accelerated growth rates were primarily due to the decreased agar content (solidity) of the media. GC Medium Base was introduced in 1947 with reduced agar content. While investigating the growth rate of some gonococcal strains, a medium containing the growth factors glutamine and cocarboxylase, was found to improve recovery.[2,3] From this discovery, Supplement B was developed. In a comparative study[4] of 12 different media, an enriched Chocolate Agar prepared with GC Medium Base, Hemoglobin and Supplement B proved superior for isolating *N. gonorrhoeae.*

Supplement B w/ Reconstituting Fluid is a sterile yeast concentrate for use in supplementing media for microorganisms with exacting growth requirements. It is recommended for use in the preparation of chocolate agar described by Christensen and Schoenlein.[5]

Supplement VX w/ Reconstituting Fluid is a sterile lyophilized concentrate. Supplement B and Supplement VX are recommended for enriching GC Medium Base, Proteose No. 3 Agar, Thayer-Martin Medium and Modified Thayer-Martin Medium.

Hemoglobin, an autoclavable preparation of beef blood, is prepared according to described by Spray.[6] Hemoglobin provides hemin, which is required by *Haemophilus* species and enhances growth of *Neisseria* species.

In 1964, Thayer and Martin[7] formulated a selective medium incorporating the antibiotics polymyxin B and ristocetin into GC Agar with added hemoglobin and yeast supplement B. Thayer and Martin[8] improved their medium by replacing the two original antibiotics with a new microbial solution of colistin, vancomycin and nystatin (CVN). In 1970, Martin and Lester[9] improved the new Thayer-Martin (TM) medium by increasing the agar and glucose content and by incorporating an additional antibiotic, trimethoprim lactate (T) into the formulation. This improved medium is called Modified Thayer-Martin (MTM) Medium. Antimicrobic Vial CNV and Antimicrobic Vial CNVT are used in the preparation of Thayer-Martin (TM) Medium and Modified Thayer-Martin, respectively.

Martin and Lewis[10] further improved selectivity of MTM by increasing the concentration of vancomycin and replacing nystatin with anisomycin for greater inhibition of yeasts; this is known as Martin-Lewis (ML) Agar Medium. Transgrow Medium is a transport medium system incorporating either MTM or ML formulations.[11]

Principles of the Procedure

GC Medium Base is employed as the basal medium in the preparation of Chocolate Agar Enriched, Thayer-Martin Medium and Modified Thayer-Martin Medium.

Proteose Peptone No. 3 provides nitrogen, vitamins and amino acids in GC Medium Base. Corn Starch absorbs any toxic metabolites that are produced, Potassium Phosphate, Dibasic and Monobasic buffer the medium. Sodium Chloride maintains osmotic balance. Bacto Agar is a solidifying agent.

Chocolate Agar is prepared from GC Medium Base with the addition of 2% Hemoglobin. Hemoglobin provides hemin (X factor) required for growth of *Haemophilus* and enhanced growth of *Neisseria*.

The growth rate of *Neisseria* and *Haemophilus* is improved with the addition of 1% Supplement B or VX, providing the growth factors glutamine and cocarboxylase. Supplement B contains yeast concentrate, glutamine, coenzyme, cocarboxylase, hematin and growth factors. Supplement VX is a sterile, defined lyophilized concentrate of essential growth factors. Supplement VX supplies vitamins, amino acids, coenzymes, dextrose and other factors to improve the growth of *Haemophilus* and *Neisseria* species.

Antimicrobic Vial CNV and Antimicrobic Vial CNVT are antimicrobial agents used as inhibitors in the selective media, Thayer-Martin Medium and Modified Thayer-Martin Medium.

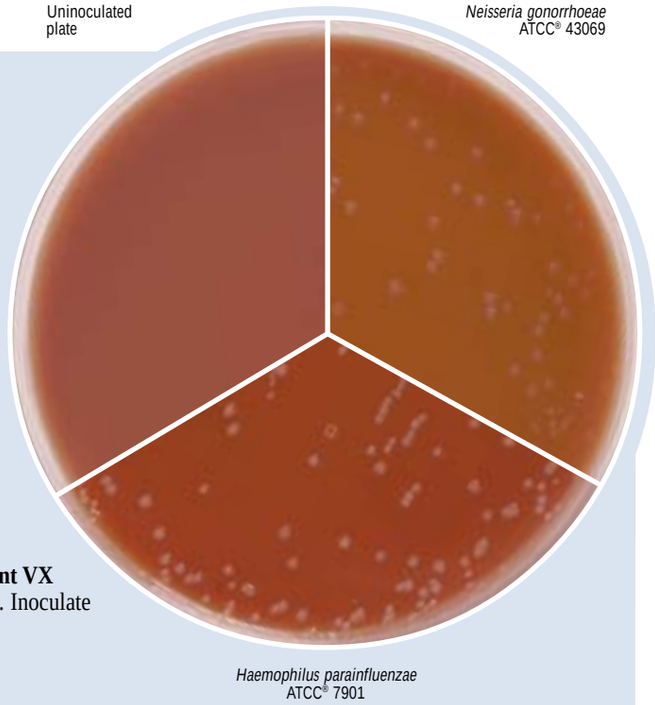
Formula

GC Medium Base

Formula Per Liter

Bacto Proteose Peptone No. 3	15 g
Corn Starch	1 g
Potassium Phosphate, Dibasic	4 g
Potassium Phosphate, Monobasic	1 g
Sodium Chloride	5 g
Bacto Agar	10 g

Final pH 7.2 ± 0.2 at 25°C



User Quality Control cont.

Antimicrobic Vial CNV

Lyophilized Appearance:	Pale yellow, dry cake or powder.
Rehydrated Appearance:	Off-white to pale yellow, opalescent to opaque even suspension.
Solubility:	Not completely soluble in distilled water, but must be evenly suspendable.
Microbial Limits Test:	Negative.

Antimicrobic Vial CNVT

Lyophilized Appearance:	Pale yellow, dry cake or powder.
Rehydrated Appearance:	Off-white to pale yellow, opalescent to opaque even suspension.
Solubility:	Not completely soluble in distilled water, but must be evenly suspendable.
Microbial Limits Test:	Negative.

Cultural Response

GC Medium Base, Hemoglobin 2%, Supplement B or Supplement VX

Prepare Chocolate Agar with GC Medium Base, per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Haemophilus influenzae	10211	30-300	good
Neisseria gonorrhoeae	43069	30-300	good

GC Medium Base, Hemoglobin 2%, Supplement B or Supplement VX, Antimicrobic Vial CNV or CNVT

Prepare Thayer-Martin Medium or Modified Thayer-Martin Medium with GC Medium Base per label directions, enriched with Antimicrobic Vial CNV or Antimicrobic Vial CNVT. Inoculate and incubate at 35 ± 2°C under CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Candida albicans	60193	1,000	partial inhibition
Escherichia coli	25922*	1,000	marked to complete inhibition
Neisseria gonorrhoeae	43069	100-1,000	good
Neisseria meningitidis	13090*	100-1,000	good
Neisseria sicca	9913*	100-1,000	marked to complete inhibition
Staphylococcus epidermidis	12228*	1,000	marked to complete inhibition

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disk Technical Information.

Hemoglobin

An autoclavable preparation of beef blood prepared according to the procedure described by Spray.[6]

Supplement B

Processed to preserve both the thermolabile and thermostable growth accessory factors of fresh yeast, contains glutamine, coenzyme (V factor), cocarboxylase and other growth factors, as well as hematin (X factor).

Supplement VX

Ingredients per 10 ml Vial

Adenine Sulfate	10 mg
p-Aminobenzoic Acid	0.25 mg
Cocarboxylase	2 mg
L-Cysteine HCl	259 mg
L-Cystine	11 mg
Diphosphopyridine Nucleotide	3.5 mg
Ferric citrate	0.3 mg
L-Glutamine	200 mg
Guanine HCl	0.3 mg
Thiamine HCl	0.06 mg
Vitamin B_{12} (Cyanocobalamin)	0.2 mg
Bacto Dextrose	1 mg

Antimicrobial Vial CNV

A sterile, lyophilized preparation containing 7,500 µg Colistin Sulfate, 12,500 units Nystatin and 3,000 µg Vancomycin per 10 ml.

Antimicrobial Vial CNVT

A sterile lyophilized preparation containing 7,500 µg Colistin Sulfate, 12,500 units Nystatin, 3,000 µg Vancomycin and 5,000 µg Trimethoprim per 10 ml.

Precautions

1. For Laboratory Use.
2. **Antimicrobic Vial CNV**

   HARMFUL. MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Kidney, Ears, Lungs, Thorax.

   Antimicrobic Vial CNVT

   HARMFUL. HARMFUL BY INHALATION AND IF SWALLOWED. (US) MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Lungs, Thorax.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store GC Medium Base below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Hemoglobin below 30°C. Store Hemoglobin 2% at 15-30°C.

Store Supplements B and VX at 2-8°C.

Store Antimicrobic Vials CNV and CNVT at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

GC Medium Base
Hemoglobin, Hemoglobin 2%
Supplement B or VX
Antimicrobial Vial CNV or CNVT

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C).
Sterile Petri dishes or tubes

Method of Preparation

Supplement B
Supplement VX

1. Aseptically rehydrate Supplement B and Supplement VX with 10 ml or 100 ml of the corresponding Reconstituting Fluid, as appropriate.
2. Rotate the vial to dissolve completely.

Hemoglobin

1. Place 10 grams of Hemoglobin in a dry beaker.
2. Measure 500 ml distilled or deionized water.
3. Add approximately 100 ml amounts to the Hemoglobin, stirring well after each addition. Use a spatula to break up clumps.
4. Transfer to flasks as desired for autoclaving.
5. Autoclave at 121°C for 15 minutes.
6. Cool to 45-50°C.
7. Swirl flask to reestablish complete solution and add to an equal amount of double-strength sterile agar base cooled to 45-50°C.

Hemoglobin 2%

1. Shake the bottle to resuspend any sedimented hemoglobin before use.

Antimicrobial Vial CNV
Antimicrobial CNVT

1. Aseptically rehydrate Antimicrobial Vial CNV or Antimicrobial CNVT with the appropriate amount of sterile distilled or deionized water, as indicated on the product label.
2. Rotate the vial to dissolve completely.

Chocolate Agar, Enriched

1. Suspend 7.2 grams GC Medium Base in 100 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 ml Hemoglobin Solution 2%.
5. Aseptically add 2 ml Supplement B or Supplement VX. Mix well.
6. Dispense into sterile Petri dishes or tubes as desired.

Thayer-Martin Medium

1. Suspend 7.2 grams GC Medium Base in 100 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 ml Hemoglobin Solution 2%.
5. Aseptically add 2 ml Supplement B or Supplement VX.
6. Aseptically add 2 ml rehydrated Antimicrobial Vial CNV to the medium.
7. Dispense into sterile Petri dishes.

Modified Thayer-Martin Medium

1. Suspend 7.2 grams GC Medium Base in 100 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Aseptically add 100 ml Hemoglobin Solution 2% and 0.3 grams dextrose to the medium.
5. Aseptically add 2 ml Supplement B or Supplement VX.
6. Aseptically add 2 ml of rehydrated Antimicrobial Vial CNVT to the medium.
7. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion on the isolation and identification of *Neisseria* and *Haemophilus*, consult the procedures outlined in the references.[12,13,14]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. GC Medium Base is intended for use with supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.
3. Improper specimen collection, environment, temperature, CO_2 level, moisture and pH can adversely affect the growth and viability of the organism.
4. Inactivation or deterioration of antibiotics in Thayer-Martin or Modified Thayer- Martin may allow growth of contaminants.
5. GC Medium Base has sufficient buffering capacity to offset the very low pH of the small amount of Supplement VX added. The pH of some media has to be adjusted with 1% NaOH after the addition of Supplement VX.

References

1. **Johnston, J.** 1945. Comparison of gonococcus cultures read at 24 and 48 hours. J. Venera. Dis. Inform. **26**:239.
2. **Lankford, C. E., V. Scott, M. F. Cox, and W. R. Cooke.** 1943. Some aspects of nutritional variation of the gonococcus. J. Bacteriol. **45**:321.
3. **Lankford, C. E., and E. E. Snell.** 1943. Glutamine as a growth factor for certain strains of *Neisseria gonorrhoeae*. J. Bacteriol. **45**:421.
4. **Carpenter, C. M., M. A. Bucca, T. C. Buck, E. P. Casman, C. W. Christensen, E. Crowe, R. Drew, J. Hill, C. E. Lankford, H. E. Morton, L. R. Peizer, C. I. Shaw, and J. D. Thayer.** 1949. Am. J. Syphil. Gonorrh. Vener. Dis. **33**:164
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6. **Spray.** 1930. J. Lab. Clin. Med. **16**:166.
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8. **Thayer, J. D., and A. Lester.** 1971. Transgrow, a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis*. HSMHA Health Service Rep., **86**:30.
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10. **Martin, J. E., Jr., and J. S. Lewis**. 1977. Anisomycin: improve anti-mycotic activity in modified Thayer-Martin Medium. Public Health Rep., **35**:53.
11. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, M.D.
12. **Isenberg, H. D. (ed.)**. 1992. Clinical microbiology procedures handbook, vol 1. American Society for Microbiology, Washington, D.C.
13. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
14. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

GC Medium Base	100 g	0289-15
	500 g	0289-17
	2 kg	0289-07
	10 kg	0289-08
Hemoglobin	100 g	0136-15
	500 g	0136-17
	2 kg	0136-07
	10 kg	0136-08
Hemoglobin 2% Solution	6 x 100 ml	3248-73
Supplement B w/Reconstituting Fluid	6 x 100 ml	0276-60
	100 ml	0276-72
Supplement VX w/Reconstituting Fluid	6 x 10 ml	3354-60
	100 ml	3354-72
Antimicrobic Vial CNV	6 x 10 ml	3260-60
Antimicrobic Vial CNVT	6 x 10 ml	3198-60
	100 ml	3198-72

Bacto® GN Broth, Hajna

Intended Use

Bacto GN Broth, Hajna is used for isolating and cultivating gram-negative microorganisms.

Also Known As

Gram Negative (GN) Broth[1]

Hajna GN Broth[1]

Gram Negative Enrichment Broth[1]

Summary and Explanation

Hajna[2,3] formulated Gram Negative (GN) Broth as an enrichment medium for enteric gram-negative bacilli, especially *Salmonella* and *Shigella*, from clinical and non-clinical specimens. Croft and Miller[4] demonstrated improved recovery of *Shigella* using GN Broth enrichment compared to direct inoculation of agar media. Taylor and Schelhart[5] reported improved recovery of both *Salmonella* and *Shigella* when using GN Broth enrichment compared to direct inoculation of agar media. Taylor and Schelhart[6] showed GN Broth to be superior to selenite enrichment medium for recovering *Shigella*.

GN Broth, Hajna is recommended as an enteric enrichment broth for clinical specimens[7,8] and as a nonselective enrichment broth for foods[9] to recover *Salmonella* and *Shigella*.

Principles of the Procedure

GN Broth, Hajna contains Tryptose as a source of carbon, nitrogen, vitamins and minerals. Dextrose and D-Mannitol are carbohydrates. Sodium Citrate and Sodium Desoxycholate inhibit growth of gram-positive bacteria and of coliforms other than *Salmonella* and *Shigella*. Dipotassium Phosphate and Monopotassium Phosphate buffer the medium.

The higher concentration of mannitol over dextrose favors growth of mannitol-fermenting *Salmonella* and *Shigella* over mannitol non-fermenting species, such as *Proteus*.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white to light tan, free-flowing, homogeneous.
Solution:	3.9% solution, soluble in distilled or deionized water. Solution is light amber, clear to very slightly opalescent.
Prepared Medium:	Light amber, clear to very slightly opalescent.
Reaction of 3.9% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare GN Broth, Hajna per label directions. Inoculate the medium and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	100-1,000	good
Salmonella typhimurium	14028*	100-1,000	good
Shigella flexneri	12022*	100-1,000	good
Enterococcus faecalis	19433*	1,000-2,000	none to poor

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

GN Broth, Hajna

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	1 g
Bacto D-Mannitol	2 g
Sodium Citrate	5 g
Sodium Desoxycholate	0.5 g
Dipotassium Phosphate	4 g
Monopotassium Phosphate	1.5 g
Sodium Chloride	5 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

GN Broth, Hajna

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Dissolve 39 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes. Avoid overheating.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Growth of gram-negative organisms, especially *Salmonella* and *Shigella* species, is enhanced.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol 1, p. 357-359. Williams & Wilkins, Baltimore, MD.
2. **Hajna, A. A.** 1955. A new specimen preservative for gram-negative organisms of the intestinal group. Public Health Lab. **13**:59-62.
3. **Hajna, A. A.** 1955. A new enrichment broth medium for gram-negative organisms of the intestinal group. Public Health Lab. **13**:83-89.
4. **Croft, C. C., and M. J. Miller.** 1956. Isolation of *Shigella* from rectal swabs with Hajna "GN" broth. Am. J. Clin. Path. **26**:411-417.
5. **Taylor, W. I., and D. Schelhart.** 1967. Isolation of shigellae, IV. Comparison of plating media with stools. Am. J. Clin. Path. **48**:356-362.
6. **Taylor, W. I., and D. Schelhart.** 1968. Isolation of shigellae, V. Comparison of enrichment broths with stools. Appl. Microbiol. **16**:1383-1386.
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8. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, 1.10.8. American Society for Microbiology, Washington, D.C.
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Packaging

GN Broth, Hajna	500 g	0486-17

Bacto® Gelatin
Bacto Gelatone

Intended Use

Bacto Gelatin is used in preparing microbiological culture media.

Bacto Gelatone is used in preparing microbiological culture media.

Also Known As

Gelatone is also referred to as Gelatin Peptone.

Summary and Explanation

Gelatin is a protein of uniform molecular constitution derived chiefly by the hydrolysis of collagen.[1] Collagens are a class of albuminoids found abundantly in bones, skin, tendon, cartilage and similar animal tissues.[1]

Koch[1] introduced gelatin into bacteriology when he invented the gelatin tube method in 1875 and the plate method in 1881. This innovation, a solid culture method, became the foundation for investigation of the propagation of bacteria.[1] However, gelatin-based media were soon replaced by media containing agar as the solidifying agent.

Gelatin is used in culture media for determining gelatinolysis (elaboration of gelatinases) by bacteria. Levine and Carpenter[2] and Levine and Shaw[3] employed gelatin media in their studies of gelatin liquefaction. Garner and Tillett[4] used culture media prepared with gelatin to study the fibrinolytic activity of hemolytic streptococci.

Gelatin is a high grade gelatin in granular form which may be used as a solidifying agent or may be incorporated into culture media for various uses. Gelatin is used in Nutrient Gelatin, Motility GI Medium, Motility Medium S, Stock Culture Agar and Dextrose Starch Agar. Media containing gelatin are specified in Standard Methods[5,6] for multiple applications.

Gelatone, a granular pancreatic digest of gelatin, is deficient in carbohydrates. It is distinguished by low cystine and tryptophan content. Gelatone is used as an ingredient in media for fermentation studies and, by itself, to support growth of non-fastidious microorganisms.

Principles of the Procedure

The melting point of a 12% concentration of Gelatin is between 28 and 30%C, which allows it to be used as a solidifying agent. Certain microorganisms elaborate gelatinolytic enzymes (gelatinases) which hydrolyze gelatin, causing liquefaction of a solidified medium or preventing the gelation of a medium containing gelatin. Gelatin is also used as a source of nitrogen and amino acids.

Gelatone is a peptone from gelatin obtained by digesting gelatin with pancreatin.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated product below 30°C. The dehydrated product is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Gelatin

Gelatone

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Preparation varies depending on the medium being prepared.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Gelatin or Gelatone.

Results

Refer to appropriate references and procedures for results.

References

1. **Gershenfeld, L., and L. F. Tice.** 1941. Gelatin for bacteriological use. J. Bacteriol. **41**:645-652.
2. **Levine and Carpenter.** 1923. J. Bacteriol. **8**:297.
3. **Levine and Shaw.** 1924. J. Bacteriol. **9**:225.
4. **Garner and Tillett.** 1934. J. Exp. Med. **60**:255.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Packaging

Gelatin	100 g	0143-15
	500 g	0143-17
	10 kg	0143-08
Gelatone	500 g	0657-17

User Quality Control

Identity Specifications

Gelatin

Dehydrated Appearance:	Light beige, free-flowing, homogeneous granules.
Solution:	12% solution, soluble in distilled or deionized water on slight heating in a 50-55°C waterbath. Solution is light amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Gel:	Very light amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 12% Solution at 25°C:	pH 6.8 ± 0.2

Gelatone

Dehydrated Appearance:	Tan, free-flowing granules.
Solution:	10% solution, soluble in distilled or deionized water: 1%-Very light to light amber, clear; 2%-Light to medium amber, clear; 10%-Medium to dark amber, clear to very slightly opalescent.
Reaction of 2% Solution at 25°C:	pH 6.3-7.6

Uninoculated tube — *Bacillus subtilis* ATCC® 6633

Cultural Response

Gelatin

Prepare a 12% Gelatin solution in 0.8% Nutrient Broth and sterilize. Inoculate and incubate at 35 ± 2°C under appropriate atmospheric conditions for 18-48 hours or for up to two weeks for the gelatinase test. To read gelatinase, refrigerate until well chilled and compare to uninoculated tubes. Tubes positive for gelatinase will remain liquid.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	GELATINASE
Escherichia coli	25922*	100-1,000	good	–
Clostridium sporogenes	11437	100-1,000	good	+
Bacillus subtilis†	6633	100-1,000	good	+

†*Bacillus subtilis* is available as Bacto Subtilis Spore Suspension.

Gelatone

Prepare a 2% Gelatone solution in 0.5% saline; adjust pH to 7.2-7.4; add 1.5% Bacto Agar, boil and sterilize. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Brucella suis	4314	100-1,000	good growth
Escherichia coli	25922*	100-1,000	good growth
Staphylococcus aureus	25923*	100-1,000	good growth

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Bacto® Giolitti-Cantoni Broth Base
Bacto Potassium Tellurite Solution 3.5%

Intended Use

Bacto Giolitti-Cantoni Broth Base is used with Bacto Potassium Tellurite Solution 3.5% in enriching *Staphylococcus aureus* from foods during isolation procedures.

Summary and Explanation

Giolitti and Cantoni[1] described a broth medium with added potassium tellurite and a test procedure for enriching small numbers of staphylococci in foods. Mossel et al[2] recommended Giolitti-Cantoni Broth for detecting *Staphylococcus aureus* in dried milk and other infant foods where the organism should be absent from 1 g of test material.

The International Dairy Federation (IDF) and American Public Health Association recommend a procedure for detecting *S. aureus* in dairy products using Giolitti-Cantoni Broth as an enrichment medium from which selective media are inoculated.[3, 4]

Principles of the Procedure

Giolitti-Cantoni Broth Base contains Tryptone and Beef Extract as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. D-Mannitol is the carbohydrate source. Sodium Pyruvate stimulates growth of staphylococci. Lithium Chloride inhibits gram-negative bacilli. Potassium Tellurite Solution 3.5% supplies potassium tellurite, which in combination with glycine, inhibits gram-positive bacteria other than staphylococci.

Formula

Giolitti-Cantoni Broth Base

Formula Per Liter

Bacto Tryptone	10 g
Bacto Beef Extract	5 g
Bacto Yeast Extract	5 g
Bacto D-Mannitol	20 g
Sodium Chloride	5 g
Lithium Chloride	5 g
Glycine	1.2 g
Sodium Pyruvate	3 g

Final pH 6.9 ± 0.2 at 25°C

Potassium Tellurite Solution 3.5%

A filter-sterilized solution of potassium tellurite in distilled water.

Precautions

1. For Laboratory Use.

User Quality Control

Identity Specifications

Giolitti-Cantoni Broth Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	5.42% solution, soluble in distilled or deionized water on warming. Solution is medium amber, clear without significant precipitate.
Prepared Medium:	Medium amber, clear without significant precipitate.
Reaction of 5.42% Solution at 25°C:	pH 6.9 ± 0.2

Potassium Tellurite Solution 3.5%

Appearance:	Colorless, clear solution, may have a fine precipitate.

Cultural Response

Prepare Giolitti-Cantoni Broth per label directions. Inoculate per Test Procedure and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Escherichia coli	25922*	1,000-2,000	inhibited	no blackening
Micrococcus luteus	10240	1,000-2,000	inhibited	no blackening
Staphylococcus aureus	6538	100-1000	good	blackening
Staphylococcus aureus	25923*	100-1000	good	blackening

Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Staphylococcus aureus* ATCC® 6538

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

2. **Giolitti-Cantoni Broth Base**

   HARMFUL. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGANS: Blood, Kidneys, Nerves.

   Potassium Tellurite Solution

   WARNING! HARMFUL IF SWALLOWED. CAUSES IRRITATION.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store dehydrated Giolitti-Cantoni Broth Base below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Potassium Tellurite Solution 3.5% at 15-30°C

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Giolitti-Cantoni Broth Base
Potassium Tellurite Solution 3.5%

Materials Required but not Provided

Glassware
Tubes 20 X 200 mm
Distilled or deionized water
Autoclave
Incubator (35°C)
Sterile paraffin wax or sterile mineral oil

Method of Preparation

1. Suspend 54.2 grams Giolitti-Cantoni Broth Base in 1 liter distilled or deionized water.
2. Warm gently to dissolve completely.
3. Dispense 19 ml amounts into 20 x 200 mm tubes.
4. Autoclave at 121°C for 15 minutes. Cool to 15-30°C.
5. Aseptically add 0.3 ml Potassium Tellurite Solution 3.5% per 19 ml tube or 0.03 ml when testing meat products or quality control organisms.
6. Mix well.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate 1 gram or 1 ml of test sample (0.1 gram or 0.1 ml when testing meat or meat products) and 1 ml aliquots of each of a suitable decimal dilution series of the test sample into duplicate tubes.
2. Overlay each tube with 5 ml sterile molten paraffin wax to an approximate height of 2 cm.
3. Incubate at 35 ± 2°C 40-48 hours.
4. Examine daily.

Results

Read tubes for blackening of the medium (a positive reaction) or no blackening (a negative reaction). If blackening occurs, subculture to Baird Parker Agar to confirm the isolation of *S. aureus*.

References

1. **Giolitti, G., and C. Cantoni**. 1966. A medium for the isolation of staphylococci from foodstuffs. J. Appl. Bacteriol. **29**:395-398.
2. **Mossel, D. A. A., G. A. Harrewijn, and J. M. Elzebroek**. 1973. UNICEF.
3. **International Dairy Federation**. 1978. IDF Standard 60A:1978. International Dairy Federation.
4. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall (ed.). Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Packaging

Giolitti-Cantoni Broth Base	500 g	1809-17
Potassium Tellurite Solution 3.5%	25 ml	1814-65

Bacto® HC Agar Base

Intended Use

Bacto HC Agar Base, when supplemented with Polysorbate 80, is used for enumerating molds in cosmetic products.

Summary and Explanation

Methods for isolating molds from cosmetic products require incubation for 5 to 7 days using traditional agar media.[1] In 1986, Mead and O'Neill[2] described a new medium, HC Agar, for enumerating molds in cosmetic products that decreased incubation time to 3 days at 27.5 ± 0.5°C. HC Agar Base, based on the HC Agar formula of Mead and O'Neill, is supplemented with Polysorbate 80 to prepare HC Agar.

Principles of the Procedure

HC Agar Base contains Tryptone and Proteose Peptone as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose provides a source of fermentable carbohydrate. Ammonium Chloride and Magnesium Sulfate provide essential ions. Disodium and Monopotassium Phosphates

buffer the pH to near neutrality. Sodium Carbonate inactivates low levels of preservatives that are active at a more acidic pH (e.g., benzoic acid). Chloramphenicol inhibits bacteria, including *Pseudomonas aeruginosa* and *Serratia marcescens*, that are potential contaminants of cosmetic products. Polysorbate 80 neutralizes preservatives and sequesters surfactants that may be present in residual amounts from the product sample.[2] Bacto Agar is the solidifying agent.

Formula

HC Agar Base

Formula Per Liter

Bacto Tryptone	2.5 g
Bacto Proteose Peptone	2.5 g
Bacto Yeast Extract	5 g
Bacto Dextrose	20 g
Disodium Phosphate	3.5 g
Monopotassium Phosphate	3.4 g
Ammonium Chloride	1.4 g
Magnesium Sulfate	0.06 g
Chloramphenicol	0.1 g
Sodium Carbonate	1 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **TOXIC.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE CANCER. POSSIBLE RISK OF HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Eye/Ear, Muscles, Nerves, Urogenital.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light to light beige, free-flowing, homogeneous.
Solution:	5.45% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, slightly opalescent to opalescent, may have a slight precipitate.
Prepared Medium:	Medium amber with yellow tint, very slightly to slightly opalescent, no significant precipitate.
Reaction of 5.45% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare HC Agar Base per label directions. Supplement with Polysorbate 80. Inoculate and incubate the plates at 27.5 ± 0.5°C for 65-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1000	good
Pseudomonas aeruginosa	10145	1,000-2,000	none to poor
Serratia marcescens	13880	1,000-2,000	none to poor

The cultures listed are the minimum that should be used for performance testing.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

HC Agar Base

Materials Required but not Provided

Polysorbate 80
Glassware
Sterile Petri dishes
Autoclave
Incubator (27.5 ± 0.5°C)

Method of Preparation

1. Suspend 54.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Add 20 ml Polysorbate 80.
4. Autoclave at 121°C for 15 minutes.
5. Dispense into petri dishes.

Specimen Collection and Preparation

Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory in accordance with recommended guidelines.[1]

Test Procedure

1. Process each specimen as appropriate for that specimen and inoculate directly onto the surface of the medium.[1] Inoculate duplicate plates.
2. Incubate plates aerobically at 27.5 ± 0.5°C.
3. Examine plates for growth and recovery after 72 hours incubation.
4. Count mold colonies from duplicate plates and record average count as mold count per gram or milliliter of sample.

Results

Mold cultures should yield good growth and recovery. Bacteria should be inhibited.

Limitations of the Procedure

1. The 27.5 ± 0.5°C incubation temperature is critical for obtaining statistically significant mold counts after three days using this medium.
2. Nutritional requirements of organisms vary. Some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Hitchins, A. D., T. T. Tran, and J. E. McCarron**. 1995. Microbiological methods in cosmetics, p. 23-01-23.12. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
2. **Mead, C., and J. O'Neill**. 1986. A three-day mold assay for cosmetics and toiletries. J. Soc. Cosmet. Chem. **37**:49-57.

Packaging

HC Agar Base	500 g	0685-17

Bacto® m HPC Agar

Intended Use

Bacto m HPC Agar is used for enumerating heterotrophic organisms in treated potable water and other water samples with low counts by membrane filtration.

Also Known As

m HPC Agar is also known as m-Heterotrophic Plate Count Agar and previously as membrane filter Standard Plate Count Agar, m-SPC Agar.

Summary and Explanation

m HPC Agar was developed by Taylor and Geldreich in 1979 in their pursuit of a suitable Standard Methods medium to use with the membrane filter procedure.[1] m HPC Agar was evaluated by many investigators who reported it as a suitable alternate medium for standard plate counts.[2,3,4] This medium is recommended for the membrane filter method in the 19th edition of *Standard Methods for the Examination of Water and Wastewater.*[5]

The advantages of the membrane filter procedure over the standard plate count method have been described by many investigators.[6,7,8] The volume of inoculum is limited with both pour and spread plate techniques while the membrane filter method enables the use of large samples, which is desirable for water with low counts.

Principles of the Procedure

In m HPC Agar, Bacto Peptone provides sufficient nitrogen and carbon as well as other nutrients. Gelatin at 2.5% concentration eliminates problems of liquefaction and spreading colonies. Bacto Agar is a solidifying agent.

Formula

m HPC Agar

Formula Per Liter

Bacto Peptone	20 g
Bacto Gelatin	25 g
Bacto Agar	15 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.

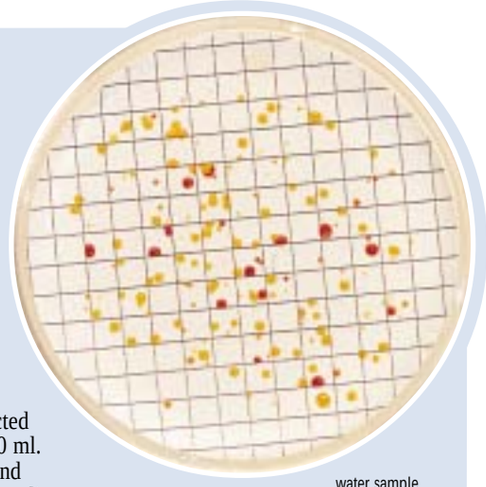
User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	Soluble in distilled or deionized water on boiling. (Add 1% glycerol after boiling). Light amber, slightly opalescent to opalescent, may have a precipitate.
Reaction of 6% Solution at 25°C:	pH 7.1 ± 0.2 with 1% added glycerol and after autoclaving 5 minutes at 121-124°C.
Prepared Plates:	Light amber, opalescent, may have a precipitate.

Cultural Response

Prepare m HPC Agar per label instructions. Dilute ten chlorinated water samples collected as recommended by Standard Methods[5] from different sources to yield 20-200 CFUs/10 ml. Filter the dilutions through a membrane filter. Place the filters on m-HPC Agar plates and incubate at 35 ± 2°C for 40-72 hours. Therecovery and morphology of bacteria on test medium should be comparable to that of a reference lot.



water sample

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

m HPC Agar

Materials Required But Not Provided

Sterile Petri dishes, 50 x 9 mm
Membrane filter equipment
Dilution blanks
Pipettes or glass rods
Incubator (35°C)
Stereoscopic microscope
Sterile 47 mm, 0.45 µm, gridded membrane filters
Close fitting box or plastic bag containing moistened paper towels
Bacto Glycerol

Method of Preparation

1. Suspend 6 grams in 100 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Add 1 ml glycerol.
4. Autoclave at 121-124°C for 5 minutes.
5. Dispense 5 ml aliquots into Petri dishes.

Specimen Collection and Preparation

Water samples should be collected as described in *Standard Methods for the Examination of Water and Wastewater*, Section 9060A.[5]

To minimize changes in bacterial population, water samples should be tested as soon as possible after collection. The recommended maximum elapsed time between collection and analysis of samples is 8 hours (maximum transit time of 6 hours, maximum processing time of 2 hours). When analysis cannot begin within 8 hours, maintain sample at a temperature below 4°C but do not freeze. Maximum elapsed time between collection and analysis must not exceed 24 hours.[5]

Test Procedure

1. The volume to be filtered will vary with the sample. Select a maximum sample size to give 20 to 200 CFU per filter.
2. Filter appropriate volume through a sterile 47 mm, 0.45 µm, gridded membrane filter, under partial vacuum. Rinse funnel with three 20 to 30 ml portions of sterile dilution water. Place filter on agar in Petri dish.
3. Place dishes in close-fitting box or plastic bag containing moistened paper towels.
4. Incubate at 35 ± 0.5°C for 48 hours. Duplicate plates may be incubated at other conditions as desired.

Results

Count all colonies on the membrane when there are 2 or less colonies per square. For 3 to 10 colonies per square, count 10 squares and obtain average count per square. For 10 to 20 colonies per square, count 5 squares and obtain average count per square. Multiply average count per square by 100 and divide by the sample volume to give colonies per milliliter. If there are more than 20 colonies per square, record count as > 2,000 divided by the sample volume. Report averaged counts as estimated colony-forming units. Make estimated counts only when there are discrete, separated colonies.[5]

Limitations of the Procedure

1. m HPC Agar is intended for use only with the membrane filter method.
2. m HPC Agar is recommended for testing treated water.
3. Longer incubation times may be necessary to recover slow-growing bacteria.

References

1. **Taylor, R. H., and E. E. Geldreich.** 1979. A new membrane filter procedure for bacterial counts in potable water and swimming pool samples. J. Amer. Water Works Assoc. **71**:402-405.
2. **Means, E. G., L. Hanami, H. F. Ridgway, and B. H. Olson.** 1981. Evaluating mediums and plating techniques for enumerating bacteria in water distribution systems. J. Amer. Water Works Assoc. **73**:585-590.
3. **Nagy, L. A., and B. H. Olson.** 1982. The occurrence of filamentous fungi in drinking water distribution systems. Can. J. Microbiol. **28**:667-671.
4. **Haas, C. N., M. A. Meyer, and M. S. Paller.** 1982. Analytical note: evaluation of the m-SPC method as a substitute for the standard plate count in water microbiology. J. Amer. Water Works Assoc. **74**:322.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **Lechevallier, M. W., R. J. Seidler, and T. M. Evans.** 1980. Enumeration and characterization of standard plate count bacteria in chlorinated and raw water supplies. App. And Environ. Microbiol. **40**:922-930.
7. **Stapert, E. M., W. T. Sokolski, and J. I. Northam.** 1962. The factor of temperature in the better recovery of bacteria from water by filtration. Can. Journal Microbiol. **8**:809-810.
8. **Saleem, M., and R. L. Schlitzer.** 1983. Comparative recovery of bacteria from purified water by the membrane filter technique and the standard plate count methods, p. 281. Abs. Ann. Meeting, ASM.

Packaging

m HPC Agar	100 g	0752-15
	500 g	0752-17
Glycerol	100 g	0282-15
	500 g	0282-17

Bacto® Heart Infusion Broth
Bacto Heart Infusion Agar

Intended Use

Bacto Heart Infusion Broth is used for cultivating fastidious microorganisms.

Bacto Heart Infusion Agar is an infusion medium used for cultivating a wide variety of fastidious microorganisms and as a base for preparing blood agar.

Also Known As

Heart Infusion Broth is abbreviated as HIB, Heart Infusion Agar as HIA.

Summary and Explanation

Heart Infusion Broth and Heart Infusion Agar are non-selective general purpose media used for the isolation of nutritionally fastidious microorganisms. One of the first media used for the cultivation of bacteria was a liquid medium containing an infusion of meat. Huntoon[1] using fresh beef heart and Bacto Peptone, prepared a "hormone" broth to retain growth promoting substances. Highly pathogenic organisms, such as meningococci and pneumococci, could be grown on infusion medium without enrichments.[1] The formulas for HIA and HIB contain Tryptose, which is better suited to the nutritional requirements of pathogenic bacteria than Bacto Peptone.

Heart Infusion Agar can be used as a base for the preparation of blood agar in determining hemolytic reactions, and for mass cultivation of microorganisms in the preparation of vaccines. Heart Infusion Media are specified for the isolation of *Vibrio cholerae* and *Vibrio* species.[2,3]

User Quality Control

Identity Specifications

Heart Infusion Broth

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	2.5% solution, soluble in distilled or deionized water; light to medium amber in color, clear.
Prepared Medium:	Light to medium amber, clear.
Reaction of 2.5% Solution at 25°C	pH 7.4 ± 0.2

Heart Infusion Agar

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	4% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Plain - Light to medium amber, slightly opalescent with no precipitate. With 5% sheep blood - cherry red, opaque.
Reaction of 4% Solution at 25°C:	pH 7.4 ± 0.2

continued on following page

Heart Infusion Broth may be used as the base in carbohydrate fermentation tests.[4]

Several modifications of Heart Infusion media have been described.[5] The addition of carbohydrates, blood or other ingredients result in media used for a variety of purposes. The methodologies for the multiple applications using Heart Infusion Agar and Heart Infusion Broth are outlined in the references.

Principles of the Procedure

Infusion from Beef Heart and Tryptose supply the nutritional requirements for growth of microorganisms in Heart Infusion Media. Sodium chloride maintains the osmotic balance of the medium, and Bacto Agar is the solidifying agent. The addition of 5% sheep blood provides additional growth factors and is used to determine hemolytic reactions.

Formula

Heart Infusion Broth

Formula Per Liter

Beef Heart, Infusion from	500 g
Bacto Tryptose	10 g
Sodium Chloride	5 g

Final pH 7.4 ± 0.2 at 25°C

Heart Infusion Agar

Formula Per Liter

Beef Heart, Infusion from	500 g
Bacto Tryptose	10 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Heart Infusion Broth
Heart Infusion Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Sterile Petri dishes
Sterile tubes with closures

Method of Preparation

Heart Infusion Broth

1. Dissolve 25 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Cool to room temperature.

Heart Infusion Agar

1. Suspend 40 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

   OPTIONAL: To prepare blood agar, aseptically add 5% sterile defibrinated blood to Heart Infusion Agar at 45-50°C. Mix well.
4. Dispense into Petri dishes.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Huntoon, F. M.** 1918. "Hormone" Medium. A simple medium employable as a substitute for serum medium. J. of Infect. Dis. **23**:169-172.
2. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel**. 1995. p. 9.01-9.24. App. 3.24-3.25. FDA Bacteriological Analytical Manual, 8th ed. AOAC International, Arlington, VA.

User Quality Control cont.

Cultural Response

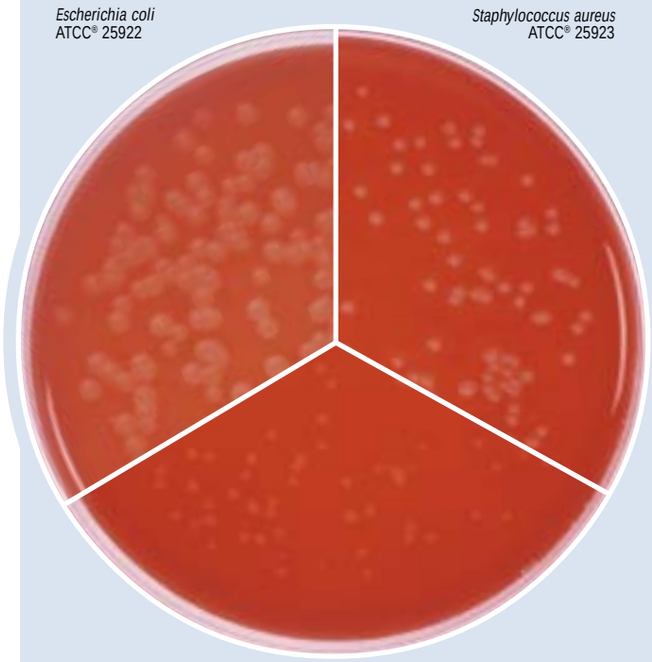
Prepare Heart Infusion Broth per label directions. Prepare Heart Infusion Agar with and without 5% sheep blood. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

Heart Infusion Broth

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	100-1,000	good
Staphylococcus aureus	25923	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

Uninoculated tube

Escherichia coli ATCC® 25922



Escherichia coli ATCC® 25922

Staphylococcus aureus ATCC® 25923

Streptococcus pyogenes ATCC® 19615

All with blood on Heart Infusion Agar

Heart Infusion Agar

ORGANISM	ATCC®	INOCULUM CFU	GROWTH PLAIN	GROWTH w/5% SHEEP BLOOD	HEMOLYSIS w/5% SHEEP BLOOD
Escherichia coli	25922*	100-1,000	good	good	beta
Staphylococcus aureus	25923*	100-1,000	good	good	beta
Streptococcus pneumoniae	6305	100-1,000	fair	good	alpha
Streptococcus pyogenes	19615*	100-1,000	fair	good	beta

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

3. **Vanderzant, C. and D. F. Splittstoesser (ed.).** 1992. p. 451-469. 1132. Compendium of Methods for the Microbiological Examination of Food, 3rd ed. American Public Health Association, Washington, D.C.
4. **Ruoff, K. L.** 1995. *Streptococcus*, p.305. *In* Murray, P.R., Baron E.J., Pfaller, M.A. Tenover, F.C., and R.H. Yolken (ed.)., Manual of Clinical Microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Atlas, R. M.** 1993. Handbook of Microbiological Media, p. 426-431, CRC Press, Boca Raton, FL.

Packaging

Heart Infusion Agar	100 g	0044-15
	500 g	0044-17
	2 kg	0044-07
	10 kg	0044-08
Heart Infusion Broth	100 g	0038-15
	500 g	0038-17
	2 kg	0038-07

Bacto® Hektoen Enteric Agar

Intended Use

Bacto Hektoen Enteric Agar is used for the isolating and differentiating gram-negative enteric bacilli.

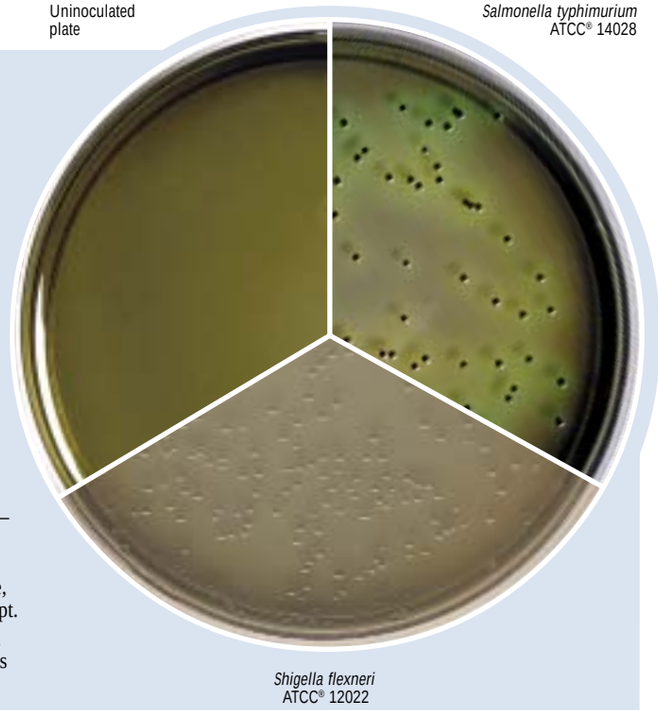
Also Known As

Hektoen Enteric Agar is also known as HE Agar or HEA.

Summary and Explanation

Hektoen Enteric Agar was developed in 1967 by King and Metzger.[1, 2] Compared to other enteric differentiating media commonly used in clinical laboratories at that time, Hektoen Enteric Agar increased the frequency of isolation of *Salmonella* and *Shigella* organisms. This was accomplished by increasing the carbohydrate and peptone content of the medium in order to counteract the inhibitory effects of the bile salts and indicators. King and Metzger formulated a medium that only slightly inhibited the growth of *Salmonella* and *Shigella* while at the same time ensuring the adequate inhibition of gram-positive microorganisms.

Hektoen Enteric Agar is used to isolate and differentiate *Salmonella* and *Shigella*, which cause a variety of serious human gastrointestinal illnesses.[3] *Salmonella* is the most frequently reported cause of foodborne outbreaks of gastroenteritis in the United States.[4] Foods containing poultry, eggs, or dairy products are the most frequent vehicles for foodborne salmonellosis. For food samples, a variety of procedures have been developed using Hektoen Enteric Agar as part of the multi-step procedure to isolate *Salmonella*.[5-8]



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light purplish beige, free-flowing, homogeneous.
Solution:	7.6% solution soluble in distilled or deionized water upon boiling.
Prepared Plates:	Green with yellowish cast, slightly opalescent.
Reaction of 7.6% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

Prepare Hektoen Enteric Agar per label directions. Inoculate and incubate plates at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Enterococcus faecalis	29212*	1,000-2,000	markedly inhibited	–
Escherichia coli	25922*	100-1,000	partial inhibition	salmon-orange, may have bile ppt.
Salmonella typhimurium	14028*	100-1,000	good	greenish blue, w/black centers
Shigella flexneri	12022*	100-1,000	good	greenish blue

The cultures listed are the minimum that should be used for performance.

*****These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Novobiocin (15 mg/liter) can be added to Hektoen Enteric Agar to inhibit growth of *Citrobacter* and *Proteus* colonies, which may resemble those of *Salmonella*.[9]

Principles of the Procedure

Proteose Peptone is a source of nitrogen and other nutrients in Hektoen Enteric Agar. Bile Salts and the dyes, brom thymol blue and acid fuchsin, inhibit gram-positive organisms. Lactose, saccharose and salicin are sources of fermentable carbohydrates. Ferric ammonium citrate, a source of iron, allows production of hydrogen sulfide (H_2S) from sodium thiosulfate. H_2S-positive colonies have black centers. Yeast Extract provides vitamins and cofactors required for growth and additional nitrogen and carbon. Bacto Agar is used as a solidifying agent.

Formula

Hektoen Enteric Agar

Formula Per Liter

Bacto Proteose Peptone	12 g
Bacto Yeast Extract	3 g
Bacto Bile Salts No. 3	9 g
Bacto Lactose	12 g
Bacto Saccharose	12 g
Bacto Salicin	2 g
Sodium Chloride	5 g
Sodium Thiosulfate	5 g
Ferric Ammonium Citrate	1.5 g
Bacto Agar	14 g
Brom Thymol Blue	0.065 g
Acid Fuchsin	0.1 g

Final pH 7.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Hektoen Enteric Agar

Materials Required but not Provided

Glassware
Autoclave
Incubator
Petri dishes

Method of Preparation

1. Suspend 76 grams in 1 liter distilled or deionized water.
2 Heat to boiling with frequent agitation to dissolve completely. Do not overheat. **DO NOT AUTOCLAVE**.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Do not autoclave this medium because excessive heat may alter the ingredients.
2. *Proteus* species may resemble salmonellae or shigellae. Further testing should be conducted to confirm the presumptive identification of organisms isolated on this medium.

References

1. **King, S., and W. I. Metzger**. 1968. A new plating medium for the isolation of enteric pathogens. Appl. Microbiol. **16**:577-578.
2. **King, S., and W. I. Metzger**. 1968. A new plating medium for the isolation of enteric pathogens. II. Comparison of Hektoen Enteric Agar with SS and EMB Agar. Appl. Microbiol. **16**:579-581.
3. **Gray, L .D.** 1995. *Escherichia*, *Salmonella*, *Shigella*, and *Yersinia*, p. 450-456. *In* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Centers for Disease Control**. 1991. Summary of notifiable diseases. Morbid. Mortal. Weekly Rep. **40** (53):3.
5. **Flowers, R. S., J-Y. D'Aoust, W. H. Andrews, and J. S. Bailey**. 1992. *Salmonella*, p. 371-422. *In* Vanderzant, C., and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
6. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig**. 1993. Pathogens in milk and milk products, p. 103-212. *In* Marshall, R. T. (ed.), Standard methods for the examination of dairy products. 16th ed. American Public Health Association, Washington, D.C.
7. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*, p. 5.01-5.20. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
8. **Association of Official Analytical Chemists**. 1996 official methods of analysis of AOAC International, Supplement March 1996. AOAC International, Arlington, VA.

9. **Hoben, D. A., D. H. Ashton, and A. C. Peterson**. 1973. Some observations on the incorporation of novobiocin into Hektoen Enteric Agar for improved *Salmonella* isolation. Appl. Microbiol. **26**:126-127.

Packaging

Hektoen Enteric Agar	100 g	0853-15
	500 g	0853-17
	2 kg	0853-07
	10 kg	0853-08

Bacto® Hemoglobin

Intended Use

Bacto Hemoglobin is used in preparing microbiological culture media.

Summary and Explanation

Hemoglobin, an autoclavable preparation of beef blood, is prepared according to the procedure described by Spray.[1]

Hemoglobin is used with GC Medium Base is in the preparation of Chocolate Agar Enriched, Thayer-Martin Medium and Modified Thayer-Martin Medium. Supplemented with Hemoglobin and Supplement B or VX, the enriched media are used for the isolation and cultivation of fastidious microorganisms, especially *Neisseria* and *Haemophilus* species. With the exception of some laboratory-adapted strains of *Haemophilus aphrophilus*, *Haemophilus* species require either exogenous hemin (X factor), nicotinamide adenine dinucleotide (NAD) (V factor), or both.[2]

Principles of the Procedure

Hemoglobin provides the hemin (X factor) required for growth of *Haemophilus* and for enhanced growth of *Neisseria* species.

Formula

Hemoglobin is obtained from beef blood, desiccated.

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Dark brown, fine, free-flowing.
Solution:	2% solution, insoluble in distilled or deionized water. Solution is chocolate brown, opaque with a dispersed precipitate.
Reaction of 2% Solution at 25°C:	pH 8.2 ± 0.2

Cultural Response

Prepare GC Medium enriched with 2% Hemoglobin and Supplement B or VX per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Haemophilus influenzae	10211	100-1,000	good
Neisseria gonorrhoeae	43069	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Storage

Store Hemoglobin below 30°C. The dehydrated ingredient is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Hemoglobin

Materials Required But Not Provided

Glassware
Autoclave
GC Medium Base (for the cultivation of *Neisseria* and *Haemophilus* species)
Supplement B or VX, depending on the medium being prepared
Antimicrobic Vial CNV or CNVT, depending on the medium being prepared

Method of Preparation

1. Place 10 grams of Hemoglobin in a dry beaker.
2. Measure 500 ml distilled or deionized water.
3. Add the water in approximately 100 ml amounts, stirring well after each addition. Use a spatula to break up clumps.
4. Transfer to flasks, as desired, for autoclaving.
5. Autoclave at 121°C for 15 minutes.
6. Cool to 45-50°C.
7. Swirl the flask to reestablish complete solution, then add to an equal amount of double-strength sterile agar base cooled to 45-50°C.

Test Procedure

For a complete discussion on the isolation and identification of *Neisseria* and *Haemophilus* species, refer to procedures outlined in appropriate references.[2,3,4]

Results

Refer to appropriate references and procedures for results.

References

1. **Spray.** 1930. J. Lab Clin. Med. **16**:166.
2. **Campos, J. M.** 1995. *Haemophilus*, p. 556-565. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

3. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
4. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Hemoglobin	100 g	0136-15
	500 g	0136-17
	2 kg	0136-07
	10 kg	0136-08

Bacto® Horse Serum, Desiccated

Intended Use

Bacto Horse Serum, Desiccated is used as an enrichment in bacteriological culture media.

Summary and Explanation

Horse Serum, Desiccated is an enrichment prepared from filter-sterilized, normal horse serum. When used in the preparation of Mycoplasma Supplement, Horse Serum supplies cholesterol, a growth stimulant for *Mycoplasma*.[1] Loeffler[2] used dextrose broth enriched with horse serum for cultivating *Corynebacterium diphtheriae*.

A medium supplemented with horse serum or lysed horse blood is usually sufficient to enhance the growth of fastidious anaerobes.[3] Broth media supplemented with horse serum are used in the microdilution susceptibility testing of anaerobic bacteria.[3]

Principles of the Procedure

Horse Serum, Desiccated provides essential nutritional factors that stimulate organism growth.

Reagent

Horse Serum, Desiccated is sterile, lyophilized horse serum.

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Lyophilized Appearance:	Brown, lyophilized cake or powder.
Solution:	Soluble in 10 ml distilled or deionized water.
Rehydrated Appearance:	Light to medium amber, clear to slightly opalescent.

Cultural Response

Prepare Tryptose Blood Agar Base with 10% Horse Serum (rehydrated) per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Streptococcus mitis	9895	100-1,000	good
Streptococcus pneumoniae	6303*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store Horse Serum, Desiccated and reconstituted Horse Serum at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Horse Serum, Desiccated

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Horse Serum, Desiccated specified in the formula of the medium or enrichment being prepared. Add as required.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Horse Serum, Desiccated.[1-3]

Results

Refer to appropriate references and procedures for results.

References

1. **Taylor-Robinson, D.** 1995. *Mycoplasma* and *Ureaplasma*, p. 652-661. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Loeffler, F.** 1887. Darauf theilte HeuLoeffer en einem Zweiten Vortrag die ergebnisse seiner weiteren untersuchungen uber die Diphtherie-Bacillen mit. Zentralb. Bacteriol. **2**:105.
3. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Packaging

Horse Serum, Desiccated	12 x 10 ml	0261-61

Bacto® ISP Medium 1 · Bacto ISP Medium 2 Bacto ISP Medium 4

Intended Use

Bacto ISP Medium 1, Bacto ISP Medium 2 and Bacto ISP Medium 4 are used for characterizing *Streptomyces* species according to the International Streptomyces Project (ISP).[1]

User Quality Control

Identity Specifications

ISP Medium 1

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 0.8% solution, soluble in distilled or deionized water on boiling; light amber, clear to very slightly opalescent.

Prepared Medium: Light amber, clear to very slightly opalescent, w/o significant precipitation.

Reaction of 0.8% Solution at 25°C: pH 7.0 ± 0.2

ISP Medium 2

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 3.8% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent.

Prepared Medium: Light to medium amber, slightly opalescent, without precipitate.

Reaction of 3.8% Solution at 25°C: pH 7.2 ± 0.2

ISP Medium 4

Dehydrated Appearance: White to light beige, free-flowing, homogeneous.

Solution: 3.7% solution, soluble in distilled or deionized water on boiling; white to off-white, opaque with precipitate.

Prepared Medium: White to off-white, opaque, may have a precipitate.

Reaction of 3.7% Solution at 25°C: pH 7.2 ± 0.2

Cultural Response

Prepare ISP Medium 1, ISP Medium 2 and ISP Medium 4 per label directions. Inoculate tubes of prepared ISP Medium 1, and incubate at 30 ± 2°C for 48-96 hours.

Inoculate prepared ISP Medium 2 and ISP Medium 4 with the test organisms by placing a drop of inoculum near the edge of the plate. Five parallel streaks across the plate are made from this drop, followed by four perpendicular streaks. Incubate inoculated plates at 30 ± 2°C for 48-96 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Streptomyces albus	3004	100-1,000	good
Streptomyces lavendulae	8664	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Also Known As

ISP Medium 1 is also referred to as Tryptone Yeast Extract Broth.

ISP Medium 2 is also referred to as Yeast Malt Extract Agar.

ISP Medium 4 is also referred to as Inorganic Salts Starch Agar.

Summary and Explanation

ISP media were developed by Difco Laboratories for the International Streptomyces Project (ISP) in order to select stable properties and reproducible procedures for characterization of *Streptomyces* species.[1]

Principles of the Procedure

Tryptone and Yeast Extract are the nitrogen, vitamin, carbon and amino acid source in ISP Medium 1.

Yeast Extract and Malt Extract provide nitrogen, amino acids and vitamins in ISP Medium 2. Dextrose is the carbon source, and Bacto Agar is the solidifying agent.

ISP Medium 4 is composed of many inorganic salts and Soluble Starch to provide essential nutrients for organism growth. Bacto Agar is the solidifying agent.

Formula

ISP Medium 1

Formula Per Liter

Bacto Tryptone	5 g
Bacto Yeast Extract	3 g

Final pH 7.0 ± 0.2 at 25°C

ISP Medium 2

Formula Per Liter

Bacto Yeast Extract	4 g
Bacto Malt Extract	10 g
Bacto Dextrose	4 g
Bacto Agar	20 g

Final pH 7.2 ± 0.2 at 25°C

ISP Medium 4

Formula Per Liter

Bacto Soluble Starch	10 g
Potassium Phosphate, Dibasic	1 g
Magnesium Sulfate USP	1 g
Sodium Chloride	1 g
Ammonium Sulfate	2 g
Calcium Carbonate	2 g
Ferrous Sulfate ($FeSO_4 \cdot 7H_2O$)	0.001 g
Manganous Chloride ($MnCl_2 \cdot 7H_2O$)	0.001 g
Zinc Sulfate ($ZnSO_4 \cdot 7H_2O$)	0.001 g
Bacto Agar	20 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.

2. **ISP Medium 4**

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breath dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Lungs, Intestines.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

ISP Medium 1
ISP Medium 2
ISP Medium 4

Materials Required But Not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (30°C)
Sterile tubes
Sterile Petri dishes

Method of Preparation

1. Suspend the appropriate amount of medium in 1 liter distilled or deionized water:

ISP Medium 1	8 g/l
ISP Medium 2	38 g/l
ISP Medium 4	37 g/l

2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Mix thoroughly while dispensing.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion on the isolation and maintenance of *Streptomyces* species refer to appropriate references.[2,3]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on the medium.

References

1. **Shirling, E. B., and D. Gottlieb.** 1966. Methods for characterization of *Streptomyces* species. Int. J. Syst. Bacteriol. **16**:313-340.
2. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

ISP Medium 1	500 g	0769-17
ISP Medium 2	500 g	0770-17
ISP Medium 4	500 g	0772-17

Bacto® Inositol Assay Medium

Intended Use

Bacto Inositol Assay Medium is used for determining inositol concentration by the microbiological assay technique.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test.

Inositol Assay Medium, a modification of the formula described by Atkin et al.,[1] is used in the microbiological assay of inositol using *Saccharomyces cerevisiae* ATCC® 9080 (*Saccharomyces uvarum*) as the test organism.

Principles of the Procedure

Inositol Assay Medium is an inositol-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *S. cerevisiae* ATCC® 9080. The addition of inositol in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

Formula

Inositol Assay Medium

Formula Per Liter	
Bacto Dextrose	100 g
Potassium Citrate	10 g
Citric Acid	2 g

Monopotassium Phosphate	1.1	g
Potassium Chloride	0.85	g
Magnesium Sulfate	0.25	g
Calcium Chloride	0.25	g
Manganese Sulfate	50	mg
Ferric Chloride	50	mg
DL-Tryptophane	80	mg
L-Cystine	0.1	g
L-Isoleucine	0.5	g
L-Leucine	0.5	g
L-Lysine	0.5	g
L-Methionine	0.2	g
DL-Phenylalanine	0.2	g
L-Tyrosine	0.2	g
L-Asparagine	0.8	g
DL-Aspartic Acid	0.2	g
DL-Serine	0.1	g
Glycine	0.2	g
DL-Threonine	0.4	g
L-Valine	0.5	g
L-Histidine	0.124	g
L-Proline	0.2	g
DL-Alanine	0.4	g
L-Glutamic Acid	0.6	g
L-Arginine	0.48	g
Thiamine Hydrochloride	500	µg
Biotin	16	µg
Calcium Pantothenate	5	mg
Pyridoxine Hydrochloride	1	mg

Final pH 5.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
3. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.
4. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous.
Solution:	6.1% (single strength) or 12.2% (double strength) solution, soluble in distilled or deionized water on boiling. Light amber, clear, may have a slight precipitate.
Prepared Medium:	Light amber, clear, may have a slight precipitate.
Reaction of 6.1% Solution at 25°C:	pH 5.2 ± 0.2

Cultural Response

Prepare Inositol Assay Medium per label directions. Dispense medium into 50 ml flasks with a titration from 0 to 10 µg of Inositol. Inoculate flasks with one drop of *S. cerevisiae* ATCC® 9080 inoculum suspension (washed three times and diluted 1:1000). Incubate flasks at 25-30°C for 20-24 hours. The curve obtained from turbidimetric readings should be typical.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Inositol Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Saccharomyces cerevisiae* ATCC® 9080
Inositol
Sterile tubes
Sterile 0.85% saline
Distilled or deionized water
Lactobacilli Agar AOAC
Centrifuge
Spectrophotometer

Method of Preparation

1. Suspend 12.2 grams in 100 ml distilled or deionized water.
2. Boil to dissolve.
3. Dispense 5 ml amounts into flasks.
4. Add standard or test samples.
5. Adjust flask volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 5 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. The samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Remove a loopful of culture from a stock culture slant of *S. cerevisiae* ATCC® 9080 and suspend it in 10 ml sterile 0.85% saline. Centrifuge cells at moderate speed for 10 minutes. Decant the supernatant and resuspend cells in 10 ml 0.85% sterile saline. Wash the cells three times with 10 ml sterile 0.85% saline. After the third wash, resuspend the

cells in 10 ml 0.85% saline. Dilute 1 ml of the cell suspension in 1000 ml of sterile 0.85% saline. This diluted suspension is the inoculum. Use 1 drop of inoculum suspension to inoculate each assay flask.

The concentrations of inositol required for the preparation of the standard curve may be prepared by dissolving 200 mg inositol in 100 ml distilled water. Mix thoroughly. Dilute 1 ml of this solution with 999 ml distilled water to make a final solution containing 2 µg inositol per ml. Use 0.0, 0.5, 1, 2, 3, 4 and 5 ml per flask. Prepare this stock solution fresh daily.

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can impact the standard curve readings and cannot always be duplicated. The standard curve is obtained by using inositol at levels of 0.0, 1, 2, 4, 6, 8 and 10 µg per assay flask (10 ml).

Following inoculation, flasks are incubated at 25-30°C for 20-24 hours. Place flasks in the refrigerator for 15-30 minutes to stop growth. Growth is measured turbidimetrically using any suitable spectrophotometer.

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ± 10% from the average. Use the results only if two thirds of the values do not vary more than ± 10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be grown and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

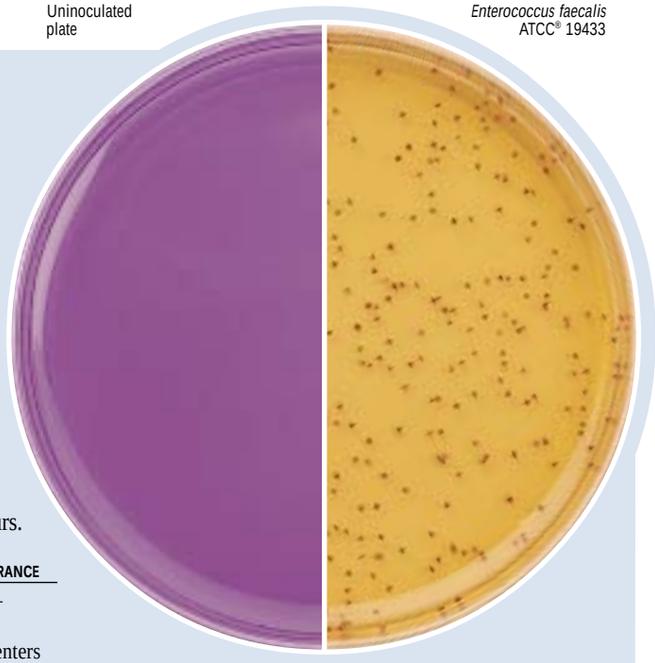
References

1. **Atkin, Schultz, Williams, and Frey.** 1943. End. & Eng. Chem., Ann. Ed. **15**:141.

Packaging

Inositol Assay Medium	100 g	0995-15

Bacto® KF Streptococcus Agar



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light greenish-beige, free-flowing, homogeneous.
Solution:	7.64% solution, soluble in distilled or deionized water on boiling. Solution is light purple, very slightly to slightly opalescent.
Prepared Medium:	Light purple, very slightly to slightly opalescent.
Reaction of 7.64% Solution at 25°C:	7.2 ± 0.2

Cultural Response

Prepare KF Streptococcus Agar per label directions. Inoculate using the pour plate technique and incubate at 35 ± 2°C for 46-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterobacter aerogenes	13048*	1,000-2,000	marked to complete inhibition	–
Enterococcus faecalis	19433*	30-300	good	red centers
Enterococcus faecalis	29212*	30-300	good	red centers
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and are to be used as directed per Bactrol Disk Technical Information.

Intended Use

Bacto KF Streptococcus Agar is used with Bacto TTC Solution 1% in isolating and enumerating fecal streptococci according to APHA.

Also Known As

Kenner Fecal Streptococcus Agar

Summary and Explanation

Kenner et al. developed KF Streptococcal Agar for use in detecting streptococci in surface waters by direct plating or by the membrane filtration method.[1] These investigators compared the performance of their formulation to other media used for enumerating fecal streptococci and achieved greater recoveries with KF Streptococcal Agar.

This medium is currently recommended for use in determining counts of fecal streptococci in foods and water.[2,3]

Principles of the Procedure

Peptone provides a source of nitrogen, amino acids and carbon. Yeast Extract is a source of trace elements, vitamins and amino acids. Maltose and Lactose are fermentable carbohydrates and carbon sources. Sodium Azide is a selective agent. Brom Cresol Purple is an indicator dye.

The addition of 1% triphenyltetrazolium chloride (TTC) causes enterococci to develop a deep red color following reduction of tetrazolium to an acid azo dye.

Formula

KF Streptococcus Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Yeast Extract	10 g
Sodium Chloride	5 g
Sodium Glycerophosphate	10 g
Bacto Maltose	20 g
Bacto Lactose	1 g
Sodium Azide	0.4 g
Bacto Brom Cresol Purple	0.015 g
Bacto Agar	20 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use
2. **HARMFUL.** HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Cardiovascular, Lungs, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

KF Streptococcus Agar
TTC Solution 1%

Materials Required But Not Provided

Glassware
Incubator (35°C)
Pipettes
Sterile Petri dishes, 50 x 9 mm
Membrane filter equipment
Sterile 47 mm, 0.45 µm, gridded membrane filters
Dilution blanks
Stereoscopic microscope

Method of Preparation

1. Suspend 76.4 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
2. Heat an additional 5 minutes. Avoid overheating which could decrease the productivity of the medium. DO NOT AUTOCLAVE.
3. Add 10 ml TTC Solution 1% to the medium at 50°C and mix well.
4. Pour medium into sterile Petri dishes if using the Membrane Filter procedure. If using the Pour Plate technique, hold the liquid medium at 45°C.

Specimen Collection and Preparation

Consult appropriate references for specific procedures using KF Streptococcus Agar in the examination of waters and food.[2,3] The pour plate technique or the membrane filter procedure can be used for detection and enumeration of enterococci.

Test Procedure

Pour Plate Technique

1. Prepare appropriate dilutions of the test material.
2. Place the selected volume of sample in a Petri dish.
3. Pour 15 ml of the prepared medium at 45°C into each plate.
4. Thoroughly mix the medium and sample to uniformly disperse the organisms.
5. Allow the agar to solidify.
6. Incubate plates in the inverted position at 35 ± 2°C for 46-48 hours.

Membrane Filter Procedure

1. Filter a suitable volume of sample through a sterile membrane, as directed.
2. Place the inoculated membrane filter on the solidified agar in the Petri dish, inoculum side up.
3. Incubate the plates, inverted, at 35 ± 2°C for 46-48 hours.

Results

Enterococci will appear as red or pink colonies. The use of a stereoscopic microscope with 15X magnification can aid in counting colonies.

Limitations of the Procedure

1. Many strains of *S. bovis* and *S. equinus* are inhibited by azide.
2. Overheating may lower the pH, causing a decrease in the productivity of the medium.

References

1. **Kenner, B. A., H. F. Clark, and P. W. Kabler.** 1961. Fecal streptococci. I. Cultivation and enumeration of streptococci in surface waters. Appl. Microbiol. **9**:15.
2. **Donnelly, C. W., R. E. Bracket, D. Doores, W. H. Lee, and J. Lovett.** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Bordner, R., and J. Winter.** 1978. Microbiological methods for monitoring the environment, water and wastes. EPA, Cincinnati, OH.
4. **MacFadden, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. I. Williams & Wilkens, Baltimore, MD.

Packaging

KF Streptococcus Agar	500 g	0496-17

Bacto® KF Streptococcus Broth

Intended Use

Bacto KF Streptococcus Broth is used for isolating fecal streptococci.

Also Known As

Kenner Fecal Streptococcus Broth

Summary and Explanation

Kenner et al. developed KF Streptococcal Broth for the detection and enumeration of enterococci in waters.[1,2] They found that this formulation was superior to other liquid media in the recovery of enterococci in Most Probable Number (MPN) test systems. The medium is not specific for presumptive identification of group D streptococci. Other tests are required.[2-4]

This medium currently is recommended for use in enumerating enterococci in foods.[5]

Principles of the Procedure

Proteose Peptone No. 3 provides a source of nitrogen, amino acids and carbon. Yeast Extract is a source of trace elements, vitamins and amino acids. Maltose and Lactose are the fermentable carbohydrates and carbon source. Sodium Azide is the selective agent. Brom Cresol Purple is the indicator dye.

The addition of 1% triphenyltetrazolium chloride, in the membrane filter procedure, causes the enterococci to have a deep red color as a result of tetrazolium reduction to an acid azo dye.

User Quality Control

Identity Specifications

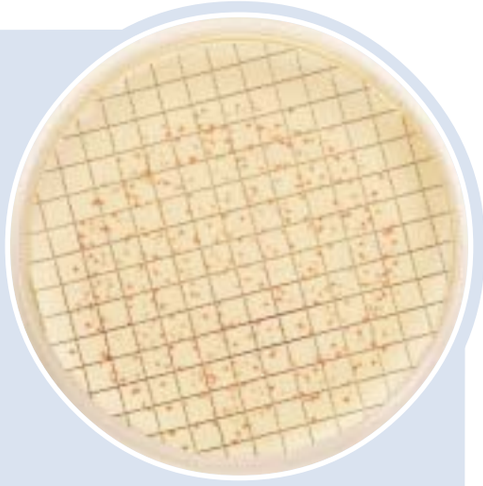
Dehydrated Appearance:	Light greenish-beige, free-flowing, homogeneous.
Solution:	5.64% solution, soluble in distilled or deionized water with frequent agitation on boiling. Solution is reddish to light purple, clear to very slightly opalescent.
Prepared Tubes:	Purple, clear to very slightly opalescent.
Reaction of 5.64% Solution at 25°C:	7.2 ± 0.2.

Cultural Response

Prepare KF Streptococcus Broth per label directions. Supplement with TTC Solution 1%. Using the membrane filter technique, inoculate and incubate at 35 ± 1°C in an atmosphere saturated with water vapor for 46-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterobacter aerogenes	13048*	300-1,000	inhibited	–
Enterococcus faecalis	19433*	30-200	good	red
Enterococcus faecalis	29212*	30-200	good	red
Escherichia coli	25922*	300-1,000	inhibited	–

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Enterococcus faecalis
ATCC® 29212

Formula

KF Streptococcus Broth

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Yeast Extract	10 g
Sodium Chloride	5 g
Sodium Glycerophosphate	10 g
Bacto Maltose	20 g
Bacto Lactose	1 g
Sodium Azide	0.4 g
Bacto Brom Cresol Purple	0.015 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Cardiovascular, Lungs, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

KF Streptococcus Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Pipettes
Culture tubes
Membrane filter equipment
TTC Solution 1%
Incubator (35°C), saturated with water vapor
Sterile Petri dishes, 50 x 9 mm
Sterile 47 mm, 0.45 µm, gridded membrane filters
Sterile absorbent pads
Stereoscopic microscope

Method of Preparation

MPN Procedure

1. For an inoculum of 1 ml or less, suspend 56.4 g in 1 liter distilled or deionized water.

   For an inoculum of 10 ml, suspend 84.6 g in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. For an inoculum of 1 ml or less, dispense 10 ml amounts into culture tubes.

   For an inoculum of 10 ml, dispense 20 ml amounts into culture tubes.
4. Autoclave at 121°C for 10 minutes.

Membrane Filter Procedure

1. Suspend 56.4 g in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense 100 ml amounts into flasks and autoclave at 121°C for 10 minutes.
4. Cool to 60°C.
5. Add 1 ml TTC Solution 1% per 100 ml of medium.

Specimen Collection and Preparation

Water or food samples should be collected and prepared according to appropriate references.

Test Procedure

MPN Procedure

1. Inoculate tubes of the KF Streptococcus Broth with the appropriate amount of inoculum.
2. Incubate tubes at 35 ± 1°C, with loosened caps, for 46-48 hours.

Membrane Filter Procedure

1. Place a sterile absorbent pad in each sterile Petri dish.
2. Saturate the pads with the sterile medium containing TTC.
3. Place an inoculated membrane filter, inoculated side up, on the saturated pad.
4. Incubate at 35 ± 1°C in an atmosphere saturated with water vapor for 46-48 hours.

Results

MPN Procedure

MPN tubes positive for enterococci are turbid with growth that appears yellow in color and does not produce foaming. When foaming occurs, confirmation for enterococci should be made by Gram staining.

Membrane Filter Procedure

All red or pink colonies visible with 15x magnification are counted as enterococci colonies.

Limitations of the Procedure

1. Many strains of *S. bovis* and *S. equinus* are inhibited by azide.
2. The pH of KF Streptococcus Broth should be between 7.2 and 7.3. If below 7.0, it should not be used.
3. Overheating may lower the pH, resulting in a decrease in productivity of the medium.

References

1. **Kenner, B. A., H. F. Clark, and P. W. Kabler.** 1960. Fecal streptococci. II. Quantification of streptococci in feces. Am. J. Public Health **50**:1553.
2. **Kenner, B. A., H. F. Clark, and P. W. Kabler.** 1961. Fecal streptococci. I. Cultivation and enumeration of streptococci in surface waters. Appl. Microbiol. **9**:15.
3. **MacFadden, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkens, Baltimore, MD.
4. **Facklam, R. R., and M. D. Moody.** 1970. Presumptive identification of group D streptococci: the bile-esculin test. Appl. Microbiol. **20**:245-250.
5. **Donnelly, C. W., R. E. Bracket, D. Doores, W. H. Lee, and J. Lovett.** 1992. Compendium of Methods for the Microbiological Examination of Foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

KF Streptococcus Broth	500 g	0997-17

KL Virulence Agar

Bacto® KL Virulence Agar · KL Virulence Enrichment KL Antitoxin Strips

Intended Use

Bacto KL Virulence Agar is used with Bacto KL Virulence Enrichment, Bacto Chapman Tellurite Solution 1% and Bacto KL Antitoxin Strips in differentiating virulent (toxigenic) from nonvirulent strains of *Corynebacterium diphtheriae.*

Also Known As

KL Virulence Agar conforms with Klebs-Loeffler Virulence Agar.

Summary and Explanation

Elek[2] was the first to describe the agar plate diffusion technique for demonstrating the in vitro toxigenicity (virulence) of *Corynebacterium diphtheriae*. King, Frobisher, and Parsons[3] expanded on Elek's technique and, by using a carefully standardized medium, obtained results in agreement with animal inoculation tests. These authors demonstrated that Difco Proteose Peptone possessed properties essential for toxin production. Incorporating Difco Proteose Peptone into the test medium assured consistent results. The authors used rabbit, sheep and horse serum as enrichments, finding human serum to be unsatisfactory. To overcome irregularities encountered in previous formulations, Hermann, Moore, and Parsons[1] refined the medium used for the in vitro KL Virulence Test, simplifying the basal medium and developing a nonserous enrichment. The medium and enrichment described by these authors have been standardized for use in the KL Virulence Test.

KL Virulence Agar and KL Virulence Enrichment are prepared according to the formulation of Hermann, Moore and Parsons.[1]

Principles of the Procedure

Proteose Peptone provides the carbon and nitrogen sources required for good growth of a wide variety of organisms and for toxin production. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is incorporated as a solidifying agent. KL Virulence Enrichment, composed of Casamino Acids, Glycerol and Tween® 80, provides a source of nonserous enrichment. Casamino Acids is derived from acid-hydrolyzed casein that has low sodium chloride and iron concentrations. The low iron concentration is beneficial because iron is known to prevent the production of diph theria toxin when present in more than minute amounts. Glycerol (glycerine) contains no heavy metals and is used by bacteria as a source of carbon. Tween® 80 improves growth of certain strains of *Corynebacterium diphtheriae.* Toxin produced by bacteria and diffused into the medium is detected by precipitation with the antitoxin present on the KL Antitoxin Strip. Chapman Tellurite Solution 1% (1% potassium tellurite solution) inhibits gram-negative and most gram-positive bacteria except *Corynebacterium* spp., *Streptococcus mitis, S. salivarius*, enterococci, and possibly *Staphylococcus epidermidis.* This permits direct testing of mixed primary cultures.

Formula

KL Virulence Agar

Formula Per Liter

Bacto Proteose Peptone	20 g
Sodium Chloride	2.5 g
Bacto Agar	15 g

Final pH 7.8 ± 0.2 at 25°C

KL Virulence Enrichment

Formula Per 100 ml

Bacto Casamino Acids	1 g
Glycerol	1 ml
Tween® 80	1 ml

KL Antitoxin Strips

KL Antitoxin Strips are 1 x 7 cm filter paper strips containing antitoxin to diphtheria toxin.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store KL Virulence Agar below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store KL Virulence Enrichment and KL Antitoxin Strips at 2-8°C.

Store prepared plates at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

KL Virulence Agar
KL Virulence Enrichment
KL Antitoxin Strips

Materials Required But Not Provided

Glassware
Autoclave
Water bath (55-60°C)
Incubator (35°C)
Chapman Tellurite Solution 1%

Method of Preparation

1. Suspend 37.5 grams of KL Virulence Agar in 1 liter distilled or deionized water and heat to boiling to dissolve completely.
2. Autoclave at 121°C for 15 minutes.
3. Cool in a water bath to 55-60°C.
4. Aseptically dispense 10 ml of KL Virulence Agar into a Petri dish containing 2 ml KL Virulence Enrichment and 0.5 ml Chapman Tellurite Solution 1%; mix thoroughly.
5. Using aseptic technique, submerge a KL Antitoxin Strip or equivalent beneath the agar prior to solidification.

Specimen Collection and Preparation

For cases of suspected diphtheria, material for culture is obtained on a swab from the inflamed membranes of the throat and nasopharynx, or from wounds.[4] Care must be taken not to contaminate the swab with normal skin flora. The specimen should be immediately transported to a laboratory and inoculated onto the proper media. If the specimen is to be shipped to a laboratory, it should be placed in a sterile tube or a special packet containing a desiccant such as silica gel.[5]

User Quality Control

Identity Specifications

KL Virulence Agar

Dehydrated Appearance:	Light beige with some small dark specks, free- flowing, homogeneous.
Solution:	3.75% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, slightly opalescent, with a slight precipitate.
Reaction of 3.75% Solution at 25°C:	pH 7.8 ± 0.2
Prepared Medium:	Light medium amber, slightly opalescent, may have a slight precipitate.

KL Virulence Enrichment

Appearance:	Colorless to very light amber, clear liquid.

KL Antitoxin Strips

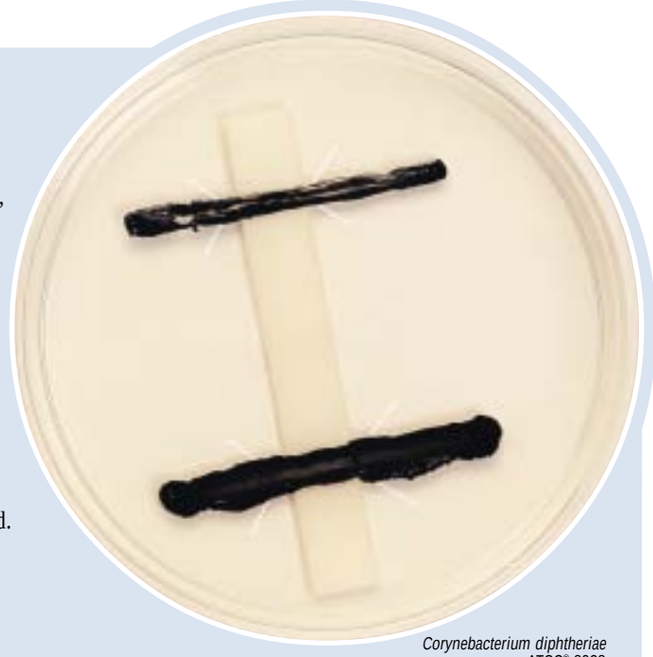
Appearance:	White, filter paper strips, 1 x 7 cm.

Cultural Response

Prepare KL Virulence Agar per label directions, including KL Virulence Enrichment, Chapman Tellurite Solution 1% and one KL Antitoxin Strip per plate. Inoculate and incubate at 35 ± 2°C under CO_2 for up to 72 hours.

ORGANISM	ATCC®	GROWTH
Corynebacterium diphtheriae Type *gravis*	8028	+
Corynebacterium diphtheriae Type *intermedius*	8032	+
Staphylococcus aureus	25923*	–

+ = positive, line of precipitation at 45% angle to the strip
– = negative, no line of precipitation



Corynebacterium diphtheriae
ATCC® 8028

Precipitate lines are graphically enhanced for demonstration purposes (see Results).

The cultures listed are the minimum that should be used for performance testing.

*This organism is available as a Bactrol™ culture and should be used as directed.

Test Procedure

Inoculate the medium by streaking a loopful of a 24-hour culture in a single line across the plate perpendicular to (right angle to) the antitoxin strip. (Do not touch the actual strip itself). As many as eight cultures may be tested on a single plate.[6] Place test isolates about 1 cm apart. Also inoculate a toxigenic (positive control) and a nontoxigenic (negative control) *C. diphtheriae* strain approximately 1 cm on either side of the test isolates.[6] Incubate the inverted plates at 37°C for 72 hours. Examine at 24-, 48- and 72-hour intervals.

Results

Toxigenic (virulent) cultures of *C. diphtheriae* will show fine lines of precipitation at approximately 45° angles from the culture streak. This line forms where toxin (from the bacteria) combines with antitoxin from the strip. Primary precipitin lines form an arc of identity with the precipitin line produced by an adjacent positive control strain.[7] Nontoxigenic strains of *C. diphtheriae* will show no lines of precipitation.

Limitations of the Procedure

1. Each test should include positive and negative controls.[5]
2. False-positive reactions may be seen after 24 hours as weak bands near the antitoxin strip. These can be recognized when compared with the positive control.[8]
3. *Corynebacterium ulcerans* and *C. pseudotuberculosis* may also produce lines of toxin-antitoxin.[9]

References

1. **Hermann, G. J., M. S. Moore, and E. I. Parsons.** 1958. A substitute for serum in the diphtheria in vitro test. Am. J. Clin. Pathol. **29**:181-183.
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4. **Clarridge, J. E., and C. A. Spiegel.** 1995. *Corynebacterium* and miscellaneous irregular gram-positive rods, *Erysipelothrix* and *Gardnerella*, p. 357-378. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Krech, T., and D. G. Hollis.** 1991. *Corynebacterium* and related organisms, p. 277-286. *In* A. Ballows, W. J. Hausler, Jr., K. Herrmann, H. D. Isenberg, and H. J. Shadomy (eds.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 410-414. Williams & Wilkins, Baltimore, MD.
7. **Washington, J. A., Jr.** 1981. Laboratory procedures in clinical microbiology. Springer-Verlag, New York, NY.
8. **Lennette, E. H., A. Balows, W. J. Hausler, Jr., and J. P. Truant (eds.).** 1980. Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
9. **Branson, D.** 1972. Methods in clinical bacteriology. Charles C. Thomas, Springfield, IL.

Packaging

KL Virulence Agar	500	g	0985-17
KL Virulence Enrichment	12 x 20	ml	0986-64
KL Antitoxin Strips	12	strips	3101-30
Chapman Tellurite Solution 1%	6 x 1 6 x 25	ml ml	0299-51 0299-66

Bacto® Kligler Iron Agar

Intended Use

Bacto Kligler Iron Agar is used for differentiating pure cultures of gram-negative bacilli based on the fermentation of dextrose and lactose and production of hydrogen sulfide.

Also Known As

Kligler Iron Agar is also known as KIA.

Summary and Explanation

Kligler Iron Agar is a modification of Kligler's[1] original formula. It is recommended to identify pure cultures of colonies picked from primary plating media, such as MacConkey Agar. Kligler's[1] original medium was a soft nutrient agar containing dextrose, Andrade indicator and lead acetate. Russell[2] devised a medium containing glucose, lactose, and an indicator for the differentiation of lactose-fermenting and nonlactose-fermenting gram negative bacilli. Kligler[3] found that lead acetate for the detection of hydrogen sulfide could be successfully combined with Russell double sugar medium for the differentiation of the typhoid, paratyphoid and dysentery groups. Bailey and Lacy[4] simplified the formula by using phenol red as the pH indicator instead of Andrade indicator. A similar medium containing saccharose, Tryptone, ferrous sulfate and thiosulfate was developed by Sulkin and Willett.[5]

Kligler Iron Agar is recommended for differentiation of enteric gram-negative bacilli from clinical specimens[6-8] and food samples.[9,10]

Principles of the Procedure

Kligler Iron Agar combines the principles of Russell double sugar agar and lead acetate agar into one medium. This combination permits the differentiation of the gram-negative bacilli both by their ability to ferment dextrose or lactose and to produce hydrogen sulfide. Beef Extract, Yeast Extract, Bacto Peptone, and Proteose Peptone provide nitrogen, vitamins and minerals. Ferrous sulfate and sodium thiosulfate are the indicators of hydrogen sulfide production. Phenol red is the pH indicator. Sodium chloride maintains the osmotic balance of the medium. Bacto® Agar is the solidifying agent.

Formula

Kligler Iron Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Yeast Extract	3 g
Bacto Peptone	15 g
Bacto Proteose Peptone	5 g
Bacto Lactose	10 g
Bacto Dextrose	1 g
Ferrous Sulfate	0.2 g
Sodium Chloride	5 g
Sodium Thiosulfate	0.3 g
Bacto Agar	12 g
Bacto Phenol Red	0.024 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared tubes at 2-8°C.

Expiration Date

The expiration date applies to the medium in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Kligler Iron Agar

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Tubes with closures
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 55 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes with closures.
4. Autoclave at 121°C for 15 minutes. Cool in slanted position with deep butts.

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[6-10]
2. Process each specimen, using procedures appropriate for that specimen or sample.[6-10]

User Quality Control

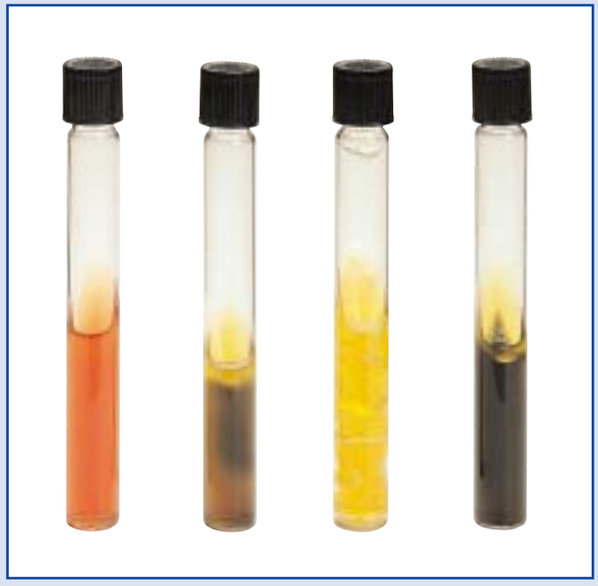
Identity Specifications

Dehydrated Appearance:	Pinkish beige, free flowing, homogeneous.
Solution:	5.5% solution; soluble in distilled or deionized water on boiling. Orange-red, slightly opalescent with precipitate.
Prepared Tubes:	Slightly orange-red, slightly opalescent, slight precipitate.
Reaction of 5.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Kligler Iron Agar per label directions. Inoculate and incubate tubes at 35°C for 18-48 hours.

ORGANISM	ATCC®	CFU	GROWTH	SLANT/ BUTT	GAS	H_2S
Citrobacter freundii	8090*	undiluted	good	A/A	+	+
Escherichia coli	25922*	undiluted	good	A/A	+	–
Proteus vulgaris	6380	undiluted	good	K/A	–	+



Uninoculated tube | *Citrobacter freundii* ATCC® 8090 | *Escherichia coli* ATCC® 25922 | *Proteus vulgatus* ATCC® 6380

A = acid reaction (yellow)
+gas = cracks, splits or bubbles in medium
+H_2S = black precipitate in butt
K = alkaline reaction (no color change)
–gas = no cracks, splits, or bubbles in medium
–H_2S = no black precipitate in butt

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Test Procedure

1. Obtain a pure culture of the organism to be tested. Select well-isolated colonies.
2. With an inoculating needle, pick the center of well-isolated colonies obtained from solid culture media.
3. Stab the center of the medium into the deep of the tube to within 3-5 mm from the bottom.
4. Withdraw the inoculating needle, and streak the surface of the slant.
5. Loosen closure on the tube before incubating.
6. Incubate at 35°C for 18-48 hours.
7. Read tubes for acid production of slant/butt, gas, and hydrogen sulfide reactions.

Results

1. An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only.
2. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose and lactose.
3. An alkaline slant-alkaline butt (red/red) indicates that neither dextrose nor lactose was fermented (non-fermenter).
4. Cracks, splits, or bubbles in the medium indicate gas production.
5. A black precipitate in the butt indicates hydrogen sulfide production.

Limitations of the Procedure

1. H_2S-producing organisms may produce a black precipitate to such a degree that the reaction in the butt is completely masked. If H_2S is produced, dextrose is fermented even if it is not observed.[11]
2. Further biochemical tests and serological typing must be performed for definite identification and confirmation of organisms.
3. Do not use an inoculating loop to inoculate a tube of Kligler Iron Agar. While stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production.[11]
4. Best reactions are obtained on freshly prepared medium.
5. A pure culture is essential when inoculating Kligler Iron Agar. If inoculated with a mixed culture, irregular observations may occur.
6. Hydrogen sulfide determinations using Kligler Iron Agar should be limited to the members of the *Enterobacteriaceae*. Other organisms may require more sensitive methods for detection of H_2S production.[11]
7. Tubes should be incubated with caps loosened to allow a free exchange of air, which is necessary to enhance the alkaline condition on the slant.[11]

References

1. **Kligler, I. J.** 1917. A simple medium for the differentiation of members of the typhoid-paratyphoid group. Am. J. Public Health. **7:**1042-1044.
2. **Russell, F. F.** 1911. The isolation of typhoid bacilli from urine and feces with the description of a new double sugar tube medium. J. Med. Res. **25:**217.
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4. **Bailey, S. F., and L. R. Lacy.** 1927. A modification of the Kligler lead acetate medium. J. Bacteriol. **13:**183.
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6. **Pezzlo, M. (ed.).** 1992. Aerobic bacteriology, p. 1.0.0-1.20.47. *In* Isenberg, H.D. (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
7. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
8. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
9. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
10. **Elliot, E. L., C. A. Kaysner, L. Jackson, and M. L. Tamplin.** 1995. *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, and other *Vibrio* spp. *In* FDA bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
11. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Kligler Iron Agar	500 g	0086-17

Bacto® Koser Citrate Medium

Intended Use

Bacto Koser Citrate Medium is used for differentiating *Escherichia coli* from *Enterobacter aerogenes* based on citrate utilization.

Also Known As

Koser's Citrate Broth.

Summary and Explanation

In 1923, the work of Koser demonstrated that coli-aerogenes bacteria could be differentiated by their use of certain salts of organic acids.[1] Koser found that the sodium salt of citric acid (sodium citrate) is used as a source of carbon by *E. aerogenes* and not by *E. coli*. Biochemical identification schemes for identifying *E. coli* frequently include Koser citrate.

E. coli is an important member of the coliform group of bacteria. The coliforms are described as aerobic and facultatively anaerobic gram-negative non-sporeforming bacilli that ferment lactose and form acid and gas at 35°C within 48 hours. Procedures to detect, enumerate and presumptively identify coliforms are used in testing foods and dairy products.[2-5] Presumptive identification is confirmed by performing biochemical tests that specifically identify *E. coli*.

Principles of the Procedure

Bacto Koser Citrate Medium is prepared with chemically pure salts and tested to determine that no sources of carbon (other than sodium citrate) or nitrogen (other than ammonium salts) are present. Bacteria that are able to use citrate as their carbon source will grow in the medium and cause turbidity.

Formula

Koser Citrate Medium

Formula Per Liter

Sodium Ammonium Phosphate	1.5 g
Monobasic Potassium Phosphate	1.0 g
Magnesium Sulfate	0.2 g
Sodium Citrate	3.0 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious material.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

User Quality Control

Identity Specifications

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	0.57% solution, soluble in distilled or deionized water. Solution is colorless, clear.
Reaction of 0.57% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterobacter aerogenes	13048*	>1,000	good
Escherichia coli	25922*	>1,000	markedly to completely inhibited

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bacto Koser Citrate Medium

Materials Required but not Provided

Flask with closure
Test tubes with caps
Distilled or deionized water
Autoclave

Method of Preparation

1. Suspend 5.7 grams in 1 liter distilled or deionized water.
2. Dispense required amount into test tubes.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Transfer growth from a single colony or a loopful of liquid suspension and inoculate the broth medium.
2. Incubate at 35 ± 2°C for 18-24 hours.

Results

Positive: Turbidity
Negative: Clear, no turbidity

Limitations of the Procedure

1. The Koser citrate test is one of many biochemical tests required to identify an isolate to genus and species.

References

1. **Koser, S. A.** 1923. Utilization of the salts of organic acids by the colon- aerogenes group. J. Bacteriol. 8:493-520.
2. **Vanderzant, C., and D. F. Splittoesser (ed.)**. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Marshall, R. T. (ed.).** 1992. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
4. **Association of Official Analytical Chemists**. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Association of Official Analytical Chemists**. 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Koser Citrate Medium	500 g	0015-17

Bacto® LB Agar, Lennox

Intended Use

Bacto LB Agar, Lennox is used for maintaining and cultivating recombinant strains of *Escherichia coli.*

Summary and Explanation

LB Agar, Lennox, a nutritionally rich medium, was developed by Lennox for the growth and maintenance of pure cultures of recombinant strains of *E. coli*.[1] These strains are generally derived from *E. coli* K12, which are deficient in B vitamin production. This strain of *E. coli* has been further modified through specific mutation to create an auxotrophic strain that is not capable of growth on nutritionally deficient media. LB Agar, Lennox provides all the nutritional requirements of these organisms. LB Agar, Lennox contains half the sodium chloride level of the Miller formulation of LB Agar.[2] This allows the researcher to select the optimal salt concentration for a specific strain.

Principles of the Procedure

Peptides and peptones are provided by Tryptone. Vitamins (including B vitamins) and certain trace elements are provided by Yeast Extract. Sodium ions for transport and osmotic balance are provided by Sodium Chloride. Bacto Agar is the solidifying agent.

Formula

LB Agar, Lennox

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water on boiling. Solution is light beige, slightly opalescent.
Prepared Plates:	Medium amber, very slightly to slightly opalescent.
Reaction of 3.5% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare LB Agar, Lennox per label directions. Inoculate and incubate at 35 ± 2°C for 18- 24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	23724	100-300	Good
Escherichia coli	33694	100-300	Good
Escherichia coli	33849	100-300	Good
Escherichia coli	39403	100-300	Good
Escherichia coli	47014	100-300	Good
Escherichia coli	53868	100-300	Good

The cultures listed are the minimum that should be used for performance testing.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

LB Agar, Lennox

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50°C)
Petri dishes
Incubator (35°C)

Method of Preparation

1. Suspend 35 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C in a waterbath.
5. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Not applicable.

Test Procedure

Consult appropriate references for recommended test procedures.[2]

Results

After sufficient incubation, the medium should show growth as evidenced by formation of colonies and/or a confluent lawn of growth.

References

1. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1:**190.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1994. Current protocols in molecular biology, vol. 1. Current Protocols, New York, N.Y.

Packaging

LB Agar, Lennox	500 g	0401-17

Bacto® LB Agar, Miller

Intended Use

Bacto LB Agar, Miller is used for maintaining and propagating *Escherichia coli* in molecular microbiology procedures.

Summary and Explanation

LB Agar, Miller is based on LB Medium as described by Miller for the growth and maintenance of *E. coli* strains used in molecular microbiology procedures.[1-3] LB Agar, Miller is a nutritionally rich medium designed for growth of pure cultures of recombinant strains. *E. coli* grows more rapidly on this rich medium because it provides the cells with amino acids, nucleotide precursors, vitamins and other metabolites that the microorganism would otherwise have to synthesize.[4]

Principles of the Procedure

Peptides and peptones are provided by Tryptone. Vitamins (including B vitamins) and certain trace elements are provided by Yeast Extract. Sodium ions for transport and osmotic balance are provided by sodium chloride. Agar is added to the medium as a gelling agent.

Formula

LB Agar, Miller

Formula Per Liter

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Sodium Chloride	10 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light to light tan, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in distilled or deionized water on boiling. Solution is very light amber, slightly opalescent.
Prepared Plates:	Very light amber, slightly opalescent.
Reaction of 4.0% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Bacto LB Agar, Miller per label directions. Inoculate plates and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	33526	100-1000	Good

The culture listed is the minimum that should be used for performance testing.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

LB Agar, Miller

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50°C)
Sterile Petri dishes
Incubator (35°C)

Method of Preparation

1. Suspend 40 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Not applicable.

Test Procedure

Consult appropriate references for recommended test procedures.[3-6]

Results

Growth should be evident by the appearance of colonies and/or a confluent lawn on the surface of the medium.

References

1. **Luria, S. E., and J. W. Burrous.** 1955. Hybridization between *Escherichia coli* and *Shigella*. J. Bacteriol. **74**:461-476.
2. **Luria, S. E., J. N. Adams, and R. C. Ting.** 1960. Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. Virology **12**:348-390.
3. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
4. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.).** 1994. Current protocols in molecular biology. Greene Publishing Associates, Inc., Brooklyn, NY.
5. **Sambrook, J., E. F. Fitsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd. ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
6. **Lennox, E. S.** 1955. Transduction of linked genetic character of the host by bacteriophage P1. Virology **1**:190-206.

Packaging

LB Agar, Miller	500 g	0445-17
	2 kg	0445-07

Bacto® LB Broth, Lennox

Intended Use

Bacto LB Broth, Lennox is used for maintaining and propagating *Escherichia coli* in molecular microbiology procedures.

Summary and Explanation

LB Broth, Lennox is a nutritionally rich medium designed for growth of pure cultures of recombinant strains. The formula is based on L Broth described by Lennox for the growth and maintenance of *E. coli* strains used in molecular microbiology procedures.[1] *E. coli* is grown to late log phase in LB Broth. Some plasmid vectors may replicate to high copy numbers without selective amplification. Some vectors may require selective amplification to reach high copy numbers. Chloramphenicol can be added to inhibit host synthesis and, as a result, prevent replication of the bacterial chromosome.[2]

LB Broth, Lennox contains ten times the sodium chloride level of Luria Broth Base, Miller and one half of that found in LB Broth, Miller.[3] This allows the researcher to select the optimal salt concentration for a specific strain. If desired, the medium may be aseptically supplemented with glucose to prepare the complete medium described by Lennox.

Principles of the Procedure

Peptides and peptones are provided by Tryptone. Vitamins (including B vitamins) and certain trace elements are provided by Yeast Extract. Sodium ions for transport and osmotic balance are provided by Sodium Chloride.

Formula

LB Broth, Lennox

Formula Per Liter

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Sodium Chloride	5 g

Final pH 7.0 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.0% solution, soluble in distilled or deionized water. Solution is very light amber, clear to very slightly opalescent.
Prepared Medium:	Very light amber, clear to very slightly opalescent.
Reaction of 2.0% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare LB Broth, Lennox per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	53868 (DH5)	100-300	Good
Escherichia coli	JM103	100-300	Good
Escherichia coli	33694 (HB101)	100-300	Good

The cultures listed are the minimum that should be used for performance testing.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

LB Broth, Lennox

Materials Required But Not Provided

Flasks with closures
Tubes with closures
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 20 grams in 1 liter of distilled or deionized water.
2. Dispense into tubes with closures.
3. Autoclave at 121°C for 15 minutes.
4. Allow to cool below 45°C.
5. If desired, aseptically add 10 ml sterile 10% glucose solution and mix thoroughly.

Specimen Collection and Preparation

Not applicable

Test Procedure

Consult appropriate references for recommended test procedures.[1,2,3]

Results

Growth is evident in the form of turbidity.

References

1. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology **1:**190.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
3. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.

Packaging

LB Broth, Lennox	500 g	0402-17
	2 kg	0402-07
	10 kg	0402-08

Bacto® LB Broth, Miller

Intended Use

Bacto LB Broth, Miller (Luria-Bertani) is used for maintaining and propagating *Escherichia coli* in molecular microbiology procedures.

Summary and Explanation

LB Broth, Miller is based on LB Medium as described by Miller for the growth and maintenance of *Escherichia coli* strains used in molecular microbiology procedures.[1-3] LB Broth, Miller is a nutritionally rich medium designed for growth of pure cultures of recombinant strains. *Escherichia coli* is grown to late log phase in LB Medium. Some plasmid vectors replicate to a high copy number without selective amplification. Some vectors do not replicate so freely, and need to be selectively amplified. Chloramphenicol has been added to inhibit host synthesis and as a result, prevents replication of the bacterial chromosome.[4]

LB Broth, Miller contains twenty times the sodium chloride level of Luria Broth Base, Miller and twice the level found in LB Broth, Lennox.[3-5] This allows the researcher to select the optimal salt concentration for a specific strain.

Principles of the Procedure

Peptides and peptones are provided by Tryptone. Vitamins (including B vitamins) and certain trace elements are provided by Yeast Extract. Sodium ions for transport and osmotic balance are provided by sodium chloride.

Formula

LB Broth, Miller

Formula Per Liter

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Sodium Chloride	10 g

Final pH 7.0 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white to beige, free-flowing, homogeneous.
Solution:	2.5% solution; soluble in distilled or deionized water. Solution is light amber, clear to very slightly opalescent.
Prepared Tubes:	Very light amber, clear to very slightly opalescent.
Reaction of 2.5% Medium at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare LB Broth, Miller per label directions. Inoculate the tubes and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	33526	100-1,000	Good

The culture listed above is the minimum that should be used for performance testing.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

LB Broth, Miller

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Dissolve 25 grams in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references for recommended test procedures.[3,4]

Results

Growth should be evident by the appearance of turbidity in the medium.

References

1. **Luria, S. E., and J. W. Burrous.** 1955. Hybridization between *Escherichia coli* and *Shigella*. J. Bacteriol. **74:**461-476.
2. **Luria, S. E., J. N. Adams, and R. C. Ting.** 1960. Transduction of lactose- utilizing ability among stains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. Virology. **12:**348-390.
3. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York.
4. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
5. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology. **1:**190-206.

Packaging

LB Broth, Miller	500 g	0446-17
	2 kg	0446-07

Bacto® LPM Agar Base
Bacto Moxalactam Antimicrobic Supplement

Intended Use

LPM Agar Base is used with Bacto Moxalactam Antimicrobic Supplement for isolating and cultivating *Listeria monocytogenes*.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[1] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[2] The first reported food-borne outbreak of listeriosis was in 1985,[3] and since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[4]

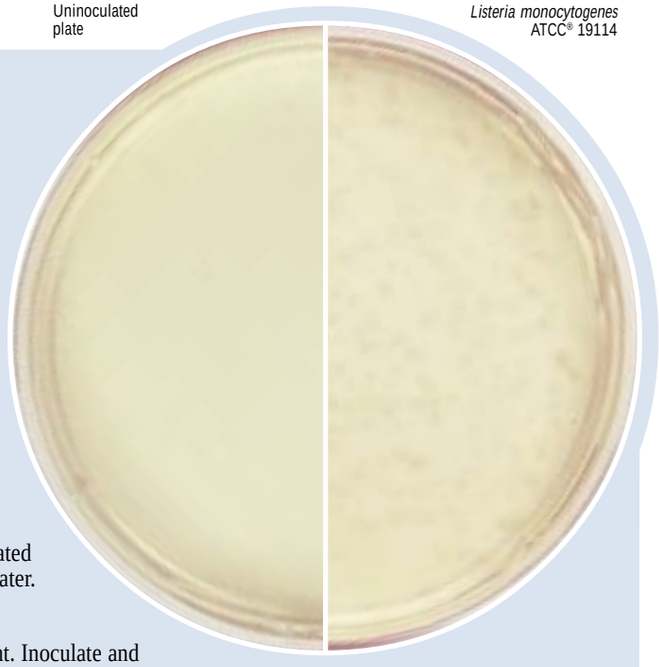
Implicated vehicles of transmission include turkey frankfurters,[5] coleslaw, pasteurized milk, Mexican-style cheese, paté, and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants, and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[6]

Listeria species grow over a pH range of 5.0-9.6, and survive in food products with pH levels outside these parameters.[7] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are: streptococci, especially the enterococci, micrococci and *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[8]

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

LPM Agar, a modification of McBride Listeria Agar, was developed by Lee and McClain[9] to recover low numbers of *Listeria monocytogenes* from samples with profusely mixed microflora. Its use is recommended when testing food and dairy samples and clinical specimens for *Listeria*.



User Quality Control

Identity Specifications

LPM Agar Base

Dehydrated Appearance:	Light tan, homogeneous, may have a tendency to form soft lumps.
Solution:	5.05% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, slightly opalescent.
Prepared Plates:	Light to medium amber, slightly opalescent.
Reaction of 5.05% Solution at 25°C:	pH 7.3 ± 0.2

Moxalactam Antimicrobic Supplement

Lyophilized Appearance:	White to off-white cake (may be broken).
Solution:	Yellow tinted, clear solution when rehydrated with 10 ml sterile distilled or deionized water.

Cultural Response

Prepare LPM Agar Base with Moxalactam Antimicrobic Supplement. Inoculate and incubate plates at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus faecalis	29212*	1,000-2,000	marked to complete inhibition
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition
Listeria monocytogenes	19114	100-1,000	good at 40-48 hours

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

In LPM Agar, Tryptose and Beef Extract provide nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance of the medium. Glycine anhydride is used for improved recovery of *Listeria*. Lithium chloride, in an increased concentration, and phenylethanol are incorporated to aid in suppression of both gram-positive and gram-negative contaminants. Agar is a solidifying agent. Moxalactam Antimicrobic Supplement is added to LPM Agar Base after autoclaving to inhibit staphylococci, bacilli and *Proteus* species.

Formula

LPM Agar Base

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	3 g
Sodium Chloride	5 g
Lithium Chloride	5 g
Glycine Anhydride	10 g
Phenylethanol	2.5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Moxalactam Antimicrobic Supplement

Formula per 10 ml

Moxalactam	20 mg

Precautions

1. For Laboratory Use.
2. **LPM Agar Base:**

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Face, Muscles, Nerves, Urogenital.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   Moxalactam Antimicrobic Supplement:

   MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Liver, Kidneys

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

1. Store LPM Agar Base at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

   Store Moxalactam Antimicrobic Supplement at 2-8°C.

   Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

LPM Agar Base

Moxalactam Antimicrobic Supplement

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50°C)
Petri dishes
Incubator (35°C)

Method of Preparation

1. Suspend 50.5 grams of LPM Agar Base in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45-50°C in a waterbath.
5. Aseptically add 10 ml Moxalactam Antimicrobic Supplement rehydrated per label instructions with sterile distilled or deionized water.
6. Mix well and dispense into Petri dishes.

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[7,10,11,12]
2. Clinical specimens obtained from nonsterile sites, foods, and specimens obtained from the environment should be selectively enriched for *Listeria* spp. before being plated.[10]
3. Process each specimen, using procedures appropriate for that specimen or sample.[7,10,11,12]

Test Procedure

Clinical specimens obtained from nonsterile sites should be selectively enriched for *Listeria* spp. before being plated. Please refer to appropriate references for the procedure to use with clinical specimens.[10] For a procedure for isolating *Listeria* from milk, milk products and food samples, refer to an appropriate reference.[7,11,12]

Results

Observe colonies under oblique transmitted light. *Listeria* colonies display a grey to blue color with a ground glass appearance.

References

1. **Murray, E. G. D., R. A. Webb, and M. B. R. Swann.** 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n. sp.). J. Path. Bact. **29:**407- 439.
2. **Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low- and high-fat, frozen and refrigerated ground beef. J. Food Prot. **57**:969-974.
3. **Wehr, H. M.** 1987. *Listeria monocytogenes* - a current dilemma. Special Report. J. Assoc. Off. Anal. Chem. **70**:769-772.
4. **Bremer, P. J., and C. M. Osborne.** 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels (*Perna canaliculus*) prepared for hot smoking. J. Food Prot. **58**:604-608.
5. **Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
6. **Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett**. 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
7. **Donnelly, C. W., R. E. Bracket, D. Doores, W. H. Lee, and J. Lovett.** 1992. *Listeria*. *In* C. Vanderzant and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
8. **Kramer, P. A., and D. Jones**. 1969. Media selective for *Listeria monocytogenes*. J. Appl. Bacteriol. **32:**381-394.
9. **Lee, W. H., and D. McClain.** 1986. Improved *Listeria monocytogenes* selective agar. Appl. Environ. Microbiol. **52**:1215-1217.
10. **Swaminathan, B., J. Rocourt, and J. Bille.** 1995. *Listeria*, p. 342-343. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R.H. Yolken (eds.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
11. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products. *In* R. T. Marshall, ed. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
12. **Hitchins, A. D.** 1992. *Listeria monocytogenes*, p. 141-151. FDA Bacteriological analytical manual, 7th ed. AOAC International, Arlington, VA.

Packaging

LPM Agar Base	500 g	0221-17
	2 kg	0221-07
Moxalactam Antimicrobic Supplement	6 x 10 ml	0216-60

Bacto® Lactobacilli Agar AOAC
Bacto Lactobacilli Broth AOAC

Intended Use

Bacto Lactobacilli Agar AOAC is used for maintaining stock cultures used in the microbiological assays of vitamins and amino acids.

Bacto Lactobacilli Broth AOAC is used for preparing inocula used in the microbiological assays of vitamins and amino acids.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For maintaining the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose.
2. Inoculum Media: To condition the test culture for immediate use.
3. Assay Media: To permit quantitation of the vitamin under test. Assay media contain all the factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Lactobacilli Agar AOAC[1] and Lactobacilli Broth AOAC[1] are prepared according to the formula recommended by Loy.[2] Lactobacilli Agar AOAC is used for maintaining stock cultures. Lactobacilli Broth AOAC is used to prepare inocula of *Lactobacillus leichmannii* ATCC® 7830, *Enterococcus faecium* ATCC 8043, *Lactobacillus plantarum* ATCC 8014, *Lactobacillus casei* ATCC 7469 and other organisms used in the microbiological assay of B vitamins.

Lactobacillus species grow poorly on non-selective culture media and require special nutrients. Mickle and Breed[3] reported the use of tomato juice in culture media for lactobacilli. Kulp,[4] while investigating the use of tomato juice on bacterial development, found that growth of *Lactobacillus acidophilus* was enhanced.

Principles of the Procedure

Peptonized Milk and Yeast Extract provides the nitrogen, amino acids and vitamins sources in Lactobacilli Agar AOAC and Lactobacilli Broth AOAC. Dextrose is a carbon source to facilitate organism growth. Tomato juice creates the proper acidic environment. Potassium Phosphate Monobasic is a buffering agent. Tween® 80 (Sorbitan Monooleate Complex) acts as an emulsifier. Bacto Agar is a solidifying agent in Lactobacilli Agar AOAC.

Formula

Lactobacilli Agar AOAC

Formula Per Liter

Peptonized Milk	15 g
Bacto Yeast Extract	5 g
Bacto Dextrose	10 g
Tomato Juice (100 ml)	5 g
Potassium Phosphate Monobasic	2 g
Sorbitan Monooleate Complex	1 g
Bacto Agar	10 g

Final pH 6.8 ± 0.2 at 25°C

Lactobacilli Broth AOAC

Formula Per Liter

Peptonized Milk	5 g
Bacto Yeast Extract	5 g
Bacto Dextrose	10 g
Tomato Juice (100 ml)	5 g
Monobasic Potassium Phosphate	2 g
Tween® 80	1 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. Take care to avoid contamination of media or glassware used for microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemical must be used.

User Quality Control

Identity Specifications

Lactobacilli Agar AOAC

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	4.8% solution, soluble in distilled or deionized water on boiling 2-3 minutes.
Prepared Medium:	Medium amber, opalescent when hot, clearer when cooled to 45-50°C.
Reaction of 4.8% Solution at 25°C:	pH 6.8 ± 0.2

Lactobacilli Broth AOAC

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in distilled or deionized water on boiling 2-3 minutes. Medium amber, clear, may have a slight precipitate.
Prepared Medium:	Medium amber, opalescent when hot, clear with a very slight precipitate when cooled.
Reaction of 3.8% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Lactobacilli Agar AOAC and Lactobacilli Broth AOAC per label directions. Inoculate Lactobacilli Agar AOAC by stabbing the medium with test organisms; incubate at 35 ± 2°C for 18-48 hours. Inoculate Lactobacilli Broth AOAC with test organisms and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus hirae	8043	100-1,000	good
Lactobacillus casei subsp. *rhamnosus*	7469	100-1,000	good
Lactobacillus delbrueckii subsp. *delbrueckii*	7830	100-1,000	good
Lactobacillus plantarum	8014	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Storage

Store the dehydrated medium below 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Lactobacilli Agar AOAC
Lactobacilli Broth AOAC

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Inoculating needle
0.9% NaCl

Method of Preparation

1. Suspend the appropriate amount of medium in 1 liter distilled or deionized water:
   Lactobacilli Agar AOAC - 48 grams/liter
   Lactobacilli Broth AOAC - 38 grams/liter
2. Heat to boiling 2-3 minutes to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Samples are prepared for assay according to references given in the specific assay procedures. The samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Stock Cultures

1. Prepare stock cultures in one or more tubes of sterile Lactobacilli Agar AOAC.
2. Inoculate the medium using an inoculating needle.
3. Incubate at 30-37°C for 18-24 hours.
4. Store at 2-8°C.
5. Transfer at weekly or twice monthly intervals.

Inoculum

1. Subculture from a 16-24 hour stock culture into 10 ml Lactobacilli Broth AOAC.
2. Incubate at 35-37°C for 16-24 hours or as specified in specific assay procedures.
3. Centrifuge the culture and decant the supernatant.
4. Resuspend cells in 10 ml of sterile 0.9% NaCl solution or sterile single-strength basal assay medium.

5. Wash the cells by centrifuging and decanting the supernatant two additional times unless otherwise indicated.
6. Dilute the washed suspension 1:100 with sterile 0.9% NaCl or sterile single- strength basal assay medium or as indicated. Where applicable, inoculum concentration should be adjusted according to limits specified in AOAC[1] or US Pharmacopeia.[5]

For a complete discussion on vitamin assay methodology refer to appropriate procedures outlined in the references.[1,5]

Results

Refer to appropriate references for vitamin assay results.[1,5]

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for that purpose.
2. Aseptic technique should be used throughout the vitamin assay procedure.
3. The use of altered or deficient media may result in mutants with different nutritional requirements that will not give a satisfactory response.
4. For a successful completion of these procedures, all conditions of the assay must be adhered to meticulously.

References

1. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
2. **Loy.** 1958. J. AOAC. **4**:61.
3. **Mickle and Breed.** 1925. Technical Bulletin 110, NY State Agriculture Ex. Station.
4. **Kulp, J. W. L., and V. White.** 1932. Modified medium for plating *Lactobacillus acidophilus*. Science **76**:17.
5. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention Inc., Rockville, MD.

Packaging

Lactobacilli Agar AOAC	100 g	0900-15*
Lactobacilli Broth AOAC	100 g	0901-15*

*Store at 2-8°C

Bacto® Lactobacilli MRS Agar
Bacto Lactobacilli MRS Broth

Intended Use

Bacto Lactobacilli MRS Agar and Bacto Lactobacilli MRS Broth are recommended for use in the isolation, enumeration and cultivation of *Lactobacillus* species.

Also Known As

"MRS" is an abbreviation for the authors' names, deMan, Rogosa and Sharpe.

Summary and Explanation

Lactobacilli MRS Agar and Lactobacilli MRS Broth are based on the formulations of deMan, Rogosa and Sharpe.[1] These media were shown by the authors to support luxuriant growth of all lactobacilli from oral, fecal, dairy and other sources.

Principles of the Procedure

Lactobacilli MRS Agar and Lactobacilli MRS Broth contain Peptone and Dextrose. These ingredients supply nitrogen, carbon and other elements necessary for growth. Polysorbate 80, Acetate, Magnesium and Manganese provide growth factors for culturing a variety of lactobacilli. The above ingredients may inhibit the growth of some organisms other than lactobacilli.

Formula

Lactobacilli MRS Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	10 g
Bacto Yeast Extract	5 g
Dextrose	20 g
Sorbitan Monooleate Complex	1 g
Ammonium Citrate	2 g
Sodium Acetate	5 g
Magnesium Sulfate	0.1 g
Manganese Sulfate	0.05 g
Potassium Phosphate, Dibasic	2 g
Bacto Agar	15 g

Final pH 6.5 ± 0.2 at 25°C

Lactobacilli MRS Broth

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	10 g
Bacto Yeast Extract	5 g
Dextrose	20 g
Sorbitan Monooleate Complex	1 g
Ammonium Citrate	2 g
Sodium Acetate	5 g
Magnesium Sulfate	0.1 g
Manganese Sulfate	0.05 g
Potassium Phosphate, Dibasic	2 g

Final pH 6.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store Lactobacilli MRS Agar and Lactobacilli MRS Broth at 2-8°C. The powders are very hygroscopic. Keep containers tightly closed. Store prepared media at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Lactobacilli MRS Agar
Lactobacilli MRS Broth

Materials Required But Not Provided

Flasks with closures
Sterile Petri dishes
Distilled or deionized water
Autoclave
Waterbath (45-50°C)
Incubator (30 or 35°C)

Method of Preparation

Lactobacilli MRS Agar

1. Suspend 70 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Lactobacilli MRS Broth

1. Suspend 55 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect samples in sterile containers and transport immediately to the laboratory following recommended guidelines.[2,3,4]
2. Process each sample using procedures appropriate for that sample.[2,3,4]

Test Procedure

Direct Counts

1. To obtain direct counts of lactobacilli, pour 15-20 ml sterile, molten (45-50°C) Lactobacilli MRS Agar into sterile Petri dishes containing 1 ml volumes of diluted test sample.
2. Distribute the inoculum throughout the medium by rotating the plate in one direction and then in the reverse direction.
3. Allow the medium to solidify on a flat surface for 5-10 minutes.
4. Alternatively, plates of Lactobacilli MRS Agar can be used for direct recovery of organisms using the streak inoculation technique.
5. Incubate agar plates at 35°C for 3 days, or at 30°C for 5 days, in an aerobic atmosphere supplemented with carbon dioxide.

Broth Enrichment

1. Samples can be inoculated directly into Lactobacilli MRS Broth.
2. Incubate broth tubes at 35°C for 3 days, or at 30°C for 5 days, in an aerobic atmosphere.
3. Subculture growth in broth tubes to appropriate solid media.

User Quality Control

Identity Specifications

Lactobacilli MRS Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	7.0% solution, soluble in distilled or deionized water upon boiling. Solution is dark amber, clear to slightly opalescent.
Prepared Medium:	Medium amber, very slightly to slightly opalescent.
Reaction of 7.0% Solution at 25°C:	pH 6.5 ± 0.2

Lactobacilli MRS Broth

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	5.5% solution, soluble in distilled or deionized water upon boiling. Solution is dark amber, clear to very slightly opalescent.
Reaction of 5.5% Solution at 25°C	pH 6.5 ± 0.2

Lactobacillus delbrueckii
ATCC® 7830

Cultural Response

Prepare Lactobacilli MRS Agar or Lactobacilli MRS Broth per label directions. Inoculate Lactobacilli MRS Agar and incubate in a 5% CO_2 atmosphere at 35°C for 24- 72 hours. Inoculate Lactobacilli MRS Broth and incubate at 35°C for 24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus delbrueckii subsp. *lactis*	7830	100-1,000	good
Lactobacillus fermentum	9338	100-1,000	good
Lactobacillus species	11506	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Results

Lactobacilli appear as large, white colonies embedded in or on Lactobacilli MRS Agar or as turbidity in Lactobacilli MRS Broth. Growth may be subcultured onto the appropriate media for use in additional procedures. Refer to appropriate references for recommendations on the culture of *Lactobacillus* spp.[2,3,4]

Limitations of the Procedure

1. Organisms other than lactobacilli may grow in these media. Isolates must be confirmed as lactobacilli by appropriate biochemical testing.

References

1. **deMan, J. C., M. Rogosa, and M. E. Sharpe**. 1960. A medium for the cultivation of lactobacilli. J. Appl. Bacteriol. **23:**130.
2. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
3. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Lactobacilli MRS Agar	500 g	0882-17
Lactobacilli MRS Broth	500 g	0881-17
	2 kg	0881-07
	10 kg	0881-08

Bacto® Lactose Broth

Intended Use

Bacto Lactose Broth is used for cultivating *Salmonella* and coliform organisms in water, foods, dairy and pharmaceutical products.

Summary and Explanation

Lactose Broth is frequently used as a pre-enrichment medium when testing foods and dairy products for *Salmonella*. In dried or processed foods, salmonellae may be sublethally injured and in low numbers. The presence of other bacteria as well as components of the food sample may hinder growth and recovery of *Salmonella*. Pre-enrichment in a nonselective medium such as Lactose Broth allows for repair of cell damage, dilutes toxic or inhibitory substances, and provides a nutritional advantage to *Salmonella* over other bacteria.[1] Lactose Broth is widely used and is included in many Standard Methods procedures for testing foods, dairy products and other materials.[1,2,3,4]

In past years, Lactose Broth was recommended for detection of coliforms in water[5] and foods[6] and for the confirmed phase in testing dairy products.[7]

Principles of the Procedure

Lactose Broth contains Beef Extract and Peptone as carbon and nitrogen sources for general growth requirements. Lactose is a carbohydrate source.

The purpose of a pre-enrichment medium is to provide a higher ratio of *Salmonella* to non-*Salmonella* bacteria after incubation. Most

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige to light tan, free-flowing, homogeneous.
Solution:	1.3% solution, soluble in distilled or deionized water with slight warming. Light to medium amber, clear without significant precipitate.
Prepared Medium:	Light to medium amber, clear without significant precipitate.
Reaction of 1.3% Solution at 25°C:	6.9 ± 0.2

Cultural Response

Prepare Lactose Broth (dehydrated) per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	GAS	ACID
Escherichia coli	25922*	100-1,000	good	+	+
Salmonella typhi	6539	100-1,000	good	–	–

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube | *Escherichia coli* ATCC® 25922

non-*Salmonella* bacteria ferment lactose while *Salmonella* does not. As lactose-fermenting bacteria metabolize lactose, the pH of the medium decreases, creating a bacteriostatic effect on competing microorganisms.

Formula

Bacto Lactose Broth

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Bacto Lactose	5 g

Final pH 6.9 ± at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Lactose Broth

Materials Required but not Provided

Flasks with closures
Distilled or deionized water
Autoclave
Incubator (35°C)
Tubes with closures
Fermentation vials
Disinfectant solution (for prepared Lactose Broth)

Method of Preparation

Lactose Broth (dehydrated)

1. Suspend 13 grams in 1 liter distilled or deionized water.
2. Warm slightly to dissolve completely.
3. Dispense into tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Lactose Broth is used in the pre-enrichment phase of the preparation of food samples for isolation of *Salmonella*. Consult standard references for specific instructions for each type of material being tested.[1,2,3,4]

1. Transfer a 25 gram or 25 ml sample of test material into a container. Add 225 ml of sterile Lactose Broth. Mix as necessary to make a homogeneous suspension. Incubate at 35°C for 24 ± 2 hours.
2. Transfer 1 ml of suspension to appropriate enrichment broths, such as Tetrathionate Broth and Selenite Cystine Broth. Incubate at 35°C for 24 ± 2 hours.
3. Transfer a loopful of suspension to appropriate selective agar media, such as Hektoen Enteric Agar, XLD Agar and Bismuth Sulfite Agar. Incubate at 35°C for 24 ± 2 hours.

Results

Pre-enrichment, selective enrichment and selective plating increase the likelihood of isolating *Salmonella* from foods and other materials.

References

1. **Flowers, R. S., J. D'Aoust, W. H. Andrews, and J. S. Bailey.** 1992. *Salmonella*, p. 371-442. *In* C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
2. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall (ed.), Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. Salmonella, p. 5.01-5.20. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **Andrews, W. H**. 1995. Microbial Methods, p. 1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International. Arlington, VA.
5. **American Public Health Association.** 1975. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D.C.
6. **American Public Health Association.** 1976. Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
7. **American Public Health Association.** 1978. Standard methods for the examination of dairy products, 14th ed. American Public Health Association, Washington, D.C.

Packaging

Lactose Broth	100 g	0004-15
	500 g	0004-17
	2 kg	0004-07
	10 kg	0004-07
Lactose Broth	10 x 90 ml	9070-73

Bacto® Lactose Peptone Broth

Intended Use

Bacto Lactose Peptone Broth is used for the detection of coliform organisms in water.

Summary and Explanation

Lactose Peptone Broth is based on the Lactose Peptone Broth formula described in German Standard Methods and German Drinking Water Regulations.[1] Lactose Peptone Broth is recommended as a non-selective broth enrichment and detection medium for *E. coli* and other coliform bacteria present in water. Lactose fermentation and gas production at 36 ± 1°C are used as the basis for this presumptive coliform test.

Principles of the Procedure

Lactose Peptone Broth contains Tryptone and Soytone which provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Lactose is provided as a source of fermentable carbohydrate. Sodium Chloride is present in the medium to provide a suitable osmotic environment. Brom Cresol Purple is used as a colorimetric indicator to show the production of acid from the fermentation of lactose.

Formula

Lactose Peptone Broth

Formula Per Liter

Bacto Tryptone	17 g
Bacto Soytone	3 g
Bacto Lactose	10 g
Sodium Chloride	5 g
Brom Cresol Purple	0.02 g

Final pH 7.4 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Medium:	Light beige, free-flowing, homogeneous.
Solution:	10.5% solution (triple-strength), soluble in distilled or deionized water. Solution is dark reddish-purple, clear to slightly opalescent.
Prepared Tubes:	Dark reddish purple, clear to slightly opalescent.
Reaction of 10.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Lactose Peptone Broth per label directions. Inoculate and incubate at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	LACTOSE FERMENTATION	GAS PRODUCTION
Escherichia coli	25922*	10-100	+	+
Salmonella typhimurium	14028*	100-1,000	–	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Lactose Peptone Broth

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Durham tubes
Autoclave
Incubator (36 ± 1°C)

Specimen Collection and Preparation

1. Collect samples in sterile containers and transport immediately to the laboratory following recommended guidelines.[1]
2. Process each sample using procedures appropriate for that sample.[1]

Method of Preparation

1. To prepare a triple strength solution, dissolve 105 grams in 1 liter of distilled or deionized water. To prepare a single strength solution, dissolve 35 grams in 1 liter of distilled or deionized water.
2. Dispense 50 ml into tubes or bottles containing a Durham tube.
3. Autoclave at 121°C for 15 minutes.

Test Procedure[1]

Direct Broth Method

1. Add 100 ml of sample to 50 ml of triple strength Lactose Peptone Broth.
2. Incubate at 36 ± 1°C for 24-48 hours.
3. Examine tubes or bottles for evidence of acid formation and gas production.

Membrane Filtration Broth Method

1. Filter 100 ml of sample through a sterile 0.45 micron membrane filter.
2. Remove filter and place in 50 ml of single strength Lactose Peptone Broth.
3. Incubate at 36 ± 1°C for 24-48 hours.
4. Examine tubes or bottles for evidence of acid formation and gas production.

Results

Acid formation is demonstrated by a change in the color of the medium from reddish-purple to yellow. Gas production is demonstrated by the displacement of the medium from the Durham tube. Production of both acid and gas is a presumptive indication of the presence of coliform organisms.

Subculture presumptive positives onto Endo Agar and MacConkey Agar. Incubate at 35 ± 2°C for 24 hours. Examine plates for the presence of typical coliform colonies. Further biochemical testing is necessary to confirm the presence and identify coliforms. Consult appropriate references for further information on identification of coliforms.[2,3]

Limitations of the Procedure

1. Detection of coliform bacteria in Lactose Peptone Broth using this method is only a presumptive test.

References

1. **DIN Deutsches Institut für Normung.** 1991. e.V.: Deutsche Einheitsverfahren zur Wasser-, Abwasser-und Schlammunter suchung: Mikrobiologische Verfahren (Gruppe k), Nachwels von *Escherichia coli* und coliformen Keimen (K6). Reference Method DIN 38411.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Lactose Peptone Broth	500 g	0665-17

Bacto® Lauryl Tryptose Broth

Intended Use

Bacto Lauryl Tryptose Broth is used for detecting coliform organisms in water and wastewater.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	3.56% solution, soluble in distilled or deionized water on warming. Light to medium amber, clear to very slightly opalescent.
Prepared Medium:	Light amber, clear to very slightly opalescent.
Reaction of 3.56% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Lauryl Tryptose Broth per label directions. Inoculate tubes containing inverted fermentation vials with the test organisms and incubate at 35 ± 2°C for 24 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	GAS PRODUCTION
Enterobacter aerogenes	13048*	30-100	good	weak + or –[†]
Escherichia coli	25922*	30-100	good	+
Salmonella typhimurium	14028*	30-100	good	–
Staphylococcus aureus	25923*	300-1,000	markedly to completely inhibited	–

[†]Gas production within 48 ± 3 hours

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Also Known As

Lauryl Tryptose Broth is also referred to as Lauryl Sulfate Broth and is abbreviated as LTB or LSB.

Summary and Explanation

The coliform group comprises aerobic and facultatively anaerobic, gram negative, nonspore-forming rods able to ferment lactose with the production of acid and gas at 35°C within 48 hours.[1] Typically these organisms are classified in the genera *Escherichia*, *Enterobacter* and *Klebsiella*. Coliforms are often used as indicators of fecal contamination in water, and bacterial contamination in food and food products.

Lauryl Tryptose Broth is prepared according to the formula of Mallmann and Darby.[2] These researchers demonstrated the value of Tryptose in the detection of coliform organisms.[3] In a 2% concentration of Tryptose, the growth rate during the early logarithmic growth phase was increased when compared with Bacto Peptone.[3] Mallmann and Darby[3] added phosphate buffers and sodium chloride. This buffered tryptose lactose broth permitted "slow lactose fermenters" to increase gas production in a shorter time frame. To improve the methods used to isolate coliforms from water, Mallmann and Darby[2] investigated many selective agents. Sodium lauryl sulfate gave the best results for inhibition of organisms other than coliforms.[2]

In a comparative study of EC Medium and Lauryl Tryptose Broth, Perry and Hajna[4] reported both media to be highly sensitive and specific for recovery of coliform bacteria from water, shellfish and sewage. A positive presumptive test with either medium was more dependable than the "confirmed" or "completed" test.

LTB is used in the presumptive phase of the Standard Total Coliform Fermentation Technique in the examination of water.[5] Lauryl Tryptose Broth is used in standard methods for coliform detection in water and foods.[1,5,6,7,8,9]

Principles of the Procedure

Tryptose provides nitrogen, vitamins, minerals and amino acids in Lauryl Tryptose Broth. Lactose is the fermentable carbohydrate for

coliforms. Potassium Phosphates are the buffering agents, and Sodium Chloride is used to maintain the osmotic balance of the medium. Sodium Lauryl Sulfate is the selective agent used to inhibit organisms other than coliforms.

Formula

Lauryl Tryptose Broth

Formula Per Liter

Bacto Tryptose	20 g
Bacto Lactose	5 g
Potassium Phosphate, Dibasic	2.75 g
Potassium Phosphate, Monobasic	2.75 g
Sodium Chloride	5 g
Sodium Lauryl Sulfate	0.1 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

NOTE: Refrigerated Lauryl Tryptose Broth generally becomes cloudy or forms precipitates. Incubate medium overnight at room temperature (20°C) before use to clear the medium.[5]

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Lauryl Tryptose Broth

Materials Required But Not Provided

Glassware
Test tubes
Incubator (35°C)
Fermentation vials
Autoclave
Distilled or deionized water

Method of Preparation

1. Suspend 35.6 grams in 1 liter distilled or deionized water.
2. Warm slightly to dissolve completely.
3. Dispense into tubes containing inverted fermentation vials.
4. Autoclave at 121°C for 15 minutes.

   NOTE: Lauryl Tryptose Broth may be prepared in single strength when examining 1 ml or less of water as an inoculum. For inocula of 10 ml consult the table below.

PREPARATION OF LAURYL TRYPTOSE BROTH[5]

Inoculum *mL*	Amount of Medium in Tube *mL*	Volume of Medium+Inoculum *mL*	Dehydrated Lauryl Tryptose Broth Required *g/L*
1	10 or more	11 or more	35.6
10	10	20	71.2
10	20	30	53.4
20	10	30	106.8
100	50	150	106.8
100	35	135	137.1
100	20	120	213.6

Specimen Collection and Preparation

Collect and process specimens according to laboratory policy or standard methods.[1,5,6,7,8,9]

Test Procedure

Follow the methods and procedures for the detection of coliform organisms as described in standard methods.[1,5,6,7,8,9]

Results

After incubation of the tubes at 35 ± 2°C for 24 hours, examine for turbidity and gas production. If no gas has formed in the inverted tube, reincubate and reexamine after 48 hours.[5,6]

Turbidity of the medium accompanied by formation of gas within 48 hours is a positive presumptive test for the presence of coliforms.[5,6] The result should be confirmed by additional standard testing.[1,5,6,7,8,9]

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Christen, G. L., P. M. Davidson, J. S. McAllister, and L. A. Roth.** 1992. Coliform and other indicator bacteria, p. 247-267. *In* R. T. Marshal (ed.). Standard methods for the examination of dairy products. 16th ed. American Public Health Association, Washington, D.C.
2. **Mallmann, W. L., and C. W. Darby.** 1941. Uses of a lauryl sulphate tryptose broth for the detection of coliform organisms. Am. J. Public Health. **31**:127.
3. **Darby, C. W., and W. L. Mallmann.** 1939. J. of Am. Water Works Assoc. **31**:689.
4. **Perry and Hajna.** 1944. Am. J. Public Health. **34**:735.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
7. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

8. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
9. **Bordner, R., and J. Winter (ed.).** 1978. Microbiological methods for monitoring the environment; water and wastes. Environmental monitoring and support laboratory, U.S. Environmental Protection Agency, Cincinnati, OH.

Packaging

Lauryl Tryptose Broth	100 g	0241-15
	500 g	0241-17
	2 kg	0241-07
	10 kg	0241-08

Leptospira Medium EMJH

Bacto® Leptospira Medium Base EMJH · Bacto Leptospira Enrichment EMJH

Intended Use

Bacto Leptospira Medium Base EMJH is used with Bacto Leptospira Enrichment EMJH in cultivating *Leptospira*.

Summary and Explanation

In 1816, Adolf Weil described the first recognized leptospiral infections in humans.[1] These cases were caused by *Leptospira icterohaemorrhagiae* and the disease was subsequently named Weil's Disease.[1] Leptospirosis is a zoonotic disease, having its reservoir in wild, domestic, and peridomestic animals. Infection usually results from direct or indirect exposure to the urine of leptospiruric animals.[2] Indirect exposure through contaminated water and soil accounts for most sporadic cases. Direct exposure occurs in pet owners, veterinarians and persons working with livestock.[3]

The basal medium and enrichment are prepared according to the formulations described by Ellinghausen and McCullough[4] as modified by Johnson and Harris.[5] They modified the formula by replacing rabbit serum medium with Tween 80-albumin. Leptospira Medium EMJH was used in cultivation studies of *Leptospira*.[6]

Leptospira Medium EMJH is recommended for the clinical isolation of *Leptospira*.[7,8]

User Quality Control

Identity Specifications

Leptospira Medium Base EMJH

Dehydrated Appearance:	White, free-flowing, homogeneous.
Basal Solution:	2.3 grams of base in 900 ml distilled or deionized water; soluble upon agitation; colorless, clear with no significant precipitate.
Prepared Medium w/Enrichment:	Very light to light amber, clear with no precipitate.
Reaction (Basal Medium) at 25°C:	pH 7.5 ± 0.2

Leptospira Enrichment EMJH

Appearance:	Medium to dark amber, clear to very slightly opalescent with no significant precipitate.

Cultural Response

Prepare the complete Leptospira Medium EMJH per label directions. Inoculate tubes with undiluted *Leptospira* and incubate at 30 ± 2°C for up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Leptospira interrogans serovar *australis*	23605	one drop	good
Leptospira interrogans serovar *canicola*	23470	one drop	good
Leptospira interrogans serovar *grippotyphosa*	23604	one drop	good

The cultures listed are the minimum that should be used for performance testing.

Uninoculated tube — *Leptospira interrogans* ATCC® 23605

Principles of the Procedure

Leptospira Medium Base EMJH contains ammonium chloride, a nitrogen source, and thiamine, a growth factor. Sodium phosphate dibasic and potassium phosphate monobasic are buffering agents. Sodium chloride maintains the osmotic balance of this formula.

Leptospira Enrichment EMJH contains albumin, polysorbate 80 and additional growth factors for *Leptospira*.

Formula

Leptospira Medium Base EMJH

Formula Per Liter

Sodium Phosphate Dibasic	1 g
Potassium Phosphate Monobasic	0.3 g
Sodium Chloride	1 g
Ammonium Chloride	0.25 g
Thiamine	0.005 g

Final pH 7.5 ± 0.2 at 25°C

Leptospira Enrichment EMJH

A solution of albumin, polysorbate 80 and additional growth factors for *Leptospira*.

Precautions

1. For Laboratory Use.
2. **Leptospira Medium Base EMJH**:
   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.
   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store Leptospira Medium Base EMJH below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Leptospira Enrichment EMJH at 2-8%C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Leptospira Medium Base EMJH or
Leptospira Enrichment EMJH

Materials Required But Not Provided

Glassware
Autoclave
Incubator (30°C) (optional)
Sterile tubes

Method of Preparation

1. Dissolve 2.3 grams Leptospira Medium EMJH in 900 ml distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Aseptically add 100 ml Leptospira Enrichment EMJH to the basal medium at room temperature. Mix thoroughly.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy. Blood, cerebrospinal fluid (CSF) and urine are the specimens of choice for the recovery of leptospires from patients with leptospirosis.[5]

Test Procedure

Culture Procedures[7]

Blood and Spinal Fluid

Freshly drawn blood is preferable; otherwise, use blood taken with SPS, sodium oxalate or heparin.

1. Inoculate four 5 ml tubes of Leptospira Medium EMJH with 1-2 drops of fluid per tube.
2. Incubate in the dark at 28-30%C or at room temperature.

Urine

A total of 12 tubes will be inoculated for each urine specimen.

1. Prepare 1:10 and 1:100 dilutions of urine using Leptospira Medium EMJH to dilute potential inhibitory substances.
2. Inoculate two 5 ml tubes each of Leptospira Medium EMJH with:
   Urine undiluted, 1 drop per tube;
   Urine diluted 1:10, 1 drop per tube;
   Urine diluted 1:100, 1 drop per tube.
3. Duplicate the above inoculations using medium containing 200 µg/ml 5- fluorouracil to inhibit contaminants.
4. Incubate the tubes in the dark at 28-30°C or at room temperature.

Results[7]

1. Examine tubes weekly for signs of growth (turbidity, haze, or a ring of growth).
2. Examine tubes microscopically each week. Take a small drop from a few millimeters below the surface, and examine it with dark-field illumination. Use 400X magnification.
3. Leptospires will be seen as tightly coiled spirochetes about 1 µm wide and 6-20 µm long. Leptospires rotate rapidly on their long axes and usually have hooked ends.
4. If the specimen is positive, subculture about 0.5 ml taken from the area of growth to two tubes of fresh medium.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Elliott, S. H.** 1980. Discussion and clinical diagnosis of Leptospirosis. J. Am. Med. Tech. **42**:37-44.

2. **Faine, S. (ed.).** 1982. Guidelines for the control of leptospirosis. W. H. O. Offset publication no. 67. World Health Organization, Geneva.
3. **Kaufmann, A. F., and R. S. Weyant**. 1995. *Leptospiraceae*, p.621-625. *In* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Ellinghausen, Jr., H. C., and W. G. McCullough.** 1965. Nutrition of *Leptospira pomona* and growth of 13 other serotypes: fractionation of oleic albumin complex (OAC) and a medium of bovine albumin and polysorbate 80. Am. J. Vet. Research **26**:45-51.
5. **Johnson, R., and V. G. Harris.** 1967. Differentiation of pathogenic of leptospires. J. Bacteriol. **94**:27-31.
6. **Rule, P. L., and A. D. Alexander.** 1986. Gellan gum as a substitute for agar in leptospiral media. J. Clin. Microbiol. 23:500-504.
7. **Isenberg, H. D.** (ed.). 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
8. **Koneman, E. W., S. D. Allen, V. R. Dowell, Jr., W. M. Janda, H. M. Sommers, and W. C. Winn, Jr.** 1988. Color atlas and textbook of diagnostic microbiology, 3rd ed. J. B. Lippincott Company, Philadelphia, PA.

Packaging

Leptospira Medium Base EMJH	500 g	0794-17
Leptospira Enrichment EMJH	6 x 100 ml	0795-73*

*Store at 2-8°C

Bacto® Letheen Agar
Bacto Letheen Broth

Intended Use

Bacto Letheen Agar is used for evaluating the bactericidal activity of quaternary ammonium compounds.

Bacto Letheen Broth is used for determining the phenol coefficient of cationic surface-active materials.

Also Known As

"AOAC Letheen Agar/Broth" and "Trypticase Glucose Extract Agar with Lecithin and Tween 80" are common terms for Letheen Agar/Broth.

Summary and Explanation

The value of a highly nutritional solid medium containing neutralizing agents for quaternary ammonium compounds in sanitizers was described by Weber and Black[1] in 1948. The addition of lecithin and Tween® 80 to Tryptone Glucose Extract (TGE) agar resulted in a medium that effectively neutralizes quaternary ammonium compounds in the testing of germicidal activity. Letheen Agar is a modification of TGE agar with the addition of lecithin and sorbitan monooleate (Tween 80).

Letheen Broth was developed as a subculture medium for the neutralization of quaternary ammonium compounds in disinfectant testing. Quisno, Gibby and Foter,[2] found that the addition of lecithin and Tween 80 to F.D.A. Broth resulted in a medium that neutralized high concentrations of quaternary ammonium salts. The resulting medium, termed "Letheen" (a combination of Lecithin and Tween) was easy to prepare and clear in appearance which aided in visual inspection for growth. Letheen Broth is recommended by the Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC)[3] for use with disinfectants containing cationic surface active materials.

Letheen Agar and Letheen Broth are specified for use by the American Society for Testing Materials (ASTM) in the Standard Test Method for Preservatives in Water Containing Cosmetics.[4]

Principles of the Procedure

Letheen Agar contains Beef Extract and Tryptone which provide the carbon and nitrogen sources required for growth of a wide variety of organisms. Dextrose is provided as a source of fermentable carbohydrate. Bacto Agar is added as a solidifying agent. Lecithin and Sorbitan Monooleate are added to neutralize surface disinfectants.[2,5,6] Lecithin is added to neutralize quaternary ammonium compounds and Sorbitan Monooleate is incorporated to neutralize phenols, hexachlorophene, formalin and, with lecithin, ethanol.[7]

Letheen Broth contains Peptamin and Beef Extract which provide the carbon and nitrogen sources necessary for growth. Lecithin and Tween 80 are added as surface active disinfectant neutralizing agents.[2,5,6] Sodium Chloride is included to maintain osmotic balance.

Formula

Letheen Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Tryptone	5 g
Bacto Dextrose	1 g
Bacto Agar	15 g
Sorbitan Monooleate	7 g
Lecithin	1 g

Final pH 7.0 ± 0.2 at 25°C

Letheen Broth

Formula Per Liter

Bacto Peptamin	10 g
Bacto Beef Extract	5 g
Lecithin	0.7 g
Tween 80	5 g
Sodium Chloride	5 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated media at 2-8°C. The dehydrated media are very hygroscopic. Keep containers tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

User Quality Control

Identity Specifications

Letheen Agar

Dehydrated Appearance:	Tan, moist appearance, with a tendency to clump.
Solution:	3.2% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, clear to slightly opalescent, may have a slight, fine precipitate.
Prepared Plates:	Light to medium amber, slightly opalescent, may have a slight precipitate.
Reaction of 3.2% Solution at 25°C:	pH 7.0 ± 0.2

Letheen Broth

Dehydrated Appearance:	Tan, moist appearance, with a tendency to clump.
Solution:	2.57% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, clear to slightly opalescent (opalescent when hot), May have a very slight precipitate.
Prepared Tubes:	Light to medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 2.57% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Letheen Agar per label directions. Using the pour plate technique, inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	11229	100-1,000	good
Staphylococcus aureus	6538	100-1,000	good

Prepare Letheen Broth per label directions. Inoculate and incubate at 35 ± 2°C for 40- 48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	11229	100-1,000	good
Staphylococcus aureus	6538	100-1,000	good
Salmonella typhi	6539	100-1,000	good

Procedure

Materials Provided

Letheen Agar
Letheen Broth

Materials Required But Not Provided

Glassware
Distilled or deionized water
Autoclave

Method of Preparation

1. **Letheen Agar:** Suspend 32 grams in 1 liter distilled or deionized water. Boil to dissolve completely.

   Letheen Broth: Suspend 25.7 grams in 1 liter distilled or deionized water. Boil to dissolve completely.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedures

Letheen Agar and Letheen Broth are used in a variety of procedures. Please consult appropriate references for further information.[3,4]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. The dehydrated Letheen Agar has a characteristic “brown sugar” appearance. This does not indicate deterioration.

References

1. **Weber, G. R., and L. A. Black**. 1948. Relative efficiency of quaternary inhibitors. Soap and Sanit. Chem. **24:**134-139.
2. **Quisno, R., I. W. Gibby, and M. J. Foter**. 1946. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am. J. Pharm. **118:**320-323.
3. **Association of Official Analytical Chemists.** 1995. Official methods of analysis, 16th ed. Association of Official Analytical Chemists, Washington, D.C.
4. **American Society for Testing Materials.** 1991. Standard test method for preservatives in water-containing cosmetics, E 640-78. Annual Book of ASTM Standards, Philadelphia, PA.
5. **Erlandson, A. L., Jr., and C. A. Lawrence**. 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science **118:**274-276.
6. **Brummer, B.** 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. App. Environ. Microbiol. **32:**80-84.
7. **Favero (chm.)**. 1967. Microbiological sampling of surfaces-a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.

Packaging

Letheen Agar	500 g	0680-17
Letheen Broth	500 g	0681-17

Bacto® Levine EMB Agar

Intended Use

Bacto Levine EMB Agar is used for isolating and differentiating lactose-fermenting from lactose-nonfermenting gram-negative enteric bacilli.

Summary and Explanation

Eosin methylene blue agar (EMB) was originally formulated by Holt-Harris and Teague.[1] The formulation contained eosin and methylene blue as inhibitors and pH indicators, and the carbohydrates lactose and sucrose. Levine[2,3] modified the formulation by using lactose at an increased concentration and omitting sucrose.

Levine EMB agar is used for detection and confirmation of coliforms, specifically enteropathogenic *E. coli* in foods, dairy products and other materials.[4,5,6,7]

Principles of the Procedure

Levine EMB Agar contains Bacto Peptone as a source of carbon and nitrogen for general growth requirements and Lactose as the carbohydrate. Eosin Y and Methylene Blue are pH indicators, as well as inhibitors of microorganisms other than gram-negative bacilli. However, some staphylococci, streptococci and yeast may grow as small pinpoint colonies. Bacto Agar is the solidifying agent.

Formula

Levine EMB Agar

Formula Per Liter

Bacto Peptone	10 g
Bacto Lactose	10 g
Dipotassium Phosphate	2 g
Bacto Agar	15 g
Eosin Y	0.4 g
Methylene Blue	0.065 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious material.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Levine EMB Agar

Materials Required but not Provided

Flask with closure
Distilled or deionized water
Autoclave
Incubator (35°C)
Petri dishes

User Quality Control

Identity Specifications

Dehydrated Appearance:	Reddish-pink free-flowing, homogeneous.
Solution:	3.75% solution soluble in distilled or deionized water on boiling; green to wine red color with an orange cast, slightly opalescent to opalescent. May have a flocculent precipitate.
Prepared medium:	Wine red color with a green to orange cast, slightly opalescent with a finely dispersed flocculent precipitate.
Reaction of 3.75% solution at 25°C:	pH 7.1 ± 0.2

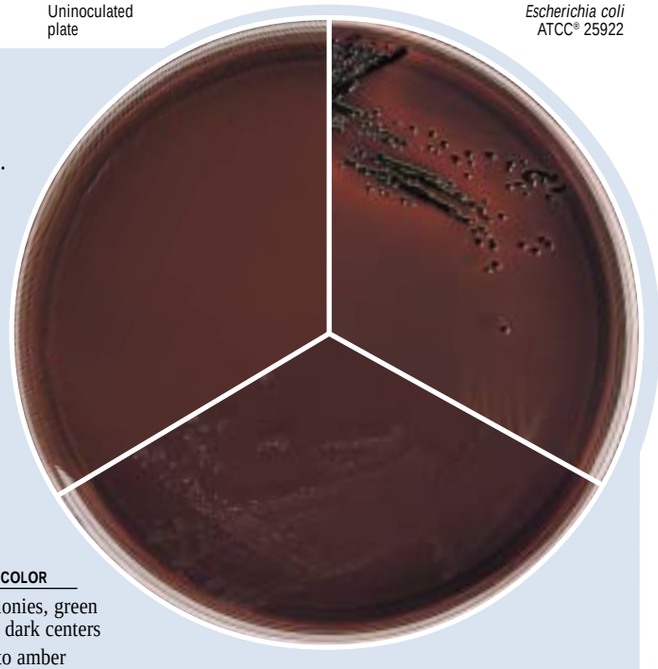
Cultural Response

Prepare Levine EMB Agar per label directions. Inoculate medium and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Escherichia coli	25922*	100-1,000	good	blue-black colonies, green metallic sheen dark centers
Salmonella typhimurium	14028*	100-1,000	good	colorless to amber
Enterococcus faecalis	29212*	1,000-2,000	partially	colorless inhibited

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Method of Preparation

1. Suspend 37.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.
4. Evenly disperse the precipitate when dispensing.

Specimen Collection and Preparation

Consult standard references for specific instructions for the type of material being tested.[4,5,6,7]

Test Procedure

Consult standard references for specific instructions for the type of material being tested.[4,5,6,7]

Results

E. coli colonies are typically dark-centered with or without a metallic sheen.

Lactose fermenters:	Blue-black to brownish colored colonies, may have dark centers with or without metallic sheen.
Lactose non-fermenters:	Colorless or transparent, amber to light-purple colonies.
Staphylococci and streptococci:	Colorless pinpoint colonies.
Yeasts:	Colorless, dull pinpoint colonies; may be "spidery" at edges.

Limitations of the Procedure

1. Levine EMB Agar is only moderately inhibitory. Some staphylococci, streptococci, and yeasts may grow as small pinpoint colonies. Perform microscopic examination and biochemical tests to identify to genus and species.
2. The medium will support growth of most gram-negative bacilli but some strains of *Salmonella* and *Shigella* may be inhibited.

References

1. **Holt-Harris, J. E., and O. Teague**. 1916. A new culture medium for the isolation of *Bacillus typhosa* from stools. J. Infect. Dis. **18**:596.
2. **Levine, M.** 1918. Differentiation of *E. coli* and *A. aerogenes* on a simplified eosin-methylene blue agar. J. Infect. Dis. **23**:43-47.
3. **Levine, M.** 1921. Bacteria fermenting lactose-the significance in water analysis. Bull. 62. Iowa State College Eng. Exp. Sta., Ames, Iowa.
4. **Hitchins, A. D., P. A. Hartman, and E. C. D. Todd.** 1992. Coliforms - *Escherichia coli* and its toxins, p. 325-369. *In* C. Vanderzant and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
6. **Hitchins, A. D., P. Feng, W. D. Watkins, S. R. Rippey, and L. A. Chandler**. 1995. *Escherichia coli* and the coliform bacteria. p. 4.01-4.29. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
7. **Andrews, W.** 1995. Microbial Methods, p. 1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Levine EMB Agar	100 g	0005-15
	500 g	0005-17
	2 kg	0005-07
	10 kg	0005-08

Bacto® Lima Bean Agar

Intended Use

Bacto Lima Bean Agar is used for cultivating fungi.

Summary and Explanation

Fungi are ubiquitous in nature.[1] Of the estimated 250,000 species, fewer than 150 are known to be primary human pathogens.[1]

Lima Bean Agar is prepared from an infusion of dry lima beans and is solidified with 1.5% agar. The nutritive properties of lima beans and the low pH of Lima Bean Agar create a suitable environment for the growth of many fungi.

Principles of the Procedure

Infusion from Lima Beans is a source of nitrogen, carbon, amino acids and vitamins. Bacto Agar is a solidifying agent.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light yellowish tan, free-flowing, homogeneous.
Solution:	2.3% solution; soluble in distilled or deionized water on boiling. Solution is light to medium amber, opalescent, may have a slight precipitate.
Prepared Media:	Light to medium amber, opalescent, may have a slight precipitate.
Reaction of 2.3% Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response

Prepare Lima Bean Agar per label directions. Inoculate and incubate at 30 ± 2° C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good
Candida albicans	10231	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Formula

Lima Bean Agar

Formula Per Liter

Lima Bean, Infusion from	62.5 g
Bacto Agar	15 g

Final pH 5.6 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Lima Bean Agar

Materials Required but not Provided

Glassware
Autoclave
Sterile Petri dishes
Waterbath

Method of Preparation

1. Suspend 23 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121° C for 15 minutes.
4. Dispense as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

Refer to appropriate references for specific procedures on the isolation and cultivation of fungi.

Results

Refer to appropriate references and procedures.

References

1. **Dixon, D. M., and R. A. Fromtling.** 1995. Morphology, taxonomy, and classification of the fungi, p. 699-708. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Lima Bean Agar	500 g	0117-17

Bacto® Listeria Enrichment Broth

Intended Use

Bacto Listeria Enrichment Broth is used to selectively enrich *Listeria* from food.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[1] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[2] The first reported food-borne outbreak of listeriosis was in 1985,[3] and since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[4]

Implicated vehicles of transmission include turkey frankfurters,[5] coleslaw, pasteurized milk, Mexican-style cheese, paté, and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants, and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[6]

Listeria species grow over a pH range of 5.0-9.6, and survive in food products with pH levels outside these parameters.[7] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are: streptococci, especially the enterococci, micrococci and *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[8]

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Listeria Enrichment Broth is based on the formula developed by Lovett *et al*.[9] in which Tryptic Soy Broth is supplemented with Yeast Extract for optimum growth of *Listeria*.

Principles of the Procedure

Tryptone, Soytone and Yeast Extract provide nitrogen, vitamins and minerals. Dextrose is a carbohydrate source. Sodium Chloride maintains the osmotic balance of the medium. Phosphate acts as a buffer. Acriflavine HCl and Nalidixic Acid are added for selectivity and Cycloheximide is used to inhibit growth of saprophytic fungi.

Formula

Listeria Enrichment Broth

Formula Per Liter

Bacto Tryptone	17 g
Bacto Soytone	3 g
Bacto Dextrose	2.5 g
Sodium Chloride	5 g
Potassium Phosphate, Dibasic	2.5 g
Bacto Yeast Extract	6 g
Cycloheximide	0.05 g
Acriflavine HCl	0.015 g
Nalidixic Acid	0.04 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** POSSIBLE RISK OF IRREVERSIBLE EFFECTS. POSSIBLE RISK OF HARM TO THE UNBORN CHILD. (US) HARMFUL IF SWALLOWED. (EC) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Cardiovascular, Liver, Lungs, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free flowing, homogeneous.
Solution:	3.61% solution, soluble in distilled or deionized water on boiling. Solution is light to medium yellowish amber with a faint green ring at the surface, clear to very slightly opalescent.
Prepared Medium:	Light yellowish amber, clear to very slightly opalescent.
Reaction of 3.61% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Listeria Enrichment Broth per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus faecalis	29212*	2,000-10,000	suppressed at 18- 24 hours
Escherichia coli	25922*	2,000-10,000	marked to complete inhibition
Listeria monocytogenes	19114	100-1000	good
Saccharomyces pastorianus	25923	2,000-10,000	marked to complete inhibition

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Listeria Enrichment Broth

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Test tubes with closures
Autoclave
Incubator (30°C)

Method of Preparation

1. Suspend 36.1 grams in l liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

1. Collect food samples in sterile containers and transport immediately to the laboratory following recommended guidelines.
2. Process each food sample, using procedures appropriate for that sample.

Test Procedure

For food samples, use recommended laboratory procedures for isolating *Listeria*.

Results

Refer to appropriate references and procedures for results.

References

1. **Murray, E. G. D., R. A. Webb, and M. B. R. Swann.** 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n. sp.). J. Path. Bact. **29:**407- 439.
2. **Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low- and high-fat, frozen and refrigerated ground beef. J. Food Prot. **57**:969-974.
3. **Wehr, H. M.** 1987. *Listeria monocytogenes* - a current dilemma. Special Report. J. Assoc. Off. Anal. Chem. **70**:769-772.

4. **Bremer, P. J., and C. M. Osborne.** 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels (*Perna canaliculus*) prepared for hot smoking. J. Food Prot. **58**:604-608.
5. **Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
6. **Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
7. **Conner, D. E., R. E. Brackett, and L. R. Beuchat.** 1986. Effect of temperature, sodium chloride, and pH on growth of *Listeria monocytogenes* in cabbage juice. Appl. Environ. Microbiol. **52:**59.
8. **Kramer, P. A., and D. Jones**. 1969. Media selective for *Listeria monocytogenes*. J. Appl. Bacteriol. **32:**381-394.
9. **Lovett, J., D. W. Frances, and J. M. Hunt.** 1987. *Listeria monocytogenes* in raw milk: detection, incidence and pathogenicity. J. Food Prot. **50:**188-192.
10. **McBride, M. E., and K. F. Girard.** 1960. A selective method for the isolation of *Listeria monocytogenes* from mixed bacterial populations. J. Lab. Clin. Med. **55:**153-157.

Packaging

Listeria Enrichment Broth	500 g	0222-17
	10 kg	0222-08

Bacto® Litmus Milk

Intended Use

Bacto Litmus Milk is used for differentiating microorganisms based on acid production and coagulation or proteolysis of casein.

Summary and Explanation

Litmus Milk has been used for many years to detect the metabolic activities of microorganisms in milk and to aid in the identification of bacterial species. It is especially useful in species differentiation within the genus *Clostridium*.[1] This medium is also of value in the maintenance and propagation of lactic acid bacteria. The reactions of litmus milk are a valuable criterion in identification.[1]

Principles of the Procedure

Skim milk is a source of nutrients. The addition of a litmus indicator to milk expands its usefulness as a differential medium. Litmus incorporated in milk is both a pH indicator and an oxidation-reduction indicator.

User Quality Control

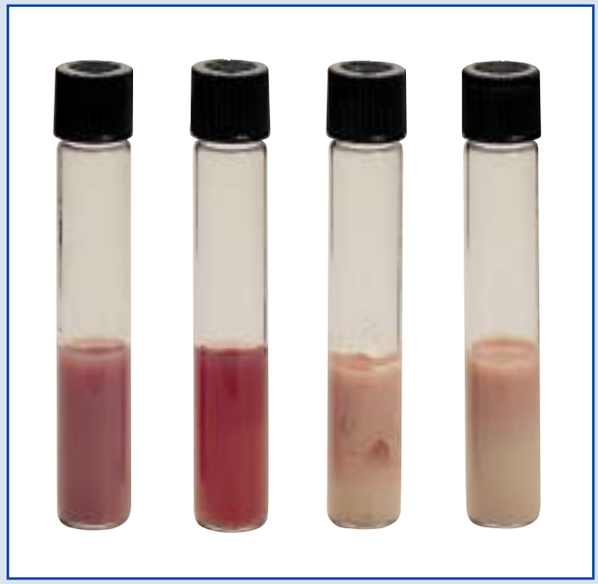
Identity Specifications

Dehydrated Appearance:	Grayish-purple, free-flowing, homogenous.
Solution:	10% solution, soluble in distilled or deionized. Solution is light purple and opaque.
Prepared tubes:	Light purple and opaque
Reaction of 10% Solution at 25°C:	pH 6.8 ± 0.2.

Cultural Response

Prepare Litmus Milk per label directions. Inoculate and incubate at 35 ± 2°C for up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	REACTION IN LITMUS MILK
Bacillus subtilis	6633	1,000-2,000	K, D
Clostridium perfringens	12924	1,000-2,000	R, C
Lactobacillus casei	7469	1,000-2,000	A, R, C



Uninoculated tube | *Bacillus subtilis* ATCC® 6633 | *Clostridium perfringens* ATCC® 12924 | *Lactobacillus casei* ATCC® 7469

A = Acid reactions:
1. Pinkish-red medium.
2. Due to fermentation of the carbohydrates lactose and glucose.

K = Alkaline reactions:
1. Blue medium.
2. No fermentation of carbohydrates.
3. Organism attacks nitrogenous substances present in medium. The breakdown of lactalbumin by proteolytic enzymes form ammonia or basic amines.

R = Reduction:
1. White medium.
2. The enzyme reductase removes oxygen from the litmus, resulting in a decolorized, milky white appearance.
3. Reduction usually begins at the bottom of the tube.

C = Clot or Curd:
1. Milk protein coagulation.
2. Coagulation due to either a precipitation of casein by acid formation or the conversion of casein to paracasein by the enzyme rennin resulting in a clear watery fluid called "whey."

D = Digestion:
1. Milk protein digested.
2. Clearing of medium and dissolution of clot by digestion of casein.

Formula

Litmus Milk

Formula Per Liter

Bacto Skim Milk	100 g
Bacto Litmus	0.75 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Litmus Milk

Materials Required but not Provided

Glassware
Autoclave

Method of Preparation

1. Dissolve 100 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Avoid overheating during sterilization, as the milk sugar will carmelize, resulting in discoloration giving an appearance atypical of the sterile medium.
2. During the sterilization period, Litmus Milk is reduced to a white colored base, however, upon cooling, the original color returns as oxygen is absorbed.
3. Reactions observed in Litmus Milk are not sufficient to speciate. Additional biochemical tests must be performed.[1]

References

1. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol 1, p.275-284, Williams & Wilkins, Baltimore, MD.

Packaging

Litmus Milk	500 g	0107-17

Bacto® Littman Oxgall Agar

Intended Use

Bacto Littman Oxgall Agar is used for isolating and cultivating fungi, especially dermatophytes.

Summary and Explanation

In 1947, Littman[1] described Littman Oxgall Agar which is neutral in reaction and suitable for growth of pathogenic fungi. It is a selective medium for the primary isolation of fungi.

Littman demonstrated that Littman Oxgall Agar is valuable for culturing the dermatophytes. Molds and yeasts form nonspreading, discrete colonies, that are easy to isolate in pure culture. He also suggested that the medium be used for estimating the normal fungal flora of feces, sputum and other human discharges. The medium can also be used for single cell isolation of fungi and plate counts of viable saprophytic fungi in foodstuffs and air.

In a comparative study, Littman[2] compared this medium with Sabouraud Dextrose Agar using a large variety of pathogenic and saprophytic fungi. He reported the isolation of three times as many fungi from feces, sputum, skin scrapings and hair on Littman Oxgall Agar and four times as many pathogenic dermatophytes on the selective medium compared with Sabouraud Dextrose Agar.

Principles of the Procedure

Bacto Peptone provides nitrogen, amino acids and carbon. Dextrose is an additional carbon source. Oxgall restricts the spreading of fungus colonies. Crystal Violet and streptomycin are selective bacteriostatic agents. Bacto Agar is a solidifying agent.

Formula

Littman Oxgall Agar

Formula Per Liter

Bacto Peptone	10 g
Bacto Dextrose	10 g
Bacto Oxgall	15 g
Bacto Agar	20 g
Bacto Crystal Violet	0.01 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breath dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh

air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Littman Oxgall Agar

Materials Required but not Provided

Glassware
Autoclave
Streptomycin

Method of Preparation

1. Suspend 55 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 46°C.
5. Add 30 mcg streptomycin per ml of medium.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Although culture techniques are primary in the identification of etiological agents of mycotic infections, they are not absolute. Often identification must be accomplished by using one or more of the following techniques to corroborate cultural findings: direct microscopic examination of the specimen; animal inoculation; biochemical determination; or serological procedures.
2. Do not use to culture *Nocardia asteroides*, *Streptomyces*, or any other microorganism sensitive to streptomycin.[3]

References

1. **Littman, M. L.** 1947. Culture medium for primary isolation of fungi. Science **106**:109-111.
2. **Littman, M. L.** 1948. Growth of pathogenic fungi on a culture medium. Am. J. Clin. Pathol. **18**:409-420.
3. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 445-447. Williams & Wilkins, Baltimore, MD.

Packaging

Littman Oxgall Agar	500 g	0294-17

User Quality Control

Identity Specifications

Dehydrated Medium	
Appearance:	Grayish-blue, free-flowing, homogeneous.
Solution:	5.5% solution, soluble in distilled or deionized water on boiling. Solution is blue, very slightly to slightly opalescent.
Reaction of 5.5% Solution at 25°C:	pH 7.0 ± 0.2

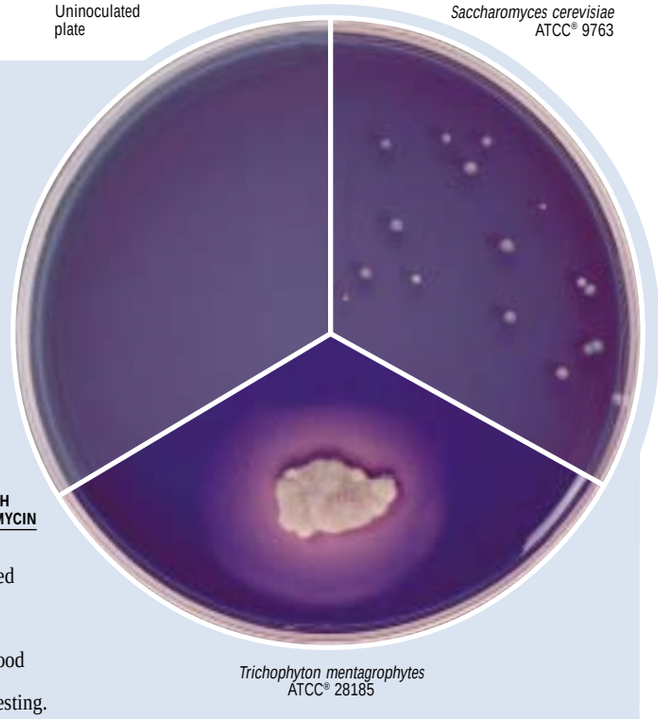
Cultural Response

Prepare Littman Oxgall Agar per label directions with and without the addition of streptomycin. Inoculate and incubate plates at 25-30°C for up to 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH PLAIN	GROWTH w/STREPTOMYCIN
Candida albicans	10231	100-1,000	good	good
Escherichia coli	25922*	100-1,000	good	inhibited
Saccharomyces cerevisiae	9763	100-1,000	good	good
Saccharomyces pastorianus	9080	100-1,000	good	good
Trichophyton mentagrophytes	28185	100-1,000	fair to good	fair to good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Bacto® Liver Infusion Agar
Bacto Liver Infusion Broth

Intended Use

Bacto Liver Infusion Agar is used for cultivating *Brucella* and other pathogenic organisms.

Bacto Liver Infusion Broth is used for cultivating a variety of organisms, particularly *Brucella* and anaerobes.

Summary and Explanation

Brucellosis is a zoonotic disease with a domestic animal reservoir. Transmission by milk, milk products, meat and direct contact with infected animals is the usual route of exposure.[1]

Most strains of *Brucella* will grow on chocolate or blood agar. However, special media such as liver infusion, tryptose, tryptone or brucella agar are preferred.[2] The nutritive factors of Liver Infusion media permit luxuriant growth of *Brucella* and other fastidious pathogens.

User Quality Control

Identity Specifications

Liver Infusion Agar

Dehydrated Appearance:	Dark beige to light tan, free-flowing, homogeneous.
Solution:	5.5% solution, in distilled or deionized water on boiling, medium to dark amber, slightly opalescent to opalescent.
Prepared Medium:	Medium to dark amber, slightly opalescent.
Reaction of 5.5% Solution at 25°C	pH 6.9 ± 0.2

Liver Infusion Broth

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water, medium to dark amber, clear to very slightly opalescent with a few particles.
Prepared Medium:	Medium to dark amber, clear to very slightly opalescent with a few particles.
Reaction of 3.5% Solution at 25°C	pH 6.9 ± 0.2

Cultural Response

Prepare Liver Infusion Agar and Liver Infusion Broth per label directions. Inoculate prepared medium and incubate under 5-10% CO_2 at 35 ± 2°C for 18-48 hours, or up to 72 hours if necessary. Incubate *Clostridium* under anaerobic conditions.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Brucella abortus	4315	100-1,000	good
Brucella melitensis	4309	100-1,000	good
Brucella suis	4314	100-1,000	good
Clostridium sporogenes	11437	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

For isolating *Brucella* strains from contaminated milk, crystal violet (gentian violet) can be added to Liver Infusion Agar to suppress gram-positive organisms.[3] Five percent (5%) heated horse or rabbit serum enhances growth of *Brucella*.[4]

Liver Infusion Agar at approximately half strength may be used to prepare Endamoeba medium for cultivating *Endamoeba histolytica*.[5]

Liver Infusion Broth maintains a degree of anaerobiosis well suited to support growth of anaerobic microorganisms, especially *Clostridium* species.

Principles of the Procedure

Infusion from Beef Liver and Proteose Peptone provide the nitrogen, amino acids, vitamins and carbon sources in Liver Infusion media. Sodium chloride maintains the osmotic balance. Bacto Agar is a solidifying agent.

Formula

Liver Infusion Agar

Formula Per Liter

Beef Liver, Infusion from	500 g
Bacto Proteose Peptone	10 g
Sodium Chloride	5 g
Bacto Agar	20 g

Final pH 6.9 ± 0.2 at 25°C

Liver Infusion Broth

Formula Per Liter

Beef Liver, infusion from	500 g
Bacto Proteose Peptone	10 g
Sodium Chloride	5 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. *Brucella* species are classified as Biosafety Level 3 pathogens. All manipulations with live cultures and antigens must be confined to a Class II biological safety cabinet (BSC).[2]
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated media below 30°C. The dehydrated media are very hygroscopic. Keep containers tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Liver Infusion Agar
Liver Infusion Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes

Method of Preparation

1. **Liver Infusion Agar**: Suspend 55 grams in 1 liter distilled or deionized water and boil to dissolve completely.

   Liver Infusion Broth: Dissolve 35 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
3. Dispense Liver Infusion Agar into sterile Petri dishes or as desired. Dispense Liver Infusion Broth into sterile tubes or as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion of the isolation and identification of *Brucella*, anaerobic microorganisms and other fastidious pathogens, refer to the procedures described in Bailey & Scott's Diagnostic Microbiology,[4] Clinical Microbiology Procedures Handbook[6] and Manual of Clinical Microbiology.[7]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Moyer, N. P., and L. A. Holcomb**. 1995. *Brucella*, p. 549-555. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Carter, G. R.** 1979. Diagnostic procedures in veterinary bacteriology and mycology, 3rd ed. Charles C. Thomas, Springfield, IL.
3. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 802-806, vol. 1. Williams & Wilkins, Baltimore, MD.
4. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
5. **Cleveland, L. R., and E. P. Sanders.** 1930. Encystation, multiple fission without encystment, encystation, metacystic development, and variation in a pure line and nine strains of *Entamoeba histolytica*. Arch. Protietenkd. **70**:223.
6. **Isenberg, H. D. (ed.).** 1995. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
7. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Liver Infusion Agar	500 g	0052-17
Liver Infusion Broth	500 g	0269-17
	10 kg	0269-08

Bacto® Liver Veal Agar

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	9.7% solution, soluble in distilled or deionized water upon boiling; medium to dark amber, opalescent, may have a slight precipitate.
Prepared Medium:	Medium to dark amber, opalescent; may have a slight precipitate.
Reaction of 9.7% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Liver Veal Agar per label directions. Inoculate medium and incubate at 35 ± 2°C under appropriate atmospheric conditions. Incubate clostridia anaerobically, *Neisseria* under increased CO_2, and streptococci aerobically.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Clostridium botulinum	25763	100-1,000	good
Clostridium tetani	10779	100-1,000	good
Neisseria meningitidis	13090*	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Intended Use

Bacto Liver Veal Agar is used for cultivating anaerobic bacteria.

Summary and Explanation

Spray[1] described a procedure using the anaerobic culture dish for the cultivation of these organisms. Liver Veal Agar is identical to the medium described by Spray.[2] Liver Veal Agar provides a rich supply of nutrients for anaerobic and fastidious aerobic pathogens. The medium supports excellent growth of sporulating anaerobes and can be used for deep tube cultures.

Liver Veal Agar is specified in the FDA Bacteriological Analytical Manual (BAM)[3] and Compendium of Methods for the Microbiological Examination of Food.[4] Liver Veal Agar can be supplemented with 50% egg yolk for the cultivation of anaerobic organisms.[5]

Principles of the Procedure

Infusion from Liver, Infusion from Veal, Proteose Peptone, Neopeptone, Tryptone, Gelatin and Isoelectric Casein provide the rich nitrogen, amino acids and vitamin content of the medium. Soluble Starch is added to enhance the growth of anaerobes and Dextrose is a carbon source. Sodium chloride maintains osmotic balance and Bacto Agar is a solidifying agent.

Formula

Liver Veal Agar

Formula Per Liter	
Bacto Liver, Infusion from	50 g
Veal, Infusion from	500 g

Bacto Proteose Peptone	20 g
Bacto Gelatin	20 g
Bacto Soluble Starch	10 g
Isoelectric Casein	2 g
Bacto Dextrose	5 g
Bacto Neopeptone	1.3 g
Bacto Tryptone	1.3 g
Sodium Chloride	5 g
Sodium Nitrate	2 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Liver Veal Agar

Materials Required But Not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes

Method of Preparation

1. Suspend 97 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion of the isolation and identification of anaerobic bacteria and other fastidious aerobic pathogens, refer to the procedure described in Clinical Microbiology Procedures Handbook[6] and Manual of Clinical Microbiology.[7]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Spray, R. S.** 1930. An improved anaerobic culture dish. J. Lab. Clin. Med. 16:203.
2. **Spray, R. S.** 1936. Semisolid media for cultivation and identification of the sporulating anaerobes. J. Bacteriol. 32:135.
3. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
5. **Atlas, R. M.** 1993. Handbook of microbiological media. CRC Press, Boca Raton, FL.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.
7. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Liver Veal Agar	500 g	0059-17

Bacto® Loeffler Blood Serum

Intended Use

Bacto Loeffler Blood Serum is used for cultivating *Corynebacterium diphtheriae* from clinical specimens and in pure culture. The medium is also used for demonstrating pigment production and proteolysis.

Also Known As

Loeffler Blood Serum is also referred to as Loeffler's Serum Agar Medium, Loeffler's Coagulated Serum Slants and LAS.

Summary and Explanation

Loeffler Blood Serum is employed in the cultural diagnosis of diphtheria. Diphtheria is an acute infectious disease primarily of the upper respiratory tract but occasionally of the skin.[1] It is caused by toxigenic strains of *Corynebacterium diphtheriae*, of which there are three biotypes: *mitis*, *intermedius*, and *gravis*.[1] The signs and symptoms of the disease are a pharyngeal membrane, sore throat, malaise, headache and nausea.[2] Death can result from respiratory obstruction by the membrane or myocarditis caused by the toxin.[2]

Loeffler Blood Serum is a modification of the horse serum, dextrose broth medium described by Loeffler[3] for cultivating *C. diphtheriae*. This lipid-rich medium supports rapid growth of *C. diphtheriae* and is useful for demonstrating colonial pigmentation and the proteolytic activities of anaerobes and other microorganisms. Loeffler Blood Serum restores virulence and other identifying properties lost after prolonged incubation or repeated subculturing.[4] Colonies grown on Loeffler medium exhibit excellent metachromatic granules under Gram stain.[2]

Cleveland and Sanders,[5] and Spector[6] used Loeffler Blood Serum in media for the cultivation of *Endamoeba histolytica*. Thompson[7] hydrolyzed Loeffler Blood Serum with sodium hydroxide and added it to a citrate agar for the isolation of *C. diphtheriae*. On Thompson's[7] medium, growth of diphtheria bacilli was stimulated while other respiratory flora were inhibited.

Principles of the Procedure

Beef Blood Serum provides the nitrogen, vitamins and amino acids necessary to support the growth of corynebacteria in Loeffler Blood Serum. Dextrose Broth is a source of fermentable carbohydrate and maintains the osmotic equilibrium of the medium.

Formula

Loeffler Blood Serum

Formula Per Liter

Beef Blood Serum	3 parts
Dextrose Broth	1 part

Final pH 7.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8%C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Medium beige, homogeneous, free-flowing.
8% Solution:	Soluble in distilled or deionized water warmed to 42-45°C.
Prepared Medium:	Coagulated in tubes - White to cream colored, slightly transparent at apex of slant.
Reaction of 8.0% Solution:	pH 7.1 ± 0.2 at 25°C

Cultural Response

Inoculate tubes with 30-300 CFU of test organism. Incubate 18-24 hours at 35 ± 2°C. Prepare slides from the growth, heat-fix, and stain with Methylene Blue, Loeffler. Stained cells will contain bipolar granules, club cells and some cells with general granulation.

ORGANISM	ATCC®	GROWTH
Corynebacterium diphtheriae type *mitis*	8024	good
Corynebacterium diphtheriae type *intermedius*	8032	good
Corynebacterium diphtheriae type *gravis*	8028	good

The cultures listed are the minimum that should be used for performance testing.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Loeffler Blood Serum

Materials Required But Not Provided

Glassware
Autoclave
Incubator

Method of Preparation

1. Suspend 80 grams in 1 liter distilled or deionized water warmed to 42-45°C. Check pH. Adjust to pH 7.1, if necessary.
2. Dispense into tubes having screw caps or other tightly sealing closures. Slant the tubes in the autoclave. Close the door loosely.
3. Coagulate the medium in constantly flowing steam for 10 minutes. Close the door tightly.
4. Autoclave at 121°C for 15 minutes.
5. Allow autoclave pressure to fall to zero before removing tubes.

Specimen Collection and Preparation

Both throat and nasopharyngeal specimens are necessary in cases of respiratory illness. If cutaneous diphtheria is suspected, collect skin, throat and nasopharynx specimens. Sterile silica gel is recommended for shipping clinical specimens when cultures are not obtained on site.[1]

Test Procedure[1]

1. If the swab appears desiccated, was collected several days prior to receipt, or is received in silica gel, place it into Todd-Hewitt Broth supplemented with 3% sterile rabbit blood. Incubate the culture overnight, then inoculate onto isolation media.
2. Inoculate the specimen onto cystine tellurite blood agar and blood agar plates, and streak for isolation on a Loeffler Blood Serum slant, leaving the swab on the slant during incubation.
3. Incubate aerobically at 35°C.
4. After 2-4 hours, prepare and heat fix a smear from the Loeffler Blood Serum slant. Flood the slide with Methylene Blue, Loeffler for 1 minute, rinse with tap water, and blot dry. Examine the smear for morphology typical of *C. diphtheriae*.
5. After 18-24 hours incubation, subculture onto a second plate of cystine tellurite blood agar.

Results

Examine all plates at 24-48 hours for colonies typical of *C. diphtheriae.* Subculture colonies that are catalase positive and exhibit typical morphology onto blood agar to provide growth for identification procedures.

Definitive identification of a *C. diphtheriae* isolate as a true pathogen requires demonstration of toxin production.[8]

For a complete discussion on the collection, isolation and identification of *Corynebacterium diptheriae* and other *Corynebacterium* species, refer to the appropriate procedures.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Loeffler Blood Serum must be used in parallel with blood agar and a tellurite- containing medium (cystine tellurite agar or modified Tinsdale medium) for selection and differentiation of *Corynebacterium*.[2]
3. Metachromatic granules that take up methylene blue are characteristic of *Corynebacterium*; however, other microorganisms may also display stained granules (e.g., *Propionibacterium*, some *Actinomyces*, and pleomorphic streptococci strains) and resemble corynebacteria. Additional culture, biochemical identification, and toxigenicity tests must be performed for differentiation and identification.[4]

References

1. **Isenberg, H. D. (ed.)** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
2. **Clarridge, J. E., and C. A. Spiegel.** 1995. *Corynebacterium* and miscellaneous irregular gram-positive rods, *Erysipelothrix*, and *Gardnerella*, p. 357-377. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover and R.H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Loeffler, F.** 1887. Darauf theilte HeuLoeffer en einem Zweiten Vortrag die ergebnisse seiner weiteren untersuchungen uber die Diphtherie-Bacillen mit. Zentralbl. Bacteriol. **2**:105.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 448-451, Williams & Wilkins, Baltimore, MD.
5. **Cleveland, L. R., and E. P. Sanders**. 1930. Encystation, multiple fission without encystment, metacystic development, and variation in a pure line and nine strains of *Entamoeba histolytica*. Arch. Protistenkd. **70**:223.
6. **Spector, B. K.** 1932. A comparative study of cultural and immunological methods of diagnosing infections with *Entamoeba histolytica*. J. Prevent. Med. **6**:117.
7. **Thompson**. 1929. J. Infect. Dis. **45**:163.
8. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.

Packaging

Loeffler Blood Serum	500 g	0070-17*

*Store at 2-8°C

Bacto® Lowenstein Medium Base · Bacto Lowenstein Medium, Gruft · Bacto Lowenstein Medium, Jensen · Bacto Lowenstein Medium, Jensen Deeps · Bacto Lowenstein Medium w/5% NaCl

User Quality Control

Identity Specification

Lowenstein Medium Base

Dehydrated Appearance:	Medium to dark green-blue, free flowing, homogenous.
Solution:	6.2% solution containing 2% glycerol, soluble in distilled or deionized water on boiling. Dark blue-green, opalescent, viscous.
Prepared Medium (as Lowenstein Medium, Jensen):	Pale green, smooth slant, opaque.

Lowenstein Medium, Gruft Tubes; Lowenstein Medium, Jensen Tubes; Lowenstein Medium w/5% NaCl

Appearance:	Pale green, opaque, smooth slants.
Reaction of Medium at 25°C:	pH 6.8-7.4

Lowenstein Medium, Jensen Deeps

Appearance:	Pale green, opaque butts.
Reaction of Medium at 25°C:	pH 7.2 ± 0.2

continued on following page

Intended Use

Bacto Lowenstein Media are prepared with fresh egg and glycerol to isolate, cultivate and differentiate mycobacteria.

Lowenstein Medium, Jensen Deeps are used for determining the catalase activity of mycobacteria.

Lowenstein Medium w/5% NaCl is used for differentiating mycobacteria on the basis of NaCl tolerance.

Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.[1] In 1985, the number of tuberculosis cases (TB) in the United States began increasing. Prior to this time, the number of US cases had been decreasing, reaching a low in 1984.[2] Non-tuberculous mycobacteria infections have also increased since 1985.[3]

The use of egg-based media for primary isolation of mycobacteria have the following significant advantages:

1. Egg-based media support a wide variety of mycobacteria.
2. The growth of mycobacteria on egg media can be used for niacin testing.

A disadvantage of egg-based media is that contaminating proteolytic organisms tend to liquefy the medium.[3]

Principles of the Procedure

Lowenstein formulations are egg-based media that contain a moderate amount of malachite green to suppress the growth of contaminating organisms. These media are commonly used in the clinical laboratory to isolate acid fast organisms from sterile and nonsterile sources.[4]

Bacto Lowenstein Medium, Jensen (LJ) is a modification of the Jensen formulation for Lowenstein Medium.[5] It contains salts and a moderate concentration of malachite green to prevent the growth of most contaminants and to allow early growth of mycobacteria.

Lowenstein Medium, Gruft is the Gruft modification of Lowenstein Medium, Jensen.[6] Ribonucleic acid is incorporated into the medium to increase the isolation of mycobacteria. Penicillin and Nalidixic Acid are added to decrease contamination.

The increased sodium chloride concentration in Lowenstein Medium, Jensen w/5% NaCl helps to differentiate rapid-growing mycobacteria from slow growers, which are inhibited in the presence of salt. Glycerol is added as a carbon source.

Formula

Lowenstein Medium Base

Formula Per Liter		
Bacto Asparagine	3.6	g
Monopotassium Phosphate	2.4	g
Magnesium Sulfate	0.24	g
Magnesium Citrate	0.6	g
Potato Flour	30	g
Malachite Green	0.4	g

Lowenstein Medium, Jensen

Formula Per Liter		
Bacto Asparagine	3.6	g
Monopotassium Phosphate	2.4	g
Magnesium Sulfate	0.24	g
Magnesium Citrate	0.6	g
Potato Flour	30	g
Malachite Green	0.4	g
Bacto Glycerol	12	ml
Distilled/Deionized Water	588	ml
Homogenized Egg	1,000	ml

Lowenstein Medium, Jensen Deeps (per 1600 ml)

Formula Per Liter		
Bacto Lowenstein Medium Base	37.2	g
Bacto Glycerol	12	ml
Distilled/Deionized Water	588	ml
Homogenized Egg	1,000	ml

Lowenstein Medium Gruft (per 1600 ml)

Formula Per Liter		
Bacto Lowenstein Medium Base	37.2	g
Bacto Glycerol	12	ml
Distilled/Deionized Water	588	ml
Homogenized Egg	1,000	ml
Penicillin	80,000	units
Nalidixic Acid	56	mg
Ribonucleic Acid	80	µg

Lowenstein Medium w/5% NaCl (per 1600 ml)

Formula Per Liter		
Bacto Lowenstein Medium Base	37.2	g
Bacto Glycerol	12	ml
Distilled/Deionized Water	588	ml
Sodium Chloride	80	g
Homogenized Egg	1,000	ml

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store Lowenstein Medium Base below 30°C.

Store prepared tubed media at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Lowenstein Medium Base
Lowenstein Medium, Gruft
Lowenstein Medium, Jensen
Lowenstein Medium, Jensen Deeps
Lowenstein Medium w/5% NaCl

Materials Required But Not Provided

Flasks
Distilled or deionized water
Screw capped tubes
Specimen digestant and decontaminant
Centrifuge
Inoculating Needles
Incubator
Fresh egg
Autoclave
Inspissator (optional)
Water bath (optional)
Glycerol
Tween-Hydrogen Peroxide 1:1

Method of Preparation

Lowenstein Medium Base

1. Suspend 37.2 grams of Lowenstein Medium Base in 600 ml distilled or deionized water containing 12 ml of Glycerol and boil with constant agitation.
2. Autoclave at 121°C for 15 minutes. Cool to 45-60°C.
3. Aseptically add the sterile base to 1 liter of a uniform suspension of fresh eggs prepared under aseptic conditions. Swirl gently to avoid introducing air into the suspension.
4. Dispense into sterile screw cap tubes or bottles. Arrange in a slanted position.
5. Place in an inspissator, water bath or autoclave at 85°C for 45 minutes to coagulate the medium.

Prepared Lowenstein Media

Prepared media are ready to use.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Observe for colonies that may or may not be pigmented. Colony morphology depends on the species isolated.

Limitations of the Procedure

Negative culture results do not rule out an active mycobacterial infection. Some factors responsible for unsuccessful cultures are:

1. The specimen was not representative of the infectious material, i.e., saliva instead of sputum.
2. The mycobacteria were destroyed during digestion and decontamination of the specimen.
3. Gross contamination interfered with the growth of mycobacteria.
4. Proper aerobic and increased CO_2 tension were not provided during incubation.

References

1. **Musser, J. M.** 1995. Antimicrobial resistance in Mycobacteria: molecular genetic insights. Clinical Microbiology Reviews **8**:496-514.
2. **Kleitmann, W.** 1995. Resistance and susceptibility testing for *Mycobacterium tuberculosis*. Clinical Microbiology Newsletter **17**:65-69.
3. **Nolte, F. S., and B. Methcock**. 1995. *Mycobacterium*, p. 400-437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
5. **Enter. Bacteriol. Parasitnek.** 1932. Abt. 1, **125**:222.
6. **J. Bact**. 1965. **90**:829.

Packaging

Lowenstein Medium Base	500 g	0444-17
	2 kg	0444-07
Lowenstein Medium, Gruft	20 tubes	1417-39
	100 ubes	1417-79
Lowenstein Medium, Jensen	20 tubes	1017-39
	100 tubes	1017-79
	100 x 1 oz	1017-57
Lowenstein Medium w/5% NaCl	20 tubes	1423-39
Lowenstein Medium, Jensen Deeps	20 tubes	1289-76

Cultural Response

Lowenstein Medium Base; Lowenstein Medium, Gruft Tubes; Lowenstein Medium, Jensen Tubes

Prepare Lowenstein Medium Base (as Lowenstein Medium, Jensen) per label directions or use prepared tubes. Inoculate and incubate at 35°C under CO_2 for up to three weeks.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Escherichia coli†	25922*	1,000-2,000	partial inhibition
Mycobacterium fortuitum	6841	100-300	good
Mycobacterium intracellulare	13950	100-300	good
Mycobacterium kansasii	12478	100-300	good
Mycobacterium scrofulaceum	19981	100-300	good
Mycobacterium tuberculosis H37Ra	25177	100-300	good

†Tested on Lowenstein Medium, Gruft, only

Lowenstein Medium w/5% NaCl

Inoculate and incubate at 35°C under CO_2 for up to three weeks.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Mycobacterium smegmatis	14468	100-300	good
Mycobacterium tuberculosis	25177	100-300	inhibited

Lowenstein Medium, Jensen Deeps

Inoculate and incubate at 35°C under CO_2 for 2 weeks. Cap loosely for 7 days, then tighten cap and incubate 1 week longer. Add 1 ml of Tween-Hydrogen Peroxide reagent to the 2-week culture. Measure the height of the column of bubbles after 5 minutes. (Tween-Hydrogen Peroxide reagent is a 1:1 final solution of 30% hydrogen peroxide in distilled water and sterile, cooled 10% Tween® 80 in distilled water.)

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Mycobacterium gordonae	14470	100-300	greater than 45 mm
Mycobacterium tuberculosis	25177	100-300	less than 45 mm

Uninoculated tube

Mycobacterium fortuitum ATCC® 6841

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Bacto® Luria Agar Base, Miller

Intended Use

Bacto Luria Agar Base, Miller is used for maintaining and propagating *Escherichia coli* in molecular microbiology procedures with or without added glucose.

Summary and Explanation

Luria Agar Base, Miller, a nutritionally rich medium designed for growth of pure cultures of recombinant strains, is based on the Luria Broth Agar formula described by Miller.[1] *E. coli* is grown to late log phase in LB Medium. Some plasmid vectors replicate to a high copy number and do not require selective amplification. Some vectors do not replicate so freely and need to be selectively amplified. Chloramphenicol may be added to inhibit host synthesis and, as a result, prevent replication of the bacterial chromosome.[2]

Luria Agar Base, Miller contains one tenth and one twentieth, respectively, the sodium chloride level of the LB Agar, Lennox and LB Agar, Miller formulations.[1-3] This allows the researcher to select the optimal salt concentration for a specific strain. The medium may be aseptically supplemented with glucose, if desired.

Principles of the Procedure

Peptides and peptones are provided by Tryptone. Vitamins (including B vitamins) and certain trace elements are provided by Yeast Extract. Sodium ions for transport and osmotic balance are provided by Sodium Chloride. Bacto Agar is the solidifying agent.

Formula

Luria Agar Base, Miller

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Sodium Chloride	0.5 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free flowing, homogeneous.
Solution:	3.05% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Medium:	Very light amber, slightly opalescent.
Reaction of 3.05% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Luria Agar Base, Miller with 10 ml sterile 20% glucose solution per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	33526	100-300	Good

The culture listed is the minimum that should be used for performance testing.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Luria Agar Base, Miller

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50°C)
Petri dishes
Incubator (35°C)
20% glucose solution (optional)

Method of Preparation

1. Suspend 30.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C in a waterbath.
4. If desired, aseptically add 10 ml sterile 20% glucose solution and mix thoroughly.
5. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references for recommended test procedures.[1,2]

Results

Growth is evident in the form of isolated colonies and/or a confluent lawn on the surface of the medium.

References

1. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
3. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology. **1:**190-206.

Packaging

Luria Agar Base, Miller	500 g	0413-17
	2 kg	0413-0

Bacto® Luria Broth Base, Miller

Intended Use

Bacto Luria Broth Base, Miller is used for maintaining and propagating *Escherichia coli* in molecular microbiology procedures with or without added glucose.

Summary and Explanation

Luria Broth Base, Miller is based on the Luria Broth formula described by Miller for the growth and maintenance of *Escherichia coli* strains used in molecular microbiology procedures.[1]

Luria Broth Base, Miller is a nutritionally rich medium designed for growth of pure cultures of recombinant strains. *Escherichia coli* is grown to late log phase in LB Medium. Some plasmid vectors may replicate to high copy numbers without selective amplification. Some vectors do not replicate so freely, and need to be selectively amplified. Chloramphenicol can be added to inhibit host synthesis and as a result, prevent replication of the bacterial chromosome.[2]

Luria Broth Base, Miller contains one tenth and one twentieth the sodium chloride level of the Lennox and Miller formulations of LB Agar, respectively.[1,2,3] This allows the researcher to select the optimal salt concentration for a specific strain. The medium may be aseptically supplemented with glucose, if desired.

Principles of the Procedure

Peptides and peptones are provided by Bacto Tryptone. Vitamins (including B vitamins) and certain trace elements are provided by Bacto Yeast Extract. Sodium ions for transport and osmotic balance are provided by sodium chloride.

Formula

Luria Broth Base, Miller

Formula Per Liter

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Sodium Chloride	0.5 g

Final pH 7.0 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	1.55% solution, soluble in distilled or deionized water. Solution is very light to light amber, clear to very slightly opalescent.
Prepared Tubes:	Very light to light amber, clear to very slightly opalescent.
Reaction of 1.55% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Luria Broth Base, Miller per label directions with 1 ml of 20% dextrose solution added to 100 ml of media. Inoculate the tubes and incubate at 35 ± 2°C for 18- 24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	33526	100-1,000	Good

The culture listed is the minimum that should be used for performance testing.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Luria Broth Base, Miller

Materials Required But Not Provided

Flasks with closures
Tubes with closures
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Dissolve 15.5 grams in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Cool to 45-50°C. If desired, aseptically add 10 ml sterile 20% glucose solution and mix thoroughly.

Specimen Collection and Preparation

Not applicable.

Test Procedure

Consult appropriate references for recommended test procedures.[1,2]

Results

Growth is evident by the appearance of turbidity in the medium.

References

1. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
3. **Lennox, E. S.** 1955. Transduction of linked genetic characters of the host by bacteriophage P1. Virology. **1:**190-206.

Packaging

Luria Broth Base, Miller	500 g	0414-17
	2 kg	0414-07

Bacto® Lysine Iron Agar

Intended Use

Bacto Lysine Iron Agar is used for differentiating microorganisms, especially *Salmonella*, based on lysine decarboxylation/deamination and H_2S production.

Summary and Explanation

Lysine Iron Agar is prepared according to the formulation of Edwards and Fife[1], who developed the medium to detect *Salmonella arizonae* (formerly *Arizona arizonae*). Because *S. arizonae* ferments lactose so rapidly, the authors found that the expected H_2S production on triple sugar iron agar was suppressed. Since *S. arizonae* strains are found occasionally in outbreaks of food borne infection, it is important to be able to detect them. By eliminating lactose and incorporating lysine, Edwards and Fife devised a medium that differentiates enteric bacilli based on their ability to decarboxylate or deaminate lysine and produce abundant hydrogen sulfide. The medium is especially recommended for detecting rapid lactose-fermenting *S. arizonae*. It is specified in Standard Methods for *Salmonella* testing.[2,3,4,5,6]

Principles of the Procedure

Lysine Iron Agar contains Bacto Peptone which provides carbon and nitrogen sources required for good growth of a wide variety of organisms. Yeast Extract provides vitamins and cofactors required for growth, as well as additional sources of nitrogen and carbon. Dextrose is an energy source. L-Lysine Hydrochloride is the substrate used to detect the lysine decarboxylase and lysine deaminase enzymes. Ferric Ammonium Citrate and Sodium Thiosulfate are indicators of hydrogen sulfide production. Brom Cresol Purple, a pH indicator, is yellow at or below pH 5.2 and purple at or above pH 6.8. Bacto Agar is a solidifying agent.

Formula

Lysine Iron Agar

Formula Per Liter

Bacto Peptone	5 g
Bacto Yeast Extract	3 g
Bacto Dextrose	1 g
L-Lysine Hydrochloride	10 g
Ferric Ammonium Citrate	0.5 g
Sodium Thiosulfate	0.04 g
Bacto Brom Cresol Purple	0.02 g
Bacto Agar	15 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.45% solution, soluble in distilled or deionized water upon boiling. Solution is reddish-purple, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Purple, slightly opalescent without precipitate.
Reaction of 3.45% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare Lysine Iron Agar per label directions. Inoculate with undiluted cultures and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH	LYSINE DECARBOXYLATION (BUTT)	LYSINE DEAMINATION (SLANT)	H_2S (APEX OF SLANT)
Proteus mirabilis	25933	good	– yellow	+ red	–
Salmonella arizonae	13314	good	+ purple	– purple	+ black
Salmonella typhimurium	14028*	good	+ purple	– purple	+ black

The cultures listed are the minimum that should be used for performance testing.

*Available as Bactrol™ Disks; use as directed in Bactrol Disks Technical Information.

Uninoculated tube | *Proteus mirabilis* ATCC® 25933 | *Salmonella typhimurium* ATCC® 14028

Procedure

Materials Provided

Lysine Iron Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Inoculating needle

Method of Preparation

1. Suspend 34.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes. Autoclave at 121°C for 12 minutes.
4. Allow medium to cool in a position that will provide a short slant and a deep butt.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Using a straight needle, pick the center of a well-isolated colony from a fresh, pure culture.
2. Inoculate by stabbing to the base of the butt and streaking the slant.
3. Cap the tube loosely to ensure aerobic conditions.
4. Incubate at 35°C for 18-48 hours.
5. Examine at 18-24 and 40-48 hours for growth and color changes in the butt and the slant of the medium and for blackening at the apex of the slant.

Results

Lysine decarboxylase reaction:
Positive: Purple (alkaline) butt, purple slant.
Negative: Yellow (acid) butt, purple (alkaline) slant.

Lysine deaminase reaction:*
Positive: Red slant.
Negative: Purple slant.

Hydrogen sulfide reaction:
Positive: Blackened medium at the apex of the slant.

* *Proteus* and *Providencia* cultures produce a red slant over a yellow (acid) butt.

Limitations of the Procedure

1. *Salmonella paratyphi A*, unlike other *Salmonella*, does not produce lysine decarboxylase and so produces an alkaline slant and an acid butt.
2. H_2S-producing *Proteus* species do not blacken the medium.[2,7] It is, therefore, suggested that Lysine Iron Agar be used in conjunction with Triple Sugar Agar or other media to confirm differentiation.
3. The reaction of *Morganella morganii* may be variable after 24 hours incubation and may require longer incubation.[7]

References

1. **Edwards, P. R., and M. A. Fife.** 1961. Lysine-iron agar in the detection of *Arizona* cultures. Appl. Microbiol. **9**:478.
2. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
3. **Russell, S. F., J. -Y. D'Aoust, W. H. Andrews and J. S. Bailey.** 1992. *Salmonella*, p. 371-422. *In* C. Vanderzant, and D. F. Splittstoesser (eds.). Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
4. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1992. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall, (ed.), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, Supplement March 1996. AOAC International, Arlington, VA.
6. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*, p. 5.01-5.20. *In* Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, MD.
7. **Finegold, S. M., and W. J. Martin.** 1982. Bailey and Scott's diagnostic microbiology, 6th ed., p. 631. The CV Mosby Company, St. Louis, MO.

Packaging

Lysine Iron Agar	100 g	0849-15
	500 g	0849-17

Bacto® Lysine Medium

Intended Use

Bacto Lysine Medium is used for isolating and enumerating "wild" yeast contaminants in brewery pitching yeasts.

Summary and Explanation

Walters and Thiselton[1] formulated a liquid synthetic medium containing lysine to study brewery yeasts. The medium separated yeasts into two groups based on their ability to grow using L-lysine as a sole source of nitrogen. Strains of brewery culture yeasts, *Saccharomyces cerevisiae* and *S. carlsbergensis*, grew poorly or not at all in the medium (lysine negative) while yeasts known as "wild" contaminants, including *S. pastorianus* and *S. cerevisiae* var. *turbidans*, grew (lysine positive).

Morris and Eddy[2] modified the formula by adding agar and confirmed the work of Walters and Thiselton using quantitative results. They showed a low level of infection of a pitching yeast with wild yeasts could be determined. They also recommended the addition of antibiotics to the medium for samples heavily contaminated with bacteria.

Principles of the Procedure

Lysine Medium contains Dextrose as the carbohydrate. L-Lysine is the nitrogen source. Essential vitamins, salts, amino acids and other nutrients are present in defined amounts. Bacto Agar is the solidifying agent.

Formula

Lysine Medium

Formula Per Liter

Bacto Dextrose	44.5 g
Potassium Dihydrogen Phosphate	1.78 g
Magnesium Sulfate	0.89 g
Calcium Chloride	0.178 g
Sodium Chloride	0.089 g
Adenine	0.00178 g
DL-Methionine	0.000891 g
L-Histidine	0.000891 g
DL-Tryptophan	0.000891 g
Boric Acid	0.0000089 g
Zinc Sulphate	0.0000356 g
Ammonium Molybdate	0.0000178 g
Manganese Sulphate	0.0000356 g
Ferrous Sulphate	0.0002225 g
Inositol	0.02 g
Calcium Pantothenate	0.002 g
Aneurine	0.0004 g
Pyridoxine	0.0004 g
p-Aminobenzoic Acid	0.0002 g
Nicotinic Acid	0.0004 g
Riboflavin	0.0002 g
Biotin	0.000002 g
Folic Acid	0.000001 g
L-Lysine	1 g
Bacto Agar	17.5 g

Final pH 4.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.6% solution containing 1 ml 50% potassium lactate solution per 100 ml medium, soluble in distilled or deionized water on gentle boiling; very light amber, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Very light amber, slightly opalescent without significant precipitate.
Final reaction of pH-adjusted 6.6% solution at 25°C:	pH 4.8 ± 0.2

Cultural Response

Prepare Lysine Medium per label directions. Inoculate and incubate at 25 ± 2°C for 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Pichia fermentans	10651	100-1,000	good
Saccharomyces pastorianus	2700	100-1,000	none to fair

The cultures listed are the minimum that should be used for performance testing.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Lysine Medium

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (25°C)
10% Lactic acid solution
50% Potassium lactate solution

Method of Preparation

1. Suspend 6.6 grams in 100 ml distilled or deionized water containing 1 ml 50% potassium lactate solution.
2. Boil gently to dissolve completely.
3. Cool to 50°C.
4. Adjust to final pH using 10% lactic acid, if necessary.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Wash and centrifuge the sample of pitching yeast three times with distilled water.
2. Resuspend the pellet in distilled water to contain approximately 10^7 cells per ml.
3. Spread 0.2 ml of the cell suspension over the surface of the prepared medium.
4. Incubate plates at 25°C. Examine daily for growth.

Results

Count the number of colonies that develop and express the degree of contamination as the number of wild cells per million cells of inoculum.

Limitations of the Procedure

1. Use of a test inoculum having less than 10^4 cells may permit growth of brewing yeast cells that can be confused with growth of wild yeasts. Use of an inoculum that exceeds 10^4 cells will restrict growth of the unwanted brewing yeasts to tiny microcolonies.[2]

References

1. **Walters, L. S., and M. R. Thiselton**. 1953. J. Inst. Brew. **59**:401-404.
2. **Morris, E. O., and A. A. Eddy**. 1957. J. Inst. Brew. **63**:34-35.

Packaging

Lysine Medium	500 g	1894-17

Bacto® M9CA Medium

Intended Use

Bacto M9CA Medium is used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

M9CA is based on M9 Minimal Salts[1] but with the addition of casamino acids. The medium may be supplemented with an appropriate carbon and energy source, such as dextrose. The Casamino Acids provide nitrogen in a readily available form. The medium will support the growth of "wild-type" and recombinant strains of *E. coli*. M9CA contains salts that supply nitrogen, phosphorus, and trace minerals.

Principles of the Procedure

Casamino Acids make this a richer medium than M9 Minimal Salts, providing all of the amino acids except tryptophan. Ammonium Chloride provides a source of nitrogen. Sodium and Potassium Phosphates buffer against pH changes due to carbohydrate metabolism. Dextrose, aseptically added to the medium, is a carbon and energy source. Magnesium Sulfate is a source of magnesium ions required in a variety of enzymatic reactions, including DNA replication.

Formula

M9CA Medium

Formula Per Liter

Bacto Casamino Acids	4 g
Sodium Phosphate, Dibasic, Anhydrous	6.8 g
Potassium Phosphate, Monobasic	3 g
Sodium Chloride	0.5 g
Ammonium Chloride	1 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1.53% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear, no significant precipitate.
Reaction of 1.53% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare M9CA Medium per label directions. Inoculate and incubate at 35°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli (B)	23226	100-300	Good

The culture listed is the minimum that should be used for performance testing.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

M9CA Medium

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Autoclave
Sterile 20% glucose solution
Sterile 1.0 M $MgSO_4$ solution
Incubator (35°C)

Method of Preparation

1. Dissolve 15.3 grams in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. After cooling to below 50°C, aseptically add 20 ml of filter-sterilized 20% glucose solution and 2 ml of filter-sterilized 1M Magnesium Sulfate solution. Mix well.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references for recommended test procedures.

Results

Growth is evident in the form of turbidity.

References

1. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Packaging

M9CA Medium	500 g	0454-17
Dextrose	500 g	0155-17

Bacto® M9 Minimal Salts, 5x

Intended Use

Bacto M9 Minimal Salts, 5x is used in preparing M9 Minimal Medium which is used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

M9 Minimal Salts, 5x is a 5x concentrate that is diluted to a 1x concentration and supplemented with an appropriate carbon and energy source, such as dextrose, to provide a minimal, chemically defined medium. The medium will support the growth of "wild-type" strains of *E. coli*. M9 Minimal Salts is useful for maintaining positive selection pressure on plasmids coding for the ability to produce essential substances such as amino acids or vitamins. M9 Minimal Medium is also used to maintain stocks of F´ containing bacteria for use with M13. The medium can be supplemented with specific amino acids or other metabolites, allowing for selection of specific auxotrophs.

Principles of the Procedure

Sodium Phosphate and Potassium Phosphate are present as buffering agents. Ammonium Chloride is a source of nitrogen for cellular systems. Sodium Chloride maintains isotonicity in the final medium. Glucose may be added as a source of carbohydrate. Supplementing the medium with magnesium and calcium increases the growth of recombinants.

Formula

M9 Minimal Salts, 5x

Formula Per Liter

Sodium Phosphate, Dibasic, Anhydrous	33.9 g
Potassium Phosphate, Monobasic	15 g
Sodium Chloride	2.5 g
Ammonium Chloride	5 g

Final pH 6.8 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	5.64% solution, soluble in distilled or deionized water. Solution is colorless, clear.
Reaction of 5.64% Solution (5x concentrate) at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare M9 Minimal Salts, 5x and dilute to 1x. Supplement with glucose per label directions. Inoculate and incubate at 35°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	23226	30-300	Good
Escherichia coli	39403	30-300	Good

The cultures listed are the minimum that should be used for performance testing.

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

M9 Minimal Salts, 5x

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Autoclave
Sterile 20% glucose solution
Sterile 1.0 M $MgSO_4$ solution
Sterile 1.0 M $CaCl_2$ solution (optional)
Incubator (35°C)

Method of Preparation

1. Dissolve 56.4 grams in 1 liter of distilled or deionized water. It is recommended that this liter be separated into 200 ml aliquots.
2. Autoclave at 121°C for 15 minutes.
3. To prepare M9 Minimal Medium, add 200 ml sterile M9 Minimal Salts, 5x to 750 ml sterile distilled or deionized water, which has been cooled to 45-50°C. Adjust final volume to 1 liter.
4. Aseptically add 20 ml filter-sterilized 20% glucose solution, 2 ml sterile 1.0 M magnesium sulfate ($MgSO_4$) solution and, if desired, 0.1 ml sterile 1.0 M calcium chloride ($CaCl_2$) solution. Mix well.
5. If desired, supplement with amino acids, as appropriate.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references for recommended test procedures.[1-2]

Results

Growth should be evident by the appearance of turbidity.

References

1. **Davis, L. G., M. D. Dibner, and J. F. Battey.** 1986. Basic methods in molecular biology. Elsevier, New York, N.Y.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Packaging

M9 Minimal Salts, 5x	500 g	0485-17

Bacto® M17 Broth
Bacto M17 Agar

User Quality Control

Identity Specifications

M17 Broth

Dehydrated Appearance:	Beige to medium tan, free-flowing, homogeneous.
Solution:	3.725% solution, soluble in distilled or deionized water. Solution is light-medium to medium amber, clear to very slightly opalescent.
Prepared Medium:	Light medium to medium amber, clear to very slightly opalescent, no significant precipitate.
Reaction 3.725% Solution at 25°C:	pH 6.9 ± 0.2

M17 Agar

Dehydrated Appearance:	Beige to medium tan, free-flowing, homogeneous.
Solution:	4.825% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly to slightly opalescent, no significant precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent, no significant precipitate.
Reaction of 4.825% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Prepare Nutrient Gelatin per label directions. Using a heavy inoculum, inoculate by stabbing the tube and incubate at 35 ± 2°C for 18-48 hours or up to two weeks, if required. To read gelatinase, refrigerate until well chilled and compare to uninoculated tube. Tilt tubes carefully to test for liquefaction. Tubes positive for gelatinase remain liquid.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus delbrueckii subsp. *bulgaricus*	11842	100-1,000	none to poor
Lactococcus lactis subsp. *cremoris*	9625	100-1,000	good
Streptococcus thermophilus	19258	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Intended Use

Bacto M17 Broth is used for isolating and enumerating lactic streptococci from yogurt, cheese starters and other dairy products.

Bacto M17 Agar is used for enumerating lactic streptococci in yogurt, cheese starters and other dairy products.

Summary and Explanation

Lactic streptococci are acid-producing bacteria. They are nutritionally fastidious and require complex culture media for optimum growth. One study showed that in a synthetic medium, all strains had an obligate requirement for at least six amino acids and three vitamins.[1] These homofermentative lactic streptococci produce large amounts of acid and, in a culture medium without an adequate buffering system, the pH decreases and adversely affects growth. Lowrie and Pearce[2] developed M16 Medium but it lacked a strong buffering system. Terzaghi and Sandine[3] worked with M16 Medium and demonstrated that the rapid drop in pH that accompanies lactic streptococcal growth can adversely affect colony size and phage plaque formation. They modified M16 Medium using disodium-ß-glycerophosphate as a buffer and called it M17.

Shankar and Davies[4] found that disodium-ß-glycerophosphate in M17 Broth suppressed *Lactobacillus bulgaricus* and selectively isolated *Streptococcus thermophilus* from yogurt. Similar results were achieved using M17 Broth solidified with agar. The International Dairy Federation recommends M17 Agar for isolating *S. thermophilus* from yogurt.[5] M17 Agar is a standard methods medium for isolating lactic streptococci.[6]

Principles of the Procedure

M17 Broth and M17 Agar contain Tryptone, Soytone, and Meat Digest as sources of carbon, nitrogen, vitamins and minerals. Yeast Digest supplies B-complex vitamins which stimulate bacterial growth. Disodium-ß-Glycerophosphate buffers the medium as acid is produced from fermentation of lactose. Ascorbic Acid stimulates growth of lactic streptococci. Magnesium Sulfate provides essential ions for growth. Bacto Agar is the solidifying agent in M17 Agar.

Formula

M17 Broth

Formula Per Liter

Bacto Tryptone	5 g
Bacto Soytone	5 g
Meat Digest	5 g
Yeast Digest	2.5 g
Ascorbic Acid	0.5 g
Magnesium Sulfate	0.25 g
Disodium-ß-glycerophosphate	19 g

Final pH 6.9 ± 0.2 at 25°C

M17 Agar

Formula Per Liter

Bacto Tryptone	5 g
Bacto Soytone	5 g
Meat Digest	5 g
Bacto Yeast Extract	2.5 g
Ascorbic Acid	0.5 g
Magnesium Sulfate	0.25 g
Disodium-ß-glycerophosphate	19 g
Bacto Agar	11 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

M17 Broth or
M17 Agar

Materials Required but not Provided

Glassware
Petri Dishes (for M17 Agar)
Distilled or deionized water
Autoclave
Incubators (30°C and 35°C)

Method of Preparation

1. M17 Broth: Dissolve 37.25 grams in 950 ml distilled or deionized water.
   M17 Agar: Suspend 48.25 grams in 950 ml distilled or deionized water and boil to dissolve completely.
2. Autoclave at 121°C for 15 minutes.
3. Cool to 50°C.
4. Add 50 ml sterile 10% lactose solution. Mix well.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Reiter, B., and J. D. Oram**. 1962. Nutritional studies on cheese starters. I. vitamin and amino acid requirements of single strain starters. J. Dairy Res. **29**:63-77.
2. **Lowrie and Pearce**. 1971. J. Dairy Sci. Technol. **6**:166.
3. **Terzaghi, B. E., and W. E. Sandine**. 1975. Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. **29**:807-813.
4. **Shankar, P. A., and F. L. Davies**. 1977. A note on the suppression of *Lactobacillus bulgaricus* in media containing ß-glycerophosphate and application of such media to selective isolation of *Streptococcus thermophilus* from yogurt. J. Soc. Dairy Tech. **30**:28-30.
5. **International Dairy Federation**. 1981. Identification and enumeration of micro- organisms in fermented milks. Joint IDF/ISO/AOAC Group E44.
6. **Vedamuthu, E. R., M. Raccach, B. A. Glatz, E. W. Seitz, and M. S. Reddy.** 1992. Acid-producing microorganisms, p. 225-238. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

M17 Broth	500 g	1856-17
M17 Agar	500 g	1857-17

Bacto® M Broth

Intended Use

Bacto M Broth is used for cultivating *Salmonella* in foods and feeds by the accelerated enrichment serology (ES) procedure.

Summary and Explanation

M Broth, prepared according to the formula of Sperber and Diebel[1], contains all the nutrients necessary for good growth and flagella development of *Salmonella*.

Fantasia, Sperber and Deibel[2] compared the enrichment serology (ES)' procedure with the traditional procedure outlined in the Bacteriological Analytical Manual[3] (BAM) and reported excellent agreement between the two. They found the ES procedure not only to be faster and less complicated but also as accurate and sensitive as the BAM procedure.

M Broth also conforms to the testing standards recommended by Compendium of Methods for the Microbiological Examination of Foods[4] (APHA) for the isolation and identification of foodborne *Salmonella*.

Both monoclonal and polyclonal enzyme immunoassay (EIA) methods have been described in AOAC Official Methods of Analysis[5] using M Broth. These methods are screening procedures for the presence of *Salmonella* and positive results must be confirmed by culture.

Principles of the Procedure

Yeast Extract is a source of B-complex vitamins. Tryptone provides organic nitrogen. D- Mannose and Sodium Citrate are fermentation energy sources. Mannose prevents fimbrial agglutination.[1] Sodium Chloride helps maintain osmotic equilibrium, while Dipotassium Phosphate acts as a buffer. The inorganic salts stimulate bacterial growth. Tween® 80 is a surfactant and dispersing agent.

Formula

M Broth

Formula per liter

Bacto Yeast Extract	5 g
Bacto Tryptone	12.5 g
Bacto D-Mannose	2 g
Sodium Citrate	5 g
Sodium Chloride	5 g
Dipotassium Phosphate	5 g
Manganese Chloride	0.14 g
Magnesium Sulfate	0.8 g
Ferrous Sulfate	0.04 g
Tween® 80	0.75 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

M Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Lactose Broth
Selenite Cystine Broth
Tetrathionate Broth
Salmonella H Antisera Spicer-Edwards Set
Salmonella H Antiserum Poly D
Salmonella H Antiserum z_6
Normal saline
NaCl
Formalin
50°C waterbath

Method of Preparation

1. Suspend 36.2 grams in 1 liter distilled or deionized water.
2. Heat to boiling for 1-2 minutes, stirring carefully.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Prepare a 10% suspension of the test sample in Lactose Broth. Incubate at 35 ± 2°C for 18-24 hours.
2. Transfer 1 ml of the above preenrichment culture to 9 ml of Selenite Cystine Broth and 1 ml to 9 ml of Tetrathionate Broth. Incubate both enrichment media at 35 ± 2°C for 24 hours.

User Quality Control

Identity Specifications

Dehydrated appearance:	Beige, homogeneous with a tendency to lump.
Solution:	3.62% solution, soluble in distilled or deionized water on boiling for 1-2 minutes. Solution is light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 3.62% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare M Broth per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Salmonella choleraesuis	12011	100-1,000	good
Salmonella typhimurium	14028*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube — *Salmonella choleraesuis* ATCC® 12011

3. Inoculate one 10 ml tube of M Broth, tempered to 35°C, with one drop from each of the above cultures. Incubate at 35 ± 2°C for 6-8 hours.
4. Prepare a formalin-salt solution by adding 4.2 grams of NaCl and 3 ml of formalin to 100 ml of distilled water. Place one drop in each of two Kahn tubes.
5. Carefully insert a pipette about 1 inch below the surface of the M Broth culture and transfer 0.85 ml of culture to each of the above Kahn tubes containing formalin-salt solution.
6. Prepare a pooled antiserum by combining together 0.5 ml each of rehydrated Salmonella H Antiserum Poly D and Salmonella H Antiserum z_6 (Salmonella H Antisera Spicer-Edwards Set) in 11.5 ml of 0.85% NaCl.
7. Add 0.1 ml pooled Salmonella H Antiserum to one of the Kahn tubes (above). Add 0.1 ml 0.85% NaCl solution to the other tube. Shake the tubes gently. Incubate in a 50°C water bath for 1 1/2 hours.

Results

Agglutination in the Kahn tube containing antiserum indicates the presence of *Salmonella*. Agglutination in the Kahn tube containing 0.85% NaCl solution (control tube) indicates a rough culture which should be streaked for isolation, passed through Motility GI Medium to enhance flagella, and then retested with pooled antiserum.

Alternative Testing Procedures

Refer to AOAC International[5] for screening procedures using enzyme immunoassay or DNA hybridization to detect *Salmonella* antigen in test samples.

References

1. **Sperber, W. H. and R. H. Deibel.** 1969. Accelerated procedure for *Salmonella* detection in dried foods and feeds involving only broth cultures and serological reactions. Appl. Microbiol. **17:**533-539.
2. **Fantasia, L. D., W. H. Sperber, and R. H. Deibel.** 1969. Comparison of two procedures for detection of *Salmonella* in food, feed, and pharmaceutical products. Appl. Microbiol. **17:**540-541.
3. **Bacteriological Analytical Manual, 2nd ed.** 1969. US HEW, Washington, D.C.
4. **Flowers, R. S., J.- Y. D'Aoust, W. H. Andrews, and J. S. Bailey.** 1992. *Salmonella*, p. 371-422. *In* C. Vanderzant, and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

M Broth	500 g	0940-17
	2 kg	0940-07

Bacto® MIL Medium

Intended Use

Bacto MIL Medium is used for differentiating *Enterobacteriaceae* based on motility, lysine decarboxylation, lysine deamination and indole production.

Also Known As

MIL Medium conforms with Motility-Indole-Lysine Medium.

Summary and Explanation

MIL Medium, prepared according to the formula of Reller and Mirrett,[1] is a single culture medium that provides four differentiating biochemical reactions. When used in conjunction with Triple Sugar Iron Agar (TSI) and Urea Agar, as many as nine reactions are provided. This combination enables reliable initial identification of *Enterobacteriaceae*.[2,3] Extensive testing of 890 enteric cultures by Reller and Mirrett[1] gave essentially the same results with MIL Medium as with the standard motility, indole and lysine decarboxylase (Moeller) test media.

Principles of the Procedure

Bacto Peptone and Tryptone provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Yeast Extract provides vitamins and cofactors required for growth. Lysine Hydrochloride is present as a substrate to detect lysine decarboxylase or lysine deaminase activity. Dextrose is an energy source. Ferric Ammonium Citrate is an H_2S indicator. Brom Cresol Purple is a pH indicator. Bacto Agar is a solidifying agent.

Formula

MIL Medium

Formula Per Liter

Bacto Peptone	10 g
Bacto Tryptone	10 g
Bacto Yeast Extract	3 g
L-Lysine Hydrochloride	10 g
Bacto Dextrose	1 g
Ferric Ammonium Citrate	0.5 g
Bacto Brom Cresol Purple	0.02 g
Bacto Agar	2 g

Final pH 6.6 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

MIL Medium

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
13 x 100 mm Screw-capped test tubes
Inoculating needle
SpotTest™ Indole Reagent Kovacs

Method of Preparation

1. Suspend 36.5 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Dispense 5 ml amounts into 13 x 100 mm screw-capped test tubes.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Using an inoculating needle, stab tubes with growth from an 18-24 hour pure culture.
2. Incubate the tubes at 35 ± 2°C for 18-24 hours.
3. After incubation, examine tubes for evidence of lysine deaminase, motility and lysine decarboxylase reactions.

Results

Lysine deaminase is indicated by a red or red-brown color in the top centimeter of the medium.

Motility is indicated by a clouding of the medium or by growth extending from the inoculation line.

Lysine decarboxylase is indicated by a purple color throughout the medium. This color may vary in intensity and may be bleached out to a pale light color due to reduction of the indicator. Lysine-negative cultures produce a yellow medium that may be purple or red on the top. Tubes that show a purple reaction with a red color on top should be incubated for a longer period of time.

After examining the medium for lysine deaminase, motility and lysine decarboxylase reactions, add 3 or 4 drops of Indole Reagent Kovacs to the top of each tube. The appearance of a pink to red color in the reagent is interpreted as a positive indole test.

Positive and negative reactions are based on 90% or more occurrences. When an aberrant reaction occurs, subcultures should be plated on differential media to ensure the purity of the culture.

Limitations of the Procedure

1. Do not add Indole Reagent Kovacs until the final lysine deaminase, lysine decarboxylase and motility results have been interpreted.
2. Occasionally, the indole test produces false-negative or falsely weak reactions.[3,4]

References

1. **Reller, L. B., and S. Mirrett.** 1975. Motility-indole-lysine medium for presumptive identification of enteric pathogens of *Enterobacteriaceae.* J. Clin. Microbiol. **2**:247-252.

User Quality Control

Identity Specifications

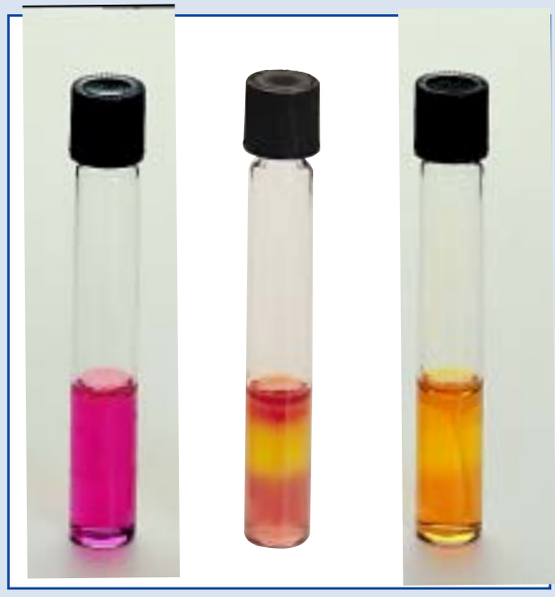
Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.65% solution, soluble in distilled or deionized water upon boiling. Solution is reddish purple, clear.
Prepared Medium:	Reddish purple, clear, semisolid.
Reaction of 3.65% Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

Prepare MIL Medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. After reading the lysine decarboxylase, motility and lysine deaminase reactions, add Indole Reagent Kovacs to determine the indole reaction.

ORGANISM	ATCC®	LYSINE DECARBOXYLASE	MOTILITY	LYSINE DEAMINASE	INDOLE PRODUCTION
Escherichia coli	25922*	+	+	–	+
Providencia alcalifaciens	9886	–	+	+	–
Salmonella enteritidis	13076	+	+	–	–
Shigella flexneri	12022*	–	–	–	–

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Shigella flexneri* ATCC® 12022

All with Indole Reagent

2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken. (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
3. **Baron, E. J., and S. M. Finegold.** 1990. Bailey and Scott's Diagnostic Microbiology, 8th ed. The C. V. Mosby Co., St. Louis, MO.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol.1. Williams & Wilkins, Baltimore, MD.

Packaging

MIL Medium	500 g	1804-17

Bacto® MIO Medium

Intended Use

Bacto MIO Medium is used for differentiating *Enterobacteriaceae* based on motility, ornithine decarboxylase activity and indole production.

Also Known As

MIO Medium conforms with Motility Indole Ornithine Medium and Ornithine Indole Motility (OIM) Medium.

Summary and Explanation

Tests for indole production, motility and ornithine decarboxylase activity play important roles in the identification of *Enterobacteriaceae.* Ederer and Clark[1] and Oberhofer and Hajkowski[2] developed MIO Medium which combines all three differentiating reactions in onemedium. Ederer and Clark stressed the advantages of MIO Medium in their extensive study comparing cultural reactions of *Enterobacteriaceae* on MIO Medium with reactions on classic media.

Principles of the Procedure

MIO Medium contains peptones which provide carbon and nitrogen. Yeast Extract provides vitamins and cofactors required for growth as well as additional sources of nitrogen and carbon. Dextrose is an energy source. Bacto Agar is added to demonstrate motility. The pH indicator, Brom Cresol Purple, facilitates detection of decarboxylase activity.

Formula

MIO Medium

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Peptone	10 g
Bacto Tryptone	10 g
Bacto L-Ornithine HCl	5 g
Bacto Dextrose	1 g
Bacto Agar	2 g
Bacto Brom Cresol Purple	0.02 g

Final pH 6.5 ± 0.2 at 25°C

User Quality Control

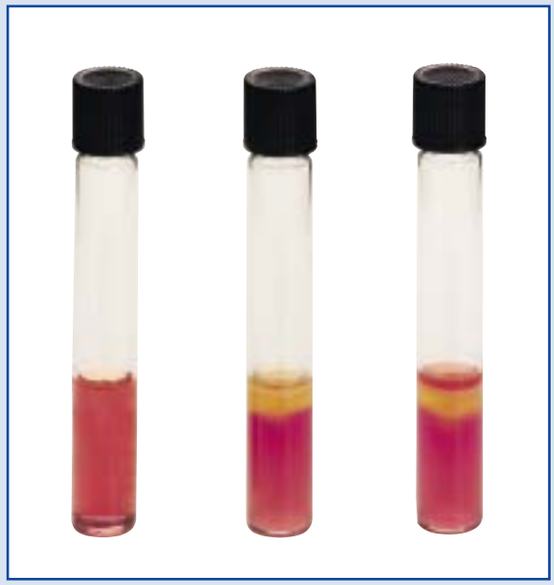
Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in distilled or deionized water upon boiling; purple, clear to slightly opalescent.
Prepared Medium:	Purple, slightly opalescent, semi-solid.
Reaction of 3.1% Solution at 25°C:	pH 6.5 ± 0.2

Cultural Response

Prepare MIO Medium per label directions. Inoculate the medium and incubate with caps loosened at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC®	GROWTH	MOTILITY	INDOLE	ORNITHINE DECARBOXYLASE
Enterobacter aerogenes	13048*	good	+	–	+
Escherichia coli	25922*	good	+	+	+
Klebsiella pneumoniae	13883*	good	–	–	–
Proteus mirabilis	25933	good	+	–	+



Uninoculated tube | *Enterobacter aerogenes* ATCC® 13048 | *Escherichia coli* ATCC® 25922

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Refer to appropriate references for typical motility, indole production and ornithine decarboxylase activity of various members of the *Enterobacteriaceae.*[3,4,5,6]

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

MIO Medium

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
SpotTest™ Indole Reagent Kovacs
Inoculating wire

Method of Preparation

1. Suspend 31 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Dispense into test tubes.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Pick isolated colonies with an inoculating wire and stab the medium to the bottom of the tube.
2. Incubate with caps loosened at 35 ± 2°C for 24-48 hours.
3. Examine tubes at 24 hours for growth, color change and motility. A color change from purple to yellow indicates a negative ornithine decarboxylase reaction; no color change indicates a positive reaction. Motility is shown by clouding of the medium or by growth extension from the inoculating line. Repeat the reading at 40-48 hours.
4. Add 3-4 drops of SpotTest™ Indole Reagent Kovacs to each tube. Record as indole positive if a pink or red color appears or as indole negative if there is no color change.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Do not add Kovacs reagent to the tubes until after final motility and ornithine results have been interpreted.
2. To prepare the stored medium for use in motility studies, loosen caps, heat to boiling and cool to 45-50°C prior to inoculation.[5]

References

1. **Ederer, G. M., and M. Clark.** 1970. Motility-Indole-Ornithine medium. Appl. Microbiol. **2**:849.
2. **Oberhofer, T. R., and R. Hajkowski.** 1970. Evaluation of non-lactose-fermenting members of the *Klebsiella-Enterobacter-Serratia* Division. I. Biochemical characteristics. Am. J. Clin. Pathol. **54**:720.
3. **Ewing, W. H.** 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, NY.
4. **Krieg, N. R., and J. G. Holt (ed.).** 1984. Bergey's manual of systematic bacteriology, vol. 1. Williams & Wilkins, Baltimore, MD.
5. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
6. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken. (ed.).** 1995. Manual of clinical microbiology. 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

MIO Medium	100 g	0735-15
	500 g	0735-17

Bacto® MYP Agar
Bacto Antimicrobic Vial P

Intended Use

Bacto MYP Agar is used with Bacto Egg Yolk Enrichment 50% and Bacto Antimicrobic Vial P for enumerating *Bacillus cereus* from foods.

Also Known As

MYP Agar is also known as Mannitol-Egg Yolk-Polymixin Agar.

Summary and Explanation

Mossel et al[1] formulated Mannitol-Egg Yolk-Polymixin (MYP) Agar to isolate and enumerate *Bacillus cereus* from foods. This medium differentiates *B. cereus* from other bacteria based on its resistance to polymixin, lack of mannitol fermentation, and presence of lecithinase.[2, 3] *B. cereus* is commonly found in nature, on vegetables, and in some processed foods.[4] Under favorable circumstances the microorganism grows to sufficient numbers and causes gastrointestinal illness.[4] Outbreaks of food borne illness have been associated with boiled and cooked rice, cooked meats and cooked vegetables.[5]

MYP Agar is a recommended medium for testing foods.[4, 5, 6]

Principles of the Procedure

MYP Agar contains Beef Extract and Bacto Peptone as sources of carbon, nitrogen, vitamins and minerals. D-Mannitol is the carbohydrate source. Phenol Red is the pH indicator. Bacto Agar is the solidifying agent. Egg Yolk Enrichment 50% provides lecithin. Antimicrobic Vial P is Polymixin B which inhibits the growth of most other bacteria.

Bacteria that ferment mannitol produce acid products and form colonies that are yellow. Bacteria that produce lecithinase hydrolyze the lecithin and a zone of white precipitate forms around the colonies. *B. cereus* is typically mannitol-negative (pink-red colonies) and lecithinase positive (zone of precipitate around the colonies).

Formula

MYP Agar

Formula Per Liter

Bacto Beef Extract	1 g
Bacto Peptone	10 g
Bacto D-Mannitol	10 g
Sodium Chloride	10 g
Bacto Phenol Red	0.025 g
Bacto Agar	15 g

Final pH 7.2 ± 0.1 at 25°C

Antimicrobic Vial P

30,000 units polymyxin B

Precautions

1. For Laboratory Use.
2. **Antimicrobic Vial P**: MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) MAY BE HARMFUL IF ABSORBED OR INTRODUCED THROUGH SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store the desiccated and rehydrated Antimicrobic Vial P at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

User Quality Control

Identity Specifications

MYP Agar

Dehydrated Appearance: Pink, free-flowing, homogeneous.

Solution: 4.6% solution, soluble in distilled or deionized water on boiling. Solution is red, slightly opalescent.

Prepared Medium: Red, slightly opalescent without significant precipitate.

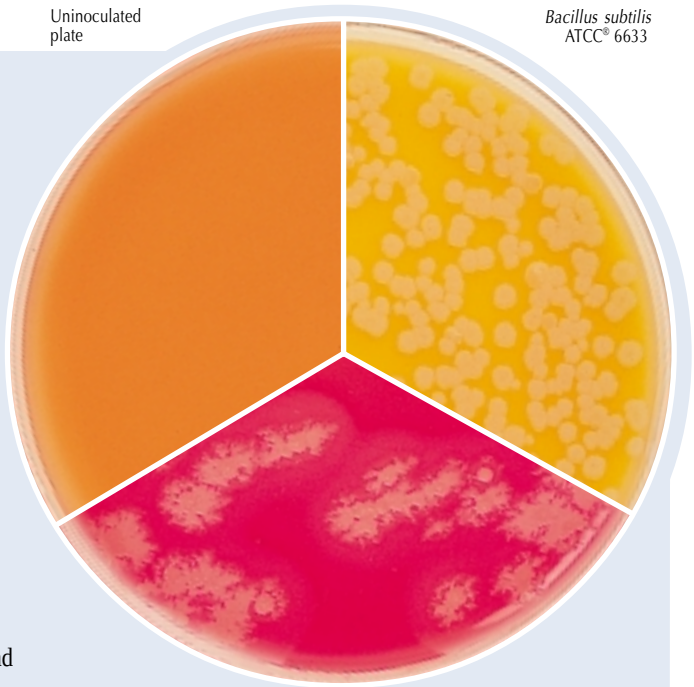
Reaction of 4.6 g/90 ml distilled or deionized water at 25°C: pH 7.2 ± 0.1

Antimicrobic Vial P

Dehydrated Appearance: White cake or powder.

Cultural Response

Prepare MYP Agar per label directions. Supplement with Egg Yolk Enrichment 50% and Antimicrobic Vial P (Polymyxin B). Inoculate and incubate at 30 ± 2°C for 18-48 hours. Lecithinase reaction is read as a zone of precipitate. Colonies that ferment mannitol are yellow.



ORGANISM	ATCC®	INOCULUM CFU	GROWTH	MANNITOL FERMENTATION	LECITHINASE REACTION
Bacillus cereus	13061	3-30 and 30-300	good	–	+
Bacillus subtilis	6633	3-30 and 30-300	good	+	–
Pseudomonas aeruginosa	27853*	1,000-2,000	inhibited	–	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Procedure

Materials Provided

MYP Agar
Egg Yolk Enrichment 50%
Antimicrobic Vial P

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

MYP Agar

1. Suspend 46 grams of MYP Agar in 900 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense 225 ml into 500 ml flasks.
4. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
5. Aseptically add 12.5 ml Egg Yolk Enrichment 50% and 4.1 ml rehydrated Antimicrobic Vial P (25,000 units of polymyxin B). Mix thoroughly.

Antimicrobic Vial P

1. Rehydrate with 5 ml sterile water.

Specimen Collection and Preparation

Consult appropriate references.[4,5,6]

Test Procedure

Consult appropriate references.[4,5,6]

Results

Consult appropriate references.[4,5,6]

References

1. **Mossel, D. A. A., M. J. Koopman, and E. Jongerius.** 1967. Enumeration of *Bacillus cereus* in foods. Appl. Microbiol. **15**:650-653.
2. **Donovan, K. O.** 1958. A selective medium for *Bacillus cereus* in milk. J. Appl. Bacteriol. **21**:100-103.
3. **Coliner, A. R.** 1948. The action of *Bacillus cereus* and related species on the lechithin complex of egg yolk. J. Bacteriol. **55**:777-785.
4. **Jeffery, E. J., and S. M. Harmon.** 1995. *Bacillus cereus*, p. 14. 01-14.08. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Harmon, S. M., J. M. Goepfert, and R. W. Bennett.** 1992. *Bacillus cereus*, p. 593-604. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
6. **Andrews, W.** 1995. Microbial methods, p. 1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International. Arlington, VA.

Packaging

MYP Agar	500 g	0810-17
Antimicrobial Vial P	6 x 10 ml	3268-60*

* Store at 2-8°C

Bacto® MacConkey Broth

User Quality Control

Identity Specifications

Dehydrated Appearance: Light beige, free-flowing, homogeneous.
Solution: 3.5% solution, soluble in distilled or deionized water; purple, clear.
Prepared Tubes: Purple, clear.
Reaction of 3.5% Solution at 25°C: pH 7.3 ± 0.1

Cultural Response

Prepare MacConkey Broth per label directions. Inoculate the medium and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	MEDIUM COLOR	GAS
Enterococcus faecalis	29212*	1,000-2,000	markedly inhibited	purple	–
Escherichia coli	25922*	100-1,000	good	yellow	+

The cultures listed are the minimum that should be used as for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube — *Escherichia coli* ATCC® 25922

Intended Use

Bacto MacConkey Broth is used for cultivating gram-negative, lactose-fermenting bacilli in water and foods as a presumptive test for coliform organisms.

Summary and Explanation

MacConkey Broth is a modification of the original bile salt broth recommended by MacConkey[1] that contained 0.5% sodium taurocholate and litmus as an indicator. In later publications,[2,3] MacConkey suggested variations of this formulation using neutral red indicator instead of litmus. Childs and Allen[4] demonstrated the inhibitory effect of neutral red and substituted the less inhibitory brom cresol purple. Oxgall in the medium replaces the original sodium taurocholates to inhibit growth of gram-positive organisms.

Principles of the Procedure

Peptone provides amino acids and other growth factors. Lactose is a carbon energy source for gram-negative lactose-fermenting bacilli. Oxgall inhibits the growth of gram-positive organisms. Brom Cresol Purple is the indicator.

Formula

MacConkey Broth

Formula Per Liter

Bacto Oxgall	5 g
Bacto Peptone	20 g
Bacto Lactose	10 g
Bacto Brom Cresol Purple	0.01 g

Final pH 7.3 ± 0.1 at 25°C

Precautions

1. For in Laboratory Use.
2. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper, established laboratory procedure in handling and disposing of infectious materials.

Storage

Store MacConkey Broth below 30°C. The powder is very hygoscopic. Keep container tightly closed.

Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

MacConkey Broth

Materials Provided But Not Required

Glassware
Autoclave
Incubator (35°C)
Tubes with closures

Method of Preparation

1. Dissolve 35 grams in 1 liter of distilled or deionized water. Rehydrate with proportionally less water when liquid inocula will exceed 1 ml.
2. Dispense into tubes.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory in accordance with recommended guidelines.
2. Process each specimen as appropriate for that specimen.
3. Inoculate specimen as appropriate for that specimen.
4. Incubate tubes for 18-24 hours at 35 ± 2°C.
5. Examine tubes.

Test Procedure

See appropriate references for specific procedures.

Results

Lactose-fermenting organisms grow very well in MacConkey Broth and produce acid, causing the medium to turn yellow. Gas is also produced. Non-fermenting organisms produce good growth but will not produce acid or gas.

References

1. **MacConkey, A.** 1901. Centr. Bakt. **29**:740.
2. **MacConkey, A.** 1905. Lactose-fermenting bacteria in faeces. J. Hyg. **5**:333-379.
3. **MacConkey, A.** 1908. Bile salt media and their advantage in some bateriological examinations. J. Hyg. **8**:322-334.
4. **Childs, E., and L. A. Allen.** 1953. Improved methods for determining the most probable number of *Bacterium coli* and of *Streptococcus faecalis*. J. Hyg. Camb. **51**:468-477.

Packaging

MacConkey Broth	500 g	0020-17

MacConkey Media

Bacto® MacConkey Agar · Bacto MacConkey Agar Base
Bacto MacConkey Agar CS · Bacto MacConkey Agar w/o CV
Bacto MacConkey Agar w/o Salt

Intended Use

MacConkey Media are selective and differential plating media mainly used for the detection and isolation of gram-negative organisms from clinical,[1] dairy,[2] food,[3,4] water,[5] pharmaceutical[6] and industrial[7] sources.

Bacto MacConkey Agar is used for isolating and differentiating lactose-fermenting from lactose nonfermenting gram-negative enteric bacilli.

Bacto MacConkey Agar Base is used with added carbohydrate in differentiating coliforms based on fermentation reactions.

User Quality Control

Identity Specifications

MacConkey Agar

Dehydrated Appearance:	Pink to pinkish beige, free-flowing, homogenous.
Solution:	5.0% solution, soluble in distilled or deionized water on boiling; reddish purple, very slightly to slightly opalescent.
Prepared Plates:	Pinkish red, slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 7.1 ± 0.2

MacConkey Agar Base

Dehydrated Appearance:	Pink to pinkish beige, free-flowing, homogenous.
Solution:	4.0% solution, soluble in distilled or deionized water upon boiling; red, very slightly to slightly opalescent without significant precipitate.
Prepared Plates:	Red, slightly opalescent without precipitate.
Reaction of 4.0% Solution at 25°C:	pH 7.1 ± 0.2

MacConkey Agar CS

Dehydrated Appearance:	Pinkish beige, homogenous, free-flowing.
Solution:	5.0% solution, soluble in distilled or deionized water on boiling; reddish purple in color, slightly opalescent, without significant precipitate.
Prepared Plates:	Reddish purple, slightly opalescent, without precipitate.
Reaction of 5.0% Solution at 25°C:	pH 7.1 ± 0.2

continued on following page

Bacto MacConkey Agar CS is used for isolating and differentiating gram-negative enteric bacilli from specimens containing swarming strains of *Proteus.*

Bacto MacConkey Agar w/o CV is used for isolating and differentiating enteric microorganisms while permitting growth of staphylococci and enterococci.

Bacto MacConkey Agar w/o Salt is used for isolating and differentiating gram-negative bacilli while suppressing the swarming of most *Proteus* species.

Also Known As

MacConkey Agar is also known as MAC.

Summary and Explanation

MacConkey Agar is based on the bile salt-neutral red-lactose agar of MacConkey.[8]

The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications improved the growth of *Shigella* and *Salmonella* strains. These modifications included the addition of 0.5% sodium chloride, decreased agar content, and altered bile salts and neutral red concentrations. The formula improvements gave improved differential reactions between these enteric pathogens and the coliform group.

MacConkey Agar contains crystal violet and bile salts that inhibit gram-positive organisms and allow gram-negative organisms to grow. Isolated colonies of coliform bacteria are brick red in color and may be surrounded by a zone of precipitated bile. This bile precipitate is due to a local pH drop around the colony due to lactose fermentation. Colonies that do not ferment lactose (such as typhoid, paratyphoid and dysentery bacilli) remain colorless. When lactose non-fermenters grow in proximity to coliform colonies, the surrounding medium appears as cleared areas.

MacConkey Agar Base is prepared without added carbohydrates, which permits their addition either individually or in combination. It is recommended that carbohydrates such as sucrose or lactose be added in a concentration of 1% to the basal medium.

MacConkey CS ("Controlled Swarming") contains carefully selected raw materials to reduce the swarming of *Proteus* species which could cause difficulty in isolating and enumerating other gram-negative bacilli.

MacConkey Agar w/o CV (Crystal Violet) is a differential medium that is less selective than MacConkey Agar. The lack of crystal violet permits the growth of *Staphylococcus* and *Enterococcus.* Staphylococci produce pale pink to red colonies and enterococci produce compact tiny red colonies either on or beneath the surface of the medium.

MacConkey Agar w/o Salt is a differential medium that restricts the swarming of *Proteus* species to aid in the detection and isolation of enteric microorganisms. In addition, this medium does not contain crystal violet, allowing *Staphyloccocus* and *Enterococcus* species to grow.

Principles of the Procedure

Bacto Peptone and Proteose Peptone are sources of nitrogen and other nutrients. Lactose is a fermentable carbohydrate. When lactose is fermented, a local pH drop around the colony causes a color change in the pH indicator (neutral red) and bile precipitation. Bile Salts, Bile Salts No. 3 and Crystal Violet are selective agents that inhibit growth of gram-positive organisms. Bacto Agar is a solidifying agent.

Formula

MacConkey Agar

Formula Per Liter

Bacto Peptone ..... 17 g
Bacto Proteose Peptone ..... 3 g
Bacto Lactose ..... 10 g
Bacto Bile Salts, No. 3 ..... 1.5 g
Sodium Chloride ..... 5 g
Bacto Agar ..... 13.5 g
Neutral Red ..... 0.03 g
Bacto Crystal Violet ..... 0.001 g

Final pH 7.1 ± 0.2 at 25°C

MacConkey Agar Base

Formula Per Liter

Bacto Peptone ..... 17 g
Bacto Proteose Peptone ..... 3 g
Bacto Bile Salts, No. 3 ..... 1.5 g
Sodium Chloride ..... 5 g
Bacto Agar ..... 13.5 g
Neutral Red ..... 0.03 g
Bacto Crystal Violet ..... 0.001 g

Final pH 7.1 ± 0.2 at 25°C

MacConkey Agar CS

Formula Per Liter

Bacto Peptone ..... 17 g
Bacto Proteose Peptone ..... 3 g
Bacto Lactose ..... 10 g
Bacto Bile Salts ..... 5 g
Sodium Chloride ..... 5 g
Bacto Agar ..... 13.5 g
Neutral Red ..... 0.03 g
Bacto Crystal Violet ..... 0.001 g

Final pH 7.1 ± 0.2 at 25°C

MacConkey Agar w/o CV

Formula Per Liter

Bacto Peptone ..... 20 g
Bacto Lactose ..... 10 g
Bacto Bile Salts ..... 5 g
Sodium Chloride ..... 5 g
Neutral Red ..... 0.05 g
Bacto Agar ..... 12 g

Final pH 7.4 ± 0.2 at 25°C

MacConkey Agar w/o Salt

Formula Per Liter

Bacto Peptone ..... 20 g
Bacto Lactose ..... 10 g
Bacto Bile Salts ..... 5 g
Neutral Red ..... 0.075 g
Bacto Agar ..... 12 g

Final pH 7.4 ± 0.2 at 25°C

User Quality Control cont.

MacConkey Agar w/o CV

Dehydrated Appearance:	Pinkish beige, free-flowing, homogenous.
Solution:	5.2% solution, soluble in distilled or deionized water upon boiling; reddish orange, clear to very slightly opalescent without significant precipitate.
Prepared Plates:	Reddish orange, slightly opalescent without significant precipitate.
Reaction of 5.2% Solution at 25°C:	pH 7.4 ± 0.2

MacConkey Agar w/o Salt

Dehydrated Appearance:	Pinkish beige, free-flowing, homogenous.
Solution:	4.7% solution, soluble in distilled or deionized water upon boiling; reddish orange, slightly opalescent.
Prepared Plates:	Reddish orange, slightly opalescent.
Reaction of 4.7% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare MacConkey media per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

MacConkey Agar

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	BILE PPT.
Enterococcus faecalis	29212*	1,000-2,000	markedly to completely inhibited	–	–
Escherichia coli	25922*	100-1,000	good	pink	+
Proteus mirabilis	12453	100-1,000	good	colorless	–
Salmonella typhimurium	14028*	100-1,000	good	colorless	–

continued on following page

Precautions

1. For Laboratory Use.
2. **For MacConkey Agar w/o CV**
   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

MacConkey Agar w/o Salt

IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is

User Quality Control cont.

MacConkey Agar Base

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	BILE PPT.
Enterococcus faecalis	29212*	1,000-2,000	markedly to completely inhibited	–	–
Escherichia coli	25922*	100-1,000	good	w/o lactose: colorless w/lactose: pink	+ +
Proteus mirabilis	12453	100-1,000	good	colorless	–
Salmonella typhimurium	14028*	100-1,000	good	colorless	–

MacConkey Agar CS

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	BILE PPT.
Enterococcus faecalis	29212*	1,000-2,000	markedly to completely inhibited	–	–
Escherichia coli	25922*	100-1,000	good	pink to red	–/+
Proteus mirabilis	12453	100-1,000	good	colorless, swarming markedly to completely inhibited	–
Salmonella typhimurium	14028*	100-1,000	good	translucent, colorless	–

MacConkey Agar w/o CV

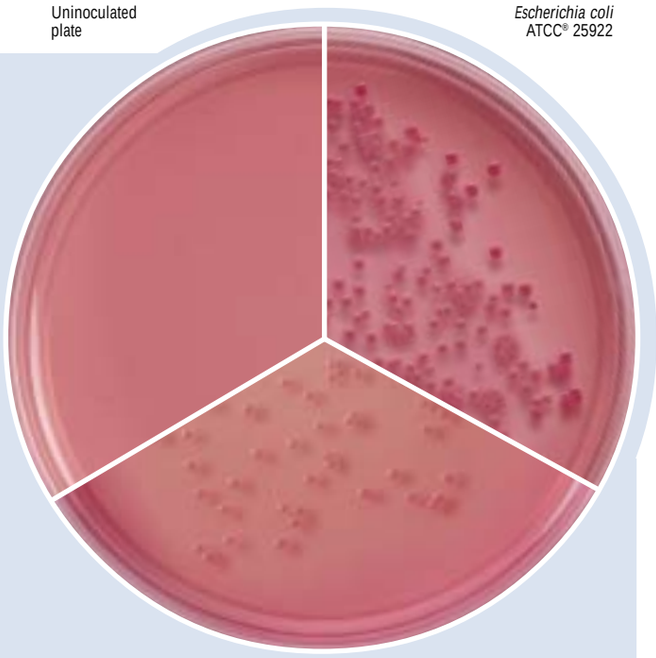
ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	BILE PPT.
Enterococcus faecalis	29212*	100-1,000	good	red	–
Escherichia coli	25922*	100-1,000	good	pink or red	–
Proteus mirabilis	12453	100-1,000	good	colorless	–
Salmonella typhimurium	14028*	100-1,000	good	colorless	–

MacConkey Agar w/o Salt

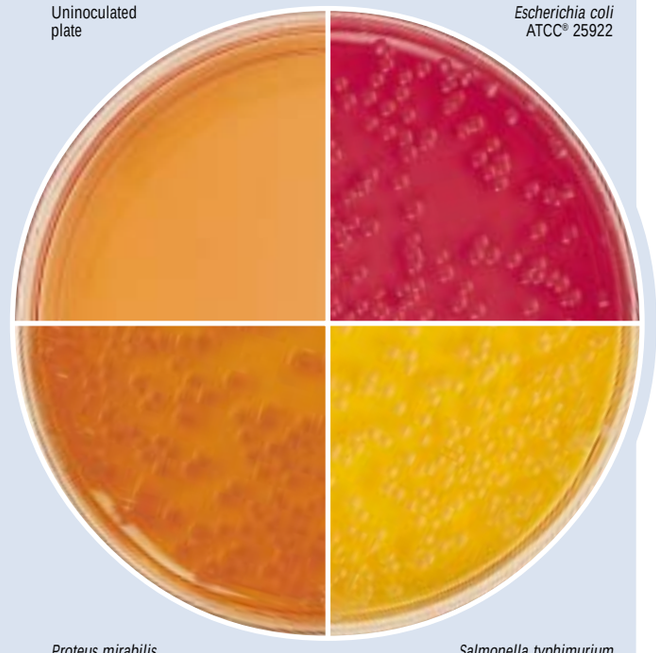
ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE	BILE PPT.
Enterococcus faecalis	29212*	100-1,000	good	red	–
Escherichia coli	25922*	100-1,000	good	pink to red	–
Proteus mirabilis	12453	100-1,000	good	colorless; swarming markedly to completely inhibited	– –
Salmonella typhimurium	14028*	100-1,000	good	colorless	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



MacConkey Agar CS



MacConkey Agar w/o CV

difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

MacConkey Agar
MacConkey Agar Base
MacConkey Agar CS
MacConkey Agar w/o CV
MacConkey Agar w/o Salt

Materials Required But Not Provided

Glassware
Autoclave
35°C incubator
50°C waterbath (optional)
Carbohydrate (lactose, sucrose, etc.) (optional)

Method of Preparation

For MacConkey Agar, MacConkey Agar CS, MacConkey Agar w/o CV or MacConkey Agar w/o Salt:

1. Suspend the medium in 1 liter distilled or deionized water:

MacConkey Agar	50 grams
MacConkey Agar CS	50 grams
MacConkey Agar w/o CV	52 grams
MacConkey Agar w/o Salt	47 grams

2. Heat to boiling to dissolve completely. Avoid overheating.
3. Autoclave at 121°C for 15 minutes. The media may be used without autoclave sterilization if the plates are to be inoculated on the day of preparation.
4. Cool to 45-50°C and dispense into sterile Petri dishes.
5. The surface of the medium should be dry when inoculated. Dry the plates for 1-2 hours with the lids slightly ajar.

For MacConkey Agar Base:

1. Suspend 40 grams of medium in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. Avoid overheating.
3. Add 10 grams lactose or other desired carbohydrate before or after sterilization, depending on heat lability.
4. Autoclave at 121°C for 15 minutes. The media may be used without autoclave sterilization if the plates are to be inoculated on the day of preparation. In this case, boiling the medium gently for 5 minutes is sufficient.
5. Cool to 45-50°C and dispense into sterile Petri dishes.
6. The surface of the medium should be dry when inoculated. Dry the plates for 1-2 hours with the lids slightly ajar.

Specimen Collection and Preparation

For a complete discussion on the isolation and identification of enteric organisms consult the appropriate references.

Test Procedure

For procedures on the isolation and identification of enteric organisms consult the appropriate references.

Results

Lactose-fermenting organisms grow as pink to brick-red colonies with or with out a zone of precipitated bile. Non-lactose fermenting organisms grow as colorless or clear colonies.

Swarming by *Proteus* spp. is reduced on MacConkey Agar CS and MacConkey Agar w/o Salt.

On MacConkey Agar w/o CV and MacConkey Agar w/o Salt, staphylococci produce pale pink to red colonies and enterococci produce tiny red colonies; these organisms are inhibited on MacConkey Agar and MacConkey Agar CS.

Limitations of the Procedure

1. Although MacConkey media are selective primarily for gram-negative enteric bacilli, biochemical and, if indicated, serological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.[1,3]
2. Due to the selective properties of MacConkey Agar CS, some strains of gram-negative enteric bacilli may be encountered that fail to grow or grow poorly on this medium. Some strains of gram-positive organisms may be encountered that are not inhibited or only partially inhibited on this medium; some strains of enterococci may grow on MacConkey Agar CS after prolonged incubation.
3. Incubation of MacConkey Agar plates under increased CO_2 has been reported to reduce the growth and recovery of a number of strains of gram-negative bacilli.[9]
4. For optimal performance, plates prepared from MacConkey Agar CS should be incubated under aerobic conditions.

References

1. **Gray, L. D.** 1995. *Escherichia, Salmonella, Shigella and Yersinia*, p. 450-456. *In* P. R. Murray, E. J. Baron, M. A, Pfaller, F. C. Tenover, and R. H Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig (H.M. Wehr, Tech. Comm.).** 1992. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall, (ed.). Standard methods for the examination of dairy products. 16th ed., American Public Health Association, Washington, D.C.

3. **Hitchins, A. D., P. A. Hartman, and E. C. D. Todd.** 1992. Coliforms-*Escherichia coli* and its Toxins, p. 325-369. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington. D.C.
4. **Food and Drug Administration**. 1995. Bacteriological analytical manual, 8th ed. AOAC International. Gaithersburg, MD.
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7. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
8. **MacConkey, A.** 1905. Lactose-fermenting bacteria in feces. J. Hyg. **5:**333-379.
9. **Mazura-Reetz, G. T. Neblett, and J. M. Galperin**. 1979. MacConkey Agar: CO_2 vs. ambient incubation. Abst. Ann. Mtg. American Society for Microbiology. C179.

Packaging

MacConkey Agar	100 g	0075-15-3
	500 g	0075-17-1
	2 kg	0075-07-3
	10 kg	0075-08-2
MacConkey Agar Base	500 g	0818-17-3
MacConkey Agar CS	500 g	1818-17-1
	2 kg	1818-07-3
	10 kg	1818-08-2
MacConkey Agar w/o CV	500 g	0470-17-2
MacConkey Agar w/o Salt	500 g	0331-17-1
	10 kg	0331-08-2

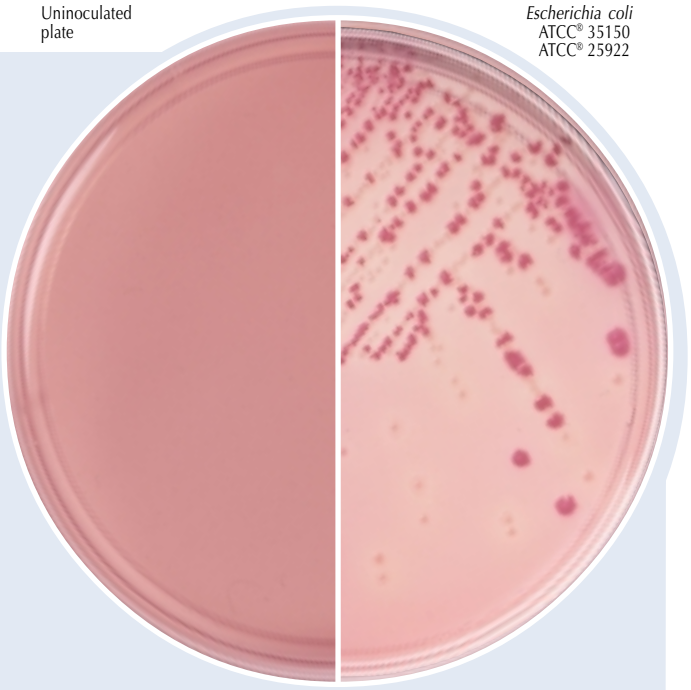
Bacto® MacConkey Sorbitol Agar

Intended Use

Bacto MacConkey Sorbitol Agar is used for isolating and differentiating enteropathogenic *Escherichia coli* serotypes.

Summary and Explanation

The original MacConkey medium was used to differentiate strains of *Salmonella typhosa* from members of the coliform group. Formula modifications used in MacConkey Agar improved the growth of *Shigella* and *Salmonella* strains as well as the differential reactions between these enteric pathogens and the coliform group. The modifications included addition of 0.5% sodium chloride, decreased agar content, and altered bile salts and neutral red concentrations.



User Quality Control

Identity Specifications

Dehydrated Appearance:	pinkish-beige, free flowing, homogeneous.
Solution:	5.0% solution, soluble in distilled or deionized water on boiling; reddish purple, very slightly to slightly opalescent.
Prepared Medium:	reddish-purple, slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 7.1 ± 0.2 at 25°C

Cultural Response

Prepare MacConkey Sorbitol Agar per label directions.
Inoculate plates and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	CFU	INOCULUM RECOVERY	COLONY COLOR	BILE PPT
Enterococcus faecalis	29212*	1,000-2,000	markedly inhibited	–	–
Escherichia coli 0157:H7	35150	100-1,000	good	colorless	–
Escherichia coli	25922*	100-1,000	good	pink-red	+

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

MacConkey Sorbitol Agar is a modification of the formula given by Rappaport and Henig[1] for isolating enteropathogenic *Escherichia coli* serotypes 011 and 055. The usefulness of this medium in detecting *E. coli* 0157:H7, a human pathogen associated with hemorrhagic colitis, has been described.[2,3,4]

This medium employs d-sorbitol rather than lactose for isolating and differentiating the enteropathogenic *E. coli* serotypes which tend to be sorbitol negative. This medium can be used for clinical and food testing.[1,5,6]

Principles of the Procedure

Bacto Peptone and Proteose Peptone are nitrogen sources in the medium. D-Sorbitol is a fermentable carbohydrate. Many hemorrhagic *E. coli* strains will not ferment d-sorbitol and appear as colorless colonies on MacConkey Sorbitol Agar. Bile salts and crystal violet are selective agents that inhibit growth of gram-positive organisms. Neutral red is a pH indicator. Bacto Agar is a gelling agent.

Formula

MacConkey Sorbitol Agar

Formula Per Liter

Bacto Peptone	15.5 g
Bacto Proteose Peptone	3 g
d-Sorbitol	10 g
Bacto Bile Salts	1.5 g
Sodium Chloride	5 g
Bacto Agar	15 g
Neutral Red	0.03 g
Bacto Crystal Violet	0.001 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

MacConkey Sorbitol Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 50 grams in 1 liter distilled or deionized water:
2. Heat to boiling to dissolve completely. Avoid overheating.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense into sterile Petri dishes.
5. Dry plates for 1-2 hours with the lids slightly ajar. The surface of the medium should be dry when inoculated.

MacConkey Sorbitol Agar may be used without autoclave sterilization if the plates are to be used on the day of preparation. Boil the medium 2-3 minutes before pouring into Petri dishes and dry before inoculation.

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs and immediately transport to the laboratory in accordance with recommended guidelines.
2. Process each specimen as appropriate for that specimen.
3. Inoculate the specimen onto medium appropriate for that specimen.
4. Incubate plates for 18-24 hours at 35 ± 2°C.
5. Examine plates.

Test Procedure

See appropriate references for specific procedures.

Results

Sorbitol-fermenting organisms produce pink colonies on MacConkey Sorbitol Agar. Organisms that do not ferment sorbitol, such as *E. coli* 0157:H7, are colorless.

Limitations of the Procedure

1. The color of sorbitol-positive colonies can fade, making them hard to distinguish from sorbitol-negative colonies.[3]
2. Upon prolonged incubation, strains of *E. coli* 0157:H7 can ferment sorbitol.[3]
3. Strains of other organisms that do not ferment sorbitol may grow on MacConkey Sorbitol Agar. It is necessary to select suspected colonies for further identification.[3]
4. The sole use of this medium can cause the microbiologist to miss other organisms that may be pathogenic.[7]
5. To isolate *E. coli* 0157:H7 from clinical specimens, inoculate fecal specimens and rectal swabs on a small area of one quadrant and streak for isolation. This will permit development of discrete colonies.

References

1. **Rappaport, F., and E. Henig**. 1952. Media for the isolation and differentiation of pathogenic *Escherichia coli* (serotypes 0111 and 055). J. Clin. Pathology. **5:**361-362.
2. **Gray, L. D**. 1995. *Escherichia, Salmonella, Shigella* and *Yersinia*, p. 450-456. *In* Murray, P.R., E. J. Baron, M.A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
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4. **March, S. B., and S. Ratnam**. 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* 0157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. **23:**869-872.
5. **Hitchins, A. D., P. A. Hartman, and E. C. D. Todd.** 1992. Coliforms-*Escherichia coli* and its toxins, p. 325-369. *In* C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington. D.C.

6. **Hitchins, A. D., P. Feng, W. D. Watkins, S. R. Rippey, and L. A. Chandler**. 1995. *Escherichia coli* and the coliform bacteria. p. 4.01-4.29. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
7. **Ewing, W. H., and P. R. Edwards**. 1954. Isolation and preliminary identification of *Escherichia coli* serotypes associated with cases of diarrhea of the newborn. Public Health Lab. **12:**75-81.

Packaging

MacConkey Sorbitol Agar	500 g	0079-17

Bacto® Malonate Broth

Intended Use

Bacto Malonate Broth is used for differentiating *Enterobacter* from *Escherichia* based on malonate utilization.

Summary and Explanation

Malonate Broth, prepared according to the formula described by Leifson[1], is a liquid medium containing ammonium sulfate as the only source of nitrogen and malonate as the only source of carbon. Leifson was able to demonstrate that the *Enterobacter* group utilizes malonate whereas the *Escherichia* group is unable to grow on the medium.

Malonate Broth is further described for differentiating *Enterobacteriaceae* in food and dairy products.[2,3,4] In some cases, however, the medium referenced is the modified Edwards and Ewing[5] formulation that contains yeast extract and dextrose. The modification permits growth of organisms that would otherwise fail on the Leifson medium.

Principles of the Procedure

Malonate Broth contains Ammonium Sulfate, which is the sole source of nitrogen in the medium; Sodium Malonate is the sole source of carbon. Dipotassium Phosphate and Monopotassium Phosphate provide buffering capability. Sodium Chloride maintains the osmotic balance of the medium. Increased alkalinity resulting from malonate utilization causes the indicator, Brom Thymol Blue, to change color from green to blue.

Formula

Malonate Broth

Formula Per Liter

Ammonium Sulfate	2 g
Dipotassium Phosphate	0.6 g
Monopotassium Phosphate	0.4 g
Sodium Chloride	2 g
Sodium Malonate	3 g
Bacto Brom Thymol Blue	0.025 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Lungs, Intestines.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light green, free-flowing, homogeneous.
Solution:	0.8% solution, soluble in distilled or deionized water. Solution is green, clear.
Reaction of 0.8% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare Malonate Broth per label directions. Inoculate the medium with a loopful of test organism and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH	APPEARANCE
Enterobacter aerogenes	13048*	good	blue
Escherichia coli	25922*	poor to fair	green

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube | *Enterobacter aerogenes* ATCC® 13048 | *Escherichia coli* ATCC® 25922

Storage
Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date
The expiration date applies to the product in its intact container. Do not use a product if it fails to meet specifications for identity and performance.

Procedure
Materials Provided
Malonate Broth

Materials Required But Not Provided
Glassware
Autoclave
Incubator (35°C)

Method of Preparation
1. Dissolve 8 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Avoid introducing extraneous carbon and nitrogen.

Specimen Collection
Refer to appropriate references for specimen collection and preparation.

Test Procedure
1. Inoculate tubes with a loopful of test organism.
2. Incubate at 35 ± 2°C for 18-48 hours.
3. Examine tubes for a change in the color of the medium from green to blue.

Results
Malonate utilization is indicated by a change in the color of the medium from green to blue:
Positive: Blue
Negative: Green

Limitations of the Procedure
1. A slight bluing (blue-green) of the medium may occur after prolonged incubation.[6] In such cases, care should be taken in interpreting results.

References

1. **Leifson, E.** 1933. The fermentation of sodium malonate as a means of differentiating *Aerobacter* and *Escherichia*. J. Bacteriol. **26**: 329.
2. **Bacteriological Analytical Manual.** 1995. 8th ed. AOAC International. Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. **Edwards, P. R., and W. H. Ewing**. 1962. *Enterobacteriaceae.* U.S. Public Health Service Bulletin No. **734**:19.
6. **Oberhofer, T. R.** 1985. Manual of nonfermenting gram-negative bacteria. Churchill Livingstone, New York, NY.

Packaging
Malonate Broth	100 g	0395-15
	500 g	0395-17

Bacto® Malonate Broth Modified

User Quality Control
Identity Specifications
Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	0.93% solution, soluble in distilled or deionized water with agitation. Solution is green, clear.
Reaction of 0.93% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response
Prepare Malonate Broth Modified per label directions. Inoculate the medium with a loopful of undiluted organism and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM	COLOR OF MEDIUM
Enterobacter aerogenes	13048*	undiluted	blue
Escherichia coli	25922*	undiluted	green
Salmonella arizonae	13314	undiluted	blue
Salmonella typhimurium	14028*	undiluted	green

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Intended Use
Bacto Malonate Broth Modified is used for differentiating *Enterobacteriaceae* based on malonate utilization.

Also Known As
Malonate Broth Modified conforms with Malonate Broth, Ewing.

Summary and Explanation
Malonate Broth Modified is essentially Malonate Broth to which Yeast Extract and Dextrose have been added.[1] These additional ingredients initiate growth of some organisms that otherwise would fail to grow on the unmodified medium and, thus, permit observation of those organisms' malonate activity.

Malonate utilization by microorganisms is indicated by an increase in alkalinity and development of a deep blue color in the medium. Malonate utilization forms a basis on which organisms can be differentiated when testing food products for *Enterobacteriaceae.*[2,3,4] It is useful in the differentiation of *Escherichia coli* from the *Klebsiella-Enterobacter* groups and is considered especially valuable in the differentiation of *Salmonella*. The majority of salmonellae do not utilize malonate whereas *Salmonella arizonae* does.[4,5,6]

This medium may be used in conjunction with phenylalanine as proposed by Shaw and Clarke[7] to detect both malonate degradation and phenylalanine deamination.

Principles of the Procedure

Yeast Extract and Dextrose provide the vitamins and cofactors, as well as minimal sources of carbon, required for good growth of a wide variety of organisms. An organism that can utilize Sodium Malonate as its carbon source while at the same time using Ammonium Sulfate as its nitrogen source causes increased alkalinity due to the formation of sodium hydroxide. This causes the indicator, Brom Thymol Blue, to change color from green to blue. Some malonate-negative strains produce a yellow color. This is due to fermentation of the dextrose alone, producing increased acidity that causes the indicator to turn yellow at pH 6.0. Dipotassium Phosphate and Monopotassium Phosphate provide buffering capability. Sodium Chloride maintains the osmotic balance of the medium.

Formula

Malonate Broth Modified

Formula Per Liter	
Bacto Yeast Extract	1 g
Ammonium Sulfate	2 g
Dipotassium Phosphate	0.6 g
Monopotassium Phosphate	0.4 g
Sodium Chloride	2 g
Sodium Malonate	3 g
Bacto Dextrose	0.25 g
Bacto Brom Thymol Blue	0.025 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Lungs, Intestines

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Malonate Broth Modified

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Distilled or deionized water

Method of Preparation

1. Suspend 9.3 grams in 1 liter distilled or deionized water and agitate to dissolve completely.
2. Dispense into tubes.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

A color change of the medium to blue indicates that malonate has been utilized.

Limitations of the Procedure

1. Some malonate-positive organisms produce only slight alkalinity. Compare any tube in question with an uninoculated malonate tube. Any trace of blue color after a 48-hour incubation period denotes a positive test. Before making a final negative interpretation, be sure that test tubes have been incubated for 48 hours.[7]

References

1. **Edwards, P. R. and W. H. Ewing.** 1962. *Enterobacteriaceae.* U.S. Public Health Service Bulletin No. **734**:19.
2. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, Supplement March 1996. AOAC International, Arlington, VA.
4. **Bacteriological Analytical Manual, 8th ed.** 1995. AOAC International, Gaithersburg, MD.
5. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology. Washington, D.C.
6. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
7. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Malonate Broth Modified	500 g	0569-17

Bacto® Malt Agar

Intended Use

Bacto Malt Agar is used for isolating and cultivating yeasts and molds from food, and for cultivating yeast and mold stock cultures.

Summary And Explanation

Malt media for yeasts and molds have been widely used for many years. In 1919, Reddish[1] prepared a satisfactory substitute for beer wort from malt extract. Thom and Church[2] used Reddish's medium for their studies of the aspergilli. Malt Agar was also employed by Fullmer and Grimes[3] for their studies of the growth of yeasts on synthetic media. Malt Agar is specified in Standard Methods[4,5] for the examination of yeasts and molds.

Principles Of The Procedure

Malt Agar contains Malt Extract which provides the carbon, protein and nutrient sources required for the growth of microorganisms. Bacto Agar is a solidifying agent. The acidic pH of Malt Agar allows for optimal growth of molds and yeasts while restricting bacterial growth.

Formula

Malt Agar

Formula per liter

Bacto Malt Extract	30 g
Bacto Agar	15 g

Final pH 5.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Malt Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator

Method of Preparation

1. Suspend 45 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. To alter the medium to pH 4.5 or pH 3.5, add the quantity of sterile 85% lactic acid USP indicated on the label. (This quantity is specific to the lot of product.) Do not reheat the medium after adding acid.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

User Quality Control

Identity Specifications

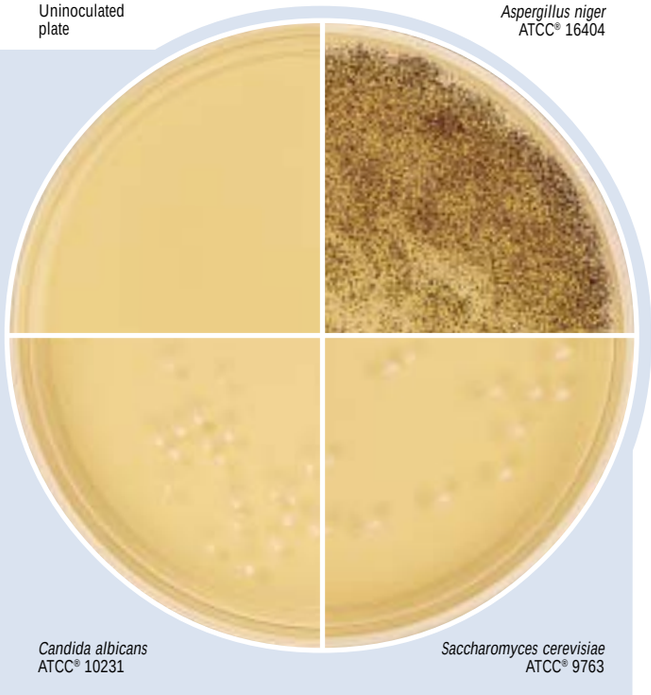
Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.5% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber in color, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, very slightly to slightly opalescent.
Reaction of a 4.5% Solution at 25°C:	5.5 ± 0.2

Cultural Response

Prepare Malt Agar per label directions. Inoculate and incubate plates at 30 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	good
Candida albicans	10231	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.



Test Procedure
See appropriate references for specific procedures.

Results
Refer to appropriate references and procedures for results.

Limitations of the Procedure
1. Do not heat the medium after addition of acid, as this will hydrolyze the agar and reduce its solidifying properties.

References

1. Abs. Bact., **3**:6, 1919.
2. **Thom, C., and M. B. Church.** 1926. The Aspergilli. Williams and Wilkins Co., Baltimore, MD.
3. **Fulmer, E. I., and M. J. Grimes.** 1923. The growth of yeasts on synthetic agar media. Bacteriol., **8**:585-588.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.)**. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Association of Official Agricultural Chemists.** 1995. Official methods of analysis, 16th ed. Association of Official Agricultural Chemists, Washington, D.C.

Packaging
Malt Agar	500 g	0024-17
	10 kg	0024-08

Bacto® Malt Extract

Intended Use
Bacto Malt Extract is used for preparing microbiological culture media for the propagation of yeasts and molds.

Summary and Explanation
Malt Extract is obtained from barley. It is a useful ingredient of culture media designed for the propagation of yeasts and molds. This product is particularly suitable for yeasts and molds because it contains a high concentration of carbohydrates, particularly maltose. The approximate percentage of reducing sugars in Malt Extract is 60-63%. Malt Extract is generally employed in culture media at concentrations between 10 to 100 grams per liter.

Malt Agar, a medium recommended for the detection and isolation of yeasts and molds from dairy products, food and as a stock culture contains Malt Extract. Wort Agar, used for the cultivation and enumeration of yeasts, has Malt Extract as one of the main ingredients in the formula. Several media containing Malt Extract are specified in standard methods.[1,2,3]

User Quality Control
Identity Specifications
Dehydrated Appearance:	Medium tan, free-flowing, homogeneous.
Solution:	2% solution, soluble in distilled or deionized water; medium amber, slightly opalescent to opalescent, may have a precipitate.
Reaction of 2% Solution at 25°C:	pH 4.5 -5.5

Cultural Response
Prepare 2% solution. Inoculate tubes with the test organisms. Incubate tubes at 30 ± 2°C for up to three days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	good
Candida albicans	10231	100-1,000	good

The cultures listed are the minimum that should be used.

Principles of the Procedure
Malt Extract provides carbon, protein and nutrients for the isolation and cultivation of yeasts and molds in bacterial culture media.

Precautions
1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage
Store the dehydrated ingredient below 30°C. The product is very hygroscopic. Keep container tightly closed.

Expiration Date
The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure
Materials Provided
Malt Extract

Materials Required But Not Provided
Materials vary depending on the medium being prepared.

Method of Preparation
Refer to the final concentration of Malt Extract in the formula of the medium being prepared. Add Malt Extract as required.

Specimen Collection and Preparation
Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure
See appropriate references for specific procedures using Malt Extract.

Results
Refer to appropriate references and procedures for results.

References

1. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
3. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Malt Extract	100 g	0186-15
	500 g	0186-17
	10 kg	0186-08

Bacto® Malt Extract Agar
Bacto® Malt Extract Broth

Intended Use

Bacto Malt Extract Agar is used for isolating, cultivating and enumerating yeasts and molds.

Bacto Malt Extract Broth is used for cultivating yeasts and molds.

User Quality Control

Identity Specifications

Malt Extract Agar

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	3.36% solution, soluble in distilled or deionized water on boiling. Solution is very light amber, slightly opalescent.
Prepared Medium:	Very light amber, slightly opalescent.
Reaction of 3.36% Solution at 25°C:	pH 4.7 ± 0.2

Malt Extract Broth

Dehydrated Appearance:	Light beige to beige, free-flowing, homogeneous.
Solution:	1.5% solution, soluble in distilled or deionized water. Solution is very light amber to light amber, clear without significant precipitate.
Prepared Medium:	Light amber, clear without significant precipitate.
Reaction of 1.5% Solution at 25°C:	pH 4.7 ± 0.2

Cultural Response

Prepare Malt Extract Agar or Malt Extract Broth per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Aspergillus niger	16404	100-1,000	good
Candida albicans	10231	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Summary And Explanation

The use of malt and malt extracts for the propagation of yeasts and molds is quite common. Reddish[1] described a culture medium prepared from malt extract that was a satisfactory substitute for wort. Thom and Church,[2] following the formula of Reddish, used Malt Extract as a base from which they prepared the complete media. Malt Extract Broth is specified in standard methods for the examination of yeasts and molds.[3,4]

Principles Of the Procedure

Malt Extract Agar contains Maltose as an energy source. Dextrin, a polysaccharide derived from high quality starch, and Glycerol are included as carbon sources. Peptone is provided as a nitrogen source. Bacto Agar is the solidifying agent.

Malt Extract Broth contains Malt Extract Base which provides the carbon, protein, and nutrient sources required for growth of microorganisms. Maltose is added as an energy source. Dextrose is included as a source of fermentable carbohydrate. Yeast Extract provides the vitamins and cofactors required for growth and additional sources of nitrogen and carbon.

The acidic pH of Malt Extract Agar and Broth allow for the optimal growth of molds and yeasts while restricting bacterial growth.

Formula

Malt Extract Agar

Formula per liter

Maltose	12.75 g
Bacto Dextrin	2.75 g
Bacto Glycerol	2.35 g
Bacto Peptone	0.78 g
Bacto Agar	15 g

Final pH 4.7 ± 0.2 at 25°C

Malt Extract Broth

Formula per liter

Malt Extract Base	6 g
Maltose	1.8 g
Bacto Dextrose	6 g
Bacto Yeast Extract	1.2 g

Final pH 4.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage
Store the dehydrated media below 30°C. The dehydrated media are very hygroscopic. Keep containers tightly closed.

Expiration Date
The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure
Materials Provided
Malt Extract Agar
Malt Extract Broth

Materials Required But Not Provided
Glassware
Autoclave
Incubator

Method of Preparation
1. **Malt Extract Agar**: Suspend 33.6 grams in 1 liter of distilled or deionized water. Heat to boiling to dissolve completely. **Malt Extract Broth**: Dissolve 15 grams in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes. Avoid overheating which could cause a softer medium.

Specimen Collection and Preparation
Refer to appropriate references for specimen collection and preparation.

Test Procedure
See appropriate references for specific procedures.

Results
Refer to appropriate references and procedures for results.

References

1. Abs. Bact., **3**:6, 1919.
2. **Thom, C., and M. B. Church.** 1926. The Aspergilli. Williams and Wilkins Co., Baltimore.
3. **MacFaddin, J.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams and Wilkins, Baltimore.
4. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International. Gaithersburg, MD.

Packaging
Malt Extract Agar	500 g	0112-17
	10 kg	0112-08
Malt Extract Broth	500 g	0113-17
	10 kg	0113-08

Bacto® Mannitol Salt Agar

Intended Use
Bacto Mannitol Salt Agar is used for isolating and differentiating staphylococci.

Summary and Explanation
Chapman[1] formulated Mannitol Salt Agar to isolate staphylococci by inhibiting the growth of most other bacteria with a high salt concentration. He added 7.5% sodium chloride to Phenol Red Mannitol Agar and noted that pathogenic strains of staphylococci (coagulase-positive staphylococci) grew luxuriantly and produced yellow colonies with yellow zones in the surrounding medium. Nonpathogenic staphylococci produced small red colonies with no color change to the surrounding medium.

Because Mannitol Salt Agar is selective, specimens from heavily contaminated sources may be streaked onto this medium without danger of overgrowth[2]. Mannitol Salt Agar is recommended for isolating pathogenic staphylococci from clinical specimens,[2] from cosmetics,[3] and for microbial limit tests.[4]

Principles of the Procedure
Mannitol Salt Agar contains Proteose Peptone No. 3 and Beef Extract as sources of carbon, nitrogen, vitamins and minerals. D-Mannitol is the carbohydrate source. Sodium Chloride, in high concentration, inhibits most bacteria other than staphylococci. Phenol Red is the pH indicator. Bacto Agar is the solidifying agent.

Bacteria that grow in the presence of a high salt concentration and ferment mannitol produce acid products which turn the phenol red pH indicator from red to yellow. Typical pathogenic staphylococci (coagulase-positive staphylococci) ferment mannitol and form yellow colonies with yellow zones around the colonies. Typical non-pathogenic staphylococci do not ferment mannitol and form red colonies.

Formula
Mannitol Salt Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	1 g
Bacto D-Mannitol	10 g
Sodium Chloride	75 g
Bacto Agar	15 g
Bacto Phenol Red	0.025 g

Final pH 7.4 ± 0.2 at 25°C

Precautions
1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage
Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date
The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Mannitol Salt Agar

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 111 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect specimens as appropriate for the specimen and transport immediately to the laboratory in accordance with recommended guidelines.
2. Process each specimen as appropriate for that specimen.

Test Procedure

Inoculate specimen onto medium as a primary isolation or inoculate isolated colonies onto medium for differentiation.

Results

Staphylococci will grow on this medium while the growth of most other bacteria will be inhibited. Coagulase-positive staphylococci will produce luxuriant growth of yellow colonies with yellow zones around them. Coagulase negative staphylococci will produce small red colonies with no color change to the medium surrounding them.

References

1. **Chapman, G. H.** The significance of sodium chloride in studies of staphylococci. J. Bacteriol. **50**:201.
2. **Kloos, W. E., and T. L. Bannerman.** 1995. Staphylococcus and Micrococcus. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Hitchins, A. D., T. T. Tran, and J. E. McCarron.** 1995. Microbiology methods for cosmetics, p. 23.01-23.12. In Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.

Packaging

Mannitol Salt Agar	100 g	0306-15
	500 g	0306-17

User Quality Control

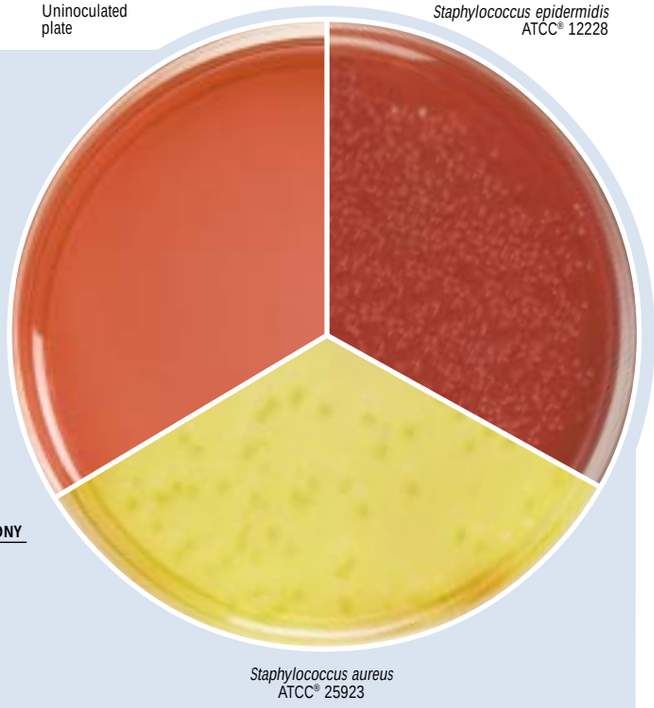
Identity Specifications

Dehydrated Appearance:	Light pink, free-flowing, homogeneous.
Solution:	11.1% solution, soluble in distilled or deionized water on boiling. Solution is red, slightly opalescent.
Prepared Medium:	Pinkish red, slightly opalescent.
Reaction of 11.1% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Mannitol Salt Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 to 48 hours. A yellow zone surrounding a colony indicates mannitol has been fermented.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE OF MEDIUM AND COLONY
Enterobacter aerogenes	13048*	1,000-2,000	marked to complete inhibition	–
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition	–
Proteus mirabilis	12453	1,000-2,000	partial inhibition	–
Staphylococcus aureus	25923*	100-1,000	good	yellow colony with yellow zone
Staphylococcus epidermidis	12228*	100-1,000	good	red colony with no zone of color change



The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Bacto® Marine Agar 2216
Bacto Marine Broth 2216

Intended Use

Bacto Marine Agar 2216 and Bacto Marine Broth 2216 are used for cultivating heterotrophic marine bacteria.

Summary and Explanation

Marine bacteria are present in nutrient sea water by the millions per ml and are essential to the life cycle of all marine flora and fauna. The enumeration and activity of marine bacteria are important to the food industry for the conservation of marine life. Marine Agar 2216 and Marine Broth 2216 are prepared according to the formula of ZoBell[1] The media contain all of the nutrients necessary for the growth of marine bacteria. The media contain minerals that nearly duplicate the major mineral composition of sea water,[2] in addition to Bacto Peptone and Yeast Extract that provide a good source of nutrients.

In the use of Marine Agar 2216, the conventional pour plate and spread plate techniques of enumeration are used. For the pour plate technique, the agar must be cooled to 42°C before inoculation because of the thermo-sensitive nature of most marine bacteria. In the spread plate technique, the agar is poured while hot and allowed to cool and solidify before inoculation. This latter method was reported by Buck and Cleverdon[3] to give higher counts than the pour plate method because of the increased growth of the thermo-sensitive bacteria.

Sizemore and Stevenson[4] used Marine Agar 2216 routinely as the upper nutrient layer of a marine agar-milk agar double-layer plate. This two layer plate was developed for isolating proteolytic marine bacteria. Marine Agar 2216 was also used in studies characterizing a marine bacterium associated with *Crassostrea virginica* (the Eastern Oyster).[5]

Principles of the Procedure

Bacto Peptone and Yeast Extract provide nitrogen, vitamins and minerals.

The high salt content helps to simulate sea water. Numerous minerals are also included to duplicate the major mineral composition of sea water. Bacto Agar is a solidifying agent.

Formula

Marine Agar 2216

Formula Per Liter

Bacto Peptone	5 g
Bacto Yeast Extract	1 g
Ferric Citrate	0.1 g
Sodium Chloride	19.45 g
Magnesium Chloride	8.8 g
Sodium Sulfate	3.24 g
Calcium Chloride	1.8 g
Potassium Chloride	0.55 g
Sodium Bicarbonate	0.16 g
Potassium Bromide	0.08 g
Strontium Chloride	0.034 g
Boric Acid	0.022 g
Sodium Silicate	0.004 g
Sodium Fluoride	0.0024 g
Ammonium Nitrate	0.0016 g
Disodium Phosphate	0.008 g
Bacto Agar	15 g

Final pH 7.6 ± 0.2 at 25°C

Marine Broth 2216

Formula Per Liter

Bacto Peptone	5 g
Bacto Yeast Extract	1 g
Ferric Citrate	0.1 g
Sodium Chloride	19.45 g
Magnesium Chloride Dried	5.9 g
Sodium Sulfate	3.24 g
Calcium Chloride	1.8 g
Potassium Chloride	0.55 g
Sodium Bicarbonate	0.16 g
Potassium Bromide	0.08 g
Strontium Chloride	0.034 g
Boric Acid	0.022 g
Sodium Silicate	0.004 g
Sodium Fluoride	0.0024 g
Ammonium Nitrate	0.0016 g
Disodium Phosphate	0.008 g

Final pH 7.6 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Marine Broth 2216**

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Kidneys, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared plates at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Marine Agar 2216
Marine Broth 2216

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50°C)
Glassware
Incubator (20-25°C)
Shaker

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water:
   Marine Agar 2216 - 55.1 grams;
   Marine Broth 2216 - 37.4 grams.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Marine Agar 2216: Cool agar to 45-50°C in a waterbath.
5. Dispense.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

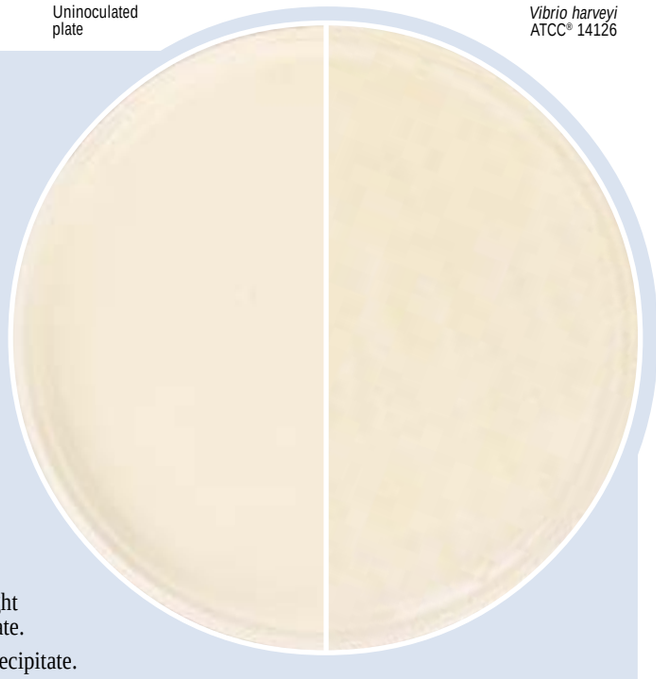
Consult appropriate references for recommended test procedures.[3,4]

Results

Refer to appropriate references and procedures for results.

References

1. **ZoBell, C. E.** 1941. Studies on marine bacteria. I. The cultural requirements of heterotrophic aerobes. J. Mar. Res. **4**:42-75.
2. **Lyman, J., and R. H. Fleming.** 1940. Composition of sea water. J. Marine Res. **3**:134.
3. **Buck, J. D., and R. C. Cleverdon.** 1960. The spread plate as a method for the enumeration of marine bacteria. Limnol. Oceanogr. **5**:78.



User Quality Control

Identity Specifications

Marine Agar 2216

Dehydrated Appearance:	Light beige with a few dark particles, free flowing, homogeneous.
Solution:	5.51% solution, soluble in distilled or deionized water on boiling. Solution is light amber, slightly opalescent to opalescent with a precipitate.
Prepared Plates:	Light amber, slightly opalescent to opalescent, may have a slight precipitate.
Reaction of 5.51% Solution at 25°C:	pH 7.6 ± 0.2

Marine Broth 2216

Dehydrated Appearance:	Light beige with a few dark particles, free flowing.
Solution:	3.74% solution, soluble in distilled or deionized water on boiling. Solution is light amber, slightly opalescent with a precipitate.
Prepared Flasks:	Light amber, slightly opalescent with a precipitate.
Reaction of 3.74% Solution at 25°C:	pH 7.6 ± 0.2

Cultural Response

Prepare Marine Agar 2216 per label directions. Inoculate and incubate at 20-25°C for 40-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Vibrio fischeri	7744	100-1,000	good
Vibrio harveyi	14126	100-1,000	good

Prepare Marine Broth 2216 per label directions. Dispense 50 ml amounts in 250 ml Erlenmeyer flasks. Inoculate and incubate at 20-25°C on a shaker for 40-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Vibrio fischeri	7744	100-1,000	good
Vibrio harveyi	14126	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

4. **Sizemore, R. K., and L. H. Stevenson.** 1970. Method for the isolation of proteolytic marine bacteria. Appl. Microbiol. **20**:991-992.
5. **Weiner, R. M., A. M. Segall, and R. R. Colwell.** 1985. Characterization of a marine bacterium associated with *Crassostrea virginica* (the Eastern Oyster). Appl. Environ. Microbiol. **49**:83-90.

Packaging

Marine Agar 2216	500 g	0979-17
Marine Broth 2216	500 g	0791-17

Bacto® Maximum Recovery Diluent

Intended Use

Bacto Maximum Recovery Diluent is an isotonic diluent containing a low level of peptone used for maintaining the viability of organisms during dilution procedures.

Summary and Explanation

Standard methods for the microbiological examination of foodstuffs require sample dilution to be carried out accurately to estimate the number of microorganisms. Diluents consisting of sterile saline, phosphate buffer solutions and distilled water have all been shown to have a lethal action on a wide range of organisms.[1,2]

The presence of low levels of peptone in the diluent at a pH of 7.0 ± 0.2 affords protection for bacteria for at least one hour during the dilution stage.[3,4] The presence of peptone also allows accurate quantitative procedures to be performed with minimal reductions in viable count in the diluent.

Principles of Procedure

Low levels of peptone help protect organisms in the diluent. Sodium Chloride maintains proper osmotic pressure.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Cream, free-flowing, homogeneous.
Solution:	0.95% solution, soluble in distilled or deionized water, colorless, clear.
Prepared Medium:	Colorless, clear.
Reaction of 0.95% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare the medium per label directions. Inoculate tubes with the test organism. At time zero and after 30 minutes, subculture 1.0 ml aliquots into Tryptic Soy Agar, using the pour plate technique. Incubate plates at 35°C for 18-24 hours.

ORGANISM	ATCC®	RECOVERY AFTER 30 MINUTES
Escherichia coli	25922*	no significant reduction
Staphylococcus aureus	25923*	no significant reduction

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

Formula per liter

Bacto Peptone	1 g
Sodium Chloride	8.5 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Maximum Recovery Diluent

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave

Method of Preparation

1. Dissolve 9.5 grams in 1 liter distilled or deionized water.
2. Dispense into final containers and cap loosely.
3. Autoclave at 121°C for 15 minutes.

Test Procedure

Consult appropriate references for dilution procedures when testing foods.[1,2,3,4]

Results

Refer to appropriate references and procedures for results.

References

1. **DeMello, G. C., I. S. Danielson, and J. S. Kiser.** 1951. The Toxic effect of buffered saline on the viability of *Brucella abortus*. J. Lab. Clin. Med. **37**:579-583.

2. **Gunter, S. E.** 1954. Factors determining the viability of selected microorganisms in inorganic media. J. Bacteriol. **67**:628-634.
3. **Straka, R. P., and J. L. Stokes.** 1957. Rapid destruction of bacteria in commonly used diluents and its eliminations. Appl. Microbiol. **5**:21-25.
4. **Patterson, J. T., and J. A. Cassells.** 1963. An examination of the value of adding peptone to diluents used in the bacteriological testing of bacon curing brines. J. Appl. Bacteriol. **26**:493-497.

Packaging

Maximum Recovery Diluent	500 g	1897-17
	5 kg	1897-03

Bacto® McBride Listeria Agar

Intended Use

Bacto McBride Listeria Agar is used for isolating *Listeria monocytogenes* with or without the addition of blood.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[1] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[2] The first reported food-borne outbreak of listeriosis was in 1985[3] and, since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[4]

Implicated vehicles of transmission include turkey frankfurters,[5] coleslaw, pasteurized milk, Mexican-style cheese, paté and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants. *Listeria* species are ubiquitous in nature, being present in a wide range of unprocessed foods, soil, sewage, silage and river water.[6]

Listeria species grow over a pH range of 5.0-9.6 and survive in food products with pH levels outside of these parameters.[7] *Listeria* species are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are streptococci, especially the enterococci, micrococci and *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[8]

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

McBride Listeria Agar is prepared according to the formulation of McBride and Girard,[9] who originally described the medium and its use in the selective isolation of *Listeria* from experimentally mixed cultures. The medium has been recommended for isolating *Listeria* from clinical specimens,[10] raw milk[11,12] and food samples.[13]

When the medium is used as a blood agar, a narrow zone of beta-hemolysis may be evident around and under *Listeria* colonies.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, homogeneous with soft lumps.
Solution:	4.6% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Medium without Blood:	Light to medium amber, slightly opalescent.
Prepared Medium with Blood:	Cherry red, opaque.
Reaction of 4.6% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare McBride Listeria Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Enterococcus faecalis	29212*	1,000-2,000	marked to complete inhibition
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition
Listeria monocytogenes	19114	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Tryptose and Beef Extract provide nitrogen, vitamins and minerals. Sodium Chloride maintains the osmotic balance of the medium. Partial selectivity is provided by Lithium Chloride, Glycine and Phenylethanol, which aid in suppressing both gram-positive and gram-negative bacteria other than *Listeria*. If increased inhibition of fungi is also needed, cycloheximide can be added after autoclaving. Bacto Agar is a solidifying agent.

Formula

McBride Listeria Agar

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	3 g
Sodium Chloride	5 g
Glycine	10 g
Lithium Chloride	0.5 g
Phenylethanol	2.5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Face, Muscles, Nerves, Urogenital.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The powder is very hygroscopic. Keep container tightly closed.

Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

McBride Listeria Agar

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50°C)
Defibrinated blood (optional)
Cycloheximide (optional)
Petri dishes
Incubator (35°C)

Method of Preparation

1. Suspend 46 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 45-50°C in a waterbath.
5. To enhance selectivity and/or differentiation, aseptically add cycloheximide (0.2 grams/liter) and/or sterile defibrinated blood to the medium. Mix well.
6. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[10,12,13]
2. Process each specimen, using procedures appropriate for that specimen or sample.[10,12,13]

Test Procedure

When testing clinical specimens for *Listeria*, inoculate directly onto primary plating media and McBride Listeria Agar.[10]

When isolating *Listeria* from raw milk and food samples, refer to appropriate references.[12,13]

Results

Observe colonies under oblique transmitted light. *Listeria* colonies should display a grey to blue color with a ground glass appearance.

References

1. **Murray, E. G. D., R. A. Webb, and M. B. R. Swann.** 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n. sp.). J. Path. Bact. **29:**407- 439.
2. **Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low- and high-fat, frozen and refrigerated ground beef. J. Food Prot. **57**:969-974.
3. **Wehr, H. M.** 1987. *Listeria monocytogenes* - a current dilemma special report. J. Assoc. Off. Anal. Chem. **70**:769-772.
4. **Bremer, P. J., and C. M. Osborne.** 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels (*Perna canaliculus*) prepared for hot smoking. J. Food Prot. **58**:604-608.
5. **Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
6. **Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
7. **Donnelly, C. W., R. E. Bracket, D. Doores, W. H. Lee, and J. Lovett.** 1992. *Listeria*, p. 637-663. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
8. **Kramer, P. A., and D. Jones**. 1969. Media selective for *Listeria monocytogenes*. J. Appl. Bacteriol. **32:**381-394.
9. **McBride, M. E., and K. F. Girard.** 1960. A selective method for the isolation of *Listeria monocytogenes* from mixed bacterial populations. J. Lab. Clin. Med. **55**:153-157.
10. **Pezzlo, M. (ed.).** 1992. Aerobic bacteria, p. 1.4.8. *In* H. D. Isenberg (ed), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
11. **Hayes, P. S., J. C. Feeley, L. M. Graves, G. W. Ajello, and D. W. Fleming**. 1986. Isolation of *Listeria monocytogenes* from raw milk. Appl. Environ. Microbiol. **51**:438-440.
12. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products. *In* R. T. Marshall (ed), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

13. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

McBride Listeria Agar	500 g	0922-17

McClung Toabe Agar

Bacto® McClung Toabe Agar Base · Bacto Egg Yolk Enrichment 50%

Intended Use

Bacto McClung Toabe Agar Base is used with Bacto Egg Yolk Enrichment 50% for isolating and detecting *Clostridium perfringens* in foods based on the lecithinase reaction.

Summary and Explanation

McClung and Toabe[1] formulated a medium for isolating *C. perfringens* from foods. With the addition of 50% egg yolk emulsion, *C. perfringens* and a few other *Clostridium* species show the lecithinase reaction.

C. perfringens is found in raw meats, poultry, dehydrated soups and sauces, raw vegetables and other foods and food ingredients, but occurrences of food borne illness are usually associated with cooked meat or poultry products.[2] Spores of some strains that may resist heat during cooking germinate and grow in foods that are not adequately refrigerated.[3] Enumerating the microorganism in food samples plays a role in epidemiological investigation of outbreaks of food borne illness.[2]

User Quality Control

Identity Specifications

McClung Toabe Agar Base

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	7.5% solution, soluble in distilled or deionized water on boiling. Solution is light amber, opalescent, with a precipitate.
Prepared Medium:	Light yellow, smooth, opaque.
Reaction of 7.5% Solution at 25°C:	pH 7.6 ± 0.2

Egg Yolk Enrichment 50%

Appearance:	Canary yellow, opaque liquid with a resuspendable precipitate.

Cultural Response

McClung Toabe Agar Base with Egg Yolk Enrichment 50%

Prepare McClung Toabe Agar Base with Egg Yolk Enrichment 50% per label directions. Inoculate and incubate the plates at 35 ± 2°C anaerobically for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	LECITHINASE REACTION
Clostridium perfringens	12919	100-1,000	good	opaque halo
Clostridium perfringens	12924	100-1,000	good	opaque halo
Staphylococcus aureus	25923*	100-1,000	good	opaque halo
Staphylococcus epidermidis	14990	100-1,000	good	none

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

McClung Toabe Agar Base contains Proteose Peptone as a source of carbon, nitrogen, vitamins and minerals. Dextrose is the carbohydrate source. Sodium Chloride maintains the osmotic balance of the medium. Magnesium Sulfate provides divalent cations and sulfate. Sodium Phosphate Dibasic and Potassium Phosphate Monobasic maintain pH balance and provide a source of phosphates. Bacto Agar is the solidifying agent. Egg Yolk Enrichment 50% provides egg yolk lecithin. Lecithinase-producing clostridia, such as *C. perfringens*, hydrolyze the lecithin and produce opaque halos.

Formula

McClung Toabe Agar Base

Formula Per Liter

Bacto Proteose Peptone	40 g
Bacto Dextrose	2 g
Sodium Phosphate Dibasic	5 g
Potassium Phosphate Monobasic	1 g
Sodium Chloride	2 g
Magnesium Sulfate	0.1 g
Bacto Agar	25 g

Final pH 7.6 ± 0.2 at 25°C

Egg Yolk Enrichment 50%

Sterile concentrated egg yolk emulsion

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated McClung Toabe Agar Base medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store the Egg Yolk Enrichment 50% at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

McClung Toabe Agar Base
Egg Yolk Enrichment 50%

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator, anaerobic (35°C)

Method of Preparation

McClung Toabe Agar Base

1. Suspend 75 grams of McClung Toabe Agar Base in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense 90 ml amounts into flasks.
4. Autoclave at 121°C for 20 minutes. Cool to 50°C.
5. Aseptically add 10 ml Egg Yolk Enrichment 50% to each flask of prepared agar base.
6. Mix thoroughly.
7. Pour into sterile Petri dishes in approximately 15 ml amounts.

Egg Yolk Enrichment 50%

1. Ready for use.
2. Shake gently to resuspend precipitate.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **McClung, L. S., and R. Toabe**. 1947. The egg yolk plate reaction for the presumptive diagnosis of *Clostridium sporogenes* and certain species of the gangrene and botulinum groups. J. Bact. **53**:139.
2. **Labbe, R. G., and S. M. Harmon.** 1992. *Clostridium perfringens*, p. 623-635. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Rhodehamel, E. J., and S. M. Harmon**. 1995. *Clostridium perfringens*, p. 16.01- 16.06. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

McClung Toabe Agar Base	500 g	0941-17
Egg Yolk Enrichment 50%	12x10 ml	3347-61*
	6x100 ml	3347-73*

*Store at 2-8°C

Methyl Red and Voges-Proskauer Tests

Bacto® MR-VP Medium · SpotTest™ Voges-Proskauer Reagent A SpotTest Voges-Proskauer Reagent B

Intended Use

Bacto MR-VP Medium is used for differentiating coliform organisms based on the methyl red and Voges-Proskauer tests.

SpotTest Voges-Proskauer Reagents A and B are used for determining the VP reaction of bacteria.

Also Known As

MR-VP Medium is also known as Methyl Red-Voges Proskauer Medium.

Summary and Explanation

In 1915, Clark and Lubs[1] demonstrated that the colon-aerogenes family of bacteria could be divided into two groups based on their action in a peptone and dextrose medium. When tested with the pH indicator methyl red, the "coli" group produced high acidity while the "aerogenes" group produced a less acid reaction. The test to detect high-acid end products is known as the Methyl Red (MR) test. The test to detect less-acid end products is based on the procedure described by Voges and Proskauer in 1898.[2] A color reaction occurs when certain cultures, incubated in a medium containing peptone and dextrose, are treated with potassium hydroxide and exposed to air. This reaction detects the formation of acetylmethylcarbinol and is known as the Voges-Proskauer (VP) Test.

The MR and VP tests appear in the identification scheme for the *Enterobacteriaceae*,[3] which are important isolates in clinical microbiology,[3] as well as in the microbiology of foods and dairy products.[4,5]

The MR and VP tests are used to complete and confirm the identification of *Escherichia coli*. [4,5,6]

Principles of the Procedure

MR-VP Medium contains Buffered Peptone as a carbon and nitrogen source for general growth requirements. Dextrose is a fermentable carbohydrate.

Members of the *Enterobacteriaceae* convert glucose to pyruvate by the Embden-Meyerhof pathway. Some bacteria metabolize pyruvate by the mixed acid pathway and produce acidic end products (pH < 4.4), such as lactic, acetic and formic acids. Other bacteria metabolize pyruvate by the butylene glycol pathway and produce neutral end products (pH > 6.0), one of which is acetoin (acetylmethylcarbinol). In the MR test, the pH indicator methyl red detects acidic end products.[7] In the VP test, acetoin is oxidized in the presence of oxygen and potassium hydroxide (KOH) to diacetyl, which produces a red color.[8]

User Quality Control

Identity Specifications

MR-VP Medium

Dehydrated Appearance:	Very light to light beige, free-flowing, homogeneous.
Solution:	1.7% solution, soluble in distilled or deionized water. Solution is light amber, clear.
Prepared Medium:	Light amber, clear.
Reaction of 1.7% Solution at 25°C:	pH 6.9 ± 0.2

SpotTest Voges-Proskauer Reagent A

Appearance:	Yellow to dark amber, clear solution inside a glass ampule contained in a plastic dispenser.

SpotTest Voges-Proskauer Reagent B

Appearance:	Colorless, clear solution inside a glass ampule contained in a plastic dispenser.

Cultural Response

MR-VP Medium, SpotTest Voges-Proskauer Reagent A or SpotTest Voges-Proskauer Reagent B

Prepare MR-VP Medium per label directions. Inoculate and incubate at 35 ± 2°C for 24-48 hours or up to 5 days. Determine the methyl red and Voges-Proskauer test reactions.

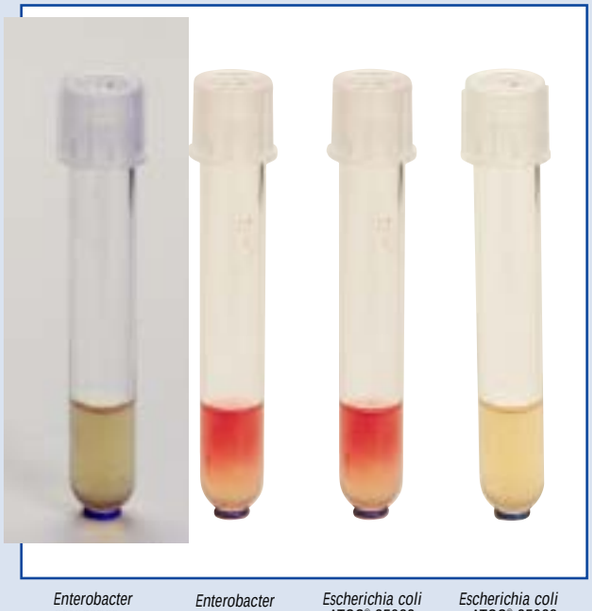
ORGANISM	ATCC®	INOCULUM	GROWTH	APPEARANCE MR TEST	APPEARANCE VP TEST
Enterobacter aerogenes	13048*	undiluted	good	–/yellow	+/red
Escherichia coli	25922*	undiluted	good	+/red	–/no change

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube | *Enterobacter aerogenes* ATCC® 13048 | *Escherichia coli* ATCC® 25922

MR-VP Medium



Enterobacter aerogenes ATCC® 13048 MR Test | *Enterobacter aerogenes* ATCC® 13048 VP Test | *Escherichia coli* ATCC® 25922 MR Test | *Escherichia coli* ATCC® 25922 VP Test

The addition of -naphthol (SpotTest Voges-Proskauer Reagent A) before KOH (SpotTest Voges-Proskauer Reagent B) enhances the sensitivity of the test.[8]

Formula

MR-VP Medium

Formula Per Liter

Buffered Peptone	7 g
Dipotassium Phosphate	5 g
Bacto Dextrose	5 g

Final pH 6.9 ± 0.2 at 25°C

SpotTest Voges-Proskauer Reagent A

Formula Per Liter

α-Naphthol	50 g
Ethyl Alcohol (absolute)	1,000 ml

SpotTest Voges-Proskauer Reagent B

Formula Per Liter

Potassium Hydroxide	400 g
Distilled Water	1,000 ml

Precautions

1. For Laboratory Use.
2. **SpotTest Voges-Proskauer Reagent A**

   HIGHLY FLAMMABLE. IRRITANT. HIGHLY FLAMMABLE. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe mist. Keep away from sources of ignition. No smoking. Keep container tightly closed. Target Organs: Liver, Blood.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   SpotTest Voges-Proskauer Reagent B

   CORROSIVE. CAUSES SEVERE BURNS. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated MR-VP Medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Store SpotTest Voges-Proskauer Reagents A and B at 15-30°C. Protect from light.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

MR-VP Medium
SpotTest Voges-Proskauer Reagent A
SpotTest Voges-Proskauer Reagent B

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35 ± 2°C)
Test tubes with caps
Test tubes, 13 x 100 mm
Methyl red indicator[7]

Method of Preparation

MR-VP Medium

1. Dissolve 17 grams in 1 liter distilled or deionized water.
2. Distribute into test tubes. Autoclave at 121°C for 15 minutes.

Methyl Red Indicator

1. Dissolve 0.1 gram of methyl red in 300 ml of 95% ethyl alcohol.
2. Add sufficient distilled or deionized water to make 500 ml.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate MR-VP Medium with growth from a single colony.
2. Incubate at 35 ± 2°C for 48 hours.
3. Test as follows.

Methyl Red Test

1. Transfer 2.5 ml of the MR-VP Medium culture to a tube (13 x 100 mm).
2. Add 5 drops of methyl red indicator and observe for a color change.

VP Test

1. Transfer 2.5 ml of the MR-VP Medium culture to a tube (13 x 100 mm).
2. Add 0.3 ml (6 drops) of SpotTest Voges-Proskauer Reagent A (5% α-naphthol).
3. Add 0.1 ml (2 drops) of SpotTest Voges-Proskauer Reagent B (40% KOH).
4. Gently agitate the tube and let stand for 10 to 15 minutes.
5. Observe for a color change.

Other Methods

SpotTest Voges-Proskauer Reagent A and Reagent B are suitable for use in other modifications of the Voges-Proskauer test requiring the use of these reagents.

Alternate VP Tube Method

1. Inoculate MR-VP Medium with the test organism and incubate at 35°C for 24-48 hours.
2. Aseptically transfer 1 ml of the incubated MR-VP Medium culture to a clean test tube.
3. Add 15 drops of Voges-Proskauer Reagent A followed by 5 drops of Voges-Proskauer Reagent B.
4. Shake gently to aerate.
5. Examine for the appearance of a red color within 20 minutes.
6. If the 24-hour test is negative, repeat the test with a 48-hour culture of the test organism. If equivocal results are obtained, repeat the test with cultures incubated for 5 days at 25-30°C.

Rapid Micro Method

1. Inoculate 0.2 ml of MR-VP Medium with the test organism.
2. Incubate for 4 hours at 35°C.
3. Add 0.1 ml of 0.3% creatine solution.
4. Add 5 drops of Voges-Proskauer Reagent A followed by 2 drops of Voges-Proskauer Reagent B.
5. Shake gently to aerate.
6. Examine for the appearance of a red color within 20 minutes.

Results

Methyl Red (MR) Test

Positive:	Bright red color.
Negative:	Yellow-orange color.

Note: If the test is negative, continue to incubate the broth without added reagent; repeat the test after an additional 18 to 24 hours incubation.

Voges-Proskauer (VP) Test

Positive:	Red color.
Negative:	No red color.

Limitations of the Procedure

1. Results of the MR and VP tests need to be used in conjunction with other biochemical tests to differentiate genus and species within the *Enterobacteriaceae*.
2. A precipitate may form in the potassium hydroxide reagent solution. This precipitate has not been shown to reduce the effectiveness of the reagent.
3. Most members of the family *Enterobacteriaceae* give either a positive MR test or a positive VP test. However, certain organisms such as *Hafnia alvei* and *Proteus mirabilis* may give a positive result for both tests.
4. Incubation time for the Methyl Red test cannot be shortened by increasing the glucose concentration in the medium or by heavily inoculating the broth.[7]
5. Incubate MR-negative tests for more than 48 hours and test again. (See **Results** section.)
6. Read the VP test at 48 hours. Increased incubation may produce acid conditions in the broth that will interfere with reading the results.[8]
7. VP reagents must be added in the order and the amounts specified or a weak-positive or false-negative reaction may occur. A weak-positive reaction may be masked by a copper-like color which may form due to the reaction of KOH and α-naphthol.[8]
8. Read the VP test within 1 hour of adding the reagents. The KOH and α-naphthol may react to form a copper-like color, causing a potential false-positive interpretation.[8]
9. Due to the possible presence of acetoin, diacetyl or related substances in certain raw materials,[9] the use of media low in these substances (such as MR-VP Medium) is recommended for this test.

References

1. **Clark, W. M., and H. A. Lubs.** 1915. The differentiation of bacteria of the colon- aerogenes family by the use of indicators. J. Infect. Dis. **17**:160-173.
2. **Voges, O., and B. Proskauer.** 1898. Z. Hyg. **28**:20-22.
3. **Farmer, J. J., III**. 1995. Enterobacteriaceae: Introduction and identification, p. 438-449. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
6 **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
7. **Isenberg, H. D. (ed.).** 1994. Clinical microbiology procedures handbook, sup. 1, 1.19.48. American Society for Microbiology, Washington, D.C.
8. **Isenberg, H. D. (ed.).** 1994. Clinical microbiology procedures handbook, sup. 1, 1.19.58. American Society for Microbiology, Washington, D.C.
9. **Barritt, M. M.** 1936. The intensification of the Voges-Proskauer reaction by the addition of alpha-naphthol. J. Pathol. **42**:441-454.

Packaging

MR-VP Medium	100 g	0016-15
	500 g	0016-17
	2 kg	0016-07
SpotTest Voges-Proskauer Reagent A	50 x 0.75 ml	3558-26
SpotTest Voges-Proskauer Reagent B	50 x 0.75 ml	3559-26

Bacto® Micro Assay Culture Agar
Bacto Micro Inoculum Broth

Intended Use

Bacto Micro Assay Culture Agar is used for cultivating lactobacilli and other organisms used in microbiological assays.

Bacto Micro Inoculum Broth is used for preparing the inoculum of lactobacilli and other microorganisms used in microbiological assays of vitamins and amino acids.

Summary and Explanation

Three types of media are used in the microbiological assay of vitamins:

1. Maintenance Media, which preserve the viability and sensitivity of the test culture for its intended purpose;
2. Inoculum Media, which condition the test culture for immediate use; and,
3. Assay Media, which permit quantitation of the vitamin under test. Assay media contain all factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Micro Assay Culture Agar is used for maintaining stock cultures of lactobacilli and other test microorganisms. This medium is also used for general cultivation of lactobacilli.

Micro Inoculum Broth is used for cultivating lactobacilli and preparing the inoculum for microbiological assays.

User Quality Control

Identity Specifications

Micro Assay Culture Agar

Dehydrated Appearance:	Light tan to tan, free-flowing, homogeneous.
Solution:	4.7% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 4.7% Solution at 25°C:	pH 6.7 ± 0.2

Micro Inoculum Broth

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	3.7% solution, soluble in distilled or deionized water. Light to medium amber in color, clear to very slightly opalescent without significant precipitate.
Prepared Medium:	Light to medium amber, clear to very slightly opalescent.
Reaction of 3.7% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare Micro Assay Culture Agar and Micro Inoculum Broth per label directions. Inoculate tubes with test organisms. Incubate Micro Assay Culture Agar at 35 ± 2°C for 18-48 hours, incubate Micro Inoculum Broth at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus hirae	804	100-1,000	good
Lactobacillus casei subsp. *rhamnosus*	7469	100-1,000	good
Lactobacillus delbrueckii subsp. *lactis*	7830	100-1,000	good
Lactobacillus plantarum	8014	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Principles of the Procedure

Proteose Peptone No. 3 provides nitrogen and amino acids in both Micro Assay Culture Agar and Micro Inoculum Broth. Yeast Extract is a vitamin source. Dextrose is a carbon source. Monopotassium phosphate is a buffering agent. Sorbitan monooleate complex (Micro Inoculum Broth) and Polysorbate 80 (Micro Assay Culture Agar) act as emulsifiers. Bacto Agar is a solidifying agent (Micro Assay Culture Agar).

Formula

Micro Assay Culture Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	20 g
Bacto Dextrose	10 g
Monopotassium Phosphate	2 g
Polysorbate 80	0.1 g
Bacto Agar	10 g

Final pH 6.7 ± 0.2 at 25°C

Micro Inoculum Broth

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	20 g
Bacto Dextrose	10 g
Monopotassium Phosphate	2 g
Sorbitan Monooleate Complex	0.1 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. Take care to avoid contamination of media and glassware used in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used.

Storage

Store the dehydrated media below 30°C. The media are very hygroscopic. Keep containers tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Micro Assay Culture Agar
Micro Inoculum Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Inoculating needle
0.9% NaCl

Method of Preparation

Micro Assay Culture Agar

1. Suspend 47 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve.
3. Dispense 10 ml amounts into 16-20 mm diameter tubes.
4. Autoclave at 121°C for 15 minutes.
5. Agitate tubes prior to solidification to disperse the flocculent precipitate.

Micro Inoculum Broth

1. Dissolve 37 grams in 1 liter distilled or deionized water.
2. Dispense 10 ml amounts into tubes of 16-20 mm diameter.
3. Autoclave at 121°C for 15 minutes.

Stock Cultures

1. Prepare stock cultures in triplicate on Micro Assay Culture Agar, inoculating tubes using a straight-wire inoculating needle.
2. Incubate tubes at 30-37°C for 18-24 hours.
3. Store at 2-8°C.
4. Transfer cultures at weekly or twice-monthly intervals.

Assay Inoculum

1. Subculture from a 16-24 hour stock culture of lactobacilli in Micro Assay Culture Agar into a 10 ml tube of Micro Inoculum Broth.
2. Incubate at 35-37°C for 16-24 hours or as specified in the assay procedure.
3. Centrifuge the culture and decant the supernatant.
4. Resuspend cells in 10 ml of sterile 0.9% NaCl solution or sterile single strength basal assay medium.
5. Wash the cells by centrifuging and decanting the supernatant two additional times unless otherwise indicated.
6. Dilute the washed suspension 1:100 with sterile 0.9% single strength basal assay medium or as indicated. Where applicable, adjust inoculum concentration according to limits specified in AOAC[1] or US Pharmacopeia.[2]

Specimen Collection and Preparation

Prepare samples for assay according to references given in the specific assay procedure. Dilute assay samples to approximately the same concentration as the standard solution.

Test Procedure

For a complete discussion of vitamin assay methodology, refer to appropriate procedures.[1,2]

Results

For test results on vitamin assay procedures, refer to appropriate procedures.[1,2]

Limitations of the Procedure

1. Test organisms used in assay procedures must be cultured and maintained on media recommended for this purpose.
2. Follow assay directions exactly. The age, preparation and size of inoculum are extremely important factors in obtaining a satisfactory assay result.
3. Although other media and methods may be used successfully for maintaining cultures and preparing inocula, uniformly good results will be obtained if the methods described are followed exactly.
4. Aseptic technique should be used throughout the microbiological assay procedure.
5. The use of altered or deficient media may create mutants having different nutritional requirements. Such organisms will not produce a satisfactory test response.

References

1. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
2. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention Inc. Rockville, MD.

Packaging

Micro Assay Culture Agar	100 g	0319-15
	500 g	0319-17
Micro Inoculum Broth	500 g	0320-17

Bacto® Microbial Content Test Agar

Intended Use

Bacto Microbial Content Test Agar is recommended for the detection of microorganisms on surfaces sanitized with quaternary ammonium compounds.

Also Known as

"Tryptic Soy Agar with Lecithin and Polysorbate 80" (TSALT) and "Casein Soy Peptone Agar with Polysorbate 80 and Lecithin" are common terms for Microbial Content Test Agar. Tween 80® is also known as Polysorbate 80.

Summary and Explanation

Microbial Content Test Agar is a modification of Tryptic Soy Agar with Lecithin and Tween 80. The formulation is recommended for determining the sanitation efficiency of containers, equipment and work areas (environmental monitoring). The Lecithin and Tween in the formula inactivate some preservatives that may inhibit bacterial

growth, reducing "preservative carryover."[1] The formulation is recommended for the Aerobic Plate Count (Microbial Limit Test) for water miscible cosmetic products containing preservatives.[1]

Principles of the Procedure

Microbial Content Test Agar contains Tryptone and Soytone which provide the carbon and nitrogen sources required for growth of a wide variety of organisms. Lecithin and Polysorbate 80 are added to neutralize surface disinfectants.[2,3,4] Lecithin is added to neutralize quaternary ammonium compounds. Polysorbate 80 is incorporated to neutralize phenols, hexachlorophene, formalin and, with lecithin, ethanol.[5] Sodium Chloride provides osmotic equilibrium. Bacto Agar has been incorporated into this medium as a solidifying agent.

Formula

Microbial Content Test Agar

Formula Per Liter

Bacto Tryptone	15 g
Bacto Soytone	5 g
Sodium Chloride	5 g
Lecithin	0.7 g
Polysorbate 80	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous, may appear moist.
Solution:	4.57% solution soluble in distilled or deionized water on boiling with frequent gentle swirling. At approximately 50°C, solution is medium amber in color, slightly opalescent, with a resuspendable precipitate. At higher temperatures, it is more opalescent.
Prepared Medium:	Medium amber, slightly opalescent, may have a precipitate.
Reaction of 4.57% Solution at 25°C	pH 7.3 ± 0.2

Cultural Response

Prepare medium per label directions. Test Microbial Content Test Agar in parallel with Plate Count Agar. Inoculate liquid media with test organisms and pour plates. After the plates dry, apply disks impregnated with varying dilutions of a quaternary ammonium compound to the medium surface. Incubate plates at 35 ± 2°C for 40-48 hours and inspect for zones of inhibition.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	11229	100-1,000	smaller zone of inhibition of growth compared to Plate Count Agar
Staphylococcus aureus	6538P	100-1,000	smaller zone of inhibition of growth compared to Plate Count Agar

Interpretation: The smaller zones of inhibition indicate neutralization of quaternary ammonium compounds by Microbial Content Test Agar.

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Microbial Content Test Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Sterile Petri dishes

Method of Preparation

1. Suspend 45.7 g in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely with frequent careful agitation to dissolve, 1-2 minutes.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Consult appropriate references.[1,4]

Test Procedures

Microbial Content Test Agar is used in a variety of procedures. Consult appropriate references for further information.[1,4]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Because the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on the medium.
2. The effectiveness of preservative neutralization with this medium depends on both the type and concentration of the preservative(s).

References

1. **Orth, D. S.** 1993. Handbook of Cosmetic Microbiology. Marcel Dekker, Inc., New York, NY.
2. **Quisno, R., I. W. Gibby, and M. J. Foter**. 1946. A neutralizing medium for evaluating the germicidal potency of the quaternary ammonium salts. Am. J. Pharm. **118:**320-323.
3 **Erlandson, A. L., Jr., and C. A. Lawrence**. 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science **118:**274-276.
4. **Brummer, B.** 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. App. Environ. Microbiol. **32:**80-84.

5. **Favero (chm.)**. 1967. Microbiological sampling of surfaces - a state of the art report. Biological Contamination Control Committee, American Association for Contamination Control.

Packaging

Microbial Content Test Agar	500 g	0553-17
	2 kg	0553-07

Bacto® Middlebrook 7H9 Broth · Bacto Middlebrook 7H10 Agar Bacto Mycobacteria 7H11 Agar · Bacto Middlebrook ADC Enrichment · Bacto Middlebrook OADC Enrichment · Bacto Middlebrook OADC Enrichment w/WR 1339 · Bacto Glycerol

Intended Use

Bacto Middlebrook 7H9 Broth is used with Bacto Middlebrook ADC Enrichment and Bacto Glycerol or Bacto Tween® 80 for cultivating pure cultures of mycobacteria and preparing the tubercle emulsion for susceptibility testing.

Bacto Middlebrook 7H10 Agar is used with Bacto Middlebrook OADC Enrichment and Bacto Glycerol for isolating, cultivating and susceptibility testing of mycobacteria. The complete, prepared medium is also available.

Bacto Middlebrook 7H10 Agar is also used with Bacto Middlebrook OADC Enrichment w/WR 1339 to demonstrate cording in differentiating *Mycobacterium tuberculosis* from atypical mycobacteria.

Bacto Mycobacteria 7H11 Agar is used with Bacto Middlebrook OADC Enrichment and Bacto Glycerol for isolating, cultivating and susceptibility testing of fastidious strains of mycobacteria. The complete, prepared medium is also available.

Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.[1-3]

Because mycobacteria grow more rapidly in a broth medium, primary culture of all specimens in a broth medium such as Middlebrook 7H9 Broth is recommended.[6]

There are two types of solid culture media for the primary isolation of mycobacteria, those that have coagulated egg as a base (Lowenstein formulations) and those that have an agar base (Middlebrook formulations).

Egg-base media:

1. Support a wide variety of groups and species of mycobacteria;
2. Provide mycobacterial growth that can be used for niacin testing;
3. Have long shelf lives when refrigerated.[3]

User Quality Control

Identity Specifications

Middlebrook 7H9 Broth

Dehydrated Appearance:	Light beige, free-flowing, homogenous.
Solution:	0.47% solution, soluble in distilled or deionized water. Solution is very light amber, clear.
Prepared Medium:	Colorless to very light amber, clear.
Reaction of 0.47% Solution at 25°C:	6.6 ± 0.2

Middlebrook 7H10 Agar

Dehydrated Appearance:	Light beige with green tint, free-flowing, homogenous.
Solution:	1.9% solution, soluble in distilled or deionized water on boiling. Solution is light amber with a slight green tint, slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent without precipitate.
Reaction of 1.9% Solution at 25°C:	pH 6.6 ± 0.2

Mycobacteria 7H11 Agar

Dehydrated Appearance:	Light beige with a green tint, free-flowing, homogenous.
Solution:	0.21% solution, soluble in distilled or deionized water on boiling. Solution is light yellowish green, slightly opalescent.
Prepared Medium:	Light amber with a greenish tint, slightly opalescent, no precipitate.
Reaction of 0.21% Solution:	pH 6.6 ± 0.2

Middlebrook OADC Enrichment

Appearance:	Light amber, clear solution.

Middlebrook ADC Enrichment

Appearance:	Very light to light amber, clear solution.

Middlebrook OADC Enrichment w/WR 1339

Appearance:	Light amber, clear solution.

Glycerol

Identity Test:	ID positive; IR Spectrum comparable to reference Glycerin.
Appearance:	Colorless, clear, syrupy liquid.

continued on following page

User Quality Control cont.

Cultural Response

Middlebrook 7H9 Broth with Middlebrook ADC Enrichment

Prepare medium per label directions. Inoculate and incubate at 35 ± 2°C under approximately 10% CO_2 for up to 21 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Mycobacterium fortuitum	6841	100-300	good
Mycobacterium intracellulare	13950	100-300	good
Mycobacterium kansasii	12478	100-300	good
Mycobacterium tuberculosis	25177	100-300	good
Mycobacterium scrofulaceum	19981	100-300	good

Middlebrook 7H10 Agar with Middlebrook OADC Enrichment
Mycobacteria 7H11 Agar with Middlebrook OADC Enrichment

Prepare medium per label directions or use prepared tubes. Inoculate and incubate at 35 ± 2°C under approximately 10% CO_2 for up to 21 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	1,000-2,000	markedly inhibited
Mycobacterium fortuitum	6841	100-300	good
Mycobacterium intracellulare	13950	100-300	good
Mycobacterium kansasii	12478	100-300	good
Mycobacterium scrofulaceum	25177	100-300	good
Mycobacterium tuberculosis	19981	100-300	good

Middlebrook 7H10 Agar with Middlebrook OADC Enrichment w/WR 1339

Prepare medium per label directions. Inoculate and incubate at 35 ± 2°C under approximately 10% CO_2 for up to three weeks.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Mycobacterium tuberculosis	27294	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as a Bactrol™ Disk and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube — *Mycobacterium fortuitum* ATCC® 6841

Agar-base media:

1. Tend not to liquefy in the presence of contaminating proteolytic organisms;[3]
2. Are recommended for specimens from nonsterile sites[7] because colonies of mycobacteria can be viewed in a clear medium after 10-12 days incubation using a stereo microscope even if contaminating organisms are present;
3. Retain exact concentrations of added drugs because the medium is solidified with agar rather than by inspissation of the egg. Also, there is less drug inactivation when egg ingredients are absent.

Middlebrook 7H10 Agar is prepared according to Middlebrook, Cohn, Dye, Russell and Levy.[4] This medium contains a low concentration of malachite green, which may be preferable for primary isolation.

Mycobacteria 7H11 Agar is a modification of Middlebrook 7H10 Agar Special as recommended by Cohn, Waggoner and McClately.[5] Cohn et al. demonstrated that the addition of an enzymatic digest of casein stimulates growth of the more fastidious strains of *Mycobacterium tuberculosis* and provides improved susceptibility testing.

Principles of the Procedure

L-Glutamic Acid, Ammonium Sulfate, Biotin, Sodium Citrate and Pyridoxine supply growth factors. Magnesium Sulfate, Ferric Ammonium Sulfate, Zinc Sulfate and Copper Sulfate are sources of trace ions. Disodium Phosphate and Monopotassium Phosphate help to maintain the pH of the medium. Malachite Green inhibits contaminating organisms. Pancreatic Digest of Casein, a good source of nitrogen and carbon, increases the recovery of isoniazid-resistant mycobacteria on Mycobacteria 7H11 Agar. Glycerol enhances the growth of *Mycobacterium avium* as well as other *Mycobacterium* species.[7] Bacto Agar is a solidifying agent.

Middlebrook OADC and ADC Enrichments contain Dextrose and Oleic Acid as carbon sources. Albumin Fraction V, Bovine and Catalase (Beef) are growth factors. WR 1339, Triton® encourages the demonstration of cording in *Mycobacterium tuberculosis*.[8]

Formula

Middlebrook 7H9 Broth

Formula Per Liter

Ammonium Sulfate	0.5 g
L-Glutamic Acid	0.5 g
Sodium Citrate	0.1 g
Pyridoxine	0.001 g
Biotin	0.0005 g
Disodium Phosphate	2.5 g
Monopotassium Phosphate	1 g
Ferric Ammonium Citrate	0.04 g
Magnesium Sulfate	0.05 g
Calcium Chloride	0.0005 g
Zinc Sulfate	0.001 g
Copper Sulfate	0.001 g

Final pH 6.6 ± 0.2 at 25%C

Middlebrook 7H10 Agar

Formula Per Liter

Ammonium Sulfate	0.5 g
Monopotassium Phosphate	1.5 g
Disodium Phosphate	1.5 g
Sodium Citrate	0.4 g

Magnesium Sulfate	0.025 g
Calcium Chloride	0.0005 g
Zinc Sulfate	0.001 g
Copper Sulfate	0.001 g
L-Glutamic Acid (Sodium Salt)	0.5 g
Ferric Ammonium Citrate	0.04 g
Pyridoxine Hydrochloride	0.001 g
Biotin	0.0005 g
Malachite Green	0.00025 g
Bacto Agar	15 g

Final pH 6.6 ± 0.2 at 25%C

Mycobacteria 7H11 Agar

Formula Per Liter

Pancreatic Digest of Casein	1 g
L-Glutamic Acid	0.5 g
Sodium Citrate	0.4 g
Pyridoxine	0.001 g
Biotin	0.0005 g
Ferric Ammonium Citrate	0.04 g
Ammonium Sulfate	0.5 g
Disodium Phosphate	1.5 g
Monopotassium Phosphate	1.5 g
Magnesium Sulfate	0.05 g
Bacto Agar	15 g
Malachite Green	0.001 g

Final pH 6.6 ± 0.2 at 25%C

Middlebrook ADC Enrichment

Formula Per 100 ml (For preparing 1 liter final medium)

Albumin Fraction V, Bovine	5 g
Dextrose	2 g
Catalase (Beef)	0.003 g
Distilled water	100 ml

Middlebrook OADC Enrichment

Formula Per 100 ml (For preparing 1 liter final medium)

Oleic Acid	0.05 g
Albumin Fraction V, Bovine	5 g
Dextrose	2 g
Catalase (Beef)	0.004 g
Sodium Chloride	0.85 g
Distilled water	100 ml

Middlebrook OADC Enrichment w/WR 1339

Formula Per 100 ml (For preparing 1 liter final medium)

Oleic Acid	0.05 g
Albumin Fraction V, Bovine	5 g
Dextrose	2 g
Catalase (Beef)	0.004 g
Sodium Chloride	0.85 g
Distilled water	100 ml
WR 1339, Triton®	0.25 g

Glycerol

Formula Per Liter

Glycerol	100 %

Precautions

1. For Laboratory Use.
2. **Middlebrook 7H9 Broth**
   Middlebrook 7H10 Agar
   Mycobacteria 7H11 Agar

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Middlebrook Enrichments at 2-8°C.

Store prepared media at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Middlebrook 7H9 Broth
Middlebrook 7H10 Agar
Mycobacteria 7H11 Agar
Middlebrook ADC Enrichment
Middlebrook OADC Enrichment
Middlebrook OADC Enrichment w/WR 1339
Glycerol

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)
Tween® 80 (optional)

Method of Preparation

Middlebrook 7H9 Broth

1. Dissolve 4.7 grams in 900 ml of distilled or deionized water containing 2 ml of Glycerol (or 0.5 g Tween® 80, if desired).
2. Autoclave at 121°C for 10 minutes.
3. Cool the medium to 45°C.
4. Aseptically add 100 ml Middlebrook ADC Enrichment. Mix well.

Middlebrook 7H10 Agar

1. Suspend 19 grams in 900 ml of distilled or deionized water containing 5 ml Glycerol.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 10 minutes.
4. Cool medium to 50-55°C.
5. Aseptically add 100 ml Middlebrook OADC Enrichment or Middlebrook OADC Enrichment w/WR 1339. Mix well.

Mycobacteria 7H11 Agar

1. Suspend 21 grams in 900 ml distilled or deionized water containing 5 ml glycerol.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 50-55°C.
5. Aseptically add 100 ml Middlebrook OADC Enrichment. Mix well.

Specimen Collection and Preparation[7]

1. Collect specimens in sterile containers and transport immediately to the laboratory following recommended guidelines.
2. Process each specimen as appropriate for that specimen.

Test Procedure

1. Inoculate the specimen onto the medium.
2 Incubate tubes for up to eight weeks.
3 Examine tubes for growth.

Results

Observe for colonies that may or may not be pigmented. Colony morphology is dependent on the species isolated.

Limitations of the Procedure

Negative culture results do not rule out active infection by mycobacteria. Some factors responsible for unsuccessful cultures are:

1. The specimen was not representative of the infectious material, i.e., saliva instead of sputum;
2. The mycobacteria were destroyed during digestion and decontamination of the specimen;
3. Gross contamination interfered with the growth of the mycobacteria;
4. Proper aerobic and increased CO_2 tension were not provided during incubation.

References

1. **Musser, J. M.** 1995. Antimicrobial resistance in Mycobacteria: molecular genetic insights. Clin. Microbiol. Rev. **8**:496-514.
2. **Kleitmann, W.** 1995. Resistance and susceptibility testing for *Mycobacterium tuberculosis.* Clin. Microbiol. News. **17**:65-69.
3. **Nolte, F. S., and B. Methcock.** 1995. *Mycobacterium*, p. 400-437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Middlebrook, G., M. L. Cohn, W. B. Dye, W. B. Russell, Jr., and D. Levy.** 1960. Microbiologic procedures of value in tuberculosis. Acta. Tubercul. Scand., **38:**66.
5. **Cohn, M. L., R. F. Waggoner, and J. K. McClatchy.** 1968. The 7H11 Medium for the cultivation of mycobacteria. Am. Rev. Resp. Dis., **98**:295.
6. **Tenover, F. C., J. T. Crawford, R. E. Huebner, L. J. Geiter, C. R. Horsburgh, Jr., and R. C. Good.** 1993. The resurgence of tuberculosis: is your laboratory ready? J. Clin. Microbiol. **31**:767-770.
7. **Isenberg, H. D. (ed.).** 1994. Clinical microbiology procedures handbook, sup. 1. American Society for Microbiology, Washington, D.C.
8. Am. Rev. Resp. Dis., **97**:1133.

Packaging

Middlebrook 7H9 Broth	500 g	0713-17
Middlebrook 7H10 Agar	500 g	0627-17
	20 tubes	0627-39
	100 tubes	0627-79
Mycobacteria 7H11 Agar	500 g	0838-17
	20 tubes	0838-39
	100 tubes	0838-79
Middlebrook ADC Enrichment	2 x 20 ml	0714-64
Middlebrook OADC Enrichment	12 x 20 ml	0722-64
	6 x 100 ml	0722-73
Middlebrook OADC Enrichment w/ WR 1339	6 x 20 ml	0801-63
Glycerol	100 g	0282-15
	500 g	0282-17

Bacto® Milk Agar

Intended Use

Bacto Milk Agar is recommended by the British Standards Institute[1] and the International Dairy Federation for the enumeration of microorganisms in liquid milk, ice cream, dried milk and whey.

Summary and Explanation

Liquid milk is a highly perishable foodstuff with a shelf life of only 5-10 days after pasteurization. Contamination of raw milk may arise from either the soiled or diseased udder or inadequately cleaned milking or storage equipment. Bovine mastitis or udder inflammation may cause contamination with *Staphylococcus aureus*, *Streptococcus agalactiae*, *Escherichia coli* or, more rarely, *Yersinia enterocolitica* and *Leptospira* species. Excretion of these organisms can increase the bulk milk count by 10^5 organisms/ml.

Poor cleaning of the milking equipment may cause contamination with micrococci, streptococci, coliforms or heat resistant *Bacillus* strains, giving an increase of the bulk milk count of >5 x 10^4 organisms/ml. Spoilage of pasteurized or raw milk by proteolytic psychrotrophic bacteria can occur on prolonged storage below 7°C.

Milk Agar conforms to the EEC Commission for the examination of ice cream.[2] Milk Agar is recommended for performing plate count tests on milks, rinse waters, milk products and ice cream.[3]

Principles of Procedure

Tryptone and Yeast Extract provide essential nutrients while Skim Milk Powder is a source of casein. Dextrose is the carbon energy source.

Proteolytic bacteria will be surrounded by a clear zone from the conversion of casein into soluble nitrogenous compounds.[1]

Formula

Milk Agar

Formula Per Liter

Tryptone	5 g
Yeast Extract	2.5 g
Dextrose	1 g
Skim Milk Powder (antibiotic free)	1 g
Bacto Agar	12.5 g

Final pH 6.9 ± 0.1 at 25°C

Precautions

1. For In Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Milk Agar

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.2% solution, soluble in distilled or deionized water upon boiling. Solution is light amber, clear to slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent.
Reaction of 2.2% Solution at 25°C:	pH 6.9 ± 0.1

Cultural Response

Prepare medium per label directions. Inoculate using the pour plate technique and incubate at 30°C for up to 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus casei	9595	30-300	good
Lactococcus lactis	19435	30-300	good
Staphylococcus aureus	25923*	30-300	good
Streptococcus thermophilus	19258	30-300	good

*This culture is available as a Bactrol™ Disk and should be used as directed in Bactrol Disks Technical Information.

Materials Required but not Provided

Flasks with closures
Distilled or deionized water
Hot plate
Autoclave
Dilution tubes containing 1/4-strength Ringer's solution
Petri dishes
1% hydrochloric acid or 10% acetic acid

Method of Preparation

1. Suspend 22 grams in 1 liter distilled or deionized water and boil gently to dissolve completely.
2. Dispense 10-12 ml per tube. Cap loosely.
3. Autoclave at 121°C for 15 minutes.
4. Pour Milk Agar into Petri dishes for the spread plate technique or allow tubes to cool to 45°C for the pour plate technique.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Total counts may be carried out using either pour plates or surface counting techniques.

1. Prepare milk dilutions of 1/10, 1/100, 1/1,000 in 1/4-strength Ringer's solution. Use this inoculum within 15 minutes.
2. **Pour Plates**: Pipette 1 ml of each dilution into Petri dishes. Add 10-12 ml of molten Milk Agar, cooled to 45°C, and mix thoroughly.

   Spread Plates: Spread 1 ml of milk dilution over the surface of the solidified medium in a Petri dish.
3. Incubate at 30°C for 72 hours.

Results

Select plates containing 10-300 colonies. Results are expressed as colonies per ml of product tested.

Proteolytic psychrotrophic colonies may be enhanced by flooding the plates with a solution of 1% hydrochloric acid or 10% acetic acid. Pour off the excess acid solution and count the colonies surrounded by clear zones.

References

1. **Microbiological examination for dairy purposes.** Diluents, media and apparatus and their preparation and sterilisation. BS4285, Sec. 1.2.
2. **Klose, J.** 1968. Susswaren. **14:**778-782.
3. **Dept. of Health.** 1987. Memo.139/Foods.

Packaging

Milk Agar	500 g	1859-17
	5 kg	1859-03

Bacto® Minerals Modified Glutamate Broth

Intended Use

Bacto Minerals Modified Glutamate Broth is used for enumerating coliform organisms in water.

Also Known As

Gray's Minerals Modified Glutamate Broth

Summary and Explanation

Gray[1] described a simple formate-lactose-glutamate medium that could be used as an alternative to MacConkey Broth for the presumptive identification of coliform bacteria in water. Gray's original medium gave fewer false positive results than MacConkey Broth, was suitable for use at 44°C, and gave low volumes of gas.

The medium was improved[2] by the addition of ammonium chloride that, by replacing ammonium lactate, resulted in a doubling of the gas volume. The addition of B complex vitamins, certain amino-acids and magnesium ions resulted in an increased rate of fermentation. Comparative trials of the modified glutamate medium and MacConkey Broth[3] with chlorinated and unchlorinated waters showed that Gray's Minerals Modified Glutamate Broth gave significantly higher numbers of positive results (acid and gas production) for coliform organisms and *Escherichia coli*. This was especially apparent after 48 hours of incubation but was also clearly seen with unchlorinated water samples after only 24 hours incubation. For chlorinated water samples, results with the two media were comparable. After 18-24 hours incubation, Minerals Modified Glutamate Medium gave significantly fewer false positive reactions. *Clostridium perfringens*, a common cause of false positive reactions in MacConkey media, is unable to grow in a minimal-glutamate based medium.

A major feature of Minerals Modified Glutamate Medium is its superiority in initiating growth of *Escherichia coli* after exposure to chlorine when incubated for 48 hours. In view of the known resistance to chlorination of some viruses, the ability to isolate coliform bacteria that survive marginal chlorination provides an additional safety factor in water treatment.

In a comparison to Lauryl Tryptose Lactose Broth[4], Minerals Modified Glutamate Medium gave superior isolation of *Escherichia coli* after 48 hours incubation by the multiple tube method, especially in waters containing small numbers of organisms. Minerals Modified Glutamate Medium is the medium of choice for the detection of fecal contamination in chlorinated drinking water supplies in Great Britain.[4]

Abbiss et al.[5] compared Minerals Modified Glutamate Medium and three other enrichment broths for the enumeration of coliform organisms present in soft cheese, cooked meat and patè. Minerals Modified Glutamate Medium was superior in sensitivity to Lauryl Sulfate Tryptose Broth, MacConkey Broth and Brilliant Green Bile Broth.

Minerals Modified Glutamate Broth has been used in the modified direct plate method for enumeration of *Escherichia coli* biotype 1 in foods.[6] According to this method, 15 grams of agar are added per liter of single strength broth before autoclaving. The medium is poured in 12-15 ml amounts into sterile Petri dishes. This resuscitation agar is used for the recovery of damaged cells from frozen or dried foodstuffs.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light beige, free flowing, homogeneous.
Solution:	1.77% solution, soluble in distilled or deionized water on gentle warming. Solution is purple, clear.
Reaction of 1.77% Solution at 25°C:	pH 6.7 ± 0.1

(containing 0.25 grams of ammonium chloride per 100 ml)

Cultural Response

Prepare Minerals Modified Glutamate Broth per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	CFU	GROWTH/GAS PRODUCTION
Enterobacter aerogenes	13048*	30-300	Good growth and turbidity Acid (yellow) production with gas
Enterococcus faecalis	19433*	1,000	No visible growth
Escherichia coli	25922*	30-300	Good growth and turbidity Acid (yellow) production with gas
Salmonella typhimurium	14028*	30-300	Good growth and turbidity. Negative for acid and gas.

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Sodium Glutamate and Sodium Formate are the basis of a defined minimal medium for the enumeration of coliform organisms in water. Lactose is the carbohydrate source in Minerals Modified Glutamate Broth. The addition of B complex vitamins, certain amino acids, and magnesium ions allows an increased rate of fermentation. Phosphate acts as a buffering agent. The addition of Ammonium Chloride allows increased gas production by the test organism. Bromocresol Purple is present as a pH indicator.

Formula

Minerals Modified Glutamate Broth

Formula Per Liter

Sodium Glutamate	6.4 g
Lactose	10 g
Sodium Formate	0.25 g
L-cystine	0.02 g
L(-) Aspartic Acid	0.024 g
L(+) Arginine	0.02 g
Thiamine	0.001 g
Nicotinic Acid	0.001 g
Pantothenic Acid	0.001 g
Magnesium Sulfate Heptahydrate	0.1 g
Ferric Ammonium Citrate	0.01 g
Calcium Chloride Dihydrate	0.01 g
Dipotassium Phosphate	0.9 g
Bromocresol Purple	0.01 g

Final Ph 6.7 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Minerals Modified Glutamate Broth

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Ammonium chloride
Bunsen burner or magnetic hot plate
Test tubes with closures
Fermentation vials
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 17.7 grams in distilled or deionized water, the amount of water depending on the strength of medium desired:
   Single-strength medium - 1 liter of water;
   Double-strength medium - 500 ml of water.
2. Add 2.5 grams of ammonium chloride per liter and mix well.
3. Heat gently to dissolve completely.
4. Dispense into tubes as listed below and place an inverted fermentation vial into each tube:
   Double strength medium - 1 x 5 ml;
   Double strength medium - 5 x 10 ml;
   Single strength medium - 10 x 5 ml.
5. Autoclave at 115-116°C for 10 minutes. Before opening the autoclave, allow the temperature to drop below 75°C to avoid entrapped air bubbles in the inverted fermentation vials.

Specimen Collection and Preparation

Follow laboratory procedure for specimen collection.

Test Procedure

The multiple tube method is used for the enumeration of *Escherichia coli* and coliform organisms using Minerals Modified Glutamate Broth.

For good quality water, inoculate the water sample into the medium in the following volumes:

1. 50 ml of sample into 50 ml of double-strength medium;
2. 5 x 10 ml of sample into 5 x 10 ml of double-strength medium.

For more polluted waters, inoculate the water sample into the medium in the following volumes:

1. 5 x 1 ml of sample into 5 x 5 ml of single-strength medium;
2. 5 x 1 ml of a 1:10 dilution of the sample into 5 x 5 ml of single-strength medium.

Incubate the tubes at 35 ± 2°C. Examine after 18-24 hours incubation and again at 48 hours,

Results

All tubes demonstrating acid production, indicated by the medium turning yellow, and gas, either in the inverted fermentation vial or by effervescence on shaking, may be regarded as presumptive positive reactions. Each presumptive positive tube should be confirmed in Brilliant Green Bile 2%, as well as with additional biochemical tests.

The most probable number of organisms in 100 ml of the original water sample can be calculated using the following table.[7]

Quantity of Water in Each Tube	50 ml	10 ml	Most Probable Number (MPN)
Number of Tubes Used	1	2	of coliforms in 100 ml in sample
Number of Tubes Giving Positive Reaction	0	0	0
	0	1	1
	0	2	2
	0	3	4
	0	4	5
	0	5	7
	1	0	2
	1	1	3
	1	2	6
	1	3	9
	1	4	16
	1	5	+18

Limitations of the Procedure

1. The performance of the medium is significantly affected by pH. Avoid overheating the broth. Check the pH of each lot before proceeding with testing.
2. Due to the nutritional requirements of the organisms, some organisms other than coliform bacteria may grow in the medium with production of acid and gas. Test all presumptive-positive tubes to confirm the presence of *Escherichia coli*.

References

1. **Gray, R. D.** 1959. Formate Lactose Glutamate: A chemically defined medium as a possible substitute for MacConkey Broth in the presumptive coliform examination of water. J. Hyg., Camb. **57:**249-265.
2. **Gray, R. D.** 1964. An improved formate-lactose-glutamate medium for the detection of *Escherichia coli* and other coliform organisms in water. J. Hyg., Camb. **62:**495-508.
3. **P. H. L. S. Standing Committee on the Bacteriological Examination of Water Supplies.** 1968. Comparison of MacConkey Broth, Teepol Broth and Glutamic Acid Media for the enumeration of coliform organisms in water. J. Hyg., Camb. **65:**67-82.
4. **Joint Committee of the P. H. L. S. and the Standing Committee of Analysts.** 1980. A comparison between Minerals Modified Glutamate Medium and Lauryl Tryptose Lactose Broth for the enumeration of *Escherichia coli* and coliform organisms in water by the multiple tube method. J. Hyg., Camb. **85:**35-48.

5. **Abbiss, J. S., J. M. Wilson, R. M. Blood, and B. Jarvis.** 1981. A comparison of Minerals Modified Glutamate Medium with other media for the enumeration of coliforms in delicatessen foods. J. Appl. Bact. **51:**121-127.
6. **Holbrook, R., J. M. Anderson, and A. C. Baird-Parker.** 1980. Modified Direct Plate Method for counting *Escherichia coli* in foods. Food Technol. in Aust. **32:**78- 83.
7. **Departments of the Environment, Health & Social Security, and P.H.L.S.** 1982. The Bacteriological Examination of Drinking Water Supplies, Report on Public Health and Medical Subjects No. 71. HMSO, London.

Packaging

Minerals Modified Glutamate Broth	500 g	1850-17

Bacto® Minimal Agar Davis
Bacto Minimal Broth Davis w/o Dextrose

User Quality Control

Identity Specifications

Minimal Agar Davis

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.66% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, very slightly to slightly opalescent.
Prepared Medium:	Medium amber, very slightly to slightly opalescent.
Reaction of 2.66% Solution at 25°C:	pH 7.0 ± 0.2

Minimal Broth Davis w/o Dextrose

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	1.06% solution, soluble in distilled or deionized water. Solution is colorless, clear.
Prepared Medium:	Colorless, clear.
Reaction of 1.06% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Minimal Agar Davis

Prepare medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	6883	100-1,000	good
Escherichia coli	9637	100-1,000	good

Minimal Broth Davis w/o Dextrose

Prepare medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacillus subtilis	6633	100-1,000	good
Escherichia coli	6883	100-1,000	good
Escherichia coli	9637	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Intended Use

Bacto Minimal Agar Davis is used for isolating and characterizing nutritional mutants of *Escherichia coli*.

Bacto Minimal Broth Davis w/o Dextrose is used with added dextrose in isolating and characterizing nutritional mutants of *Escherichia coli* and *Bacillus subtilis*.

Summary and Explanation

Lederberg[1] described the Davis formulation for Minimal Agar Davis. Minimal Broth Davis w/o Dextrose is the same formulation without dextrose and agar. Both media support the growth of nutritional mutants of *E. coli* while Minimal Broth Davis w/o Dextrose with added dextrose also supports the growth of nutritional mutants of *B. subtilis*.

Lederberg[1] described two techniques for isolating nutritional mutants of *E. coli*, one by random isolation and the other by delayed enrichment. Both Lederberg[1] and Davis[2] described a third technique using penicillin. Nutritional mutants of *B. subtilis* can be isolated by these three techniques and by a modification of the penicillin technique described by Nester, Schafer and Lederberg.[3]

After the mutants are isolated, they are characterized biochemically by growth in minimal broth supplemented with specific growth factors or groups of growth factors. It is generally best to classify mutants according to their requirements for amino acids, vitamins, nucleic acids or other substances. This is done by supplementing the minimal medium with Vitamin Assay Casamino Acids plus tryptophane, or a mixture of water soluble vitamins, alkaline-hydrolyzed yeast, nucleic acid or yeast extract, depending on the particular mutants desired. The supplemented minimal broth is inoculated with a slightly turbid suspension of the mutant colonies and incubated for 24 hours at 35°C. Growth with Vitamin Assay Casamino Acids indicates a vitamin requirement. When a major growth factor group response in obtained, the characterization is carried further by the same general procedure to subgroups and finally to individual growth substances.

Principles of the Procedure

Minimal Agar Davis and Minimal Broth Davis w/o Dextrose contain citrate and phosphates as buffers. Ammonium Sulfate is the carbon source. Magnesium is a cofactor for many metabolic reactions. Minimal Agar Davis contains Dextrose as the carbohydrate energy source; Bacto Agar is the solidifying agent.

Formula

Minimal Agar Davis

Formula Per Liter

Bacto Dextrose	1 g
Dipotassium Phosphate	7 g
Monopotassium Phosphate	2 g
Sodium Citrate	0.5 g
Magnesium Sulfate	0.1 g
Ammonium Sulfate	1 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Minimal Broth Davis w/o Dextrose

Formula Per Liter

Dipotassium Phosphate	7 g
Monopotassium Phosphate	2 g
Sodium Citrate	0.5 g
Magnesium Sulfate	0.1 g
Ammonium Sulfate	1 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Minimal Broth Davis w/o Dextrose**

   MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Minimal Agar Davis
Minimal Broth Davis w/o Dextrose

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

Minimal Agar Davis

1. Suspend 26.6 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Minimal Broth Davis w/o Dextrose

1. Dissolve 10.6 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Cool to room temperature.
4. Aseptically add 10 ml 10% dextrose solution at room temperature.
5. Mix thoroughly.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Random Technique

1. Irradiate a cell suspension of wild type *E. coli.*
2. Dilute the suspension 100-500 times.
3. Culture on a complete agar medium containing all the necessary growth requirements.
4. Incubate the cultures at 35°C for 24 hours.
5. Select isolated colonies and inoculate into Minimal Broth Davis and a nutritionally complete broth.
6. Incubate at 35°C for 24 hours.
7. Observe growth in both media.

Delayed Enrichment Method

1. Prepare plates of Minimal Agar Davis by pouring a 15-20 ml base layer in a 95 mm sterile Petri dish followed by a 5 ml seed layer.
2. Inoculate with a diluted irradiated *E. coli* suspension.
3. Pour a 5-10 ml layer of uninoculated Minimal Agar Davis over the seed layer.
4. Incubate for 24 hours or longer to allow for the growth of prototroph cells (wild type cells).
5. Pour a layer of a complete agar medium over the minimal agar medium to develop the mutant cells.
6. Incubate at 35°C for 6-12 hours.

Penicillin Method

1. Wash an irradiated *E. coli* suspension with sterile saline and dilute to 20 times the original volume in sterile minimal broth.
2. Dispense into tubes in desired amounts.
3. Add freshly prepared penicillin to each tube to give a final concentration of 200 units per ml.
4. Incubate at 35°C for 4-24 hours on a shaker.
5. Spread 0.1 ml, 0.01 ml and 0.001 ml samples onto complete agar plates.
6. Incubate at 35°C for 24 hours.
7. Select isolated colonies and test for growth in minimal broth.

***Bacillus subtilis* Procedure**

1. Grow cultures of *Bacillus subtilis* in Antibiotic Medium 3 for 18 hours.
2. Centrifuge to sediment the cells.
3. Aseptically decant the supernatant fluid.

4. Resuspend the cells in minimal medium and centrifuge.
5. Decant the supernatant and resuspend the pellet in minimal medium to give a cell concentration of about 2 x 10^8 cells per ml.
6. Irradiate the suspension with a low pressure mercury ultraviolet lamp for a sufficient time to give a cell survival of 1 x 10^4 cells per ml.
7. Incubate the suspension at room temperature for 4-18 hours in the minimal medium with appropriate substances added to allow for the growth of desired mutants.
8. Wash the culture in sterile minimal medium.
9. Centrifuge and resuspend in the same medium.
10. Dilute 1 to 10 with sterile minimal medium.
11. Let stand for 60 minutes to starve the mutants.
12. Add penicillin to give a concentration of 2,000 units per ml.
13. Incubate 15 minutes.
14. Plate the culture on nutrient agar for colony isolation.
15. Identify the nutrition mutants by transferring colonies by replicate plating onto plates of minimal agar which has been supplemented with the appropriate nutritional substances.

Results

Random Technique

Growth in the nutritionally complete medium and no growth in the Minimal Broth indicates a mutant.

Delayed Enrichment Method

Mutant colonies will grow as small colonies after the addition of the complete medium which diffuses through the Minimal Agar.

Penicillin Method

Mutant colonies grow after the addition of penicillin.

***B. subtilis* Method**

Mutant colonies grow on Nutrient Agar after the addition of penicillin.

Limitations of the Procedure

1. Strains vary in their sensitivity to penicillin. Adjustments to the time of treatment and concentration of penicillin may be necessary.[1]

References

1. **Lederberg, J.** 1950. Isolation and characterization of biochemical mutants of bacteria. Methods in Med. Res. **3**:5-21.
2. **Davis.** 1949. Proc. Nat'l Acad. Sci. **35**:1.
3. **Nester, Schafer, and Lederberg.** 1963. Genetics **48**:529.

Packaging

Minimal Agar Davis	500 g	0544-17
Minimal Broth Davis w/o Dextrose	500 g	0756-17

Bacto® Mitis Salivarius Agar
Bacto Chapman Tellurite Solution 1%

Intended Use

Bacto Mitis Salivarius Agar is used with Bacto Chapman Tellurite Solution 1% in isolating *Streptococcus mitis*, *S. salivarius* and enterococci, particularly from grossly contaminated specimens.

Summary and Explanation

Streptococcus mitis, *Streptococcus salivarius* and *Enterococcus* species are part of the normal human flora. *S. mitis* and *S. salivarius* are known as viridans streptococci. These organisms play a role in cariogenesis and infective endocarditis and cause an increasing number of bacteremias.[1] Enterococci cause urinary tract infections, wound infections and bacteremia.[2] These organisms can colonize the skin and mucous membranes.

Chapman[3,4,5] investigated methods for isolating streptococci and formulated Mitis Salivarius Agar. The medium facilitates isolation of *S. mitis* (*Streptococcus viridans*), *S. salivarius* (non-hemolytic streptococci) and enterococci from mixed cultures.[6]

Principles of the Procedure

Mitis Salivarius Agar contains Tryptose, Proteose Peptone No. 3 and Proteose Peptone as sources of carbon, nitrogen, vitamins and minerals. Dextrose and Saccharose are carbohydrate sources. Crystal Violet and Potassium Tellurite (from Chapman Tellurite Solution 1%) inhibit most gram-negative bacilli and most gram-positive bacteria except streptococci. Trypan Blue gives the colonies a blue color. Bacto Agar is the solidifying agent.

Formula

Mitis Salivarius Agar

Formula Per Liter

Bacto Tryptose	10 g
Bacto Proteose Peptone No. 3	5 g
Bacto Proteose Peptone	5 g
Bacto Dextrose	1 g
Bacto Saccharose	50 g
Dipotassium Phosphate	4 g
Trypan Blue	0.075 g
Bacto Crystal Violet	0.0008 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Chapman Tellurite Solution 1%

Sterile 1% solution of Potassium Tellurite

Precautions

1. **Mitis Salivarius Agar**: For Laboratory Use.
   Chapman Tellurite Solution 1%: For Laboratory Use.
2. **Chapman Tellurite Solution 1%**

   MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe mist.

Wear suitable protective clothing. Keep container tightly closed.

FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Chapman Tellurite Solution 1% at 15-30°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Mitis Salivarius Agar
Chapman Tellurite Solution 1%

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 90 grams of Mitis Salivarius Agar in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50-55°C.
4. Just prior to dispensing, add 1 ml Chapman Tellurite Solution 1%.
5. DO NOT HEAT THE COMPLETE MEDIUM.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure

See appropriate references for specific procedures.

Results

S. mitis produces small or minute blue colonies. These colonies may become easier to distinguish with longer incubation. *S. salivarius* produces blue, smooth or rough "gum drop" colonies, 1-5 mm in diameter depending on the number of colonies on the plate. *Enterococcus* species form dark blue or black, shiny, slightly raised, 1-2 mm colonies.

Limitations of the Procedure

1. If coliforms grown on the medium, they produce brown colonies.

User Quality Control

Identity Specifications

Mitis Salivarius Agar

Dehydrated Appearance:	Bluish beige, free-flowing, homogeneous.
Solution:	9.0% solution, soluble in distilled or deionized water on boiling. Solution is deep royal blue, very slightly opalescent.
Prepared Medium:	Deep royal blue, slightly opalescent.
Reaction of 9.0% Solution at 25°C:	pH 7.0 ± 0.2

Chapman Tellurite Solution 1%

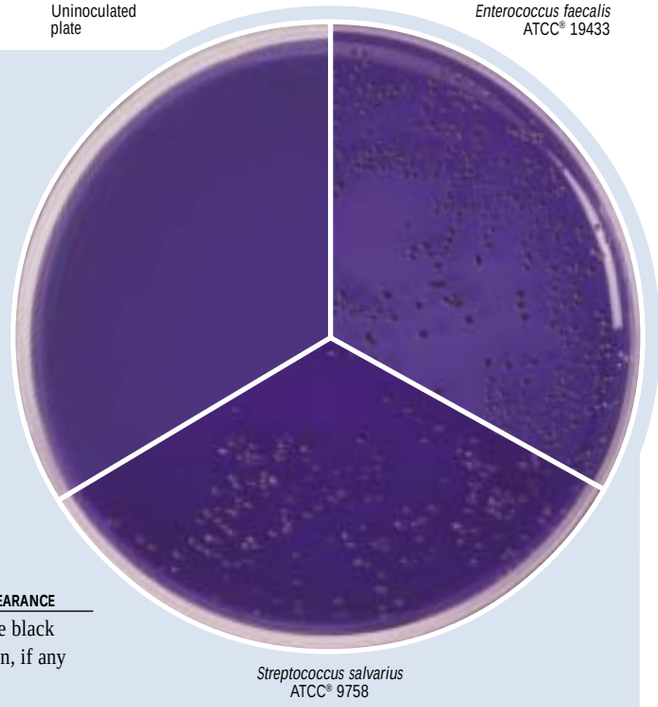
Appearance:	Colorless, clear, may have a slight precipitate.

Cultural Response

Prepare the complete medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	19433	100-1,000	good	blue black
Escherichia coli	25922*	1,000-2,000	partial to complete inhibition	brown, if any
Staphylococcus aureus	25923*	1,000-2,000	partial to complete inhibition	–
Streptococcus mitis	9895	100-1,000	good	blue
Streptococcus salivarius	9758	100-1,000	good	blue "gum drop" shape

The cultures listed are the minimum that should be used for performance testing



*This culture is available as a Bactrol™ Disk and should be used as directed in Bactrol Disks Technical Information.

2. Molds will grow on the medium after two days incubation.
3. *Erysipelothrix rhusiopathiae* produces colorless, circular, convex colonies.
4. Beta-hemolytic streptococci produce colonies that resemble *S. mitis*.

References

1. **Facklam, R. R., and J. A. Washington II.** 1991. *Streptococcus* and related catalase-negative gram-positive cocci. p. 238-257. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology. Washington, D.C.
2. **Facklam, R.R., and D. F. Sahm.** 1995. *Enterococcus*, p. 308-314. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Chapman, G. H.** 1944. The isolation of streptococci from mixed cultures. J. Bacteriol. **48**:113.
4. **Chapman, G. H.** 1946. The isolation and testing of fecal streptococci. Am. J. Dig. Dis. **13**:105.
5. **Chapman, G. H.** 1947. Relationship of nonhemolytic and viridans streptococci in man. Trans. N.Y. Acad. Sci. (Series 2) **10**:45.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 522-526. Williams & Wilkins, Baltimore, MD.

Packaging

Mitis Salivarius Agar	500 g	0298-17
Chapman Tellurite Solution 1%	6 x 1 ml	0299-51
	6 x 25 ml	0299-66

Bacto® Modified EC Medium
Bacto Novobiocin Antimicrobic Supplement

Intended Use

Bacto Modified EC Medium is used with Bacto Novobiocin Antimicrobic Supplement in the detection of *Escherichia coli* O157:H7 in meat and poultry products.

User Quality Control

Identity Specifications

Modified EC Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.66% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear, with no significant precipitate.
Reaction of 3.66% Solution at 25°C:	pH 6.9 ± 0.2

Novobiocin Antimicrobic Supplement

Lyophilized Appearance:	White cake.
Rehydrated Appearance:	Colorless solution.

Cultural Response

Prepare Modified EC Medium per label directions. Add 10 ml of Novobiocin Antimicrobic Supplement per liter. Inoculate the tubes and incubate at 35 ± 2°C for 24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus faecalis	33186	1,000-2,000	none to poor
Escherichia coli O157:H7	35150	10-100	good

The cultures listed above are the minimum that should be used for performance testing.

Also Known As

mEC+n or Modified *E. coli* Medium

Summary and Explanation

Modified EC Medium and Novobiocin Antimicrobic Supplement are based on the formula for modified EC broth with novobiocin (mEC+n) as described by Okrend and Rose.[1] In modifying the EC Medium formula, Okrend and Rose reduced the Bile Salts No. 3 from 1.5 grams per liter to 1.12 grams per liter and added 20 milligrams per liter of sodium novobiocin. Okrend, Rose *et al*. reported that mEC+n was useful in the enrichment and detection of *E. coli* O157:H7 from meats and poultry products.[2-4]

Principles of the Procedure

Tryptone supports good growth of *E. coli* O157:H7 and is rich in peptides and nitrogen. Lactose is an additional source of carbon for organisms, such as *E. coli*, that can ferment this sugar. Potassium Phosphate Dibasic and Monobasic are buffers that facilitate recovery of injured cells. Sodium Chloride provides a suitable ionic environment for growth of microorganisms.

Selectivity of the medium is achieved by the incorporation of Bile Salts No. 3 into the base medium and by the addition of Sodium Novobiocin to the complete medium. These agents suppress the growth of nuisance organisms commonly found in foods. The Sodium Novobiocin is provided in the freeze-dried state as Novobiocin Antimicrobic Supplement. This supplement is rehydrated before use with sterile distilled or deionized water.

Formula

Modified EC Medium

Formula per liter

Bacto Tryptone ..... 20 g

Bacto Bile Salts No. 3	1.12	g
Bacto Lactose	5	g
Potassium Phosphate, Dibasic	4	g
Potassium Phosphate, Monobasic	1.5	g
Sodium Chloride	5	g

Final pH 6.9 ± 0.2 at 25°C

Novobiocin Antimicrobic Supplement

Formula per 10 ml vial

Sodium Novobiocin	20	mg

Precautions

1. For Laboratory Use.
2. **Modified EC Medium**

   IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed, seek medical advice immediately and show this container or label.

   Novobiocin Antimicrobic Supplement

   HARMFUL. HARMFUL BY INHALATION AND IF SWALLOWED. (EC) MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed, seek medical advice immediately and show container or label.
3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

1. Store Modified EC Medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.
2. Store Novobiocin Antimicrobic Supplement at 2-8°C.
3. Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Modified EC Medium

Novobiocin Antimicrobic Supplement

Materials Required But Not Provided

MacConkey Sorbitol Agar
EMB Agar
Flasks with closures
Sterile distilled or deionized water
Autoclave
Incubator (35°C)
Incubator (42°C)
Butterfield's Phosphate Diluent
Phenol Red Sorbitol Agar with MUG

Method of Preparation

Rehydrate Novobiocin Antimicrobic Supplement with 10 ml sterile distilled or deionized water.

1. Dissolve 36.6 grams Modified EC Medium in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Cool to room temperature.
4. Aseptically add 10 ml rehydrated supplement to 1 liter sterile basal medium. Mix well.

Specimen Collection and Preparation

1. Collect food samples in sterile containers and transport immediately to the laboratory following recommended guidelines.
2. Process each food sample using procedures appropriate for that sample.

Test Procedure

Many procedures and systems have been described for the use of Modified EC Medium with Novobiocin in the selective and differential enrichment of *E. coli* O157:H7 in meat and poultry samples. Please consult appropriate references.[4-6] Listed below is the USDA's recommended procedure for the enrichment and detection of *E. coli* O157:H7 in meat and poultry samples using Modified EC Medium with Novobiocin.[2-4]

1. Inoculate 25 grams of meat sample into 225 ml of Modified EC Medium with Novobiocin in a stomacher bag. Blend or stomach as required (i.e., 2 minutes) for thorough mixing.
2. Incubate at 35°C for 24 hours.
3. Dilute cultures 10-fold in Butterfield's Phosphate Diluent and inoculate 0.1 ml of appropriate dilutions using a spread plate technique onto MacConkey Sorbitol Agar (MSA) and MacConkey Sorbitol Agar with BCIG (MSA-BCIG) agar plates.
4. Incubate plates at 42°C for 24 hours.
5. Examine MSA plates for sorbitol-negative colonies (white) and MSA-BCIG plates for sorbitol-negative, BCIG-negative colonies (white).
6. Subculture sorbitol-negative colonies to respective plates of EMB Agar and Phenol Red Sorbitol Agar containing MUG (PRS-MUG).
7. Incubate EMB and PRS-MUG Agar plates at 35°C for 18-24 hours. Examine plates for sorbitol fermentation, MUG reaction (fluorescence), and typical *E. coli* growth on EMB Agar.

Results

Growth in Modified EC Medium with Novobiocin is demonstrated as an increase in turbidity. Colonies of *E. coli* O157:H7 appear white on MacConkey Sorbitol and MacConkey Sorbitol-BCIG Agars. Fermentation of sorbitol in Phenol Red Sorbitol Broth is demonstrated by the production of a yellow color in the medium. With sorbitol non-fermenters, the color of the medium remains red to reddish purple. Positive MUG reactions are demonstrated as a blue fluorescence in the medium under long-wave UV light. Colonies of *E. coli* on EMB Agar appear blue-black to dark purple. A green metallic sheen may also be present.

Cultures that are sorbitol-negative, MUG-negative, and produce blue-black to dark purple colonies with a green metallic sheen on EMB Agar are indicative of *E. coli* O157:H7. These cultures should be tested serologically and with additional biochemical testing to confirm their identity as *E. coli* O157:H7.

Limitations

1. Nutritional requirements may vary from strain to strain.

References

1. **Okrend, A. J. G., and B. E. Rose.** 1989. Isolation and identification of *E. coli* O157:H7 from meat. USDA Food Safety Inspection Service. Rev. 3 of Laboratory Communication no. 38. *E. coli* O157:H7. 20 December 1989. U.S. Department of Agriculture, Washington, D.C.
2. **Okrend, A. J. G., B. E. Rose, and B. Bennett.** 1990. A screening method for the isolation of *E. coli* O157:H7 from ground beef. J. Food Prot. **53:**249-252.
3. **Okrend, A. J. G., B. E. Rose, and C. P. Lattuada.** 1990. Use of 5-bromo-4-chloro-3-indoxyl-ß-D-glucuronide in MacConkey Sorbitol Agar to aid in the isolation of *E. coli* O157:H7 from ground beef. J. Food Prot. **53:**941-943.
4. **Okrend, A. J. G., B. E. Rose, and R. Matner.** 1990. An improved screening method for the detection and isolation of *E. coli* O157:H7 from meat, incorporating the 3M Petrifilm Test Kit-HEC-for hemorrhagic *Escherichia coli* O157:H7. J. Food Prot. **53:**936-940.
5. **Hawkins, E. W., and L. E. Orme.** 1995. Rapid testing methodology for *Escherichia coli* O157:H7 using commercially available products. Proc. West. Sec., Amer. Soc. Animal Sci. vol. 46.
6. **Johnson, R. P., R. J. Durham, S. T. Johnson, and L. A. MacDonald.** 1995. Detection of *E. coli* O157:H7 in meat by an enzyme-linked immunosorbent assay, EHEC-Tek. Appl. Environ. Microbiol. **61:**386-388.

Packaging

Modified EC Medium	500 g	0340-17
Novobiocin Antimicrobic Supplement	6x10 ml	3197-60*

*Store at 2-8°C.

Bacto® Modified Letheen Agar
Bacto Modified Letheen Broth

Intended Use

Bacto Modified Letheen Agar and Bacto Modified Letheen Broth are used for the microbiological testing of cosmetics.

Summary and Explanation

Modified Letheen Agar and Modified Letheen Broth are based on Letheen Agar, Modified and Letheen Broth, Modified as described in the 7th edition of the U.S. FDA Bacteriological Analytical Manual.[1] Letheen Agar, Modified and Letheen Broth, Modified are recommended by the FDA for use in the microbiological testing of cosmetics.[2]

Principles of the Procedure

Beef Extract and Tryptone provide carbon and nitrogen sources required for good growth of a wide variety of bacteria and fungi. The Tryptone level was increased in the Modified Letheen Agar and Broth formulas to provide for better growth. Vitamins and cofactors, required for growth as well as additional sources of nitrogen and carbon, are provided by Yeast Extract. Sodium Chloride provides a suitable osmotic environment. In Modified Letheen Broth, Sodium Chloride is provided by the Letheen Broth component. Both media also contain polysorbate 80, lecithin and sodium bisulfite to partially neutralize the preservative systems commonly found in cosmetics. Bacto Agar is included in Modified Letheen Agar as a solidifying agent.

Formula

Modified Letheen Agar

Formula Per Liter

Bacto Letheen Agar	32 g
Bacto Tryptone	5 g
Bacto Proteose Peptone No. 3	10 g
Bacto Yeast Extract	2 g
Sodium Chloride	5 g
Sodium Bisulfite	0.1 g
Bacto Agar	5 g

Final pH 7.2 ± 0.2 at 25°C

Modified Letheen Broth

Formula Per Liter

Bacto Letheen Broth	26.7 g
Bacto Tryptone	5 g
Bacto Proteose Peptone No. 3	10 g
Bacto Yeast Extract	2 g
Sodium Bisulfite	0.1 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated media at 2-8°C. The dehydrated media are very hygroscopic. Keep containers tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed.

Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Modified Letheen Agar
Modified Letheen Broth

User Quality Control

Identity Specifications

Modified Letheen Agar

Dehydrated Appearance:	Tan, homogeneous, appears moist with a tendency to clump.
% Solution:	5.91% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, after cooling in approx. 45-50°C waterbath, clear to slightly opalescent, may have slight fine precipitate.
Prepared Medium:	Light-medium amber, slightly opalescent, may have a slight fine precipitate.
Reaction of 5.91% Solution at 25°C:	pH 7.2 ± 0.2

Modified Letheen Broth

Dehydrated Appearance:	Tan, homogeneous, appears moist with a tendency to clump.
% Solution:	4.38% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, after cooling, clear to slightly opalescent, may have slight fine precipitate.
Prepared Medium:	Medium-dark amber after cooling, slightly opalescent, may have slight fine precipitate.
Reaction of 4.38% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Modified Letheen Agar or Broth per label directions. Inoculate and incubate at 35°C for 24-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH (AGAR/BROTH)
Staphylococcus aureus	6538	25-100	Good

The culture listed above is the minimum that should be used for performance testing.

Materials Required But Not Provided

Glassware
Autoclave
Incubators (35°C, 30°C)
Selective media

Method of Preparation

1. Suspend the medium in 1 liter of distilled or deionized water:
   Modified Letheen Agar - 59.1 grams;
   Modified Letheen Broth - 43.8 grams.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Dispense as desired.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure [2]

1. Prepare and dilute samples in Modified Letheen Broth in accordance with established guidelines.
2. Using the spread plate technique, inoculate in duplicate 0.1 ml of the diluted samples onto Modified Letheen Agar, Potato Dextrose Agar (or Malt Extract Agar) containing chlortetracycline, Baird Parker Agar (or Vogel-Johnson Agar, optional), Anaerobic Agar, and a second set of Modified Letheen Agar plates.
3. Incubate one set of Modified Letheen Agar plates at 30 ± 2°C for 48 hours and the other set at 35 ± 2°C under anaerobic conditions for 2-4 days. Incubate the Potato Dextrose Agar (or Malt Extract Agar) plates at 30 ± 2°C for 7 days and the Baird Parker Agar (or Vogel-Johnson Agar) plates, if inoculated, at 35 ± 2°C for 48 hours.
4. Incubate the diluted samples from step 1 at 35 ± 2°C for 7 days. Subculture enriched samples onto Modified Letheen Agar only if there is no growth on the primary Modified Letheen Agar plates.

Results

Examine plates for evidence of growth and characteristic colonial morphology. Determine colony counts and subculture each colony type onto Modified Letheen Agar and MacConkey Agar (also Baird Parker or Vogel-Johnson Agar, if used in step 2).

Determine Gram reaction, cell morphology and catalase reactions. Identify bacterial isolates in accordance with established procedures.[2]

References

1. **Tomlinson, L. (ed.).** 1992. FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.
2. **Hitchins, A. D, T. T. Tran, and J. E. McCarron**. 1992. *In* L.A. Tomlinson (ed.), FDA Bacteriological Analytical Manual, 7th Ed. AOAC International, Arlington, VA.

Packaging

Modified Letheen Agar	500 g	0631-17-0
Modified Letheen Broth	500 g	0630-17-0

Bacto® Modified Listeria Enrichment Broth

Intended Use

Bacto Modified Listeria Enrichment Broth is used for selectively enriching *Listeria* from raw and pasteurized milk according to the International Dairy Federation.[1]

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[2] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[3] The first reported food-borne outbreak of listeriosis was in 1985.[4] Since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[5]

Implicated vehicles of transmission include turkey frankfurters,[6] coleslaw, pasteurized milk, Mexican-style cheese, paté and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants. It is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[7]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free flowing, homogeneous.
Solution:	3.61% solution, soluble in distilled or deionized water on boiling. Solution is light to medium yellowish-amber with a faint green ring at the surface, clear to very slightly opalescent.
Prepared Tubes:	Light yellowish-amber, clear to very slightly opalescent.
Reaction of 3.61% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Modified Listeria Enrichment Broth per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus faecalis	29212*	2,000-10,000	partially suppressed at 18- 24 hours
Escherichia coli	25922*	2,000-10,000	marked to complete inhibition
Listeria monocytogenes	19114	100-1,000	good
Saccharomyces pastorianus	9080	2,000-10,000	marked to complete inhibition

The cultures listed are the minimum that should be used for performance.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Listeria species grow over a pH range of 5.0-9.6 and survive in food products with pH levels outside these parameters.[8] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C. Many common food contaminants such as streptococci, enterococci, *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* interfere with the isolation of *Listeria monocytogenes*.[9]

Listeria Enrichment Broth is based on the formula developed by Lovett et al.[10] in which Tryptic Soy Broth was supplemented with yeast extract for optimum growth of *Listeria*. Modified Listeria Enrichment Broth is a modification of Listeria Enrichment Broth in which the acriflavine content has been reduced from 15 mg to 10 mg per liter. This modification reflects the lower concentration specified by the International Dairy Federation[1] for isolation of *L. monocytogenes* from milk and milk products.

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Principles of the Procedure

Modified Listeria Enrichment Broth contains Tryptone, Soytone and Yeast Extract as nitrogen and vitamin sources. Dextrose provides an energy source. Sodium Chloride maintains the osmotic balance of the medium. Potassium Phosphate is a buffering agent. Cycloheximide is incorporated to inhibit saprophytic fungi, while Nalidixic Acid inhibits growth of gram-negative organisms. Acriflavine HCl is added at 10 mg per liter to suppress growth of gram-positive bacteria.

Formula

Modified Listeria Enrichment Broth

Formula Per Liter

Bacto Tryptone	17 g
Bacto Soytone	3 g
Dextrose	2.5 g
Sodium Chloride	5 g
Potassium Phosphate, Dibasic	2.5 g
Bacto Yeast Extract	6 g
Cycloheximide	0.05 g
Acriflavine HCl	0.01 g
Nalidixic Acid	0.04 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **TOXIC.** HARMFUL BY INHALATION AND IF SWALLOWED. (EC) MAY CAUSE CANCER. POSSIBLE RISK OF HARM TO THE UNBORN CHILD. Do not breathe dust. In case of accident or if you feel unwell, seek medical advice immediately. (Show label where possible.) Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Cardiovascular, Face, Lungs, Nerves, Skin, Thorax.

FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed, induce vomiting; seek medical advice immediately and show this container or label.

3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Modified Listeria Enrichment Broth

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Test tubes with closures
Autoclave
Incubator (30°C)

Method of Preparation

1. Suspend 36.1 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[1]
2. For specific information about sample preparation and inoculation, consult appropriate reference.[1]

Test Procedure

For dairy samples, the IDF[1] selective enrichment method is as follows:

1. Add 25 ml liquid or 25 grams of solid test material to 225 ml Modified Listeria Enrichment Broth and mix or blend thoroughly.
2. Incubate for 48 hours at 30°C.[1]
3. At 48 hours, streak the Modified Listeria Enrichment Broth culture onto plates of Oxford Medium or Palcam Medium.
4. Incubate the agar plates at 37°C for 48 ± 2 hrs.

Results

1. Examine agar plates for typical *Listeria* colonies.
2. Consult appropriate references for selection of biochemical or serological tests for confirmation of *Listeria* sp.[1,8,11,12]

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains of *Listeria* may be encountered that fail to grow or grow poorly on this medium.
2. Modified Listeria Enrichment Broth is a partially selective medium. Growth of some contaminating strains will be markedly but not totally inhibited.

References

1. **International Dairy Federation.** 1990. Milk and milk products - detection of *Listeria monocytogenes*. IDF Provisional International Standard No. 143. International Dairy Federation, Brussels.
2. **Murray, E. G. D., R. A. Webb, and M. B. R. Swann.** 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n. sp.). J. Path. Bact., **29:**407- 439.
3. **Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low- and high-fat, frozen and refrigerated ground beef. J. Food Prot. **57**:969-974.
4. **Wehr, H. M.** 1987. *Listeria monocytogenes* - a current dilemma Special Report. J. Assoc. Off. Anal. Chem. **70**:769-772.
5. **Bremer, P. J., and C. M. Osborne.** 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels (*Perna canaliculus*) prepared for hot smoking. J. Food Prot. **58**:604-608.
6. **Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
7. **Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
8. **Donnelly, C. W., R. E. Bracket, D. Doores, W. H. Lee, and J. Lovett.** 1992. *Listeria*, p. 637-663. *In* C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
9. **Kramer, P. A., and D. Jones**. 1969. Media selective for *Listeria monocytogenes*. J. Appl. Bacteriol. **32:**381-394.
10. **Lovett, J., D. W. Frances, and J. M. Hunt.** 1987. *Listeria monocytogenes* in raw milk: detection, incidence and pathogenicity. J. Food Prot. **50:**188-192.
11. **Swaminathan, B., J. Rocourt, and J. Bille.** 1995. *Listeria*, p. 342-343. *In* P.R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
12. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products. *In* R. T. Marshall (ed.). Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Packaging

Modified Listeria Enrichment Broth	500 g	0205-17
	10 kg	0205-08

Bacto® Motility GI Medium

Intended Use

Bacto Motility GI Medium is used for detecting motility of microorganisms and for separating organisms in their motile phase.

Summary and Explanation

Motility GI Medium is prepared according to the formulation of Jordan, Caldwell and Reiter.[1] It is a semisolid gelatin-heart infusion medium that is adaptable to use in both tubes and plates for motility studies.

Principles of the Procedure

Heart Infusion Broth and Gelatin provide nitrogen, vitamins, and amino acids. Bacto Agar is the solidifying agent. Motility is evidenced by the presence of diffuse growth away from the line or spot of inoculation. Nonmotile organisms grow only along the line of inoculation.

Formula

Motility GI Medium

Formula Per Liter

Bacto Heart Infusion Broth	25 g
Bacto Gelatin	53.4 g
Bacto Agar	3 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store the prepared medium at 15-30°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Motility GI Medium

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (50-55°C)

Method of Preparation

1. Suspend 81.4 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. If tubes are desired, dispense medium to a depth of 60-75 mm.
4. Autoclave at 121°C for 15 minutes.
5. Cool tubes by placing in cold water up to the depth of the medium. Cool flasks of medium to 50-55°C; pour into sterile Petri dishes to a depth of 1/8 inch or more and allow to solidify.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	8.14% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, very slightly opalescent, without precipitate.
Prepared Medium:	Medium amber, slightly opalescent.
Reaction of 8.14% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Motility GI Medium per label instructions. Inoculate the medium and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	GROWTH	MOTILITY
Escherichia coli	25922*	good	+
Klebsiella pneumoniae	13883*	good	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Klebsiella pneumoniae* ATCC® 13883

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate with growth from an 18-24 hour pure culture.
2. If tubes are used, inoculate by stab inoculation. If plates are used, spot the inoculum on the surface or stab just below the medium surface.
3. Incubate at a temperature and duration appropriate for the suspected organism being tested.
4. Examine tubes or plates for growth and signs of motility.

Results

Motility is evidenced by the presence of diffuse growth away from the line or spot of inoculation. Nonmotile organisms grow only along the line of inoculation.

Limitations of the Procedure

1. All weak or questionable motility test results should be confirmed by flagella stain or by direct wet microscopy.[2]
2. Some flagellar proteins are not synthesized at higher temperatures.[3]
3. Some isolates of *Yersinia enterocolitica* demonstrate motility at 35°C while others may be nonmotile at 25°C.[2] The motility of *Proteus* is also temperature dependent. This effect of temperature on motility needs to be taken into account when deciding on a testing regimen.
4. Due to the temperature dependency of motility in some organisms, a negative test tube or plate should be incubated an additional 5 days at a lower temperature of 22-25°C.[3]

References

1. **Jordan, E. O., M. E. Caldwell, and D. Reiter.** 1934. Bacterial motility. J. Bacteriol. **27**:165.
2. **D'Amato, R. F., and K. M. Tomfohrde.** 1981. Influence of media on temperature-dependent motility test for *Yersinia enterocolitica*. J. Clin. Microbiol. **14**:347-348.
3. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore, MD.

Packaging

Motility GI Medium	500 g	0869-17

Bacto® Motility Medium S

Intended Use

Bacto Motility Medium S is used with Bacto TTC Solution 1% in detecting bacterial motility.

Also Known As

Motility Medium S conforms with Motility S Medium and Motility-Nitrate Medium.

Summary and Explanation

Motility Medium S is prepared according to the formula of Ball and Sellers.[1] The composition of the medium offers no more resistance to motility during incubation than would a broth culture, yet it preserves the stab line. The medium also permits further testing fornitrate reduction, nitrogen gas production and gelatin liquefaction.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	6.0% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, slightly opalescent, without significant precipitate.
Prepared Medium:	Medium amber, slightly opalescent.
Reaction of 6.0% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Motility Medium S with added TTC Solution 1% per label directions. Stab inoculate the medium and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH	MOTILITY	TTC REDUCTION
Escherichia coli	25922*	good	+	+
Klebsiella pneumoniae	13883*	good	–	+
Shigella flexneri	12022	good	–	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ disks and should be used according to the technical information.

Uninoculated tube | *Escherichia coli* ATCC® 25922

Principles of the Procedure

Infusion from Beef Heart, Gelatin and Tryptose provide nitrogen, vitamins and amino acids to support growth of fastidious microorganisms. Sodium Chloride maintains the osmotic balance of the medium and also induces the swarming of *Proteus* species.[2] Dipotassium Phosphate provides buffering capability and has been shown to have a stimulatory effect on the motility of *Proteus* species.[1] Potassium Nitrate provides trace elements necessary for bacterial growth. Organisms capable of reducing nitrate, especially nitrate-reducing obligate aerobes[1], exhibit increased motility in the presence of 0.2% Potassium Nitrate. Bacto Agar is a solidifying agent at a concentration that preserves the line of inoculation. TTC (2,3,5-triphenyltetrazolium chloride) facilitates the detection of motility. Growth along or out from the stab line is made readily visible because formazan precipitates when TTC is reduced by a microorganism.

Formula

Motility Medium S

Formula Per Liter	
Beef Heart, Infusion from	500 g
Bacto Tryptose	10 g
Bacto Gelatin	30 g
Sodium Chloride	5 g
Dipotassium Phosphate	2 g
Potassium Nitrate	2 g
Bacto Agar	1 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Motility Medium S

Materials Required But Not Provided

Glassware
Autoclave
Waterbath (60°C)
Refrigerator (2-8°C)
Incubator (35°C)
TTC Solution 1%

Method of Preparation

1. Suspend 60 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 60°C.
4. Aseptically add 10 ml of TTC Solution 1% at 60°C. Mix thoroughly.
5. Dispense into sterile test tubes.
6. Refrigerate at 2-8°C until ready to use.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Stab inoculate with growth from a pure 18-24 hour culture immediately after removing the medium from the refrigerator.
2. Incubate at 35-37°C for 18-48 hours.
3. Observe at 6, 24 and 48 hours.[3] Examine tubes for signs of growth and motility.

Results

Motility is evidenced by the presence of diffuse growth away from the line or spot of inoculation. Nonmotile organisms grow only along the line of inoculation.

Growth of microorganisms capable of reducing TTC will appear as a red color along the stab line as well as in the areas into which the cells have migrated.

Limitations of the Procedure

1. All weak or questionable motility results should be confirmed by flagella stain or by direct wet microscopy.[4]
2. Some flagellar proteins are not synthesized at higher temperatures.[3]
3. Some isolates of *Yersinia enterocolitica* demonstrate motility at 35°C while others may be nonmotile at 25°C.[4] The motility of *Proteus* is also temperature dependent. This affect of temperature on motility needs to be considered when deciding on a testing regimen.
4. Due to the fact that motility is temperature dependent in some organisms, a test tube or plate yielding a negative result should be incubated an additional 5 days at a lower temperature of 22-25°C.[3]
5. The addition of tetrazolium salts to the medium may be inhibitory to some bacteria.[5]

References

1. **Ball, R. J., and W. Sellers.** 1966. Improved motility medium. Appl. Microbiol. **14**:670-673.
2. **Schneierson, S. S.** 1961. Production of discrete nonswarming colonies of *Proteus* on medium deficient in sodium chloride and other salts. J. Bacteriol. **82**:621-622.
3. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
4. **D'Amato, R. F., and K. M. Tomfohrde.** 1981. Influence of media on temperature-dependent motility test for *Yersinia enterocolitica*. J. Clin. Microbiol. **14**:347-348.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's Diagnostic Microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Motility Medium S	500 g	0761-17
TTC Solution 1%	30 ml	3112-67*
TTC	25 g	0643-13

*Store at 2-8°C

Bacto® Motility Test Medium

Intended Use

Motility Test Medium is used for detecting microbial motility.

Summary and Explanation

In 1936, Tittsler and Sandholzer reported using a semisolid agar for the detection of bacterial motility.[1] Motility Test Medium is a modification of this formulation.

Bacterial motility is observed macroscopically by a diffuse zone of growth spreading from the line of inoculation. Certain species of motile bacteria will show diffuse growth throughout the entire medium, while others may show diffusion from one or two points only, appearing as nodular outgrowths along the stab. Tittsler and Sandholzer reported that tubes incubated for one day gave identical results with the hanging drop method and that incubation for two days permitted them to demonstrate motility in an additional 4% of the cultures tested.[1]

Motility Test Medium is recommended for the detection of microbial motility in food and dairy standard methods.[2,3,4]

Principles of the Procedure

Tryptose is a source of nitrogen, amino acids and carbon. Sodium chloride provides essential ions while maintaining osmotic balance. Agar is a solidifying agent used at a low concentration.

Formula

Motility Test Medium

Formula Per Liter

Bacto Tryptose	10 g
Sodium Chloride	5 g
Bacto Agar	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Motility Test Medium

Materials Required but not Provided

Glassware
Autoclave
Incubator (35°C)
Incubator (22-25°C)
Inoculating needle
Beef Extract (optional)

Method of Preparation

1. Suspend 20 grams in 1 liter distilled or deionized water. (To obtain more luxuriant microbial growth, add 0.1-0.3% Beef Extract prior to boiling the medium)
2. Heat to boiling to dissolve completely.
3. Dispense as desired.
4. Autoclave at 121°C for 15 minutes.

User Quality Control

Identity Specifications

Dehydrated Media	
Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2% solution, soluble in distilled or deionized water on boiling. Solution is light amber, clear to slightly opalescent with no significant precipitate.
Reaction of 2% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Motility Test Medium per label directions. Inoculate by straight stab of the test organisms and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH	MOTILITY
Escherichia coli	25922*	good	positive
Klebsiella pneumoniae	13883*	good	negative

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Klebsiella pneumoniae* ATCC® 13883

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Inoculate tubes with a pure culture by stabbing through the center of the medium with an inoculating needle to approximately one-half the depth of the medium. Incubate at the proper temperature for the organism under consideration and examine at 18-48 hours. If negative, continue incubation at 22-25°C for an additional 5 days.

Results

Motility is manifested macroscopically by a diffuse zone of growth spreading from the line of inoculation. Certain species of motile bacteria will show diffuse growth throughout the entire medium, while others may show diffusion from one or two points only, appearing as nodular growths along the stab line. Non-motile organisms grow only along the line of inoculation.

Limitations of the Procedure

1. Many organisms fail to grow deep in semisolid media, inoculating pour plates may be advantageous.[5]

References

1. **Tittsler, R. P., and L. A. Sandholzer.** 1936. The use of semi-solid agar for the detection of bacterial motility. J. Bacteriol. **31**:575-580.
2. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel**. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
3. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, p.538-543, vol 1. Williams & Wilkins, Baltimore, MD.

Packaging

Motility Test Medium	100 g	0105-15
	500 g	0105-17

Bacto® Mueller Hinton Medium
Bacto Mueller Hinton Broth

Intended Use

Bacto Mueller Hinton Medium is used for antimicrobial susceptibility testing of rapidly growing aerobic microorganisms by the disk diffusion technique.

Bacto Mueller Hinton Broth is for antimicrobial susceptibility testing of aerobic microorganisms by broth dilution methods.

Also Know As

Mueller Hinton media are abbreviated as M-H Agar and M-H Broth.

Summary and Explanation

Mueller Hinton Medium duplicates the formula recommended by Mueller and Hinton[1] for the primary isolation of *Neisseria* species. In the development of a simple transparent medium containing heat stable ingredients, Mueller and Hinton selected pea meal extract agar.[2] In their modification, starch replaced the growth-promoting properties of pea extract, acting as a "protective colloid" against toxic substances. Tryptic digest of meat was substituted with casamino acids, technical.

Bauer, Kirby, Sherris and Tuck[3] recommended Mueller Hinton Medium for performing antibiotic susceptibility tests using a single disk of high concentration. Mueller Hinton Medium is used in the disk diffusion method of susceptibility testing.[7] Mueller Hinton Broth is used for determining minimal inhibitory concentrations (MICs).[4] Mueller Hinton Medium complies with requirements of the World Health Organization.[8] Mueller Hinton Medium is specified in the FDA Bacteriological Analytical Manual[9] for food testing.

Mueller Hinton Medium is the recommended medium for testing most commonly encountered aerobic and facultatively anaerobic bacteria.[10] This unsupplemented medium has been selected by the National Committee for Clinical Laboratory Standards (NCCLS) for several reasons:[11]

- It shows good batch-to-batch reproducibility.
- It is low in sulfonamide, trimethoprim, and tetracycline inhibitors.
- It gives satisfactory growth of most non-fastidious pathogens.
- A large amount of data has been collected from antimicrobial susceptibility tests with this medium.

A variety of supplements can be added to Mueller Hinton Medium. For testing streptococci, supplementation with 5% defibrinated sheep or horse blood is recommended.[16] GC agar base with added 1% growth supplement, is used for antimicrobial susceptibility testing of *Neisseria gonorrhoeae.* Susceptibility testing of *Haemophilus* species should be performed on *Haemophilus* Test Medium. Mueller Hinton Medium should be supplemented with 2% NaCl for testing methicillin or oxacillin against staphylococci.[5] Mueller Hinton Medium with Rabbit Serum is used for the cultivation and maintenance of *Corynebacterium* species.[6]

Principles of the Procedure

Infusion from Beef and Casamino Acids, Technical provide nitrogen, vitamins, carbon and amino acids in Mueller Hinton media. Starch is added to absorb any toxic metabolites produced. Bacto Agar is the solidifying agent.

The use of a suitable medium is essential for testing the susceptibility of microorganisms to sulfonamides and trimethoprim. Antagonism to sulfonamide activity is demonstrated by para-aminobenzoic acid (PABA) and its analogs. Reduced activity of trimethoprim, resulting in smaller growth inhibition zones and innerzonal growth, is demonstrated on medium possessing high levels of thymidine. The PABA and

thymine/thymidine content of Mueller Hinton Medium and Mueller Hinton Broth are reduced to a minimum, reducing the inactivation of sulfonamides and trimethoprim.

Formula

Mueller Hinton Medium

Formula Per Liter

Beef, Infusion from	300 g
Bacto Casamino Acids, Technical	17.5 g
Starch	1.5 g
Bacto Agar	17 g

Final pH 7.3 ± 0.1 at 25°C

Mueller Hinton Broth

Formula Per Liter

Beef, Infusion from	300 g
Bacto Casamino Acids, Technical	17.5 g
Bacto Soluble Starch	1.5 g

Final pH 7.3 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store dehydrated media below 30°C. The dehydrated media are very hygroscopic. Keep containers tightly closed.

Expiration Date

Expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Mueller Hinton Medium
Mueller Hinton Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Sterile Petri dishes
Sterile 5% defibrinated blood (Optional)

User Quality Control

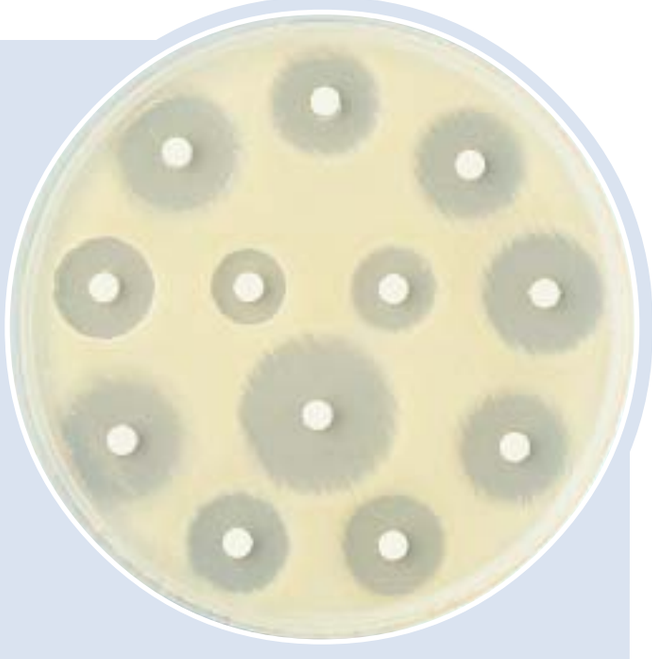
Identity Specification

Mueller Hinton Medium

Dehydrated Appearance:	Beige, homogeneous, free-flowing with few dark specks.
Prepared Medium:	Light to medium amber, slightly opalescent, no significant precipitation.
Reaction of 3.8% Solution at 25°C:	pH 7.3 ± 0.1

Mueller Hinton Broth

Dehydrated Appearance:	Light beige with a few dark specks, homogeneous, free-flowing.
Prepared Medium:	Very light amber, clear, may have slight precipitation.
Reaction of 2.1% Solution at 25°C:	pH 7.3 ± 0.1



Typical test of Mueller Hinton Medium by agar diffusion method.

Cultural Response

Mueller Hinton Medium: Prepare, inoculate and dispense antibiotic disks following the procedure described by NCCLS.[7,11] The cultures listed should have zone sizes near the middle of the range of the concentration tested.[7]

Mueller Hinton Broth: Prepare and dispense into microdilution trays or microdilution tubes described by NCCLS.[4] The cultures listed should have MIC (endpoints) near the middle of the range of the concentration tested.[4]

ORGANISM	ATCC®
Enterococcus faecalis	29212*
Escherichia coli	25922*
Pseudomonas aeruginosa	27853*
Staphylococcus aureus	25923*

The cultures listed are the minimum that should be used for performance testing.

*These organisms are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Method of Preparation

Mueller Hinton Medium

1. Suspend 38 g of medium in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. OPTIONAL: Supplement as appropriate.[16] To supplement Mueller Hinton Medium with sheep blood, aseptically add 5% sterile defibrinated blood at 45-50°C. *Haemophilus* Test Medium contains 15 mcg/ml NAD, 15 mcg/ml bovine hematin and 5 mg/ml yeast extract. The medium recommended for testing Neisseria *gonorrhoeae* consists of GC agar base with 10 ml/liter of the following supplement: 1.1 g L-cystine, 0.03 g guanine HCl, 3 mg thiamine HCl, 13 mg PABA, 0.01 g B_{12}, 0.1 g cocarboxylase, 0.25 g NAD, 1 g adenine, 10 g L- glutamine, 100 g glucose, 0.02 g ferric nitrate, 1 l distilled or deionized water.
5. Pour cooled Mueller Hinton Medium into sterile Petri dishes on a level, horizontal surface to give a uniform depth of about 4mm (60 to 70 ml of medium for 150 mm plates and 25 to 30 ml for 100 mm plates) and allow to cool to room temperature.[10]
6. Check prepared Mueller Hinton Medium to ensure the final pH is 7.3 ± 0.1 at 25°C.

Mueller Hinton Broth

1. Suspend 21 g of medium in 1 liter distilled or deionized water.
2. Warm gently to dissolve.
3. Autoclave at 121°C for 15 minutes.
4. Dispense Mueller Hinton Broth into sterile tubes.
5. Check prepared Mueller Hinton Broth to ensure the final pH is 7.3 ± 0.1 at 25°C.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

For a complete discussion on antimicrobic susceptibility testing, refer to the appropriate procedures outlined in the references.[4,7,9,10,11,12]

Results

Refer to appropriate references and procedures for results

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on these media.
2. Numerous factors can affect results: inoculum size, rate of growth, medium formulation and pH, length of incubation and incubation environment, disk content and drug diffusion rate, and measurement of endpoints. Therefore, strict adherence to protocol is required to ensure reliable results.[12]
3. Disk diffusion susceptibility testing is limited to rapidly growing organisms. Drug inactivation may result from the prolonged incubation times required by slow growers.[12]
4. Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effects of sulfonamides and trimethoprim, causing zones of growth inhibition to be smaller or less distinct.[10]
5. Variation in the concentration of divalent cations, primarily calcium and magnesium, affects results of aminoglycoside, tetracycline, and colistin tests with *P. aeruginosa* isolates.[13,14] A cation content that is too high reduces zones sizes, whereas a cation content that is too low has the opposite effect.[10]
6. When Mueller Hinton Medium is supplemented with blood, the zone of inhibition for oxacillin and methicillin may be 2 to 3 mm smaller than those obtained with unsupplemented agar.[10] Conversely, sheep blood may markedly increase the zone diameters of some cephalosporins when they are tested against enterococci.[15] Sheep blood may cause indistinct zones or a film of growth within the zones of inhibition around sulfonamide and trimethoprim disks.[10]
7. Mueller Hinton Medium deeper than 4 mm may cause false-resistant results, and agar less than 4 mm deep may be associated with a false-susceptibility report.[10]
8. A pH outside the range of 7.3 ± 0.1 may adversely affect susceptibility test results. If the pH is too low, aminoglycosides and macrolides will appear to lose potency; others may appear to have excessive activity.[10] The opposite effects are possible if the pH is too high.[10]
9. When Mueller Hinton Medium is inoculated, no droplets of moisture should be visible on the surface or on the petri dish cover.[10]
10. Mueller Hinton Medium should be inoculated within 15 minutes after the inoculum suspension has been adjusted.[10]
11. The zone of inhibition diameters of some drugs, such as the aminoglycosides, macrolides, and tetracyclines, are significantly altered by CO_2. Plates should not be incubated in increased CO_2.[10]

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Packaging

Mueller Hinton Broth	100 g	0757-15
	500 g	0757-17
	2 kg	0757-07
Mueller Hinton Medium	100 g	0252-15
	500 g	0252-17
	2 kg	0252-07
	10 kg	0252-08

Bacto® Muller Kauffmann Tetrathionate Broth Base

Intended Use

Bacto Muller Kauffmann Tetrathionate Broth Base is used for enriching *Salmonella* from water, foodstuffs and fecal samples prior to selective isolation.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white to light beige, free-flowing, homogeneous.
Solution:	10.58% solution, insoluble in distilled or deionized water.
Prepared Medium:	Very pale green with white precipitate.

Cultural Response

Prepare Muller Kauffmann Tetrathionate Broth Base per label directions, with the addition of 1.9 ml Iodine solution and 0.95 ml Brilliant Green solution per 100 ml of medium. Inoculate and incubate at 42-43°C for 18-24 hours. Subculture to Brilliant Green Agar. Incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Salmonella typhimurium	14028*	100-1,000	good	red colonies
Salmonella senftenburg (NCTC)	10384	100-1,000	good	red colonies
Escherichia coli	25922*	1,000-2,000	none to poor	–
Proteus vulgaris	13315*	1,000-2,000	none to poor	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Summary and Explanation

Muller[1] recommended Tetrathionate Broth as a selective medium for the isolation of *Salmonella*. Kauffmann[2] modified the formula to include oxbile and brilliant green as selective agents to suppress bacteria such as *Proteus* spp.

The British Standard Specification specifies Brilliant Green Tetrathionate Broth for isolating *Salmonella* from meat and meat products[3] and from poultry and poultry products.[4] It is also a recommended selective broth for isolating *Salmonella* from animal feces and sewage polluted water.[5] Using more than one selective broth increases the isolation of *Salmonella* from samples with multiple serotypes.[6]

Muller Kauffmann Tetrathionate Broth Base conforms with ISO/DIS 3565.[3]

Principles of the Procedure

Muller Kauffmann Tetrathionate Broth Base contains Bacto Peptone and Beef Extract as sources of carbon, nitrogen, vitamins and minerals. Oxgall and added Brilliant Green are selective agents which inhibit gram positive and other gram negative organisms. Calcium Carbonate is the buffer. Sodium Thiosulfate is a source of sulfur.

Formula

Bacto Muller Kauffmann Tetrathionate Broth Base

Formula Per Liter

Bacto Beef Extract	5 g
Bacto Peptone	10 g
Sodium Chloride	3 g
Calcium Carbonate	45 g
Sodium Thiosulphate (anhydrous)	38.1 g
Bacto Oxgall	4.7 g

Precautions

1. For Laboratory Use.

2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Muller Kauffmann Tetrathionate Broth Base

Materials Required but not Provided

Iodine solution (20 g iodine and 25 g potassium iodide in 100 ml water)
Brilliant Green solution (0.1 g Brilliant Green in 100 ml water)
Glassware
Distilled or deionized water
Autoclave
Incubator (43°C)
Buffered Peptone Water
Blender
Tetrathionate Broth
Selenite Brilliant Green Medium
Brilliant Green Agar Enrichment
Brilliant Green Agar

Method of Preparation-Single Strength

1. Suspend 105.8 grams in 1 liter distilled or deionized water.
2. Boil gently.
3. Cool below 45°C.
4. Add 19 ml iodine solution and 9.5 ml brilliant green solution.
5. Dispense into sterile tubes, mixing well to evenly dispense the calcium carbonate.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure

Meat and Meat Products

1. Weigh 25 grams of the sample into a sterile blender jar and add 225 ml of Buffered Peptone Water (1810) and macerate for sufficient time to give 10,000-15,000 revolutions.
2. Transfer contents of the blender jar aseptically to a 500 ml flask. Incubate at 37°C ± 0.1°C for 16-20 hours.
3. Transfer 10 ml samples to 100 ml Muller Kauffmann Tetrathionate Broth and to 100 ml Selenite Brilliant Green Medium (0661).
4. Incubate Muller Kauffmann Tetrathionate Broth at 42-43°C and the Selenite Brilliant Green Enrichment at 37°C.
5. Subculture broths after 18-24 hours and 48 hours onto Brilliant Green Agar.
6. Incubate overnight.
7. Examine for the growth of typical colonies of *Salmonella* spp.

Sewage Polluted Natural Waters

This procedure is applicable to the isolation of *Salmonella* spp. other than *S. typhi*.

1. Inoculate 25 ml aliquots of the sample into 25 ml of double strength Buffered Peptone Water (1810). Incubate at 37°C for 18 hours.
2. Transfer 1 ml samples into 10 ml of Muller Kauffmann Tetrathionate Broth.
3. Incubate at 43°C for 48 hours.
4. Subculture broths after 18-24 and 48 hours onto Brilliant Green MacConkey Agar, prepared by adding 10 ml of a 0.33% (w/v) aqueous solution of brilliant green to MacConkey Agar (0331) to give a final concentration of 0.033 g/l.
5. Incubate at 37°C overnight.
6. Examine for colonies typical of *Salmonella* spp.

Results

Salmonella spp. will produce red colonies with good growth.

Limitations of the Procedure

1. The complete medium is unstable and should be used immediately. It may be stored at 2-8°C in the dark for no more than seven days.
2. Due to the nutritional requirements and inhibitory characteristics of the organisms themselves, organisms other than salmonellae, such as *Morganella morganii* and some *Enterobacteriaceae* may grow in the medium.
3. Confirmatory tests, such as fermentation reactions and seroagglutination should be carried out on all presumptive *Salmonella* colonies that are recovered.

References

1. **Muller, L.** 1923. Un nouveau millieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. C. R. Soc. Biol. (Paris) **89**:434-443.
2. **Kauffmann, F.** 1935. Weitere erfahrungen mit dem kombininierten anreicherungsverfahren fur Salmonella bazillen. Ztschr. F. Hyg. **117**:26-32.
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5. P.H.L.S. Monograph Series No. 8. 1974.
6. **Harvey, R. W. S., and T. H. Price.** 1976. Isolation of salmonellae from sewage- polluted river water using selenite F and Muller-Kauffmann tetrathionate. J. Hyg. Camb. **77**:333-339.

Packaging

Muller Kauffmann Tetrathionate Broth Base	500 g	1853-17

Bacto® Mycobiotic Agar

Intended Use

Bacto Mycobiotic Agar is used for isolating pathogenic fungi.

Summary and Explanation

Numerous media, such as Sabouraud Dextrose Agar, Sabouraud Maltose Agar, Littman Oxgall Agar, Brain Heart Infusion Agar, and Malt Agar have been used widely in culturing pathogenic fungi. The Sabouraud media and Malt Agar are somewhat selective in nature due to low pH, which may suppress bacterial growth. It is well known that media for isolated pathogenic fungi can also be made selective by the addition of antibiotics.[1-8]

Mycobiotic Agar contains cycloheximide and chloramphenicol making it much more selective when compared to other fungal media. This medium has proven useful in the isolation of the dermatophytes and other pathogenic fungi from clinical specimens.[9]

Georg[10] recommends the use of Mycobiotic Agar exclusively for isolating dermatophytes (because none of the dermatophytes are sensitive to cycloheximide or chloramphenicol) and in parallel to media without antibiotics for isolating fungi which cause systemic disease.

Principles of the Procedure

Soytone provides carbon and nitrogen sources. Dextrose is a source of carbon. Cycloheximide suppresses the growth of saprophytic fungi. Chloramphenicol inhibits bacterial growth. Bacto Agar is the solidifying agent.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.56% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, slightly opalescent, with no significant precipitate.
Reaction of 3.56% Solution at 25° C:	pH 6.5 ± 0.2

Cultural Response

Prepare Mycobiotic Agar per label directions. Inoculate and incubate at 25-30°C for 18-48 hours. For *Trichophyton mentagrophytes*, inoculate a 1-2 week old undiluted *Trichophyton* culture directly onto a slant or plate. *Trichophyton* cultures should be incubated up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	inhibited
Candida albicans	10231	100-1,000	good
Escherichia coli	25922*	1,000-2,000	inhibited
Trichophyton mentagrophytes	9533	undiluted	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

Mycobiotic Agar

Formula Per Liter

Bacto Soytone	10 g
Bacto Dextrose	10 g
Bacto Agar	15 g
Cycloheximide	0.5 g
Chloramphenicol	0.05 g

Final pH 6.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **TOXIC.** TOXIC BY INHALATION AND IF SWALLOWED. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. POSSIBLE RISK OF HARM TO THE UNBORN CHILD. Do not breathe dust. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Eyes/Ears, Blood, Cardiovascular, Lymph Glands, Muscles, Nerves, Urogenital

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed, induce vomiting; seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Mycobiotic Agar

Materials Required but not Provided

Glassware
Autoclave
Petri dishes
Tubes with closures

Method of Preparation

1. Suspend 35.6 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 10 minutes. Avoid overheating, which will decrease selectivity.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results
Refer to appropriate references and procedures for results.

Limitations of the Procedure
1. Fungi that cause systemic disease may be sensitive to the antibiotics cycloheximide and chloramphenicol. Primary isolation should include the use of both non-selective and selective media.[11] Antibiotic-containing media should be incubated at room temperature. Additional procedures may be required for complete identification of pathogenic yeasts, particularly *Candida albicans*.
2. Although culture techniques are important in the identification of etiological agents of mycotic infections, they are not absolute. Identification must often be accomplished by using direct microscopic examination of the specimen, animal inoculation, biochemical determination, or serological procedures.

References

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Packaging
Mycobiotic Agar	100 g	0689-15
	500 g	0689-17
	2 kg	0689-07
	10 kg	0689-08

Mycological Media

Bacto® Mycological Agar · Bacto Mycological Agar w/Low pH

Intended Use
Bacto Mycological Agar is used for cultivating fungi at a neutral pH.

Bacto Mycological Agar w/Low pH is used for isolating and cultivating fungi and aciduric bacteria.

Summary and Explanation
The value of selective media for the initial cultivation of pathogenic fungi has been demonstrated by numerous investigators.[1,2,3] Earlier media for fungi generally relied on an acid pH to make the media less suitable for the growth of many bacteria.[6] More recently developed media use neutral or slightly alkaline reactions,[4,5] antibiotics, bile salts and dyes as selective agents against bacteria.

Mycological media are excellent basal media to which antifungal agents may be added to study their affect on fungi. These media may be supplemented with antibacterial substances to render them more selective for the isolation and cultivation of fungi.

Mycological Agar and Mycological Agar w/Low pH are prepared according to the formulation suggested by Huppert and Walker.[7] Mycological Agar, which has a lower dextrose content than Sabouraud Dextrose Agar, is recommended for the isolation and cultivation of fungi from clinical specimens, foods,[8] and cosmetics.[9] This medium may be adjusted to pH 4.0 after autoclaving by adding sterile lactic acid or acetic acid.

Mycological Agar at a neutral pH is recommended for working with pathogenic fungi. Mycological Agar w/Low pH is suitable for culturing saprophytic yeasts and molds, and aciduric bacteria.

Principles of the Procedure
Soytone provides a source of carbon and nitrogen. Dextrose is an additional source of carbon. Bacto Agar is the solidifying agent.

Formula
Mycological Agar

Formula Per Liter

Bacto Soytone	10 g
Bacto Dextrose	10 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Mycological Agar w/Low pH

Formula Per Liter

Bacto Soytone	10 g
Bacto Dextrose	10 g
Bacto Agar	15 g

Final pH 4.8 ± 0.2 at 25°C

Precautions

1. Mycological Agar: For Laboratory Use.
   Mycological Agar w/Low pH: For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep containers tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

User Quality Control

Identity Specifications

Mycological Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 3.5% Solution at 25°C:	pH 7.0 ± 0.2

Mycological Agar w/Low pH

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 3.5% Solution at 25°C:	pH 4.8 ± 0.2

Cultural Response

Mycological Agar, Mycological Agar w/Low pH

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH MYCOLOGICAL AGAR	GROWTH MYCOLOGICAL AGAR W/LOW PH
Aspergillus niger	16404	100-1,000	good	good
Candida albicans	10231	100-1,000	good	good
Penicillium abeanum	22346	100-1,000	good	good
Saccharomyces carlsbergensis	9080	100-1,000	good	good
Staphylococcus aureus	25923	100-1,000	good	inhibited

The cultures listed are the minimum that should be used for performance testing.

Procedure

Materials Provided

Mycological Agar
Mycological Agar w/Low pH

Materials Required but not Provided

Glassware
Autoclave
Antibacterial/antifungal agents

Method of Preparation

Mycological Agar, Mycological Agar w/low pH

1. Suspend 35 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. If desired, add antibacterial and antifungal agents after sterilizing and cooling the medium to 45-50°C.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Mycological Agar and Mycological Agar w/Low pH are used in a variety of procedures. Consult appropriate references for further information.[8,9]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Non-selective fungal media should be used concurrently with selective media when isolating fungi due to the sensitivity of some strains to cycloheximide and chloramphenicol.[10,11,12]

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7. **Huppert, M., and L. J. Walker.** 1958. The selective and differential effects of cycloheximide on many strains of *Coccidioides immitis*. Am. J. Clin. Pathol. **29:**291.
8. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1, p. 552-554. Williams & Wilkins, Baltimore, MD.
9. **Curry, A. S., J. G. Graf, and G. N. McEwen, Jr.** 1993. CTFA Microbiology Guidelines. The Cosmetic, Toiletry, and Fragrance Association, Washington, D.C.
10. **Georg, L. K., L. Ajello, and C. Papageorge.** 1954. Use of cycloheximide in the selective isolation of fungi pathogenic to man. J. Lab. Clin. Med. **44:**422.
11. **McDonough, E. S., L. Ajello, L. K. Georg, and S. Brinkman.** 1960. *In vitro* effects of antibiotics on yeast phase of *Blastomyces dermatitidis* and other fungi. J. Lab. Clin. Med. **55:**116.

12. **McDonough, E. S., L. K. Georg, L. Ajello, and S. Brinkman.** 1960. Growth of dimorphic human pathogenic fungi on media containing cycloheximide and chloramphenicol. Mycopathol. Mycol. Appl. **13:**113.

Packaging

Mycological Agar	500 g	0405-17
	2 kg	0405-07
Mycological Agar w/Low pH	500 g	0305-17

Bacto® Neopeptone

Intended Use

Bacto Neopeptone is used in preparing microbiological culture media.

Also Known As

Neopeptone is also referred to as Special Peptone.

Summary and Explanation

Neopeptone is particularly well suited to the growth requirements of fastidious miroorganisms. Certain delicate strains of microorganisms are highly susceptible to the effects of bacteriostatic substances frequently present in some peptones. The work of Dubos[1] shows clearly that a peptone free from toxic factors can support the growth of *S. pneumococci* from small inocula. Spray[2] used Neopeptone in his culture media for classification of sporulating anaerobes. Casman[3] reported Neopeptone to be best suited for use in infusion base. Eldering and Kendrick[4] reported good results with Neopeptone in cultivating *Bordetella pertussis*.

Neopeptone is valuable in culture media for the cultivation of pathogenic fungi. Growth of these microorganisms is rapid and colonial formation is uniform and typical for the various types. Bacto Sabouraud Dextrose Agar and Bacto Sabouraud Maltose Agar are prepared with Neopeptone.

Bacto Todd Hewitt Broth prepared with Neopeptone, is excellent for growing Group A streptococci for serological typing. Several media containing Neopeptone are specified in standard methods[5-7] for multiple applications.

User Quality Control

Identity Specifications

Dehydrated Appearance: Tan, free-flowing granules.

Solution: 1%, 2% and 10% solutions are soluble in distilled or deionized water:

1%-Very light to light amber, clear to very slightly opalescent, may have a precipitate.

2%-Light to medium amber, clear to very slightly opalescent, may have a precipitate.

10%-Medium to dark amber, slightly opalescent to opalescent, may have a precipitate.

Reaction of 1% Solution at 25°C: pH 6.9 - 7.5

Cultural Response

All solutions are prepared with the pH adjusted to 7.2 - 7.4.

TEST	SOLUTION	ORGANISM	ATCC®	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922*	negative
Indole Production	0.1%	*Escherichia coli*	25922*	positive
Acetylmethylcarbinol Production	0.1%	*Enterobacter aerogenes*	13048*	positive
Hydrogen Sulfide	1%	*Salmonella typhi*	6539	positive
Toxicity	2% w/0.5% NaCl & 1.5% Bacto Agar	*Escherichia coli*	25922*	good growth
Toxicity	2% w/0.5% NaCl & 1.5% Bacto Agar	*Staphylococcus aureus*	25923*	good growth

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Neopeptone is an enzymatic digest of protein. Neopeptone contains a wide variety of peptide sizes in combination with vitamins, nucleotides, minerals and other carbon sources.

Typical Analysis

Physical Characteristics

Ash (%)	7.0	Loss on Drying (%)	3.2
Clarity, 1% Soln (NTU)	1.2	pH, 1% Soln	7.4
Filterability (g/cm^2)	0.3		

Carbohydrate (%)

Total	0.8

Nitrogen Content (%)

Total Nitrogen	13.7	AN/TN (%)	23.8
Amino Nitrogen	3.3		

Amino Acids (%)

Alanine	4.03	Lysine	5.16
Arginine	4.14	Methionine	2.00
Aspartic Acid	6.19	Phenylalanine	8.67
Cystine	0.26	Proline	6.73
Glutamic Acid	13.22	Serine	4.22
Glycine	7.02	Threonine	3.69
Histidine	<0.01	Tryptophan	0.96
Isoleucine	0.36	Tyrosine	4.21
Leucine	3.65	Valine	4.96

Inorganics (%)

Calcium	0.012	Phosphate	2.209
Chloride	0.344	Potassium	0.149
Cobalt	<0.001	Sodium	2.057
Copper	<0.001	Sulfate	0.340
Iron	<0.001	Sulfur	0.657
Lead	<0.001	Tin	<0.001
Magnesium	0.006	Zinc	<0.001
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.2	PABA	2.9
Choline (as Choline Chloride)	3100.0	Pantothenic Acid	16.0
Cyanocobalamin	<0.1	Pyridoxine	2.3
Folic Acid	0.4	Riboflavin	1.3
Inositol	3600.0	Thiamine	<0.1
Nicotinic Acid	52.2	Thymidine	<14.0

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	400
Salmonella	negative	Thermophile Count	75
Spore Count	175		

Procedure

Materials Provided

Neopeptone

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Neopeptone in the formula of the medium being prepared. Add Neopeptone as required.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Neopeptone.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Dubos, R.** 1930. The bacteriostatic action of certain components of commercial peptones as affected by conditions of oxidation and reduction. J. Exp. Med. **52**:331-345.
2. **Spray, R. S.** 1936. Semisolid media for cultivation and identification of the sporulating anaerobes. J. Bacteriol. **32**:135.
3. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. **17**:281-289.
4. **Eldering, E., and P. L. Kendrick.** 1936. Some practical considerations in *B. pertussis* vaccine preparation. Am. J. Public Health. **24**:309.
5. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
6. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
7. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Packaging

Neopeptone	500 g	0119-17
	10 kg	0119-08

Bacto® Neutralizing Buffer

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, homogeneous
Solution:	0.52% solution; soluble in distilled or deionized water. Solution is very light to light amber, clear to very slightly opalescent.
Prepared tubes:	Very light to light amber, clear to slightly opalescent without significant precipitation
Reaction of 0.52% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Bacto Neutralizing Buffer per label directions. Dilute a disinfectant containing a quaternary ammonium compound such as Roccal® with Bacto Neutralizing Buffer from 1:2,500 to1:100,000. Inoculate the tubes with *Staphylococcus aureus* ATCC® 6538P. Prepare pour plates by transferring 1 ml from each dilution to Bacto Tryptone Glucose Extract Agar (Product Code 0002). Incubate the plates for 40-48 hours at 32°C. Record growth. Bacto Neutralizing Buffer inactivates the bactericidal activity which the growth pattern should reflect.

Intended Use

Bacto Neutralizing Buffer is recommended for detection of microorganisms found on dairy and food equipment disinfected with chlorine or quaternary ammonium compounds.

Summary And Explanation

Bacto Neutralizing Buffer, a modification of the Standard Methods buffered distilled water, has the ability to inactivate the bactericidal and bacteriostatic effect of chlorine as well as quaternary ammonium compounds. Neutralizing Buffer is recommended for use in the microbiological examination of surfaces in the standard methods for dairy and foods.[1,2] Neutralizing Buffer is also recommended for the digestion and decontamination of mycobacterial specimens.[3]

Principles of the Procedure

Monopotassium phosphate provides the buffering capability. Sodium thiosulfate inactivates the effect of chlorine compounds. The aryl sulfonate complex neutralizes the effects of quaternary ammonium compounds.

Formula

Neutralizing Buffer

Formula Per Liter

Monopotassium Phosphate	0.0425 g
Sodium Thiosulfate	0.16 g
Aryl Sulfonate Complex	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Neutralizing Buffer

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Tryptone Glucose Extract Agar

Method of Preparation

1. Dissolve 5.2 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate standard methods[1,2,3] for specific test methodologies.

Results

Refer to appropriate references and procedures for results.

References

1. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Cernoch, P. L., R. K. Enns, M. A. Saubolle, and R. J. Wallace, Jr.** 1994. Cumitech 16A, Laboratory diagnosis of the mycobacterioses. Coordinating ed., A. S. Weissfeld. American Society for Microbiology, Washington, D.C.

Packaging

Neutralizing Buffer	100 g	0362-15

Bacto® Niacin Assay Medium

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white, homogeneous, tendency to clump.
Solution:	3.75% (single strength) or 7.5% (double strength) solution, soluble in distilled or deionized water on boiling 2-3 minutes. Single strength solution is very light amber, clear, may have a slight precipitate.
Prepared Medium:	(Single strength) very light amber, clear, may have a slight precipitate.
Reaction of 3.75% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Prepare Niacin Assay Medium per label directions. Prepare a standard curve using nicotinic acid reference standards at 0.0 to 0.25 µg per 10 ml. The medium supports the growth of *L. plantarum* ATCC® 8014 when supplemented with nicotinic acid.

Intended Use

Bacto Niacin Assay Medium is used for determining niacin concentration by the microbiological assay technique.

Summary and Explanation

Vitamin Assay Media are used in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test.

Niacin Assay Medium is prepared according to the formula described by Snell and Wright,[1] modified by Krehl, Strong and Elvehjem[2] and Barton-Wright.[3] Niacin Assay Medium is used in the microbiological assay of nicotinic acid or nicotinamide (niacin) using *Lactobacillus plantarum* ATCC® 8014 as the test organism. The medium complies with USP[4] and AOAC.[5]

Principles of the Procedure

Niacin Assay Medium is a dehydrated medium free from nicotinic acid and its analogs but containing all other nutrients and vitamins essential for the cultivation of *L. plantarum* ATCC® 8014. The addition of nicotinic acid or its analogs in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Niacin Assay Medium

Formula Per Liter	
Bacto Vitamin Assay Casamino Acids	12 g
Bacto Dextrose	40 g
Sodium Acetate	20 g
L-Cystine	0.4 g
DL-Tryptophane	0.2 g
Adenine Sulfate	20 mg
Guanine Hydrochloride	20 mg
Uracil	20 mg
Thiamine Hydrochloride	200 µg
Calcium Pantothenate	200 µg
Pyridoxine Hydrochloride	400 µg
Riboflavin	400 µg
p-Aminobenzoic Acid	200 µg
Biotin	0.8 µg
Dipotassium Phosphate	1 g
Monopotassium Phosphate	1 g
Magnesium Sulfate	0.4 g
Sodium Chloride	20 mg
Ferrous Sulfate	20 mg
Manganese Sulfate	20 mg

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Kidney, Bladder.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
4. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.
5. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Niacin Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus plantarum* ATCC® 8014.
Sterile tubes
Distilled or deionized water
Sterile 0.85% saline
Centrifuge
Spectrophotometer
Lactobacilli Broth AOAC or Micro Inoculum Broth
Niacin

Method of Preparation

1. Suspend 7.5 grams in 100 ml distilled or deionized water.
2. Boil for 2-3 minutes.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volumes to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assay, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Follow assay procedures as outlined in USP[4] and AOAC.[5]

Stock cultures of the test organism *L. plantarum* ATCC® 8014 are prepared by stab inoculation of Lactobacilli Agar AOAC or Micro Assay Culture Agar. After 24-48 hours incubation at 35-37°C, the cultures are kept refrigerated. Transfers are made in triplicate at monthly intervals.

The inoculum for assay is prepared by subculturing a stock culture of *L. plantarum* ATCC® 8014 into 10 ml of Lactobacilli Broth AOAC or Micro Inoculum Broth. After 18-24 hours incubation at 35-37°C, the cells are centrifuged under aseptic conditions and the supernatant decanted. The cells are washed three times with 10 ml sterile 0.85% saline. After the third wash, the cells are resuspended in 10 ml sterile 0.85% saline and finally diluted 1:100 with 0.85% sterile saline. One drop of this latter suspension is used to inoculate each 10 ml assay tube.

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve reading and cannot always be duplicated. The standard curve is obtained by using niacin at levels of 0.0, 0.025, 0.05, 0.1, 0.15, 0.2 and 0.25 µg niacin per assay tube (10 ml). Niacin Assay Medium may be used for both turbidimetric and titrimetric analyses. Turbidimetric readings should be made after 18-24 hours incubation at 35-37°C. Titrimetric determinations are best made following 72 hours incubation at 35-37°C.

The concentration of niacin required for the preparation of the standard curve may be prepared by dissolving 0.05 grams of niacin in 1,000 ml

distilled water, giving a stock solution of 50 µg per ml. Dilute the stock solution by adding 1 ml to 999 ml distilled water (50 ng/ml). Use 0.0, 0.5, 1, 2, 3, 4 and 5 ml of the 50 ng/ml solution per tube. Other standard concentrations may be used provided the standard falls within the limits specified by AOAC.[5]

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average and use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results to these procedures, all conditions of the assay must be followed precisely.

References

1. **Snell and Wright.** 1941. J. Biol. Chem. **13**:675.
2. **Krehl, Strong, and Elvehjem.** 1943. Ind. & Eng. Chem., Ann. Ed., **15**:471.
3. **Barton-Wright.** 1944. J. Biochem. **38**:314.
4. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Inc., Rockville, MD.
5. **Association of Official Analytical Chemists.** 199. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Niacin Assay Medium	100 g	0322-15

Bacto® Nitrate Broth

Intended Use

Bacto Nitrate Broth is used for differentiating microorganisms based on nitrate reduction.

Summary and Explanation

Nitrate reduction is a valuable criterion for differentiating and identifying various types of bacteria. Certain bacteria reduce nitrates to nitrites only, while others are capable of further reducing nitrite to free nitrogen or ammonia.

Nitrites are colorless; however, in an acid environment, they will react with alpha-naphthylamine to produce a pink or red color. When nitrate-positive organisms reduce nitrates to nitrites, a pink color develops in the broth medium when specific reagents are added.

User Quality Control

Identity Specifications

Dehydrated Medium Appearance:	Light to medium tan, free-flowing, homogeneous.
Solution:	0.9% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Reaction of 0.9% Solution at 25°C:	pH 7.0 ± 0.2

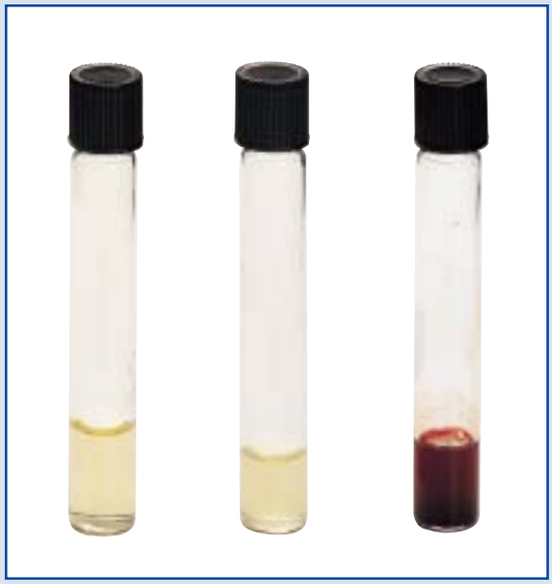
Cultural Response

Prepare Nitrate Broth per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	NITRATE REDUCTION
Acinetobacter calcoaceticus	19606	100-1,000	good	–
Enterobacter aerogenes	13048*	100-1,000	good	+
Escherichia coli	25922*	100-1,000	good	+
Salmonella typhimurium	14028*	100-1,000	good	+

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ disks and should be used as directed.



Uninoculated tube | *Acinetobacter calcoaceticus* ATCC® 19606 | *Escherichia coli* ATCC® 25922

Nitrate-negative organisms, unable to reduce nitrates, yield no color after the reagents are added. Nitrate-negative reactions are tested with zinc dust to confirm the presence of unreduced nitrate.

Principles of the Procedure

Beef Extract and Peptone are sources of carbon, protein and nutrients. Potassium Nitrate is a source of nitrate.

Formula

Nitrate Broth

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Potassium Nitrate	1 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Nitrate Broth

Materials Required but not Provided

Glassware
Autoclave
Incubator (35°C)
0.8% Sulfanilic acid (SpotTest™ Nitrate Reagent A)
N,N-Dimethyl-alpha-naphthylamine (SpotTest Nitrate Reagent B)
Zinc dust (SpotTest Nitrate Reagent C)

Method of Preparation

1. Dissolve 9 grams of Nitrate Broth in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Not applicable

Test Procedure

1. Inoculate the medium with several colonies from a pure 18-24 hour culture. Test an uninoculated control tube in parallel.
2. Incubate the tubes aerobically at 35 ± 2°C for 18-24 hours.
3. Test for nitrate by adding a few drops of 0.8% sulfanilic acid and N,N-dimethyl- alpha-naphthylamine to each tube.
4. Observe for development of a distinct red or pink color within 1-2 minutes, indicating reduction of nitrate to nitrite.
5. If there is no color development, add a pinch of zinc dust (approximately 20 mg on an applicator stick) to the tube. If there is no color development within 5-10 minutes, nitrate was reduced beyond nitrite and the test result is positive.

Results

Development of a distinct red or pink color within 1-2 minutes indicates reduction of nitrate to nitrite and is a positive test result.

Limitations of the Procedure

1. The addition of too much zinc dust may cause a false-negative reaction or a momentary color reaction.[1]
2. The nitrate test is very sensitive. An uninoculated nitrate control should be tested with reagents to determine whether the medium is nitrate free and that the glassware and reagents have not been contaminated with nitrous oxide.[1]
3. The inoculum should not be taken from a liquid or broth suspension of the organism.[1]

References

1. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, p.275-284, vol 1. Williams & Wilkins, Baltimore, MD.

Packaging

Nitrate Broth	500 g	0268-17

Bacto® Nutrient Agar

Intended Use

Bacto Nutrient Agar is used for cultivating a wide variety of microorganisms.

Summary and Explanation

In the early 1900s the American Public Health Association (APHA) suggested this formulation as a standard culture medium for use in bacterial processing for water analysis.[1] The name Nutrient Agar was later adopted for the medium. In Standard Methods of Water Analysis[2] and Standard Methods of Milk Analysis,[3] the APHA advocated the use of dehydrated media for bacterial examination of water and milk. Nutrient Agar meets APHA and Association of Official Analytical Chemists (A0AC) standard methods.[4,5,6]

Nutrient Agar continues to be a widely used general purpose medium for growing nonfastidious microorganisms. It is specified in many standard methods procedures for examining foods, dairy products, water and other materials.[4,5,6,7]

Principles of the Procedure

Nutrient Agar contains Beef Extract and Bacto Peptone as carbon and nitrogen sources for general growth requirements. Bacto Agar is added as a solidifying agent.

Formula

Nutrient Agar

Formula per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Expiration Date

The product is stable through the expiration date on the label when stored as directed. Expiration date applies to the medium in its intact container. Do not use if the medium is caked, discolored or shows other signs of deterioration.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in distilled or deionized water on boiling; light to medium amber, clear to slightly opalescent, no significant precipitate.
Prepared medium:	Light amber, very slightly to slightly opalescent, no significant precipitate.
Reaction of 2.3% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Nutrient Agar per label directions. Inoculate medium with the test organism and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Enterococcus faecalis	19433*	100-1,000	good
Escherichia coli	25922*	100-1,000	good
Pseudomonas aeruginosa	27853*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in the Bactrol Disks Technical Information.

Procedure

Materials Provided

Nutrient Agar

Materials Required but not Provided

Flask with closure
Distilled or deionized water
Autoclave
Petri dishes
Incubator

Method of Preparation

1. Suspend 23 grams in 1 liter distilled or deionized water. Heat to boiling to dissolve completely.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Process specimens according to established procedures for the type of material being tested.[4,5,6,7]

Test Procedure

1. Inoculate with growth from either a single colony on agar or a loopful of broth and streak for isolation.
2. Incubate aerobically at 35°C for 18 to 24 hours or longer if necessary.

Results

Good growth of nonfastidious organisms on Nutrient Agar will appear as translucent colonies.

References

1. **American Public Health Association**. 1917. Standard methods of water analysis, 3rd ed. American Public Health Association, Washington, D.C.
2. **American Public Health Association**. 1923. Standard methods of water analysis, 5th ed. American Public Health Association, Washington, D.C.
3. **American Public Health Association**. 1923. Standard methods of milk analysis, 4th ed. American Public Health Association, Washington, D.C.
4. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.)**. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **Marshall, R. T. (ed.).** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
6. **Association of Official Analytical Chemists**. 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
7. **Vanderzant C., and D. F. Splittstoesser (ed.)**. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Nutrient Agar	100 g	0001-15
	500 g	0001-17
	2 kg	0001-07

Bacto® Nutrient Agar 1.5%

Intended Use

Bacto Nutrient Agar 1.5% is used for cultivating a variety of microorganisms and with the addition of blood or other enrichment can be used for cultivating fastidious microorganisms.

Summary and Explanation

Nutrient Agar 1.5% is a modification of Nutrient Agar, a formula prepared according to APHA standards.[1,2] This medium is a slightly alkaline general purpose medium. Since this medium contains 0.8% sodium chloride it can be used as a base for enrichment with blood, ascitic fluid or other supplements for cultivating fastidious microorganisms.

Principles of the Procedure

Bacto Beef Extract and Bacto Peptone provide the nitrogen, vitamins, amino acids and carbon sources in Nutrient Agar 1.5%. Sodium chloride maintains the osmotic balance so that red blood cells will not rupture when blood is added as supplement.[1] Bacto Agar is the solidifying agent.

Formula

Nutrient Agar 1.5%

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Sodium Chloride	8 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

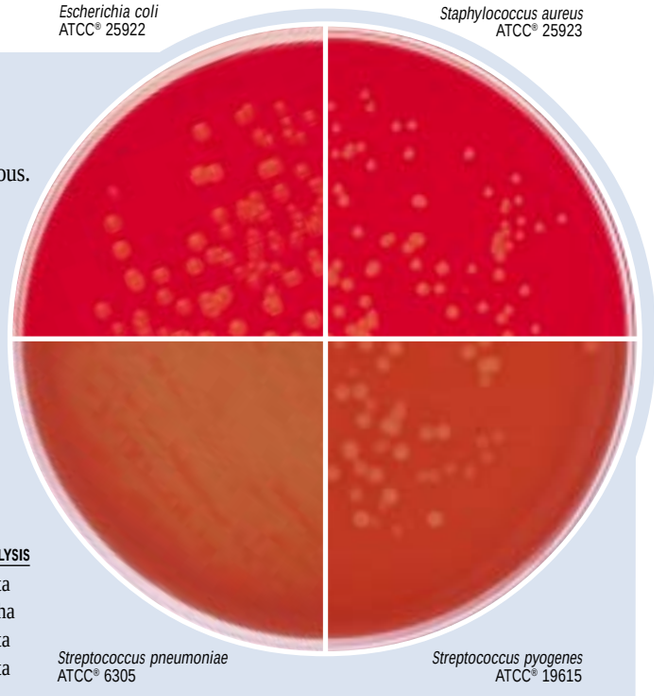
Nutrient Agar 1.5%

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath
Sterile Petri dishes

Method of Preparation

1. Suspend 31 grams in 1 liter distilled or deionized water.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige to light tan, free-flowing, homogeneous.
Solution:	3.1% solution; soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, very slightly to slightly opalescent.
Reaction of 3.1% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Bacto Nutrient Agar 1.5% per label directions.
Inoculate and incubate the plates at 32 ± 1°C for 24 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH PLAIN	GROWTH W/15% SHEEP BLOOD	HEMOLYSIS
Staphylococcus aureus	25923*	100-1,000	good	good	beta
Streptococcus pneumoniae	6305	100-1,000	good	good	alpha
Streptococcus pyogenes	19615*	100-1,000	good	good	beta
Escherichia coli	25922*	100-1,000	good	good	beta

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. To prepared an enriched medium, cool the sterile base to 45-50°C and add the desired enrichment. Mix thoroughly.
5. Dispense as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion of the isolation and identification of aerobic and anaerobic microorganisms, refer to appropriate references.

Results

Refer to appropriate references and procedures for results.

References

1. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
2. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Packaging

Nutrient Agar 1.5%	500 g	0069-17

Bacto® Nutrient Agar with MUG

Intended Use

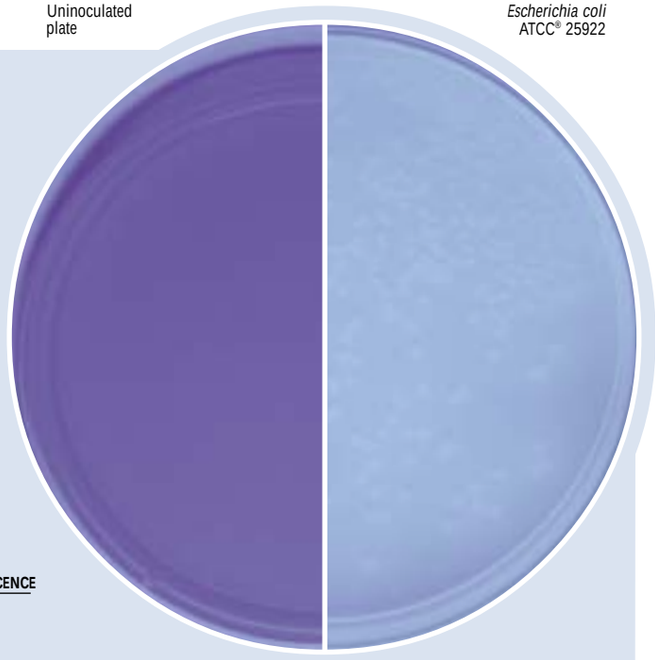
Bacto Nutrient Agar with MUG is used for detecting and enumerating *Escherichia coli* in water.

Summary and Explanation

Escherichia coli is a member of the fecal coliform group of bacteria. The presence of *E. coli* is indicative of fecal contamination.[1] Feng and Hartman[2] developed a rapid assay for *E. coli* by incorporating 4-methylumbelliferyl-ß-D-glucuronide (MUG) at a final concentration of 100 µg/ml into Lauryl Tryptose Broth. Nutrient Agar is similarly modified with the addition of MUG. Rapid quantitation and verification may be achieved with the membrane filtration procedure by transferring the membrane from a total-coliform or fecal-coliform positive sample to a Nutrient Agar substrate containing 4-methylumbelliferyl-ß-D-glucuronide (MUG).[1]

Mates and Shaffer[3] used the membrane filter-Endo Agar method, followed by incubation on Nutrient Agar with MUG, to detect and enumerate *E. coli* within 4 hours of membrane transfer. *E. coli* was recovered at a rate of 98% with no false-positive results.

Nutrient Agar with MUG is prepared according to the formula specified by US EPA[4] and Standard Methods.[1]



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.31% solution, soluble in distilled or deionized water on boiling; light amber, clear to very slightly opalescent.
Prepared Medium:	Light amber, clear to slightly opalescent without significant precipitate.
Reaction of 2.31% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Nutrient Agar with MUG per label directions. After incubation on mEndo Agar LES, aseptically transfer the membrane to Nutrient Agar with MUG. Incubate 18-24 hours at 35 ± 2°C.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH ON mENDO LES	COLONY COLOR	FLUORESCENCE
Enterobacter aerogenes	13048*	30-300	good	red	–
Escherichia coli	25922*	30-300	good	red w/sheen	+

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Beef Extract and Bacto Peptone are sources of nitrogen, vitamins, carbon and amino acids. Bacto Agar is a solidifying agent. The substrate, MUG (4-methylumbelliferyl-ß-D-glucuronide), produces a blue fluorescence when hydrolyzed by the enzyme ß-glucuronidase, which is produced by most *E. coli*.

Formula

Nutrient Agar with MUG

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Bacto Agar	15 g
MUG (4-Methylumbelliferyl-ß-D-glucuronide)	0.1 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Nutrient Agar with MUG

Materials Required But Not Provided

Distilled or deionized water
Glassware
Test tubes
Sterile pipettes
Incubator (35°C)
Longwave UV lamp (approximately 366 nm)
mEndo Agar LES
Sterile membranes
Filter apparatus
Petri dishes (50 x 9 mm)
Sterile absorbent pad

Method of Preparation

1. Suspend 23.1 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Dispense into sterile 50 x 9 mm Petri dishes.

Specimen Collection and Preparation

Collect water samples in accordance with recommended procedures.[1]

Test Procedure

Follow the methods and procedures for water testing using mEndo Agar LES in Standard Methods.[1] After incubation on mEndo Agar LES, aseptically transfer the membrane to Nutrient Agar with MUG. Incubate 18-24 hours at 35 ± 2°C. Expose the filter surface to longwave UV light.

Results

Observe for fluorescence following incubation. Positive MUG reactions exhibit a bluish fluorescence around the periphery of the colony under longwave (approximately 366 nm) UV light.

Typical strains of *E. coli* (red with a green metallic sheen on mEndo Agar LES) exhibit blue fluorescence on Nutrient Agar with MUG. Non-*E. coli* coliforms may produce a metallic sheen but do not fluoresce.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Glucuronidase-negative strains of *E. coli* have been encountered.[5,6,7] Similarly, MUG-negative strains of *E. coli* have been reported in this assay procedure but at a very low frequency.[3]
3. Strains of *Salmonella* and *Shigella* species that produce glucuronidase may infrequently be encountered.[8] These strains must be distinguished from *E. coli* on the basis of other parameters, i.e., gas production, lactose fermentation or growth at 44.5°C.

References

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **Feng, P. C. S., and P. A. Hartman.** 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. **43**:1320-1329.
3. **Mates, A., and M. Shaffer.** 1989. Membrane filtration differentiation of *E. coli* from coliforms in the examination of water. J. Appl. Bacteriol. **67**:343-346.
4. **Federal Register.** 1991. National primary drinking water regulations; analytical techniques: coliform bacteria. Fed. Regist. **56**:636-643.
5. **Chang, G. W., J. Brill, and R. Lum.** 1989. Proportion of ß-D-glucuronidase-negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. **55**:335-339.
6. **Hansen, W., and E. Yourassowsky.** 1984. Detection of ß-glucuronidase in lactose fermenting members of the family enterobacteriaceae and its presence in bacterial urine cultures. J. Clin. Microbiol. **20**:1177-1179.
7. **Kilian, M., and P. Bulow.** 1976. Rapid diagnosis of *Enterobacteriaceae*. Acta Pathol. Microbiol. Scand. Sect. B **84**:245-251.
8. **Damare, J. M., D. F. Campbell, and R. W. Johnston.** 1985. Simplified direct plating method for enhanced recovery of Escherichia coli in food. J. Food Sc. **50**:1736-1746.

Packaging

Nutrient Agar with MUG	100 g	0023-15
	500 g	0023-17

Bacto® Nutrient Broth

Intended Use

Bacto Nutrient Broth is used for cultivating nonfastidious microorganisms.

Summary and Explanation

In the early 1900s, the American Public Health Association (APHA) suggested this formulation of a standard culture medium for use in bacteriological procedures for water analysis.[1] In *Standard Methods of Water Analysis*[2] and *Standard Methods of Milk Analysis*,[3] the APHA advocated the use of dehydrated culture media for bacteriological examination of water and milk.

Nutrient Broth, a widely used medium, is included in many standard methods procedures. In *Compendium of Methods for the Microbiological Examination of Foods*[4] and *Standard Methods for the Examination of Dairy Products*,[5] Nutrient Broth is included as a satisfactory substitute for the buffered rinse solution used in sampling equipment, containers and air because it effectively neutralizes chlorine and quaternary ammonium compounds. In *Standard Methods for the Examination of Water and Wastewater*, Nutrient Broth is included in testing methods for viruses using microporous filters.[6]

Nutrient Broth is used as a pre-enrichment medium when testing certain foods and dairy products for *Salmonella*. In dried or processed foods, salmonellae may be sublethally injured and in low numbers. The presence of other bacteria and the components of the food sample may hinder growth and recovery of *Salmonella*. Preenrichment in a nonselective medium such as Nutrient Broth allows for repair of cell damage, dilutes toxic or inhibitory substances, and provides a nutritional advantage to *Salmonella* over other bacteria.[4] Nutrient Broth is included in many standard methods procedures for testing foods, dairy products and other materials.[4,5,7,8]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Medium tan, free-flowing, homogeneous.
Solution:	0.8% solution, soluble in distilled or deionized water; light to medium amber, clear with no precipitate.
Prepared Medium:	Light to medium amber, clear with no precipitate.
Reaction of 0.8% Solution at 25°C:	pH 6.8 ± 0.2 at 25°C

Cultural Response

Prepare Nutrient Broth per label directions. Inoculate medium with the test organism and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	CFU	GROWTH
Escherichia coli	25922*	100-1,000	good
Staphylococcus aureus	25923*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol Disks™ and should be used as directed in the Bactrol Disks Technical Information.

Principles of the Procedure

Nutrient Broth contains Beef Extract and Bacto Peptone as carbon and nitrogen sources for general growth requirements.

Formula

Nutrient Broth

Formula per liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Expiration Date

The product is stable through the expiration date on the label when stored as directed. Expiry date applies to the medium in its intact container. Do not use if the medium is caked, discolored or shows other signs of deterioration.

Procedure

Material Provided

Nutrient Broth

Materials Required but not Provided

Flask with closure
Distilled or deionized water
Autoclave
Containers, 225 ml
Incubator

Method of Preparation

1. Dissolve 8 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Test Procedure

Direct:

1. Inoculate the broth with specimen on a swab.
2. Incubate for 18-24 hours at 35 ± 2°C.

As a preenrichment medium when testing certain foods and dairy products for *Salmonella*, consult appropriate references for specific recommendations:[4,5,7,8]

1. Mix 25 grams of sample with 225 ml of Nutrient Broth.
2. Incubate for 18-24 hours at 35 ± 2°C.
3. Transfer a portion to one or more selective enrichment broths.

Results

Turbidity indicates growth.

References

1. **American Public Health Association**. 1917. Standard methods of water analysis, 3rd ed. American Public Health Association, Washington, D.C.

2. **American Public Health Association**. 1923. Standard methods of water analysis, 5th ed. American Public Health Association, Washington, D.C.
3. **American Public Health Association**. 1923. Standard methods of milk analysis, 4th ed. American Public Health Association, Washington, D.C.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.)**. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Marshall, R. T. (ed.)** 1993. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
6. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.)**. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
7. **Association of Official Analytical Chemists**. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
8. **Association of Official Analytical Chemists**. 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Nutrient Broth	100 g	0003-15-0
	500 g	0003-17-8
	2 kg	0003-07-0
	10 kg	0003-08-9

Bacto® Nutrient Gelatin

Intended Use

Bacto Nutrient Gelatin is used for detecting gelatin liquefaction by proteolytic microorganisms.

Summary and Explanation

Gelatin was the first gelling agent used to solidify culture media. The advantages of a solid medium were the ability to perform plate counts and to isolate microorganisms in pure culture. The disadvantages of gelatin were the limitation of incubation at 20°C, a temperature that is lower than the optimum for growing many microorganisms, and the fact that many organisms metabolize (liquefy) gelatin. Agar later replaced gelatin as a solidifying agent.

Characterizing fermentative and non-fermentative gram-negative bacilli includes the test for gelatin liquefaction. If the proteolytic enzyme gelatinase is present, gelatin is hydrolyzed and loses its gelling characteristic.[1] Edwards and Ewing include this test in the differentiation scheme for the *Enterobacteriaceae*.[2] Procedures for performing the standard tube method for gelatin liquefaction are available.[2,3,4]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Tan, free-flowing, fine granular.
Solution:	Medium amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Medium:	Medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 12.8% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Nutrient Gelatin per label directions. Using a heavy inoculum, inoculate by stabbing the tube and incubate at 35 ± 2°C for 18-48 hours or up to two weeks, if required. To read gelatinase, refrigerate until well chilled and compare to uninoculated tube. Tilt tubes carefully to test for liquefaction. Tubes positive for gelatinase remain liquid.

ORGANISM	ATCC®	RECOVERY	GELATINASE
Escherichia coli	25922*	good	–
Pseudomonas aeruginosa	27853	good	+

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Nutrient Gelatin contains Bacto Peptone and Beef Extract as carbon and nitrogen sources for general growth requirements. Gelatin is the substrate for determining if the microorganism has the proteolytic enzyme to hydrolyze (liquefy) gelatin.

Formula

Nutrient Gelatin

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Bacto Gelatin	120 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Nutrient Gelatin

Materials Required but not Provided

Flask with closure
Test tubes with caps
Distilled or deionized water
Autoclave
Incubator (35°C)
Inoculating needle
Refrigerator

Method of Preparation

1. Suspend 128 grams in 1 liter distilled or deionized water.
2. Warm to 50-55°C to dissolve completely.
3. Dispense required amount in test tubes and place caps on tubes.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure[1]

1. Using a sterile inoculating needle, touch several similar, well-isolated colonies on agar and stab directly down the center of the tube to approximately 10 mm from the bottom.
2. Incubate at 35 ± 2°C for 24-48 hours. Incubate an uninoculated control tube with the test. Incubation may be extended to 14 days for some organisms.
3. Examine at various intervals.
   a. Transfer the tubes to a refrigerator or ice bath.
   b. Do not shake the tubes when transferring from incubator to refrigerator.
   c. Gently invert the chilled tubes to test for solidity.

Results

Positive: Medium remains liquefied after refrigeration.
Negative: Medium becomes solid after refrigeration.
Uninoculated control tube: Medium becomes solid after refrigeration.

Limitations of the Procedure

1. Use this method for detecting gelatinase only if the identification procedure allows for incubation beyond 48 hours.
2. Gelatin is liquid at temperatures above 20°C. If tubes are incubated at 35°C, they must be refrigerated in order to read for liquefaction. Include an uninoculated tube in the test procedure for comparison.
3. Growth and liquefaction frequently occur only at the surface of the tube. To prevent a false-negative interpretation, handle tubes carefully when warm so that liquefied gelatin remains at the surface of the tube.

References

1. **Isenberg, H. D. (ed.).** 1994. Clinical microbiology procedures handbook, sup. 1. American Society for Microbiology, Washington, D.C.
2. **Ewing, W. H.** 1986. Edwards and Ewing's identification of Enterobacteriaceae, 4th ed. Elsevier Science Publishing Co. Inc., New York, NY.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.)**. 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Association of Official Analytical Chemists**. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

Nutrient Gelatin	500 g	0011-17

Bacto® NZCYM Broth
Bacto NZYM Broth, Bacto NZM Broth

Intended Use

Bacto NZCYM Broth, Bacto NZYM Broth, and Bacto NZM Broth are used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

NZCYM Broth was developed by Blattner et al. as an enriched medium for cultivating recombinant strains of *E. coli* and propagating λ bacteriophage.[1] *E. coli* grows rapidly in rich media, such as the NZ media, which provide amino acids, vitamins and other metabolites the cell would otherwise have to synthesize.[2] Casein is supplied as Casein Digest in NZ media, a different form than in the LB formulations, providing a good nitrogen source.

The three variations of NZ Media allow the user to select a formulation appropriate to the need.

Principles of the Procedure

Casein Digest, Yeast Extract and Casamino Acids provide the necessary nutrients and cofactors required for excellent growth of recombinant strains of *E. coli*. Due to its higher degree of digestion, Casamino Acids is an excellent source of free amino acids. Sodium Chloride is included in the medium to provide a suitable osmotic environment. Magnesium Sulfate is a source of magnesium ions required in a variety of enzymatic reactions, including DNA replication.

Formula

NZCYM Broth

Formula Per Liter

Bacto Casein Digest	10 g
Bacto Casamino Acids	1 g
Bacto Yeast Extract	5 g
Sodium Chloride	5 g
Magnesium Sulfate, Anhydrous	0.98 g

Final pH 7.0 ± 0.2 at 25°C

NZYM Broth

Formula Per Liter

Bacto Casein Digest	10 g
Bacto Yeast Extract	5 g

Sodium Chloride	5 g
Magnesium Sulfate, Anhydrous	0.98 g

Final pH 7.0 ± 0.2 at 25°C

NZM Broth

Formula Per Liter

Bacto Casein Digest	10 g
Sodium Chloride	5 g
Magnesium Chloride	0.94 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

NZCYM Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.2% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Tubes:	Light to medium amber, clear.
Reaction of 2.2% Solution at 25°C:	pH 7.0 ± 0.2

NZYM Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Tubes:	Light to medium amber, clear.
Reaction of 2.1% Solution at 25°C:	pH 7.0 ± 0.2

NZM Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1.6% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Tubes:	Light to medium amber, clear.
Reaction of 1.6% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

NZCYM Broth, NZYM Broth or NZM Broth

Prepare medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli (C600)	23724	100-300	Good

The cultures listed are the minimum that should be used for performance testing.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

NZCYM Broth
NZYM Broth
NZM Broth

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Tubes with closures
Autoclave
Incubator (35°C)

Method of Preparation

1. Dissolve the medium in 1 liter of distilled or deionized water:
   NZCYM Broth - 22 grams;
   NZYM Broth - 21 grams;
   NZM Broth - 16 grams.
2. Dispense into tubes with closures.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult an appropriate reference for recommended test procedures.[3]

Results

Growth should be evident in the form of turbidity.

References

1. **Blattner, F. R., B. G. Williams, A. E. Blechl, K. Denniston-Thompson, H. E. Faber, L. A. Furlong, D. J. Grunwald, D. O. Kiefer, D. D. Moore, J. W. Schumm, E. L. Sheldon, and O. Smithies.** 1977. Charon phages: Safer derivatives of bacteriophage λ for DNA cloning. Science **196:**161.
2. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl, (ed.).** 1994. Current protocols in molecular biology, vol. 1. Current Protocols, New York, NY.
3. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Packaging

NZCYM Broth	500 g	0404-17
NZYM Broth	500 g	0415-17
NZM Broth	500 g	0435-17

Bacto® OF Basal Medium

Intended Use

Bacto OF Basal Medium is used with added carbohydrate for differentiating gram-negative microorganisms based on oxidation-fermentation patterns.

Also Known As

OF Basal Medium conforms with Oxidation Fermentation Basal Medium.

Summary and Explanation

OF Basal Medium is based on the formula of Hugh and Leifson[1], who described the taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by gram-negative bacteria. When an organism is inoculated into two tubes of OF Basal Medium containing a carbohydrate and the medium in one tube is covered with petrolatum to exclude oxygen, reactions of differential significance may be observed. Fermentative organisms will produce an acid reaction in both the covered and uncovered tubes. Oxidative organisms produce an acid reaction in the uncovered tube while there is little or no growth and no acid formation in the covered tube. Non-oxidative, non-fermentative organisms yield no change in the covered tube and an alkaline reaction in the uncovered tube. OF Basal Medium is listed in standard procedures for the differentiation of gram-negative bacteria based on the oxidation and fermentation of carbohydrates.[2,3,4,5,6]

A modification of OF Basal Medium was developed by Knapp and Holmes[7] to detect acid production from carbohydrates by *Neisseria* spp. and *Branhamella catarrhalis*. The authors reduced the protein concentration relative to the carbohydrate concentration, substituted phenol red for brom thymol blue at a low concentration, and adjusted the initial pH to 7.2. A selective and differential medium for *Pseudomonas cepacia*, based on OF Basal Medium, was developed by Welch et al.[8] by adding agar-agar, lactose, polymyxin B and bacitracin.

Principles of the Procedure

Tryptone provides nitrogen, vitamins and minerals. A high concentration of carbohydrate is added to OF Basal Medium. This helps avoid utilization of peptone by an aerobic organism, which would produce an alkaline reaction and neutralize the slight acidity produced by oxidation.[9] Glucose is the carbohydrate commonly added to the OF Basal Medium; lactose, maltose, mannitol, saccharose and xylose may also be used. Sodium Chloride maintains the osmotic balance of the medium and enhances growth of *Brucella* species.[1] Dipotassium Phosphate provides buffering capacity. Brom Thymol Blue acts as a pH indicator, changing to yellow under acidic conditions. Bacto Agar aids in the determination of motility and also helps evenly distribute any acid produced at the surface of the medium.

Formula

OF Basal Medium

Formula Per Liter

Bacto Tryptone	2 g
Sodium Chloride	5 g
Dipotassium Phosphate	0.3 g
Bacto Brom Thymol Blue	0.08 g
Bacto Agar	2 g

Final pH 6.8 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige with green tinge, homogeneous, free-flowing.
Solution:	0.94% solution, soluble on boiling in distilled or deionized water. Solution is green, clear, with no precipitate.
Prepared Medium :	Green, clear to very slightly opalescent, with no precipitate.
Reaction of 0.94% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare OF Basal Medium per label directions. Inoculate duplicate tubes, overlay one tube with 2 ml sterile mineral oil, and incubate at 35°C for 40-48 hours.

ORGANISM	ATCC®	PLAIN OPEN	PLAIN CLOSED	w/1% DEXTROSE OPEN	w/1% DEXTROSE CLOSED
Acinetobacter calcoaceticus	19606	K	K	A	K
Escherichia coli	25922*	K	K	AG	AG
Pseudomonas aeruginosa	27853*	K	K	A	K
Shigella flexneri	12022*	K	K	A	A

K = Alkaline reaction, green to blue medium
A = Acid reaction, yellow medium
G = Gas

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and are to be used as directed in Bactrol Disks Technical Information.

Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Acinetobacter calcoaceticus* ATCC® 19606 | *Shigella flexneri* ATCC® 12022

Tubes above are closed, with Dextrose

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

OF Basal Medium

Materials Required But Not Provided

Glassware
Autoclave
Sterile melted petrolatum (mineral oil)
Carbohydrates of choice: Glucose (dextrose), Lactose, Maltose, Mannitol, Saccharose and/or Xylose
Incubator (35°C)
Test tubes with closures

Method of Preparation

1. Suspend 9.4 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Add 1% carbohydrate before or after sterilization, depending on heat lability.
5. Dispense in test tubes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate duplicate test tubes by stabbing the medium to the desired depth with an inoculating needle.
2. Overlay one tube of each set with 2 ml sterile mineral oil.
3. Incubate at a temperature appropriate for the test organism for a minimum of 48 hours.
4. Examine the medium for gas production and a color change from green to yellow.

Results

Alkaline reaction: green to blue medium
Acid reaction: yellow medium

A color change of the medium in both the plain and overlay tubes, with or without gas production, indicates fermentation of the carbohydrate tested.

A color change in the plain tube, only, indicates oxidative metabolism of the carbohydrate tested.

No color change in both tubes of the set indicates the organism is nonsaccharolytic for the carbohydrate tested.

Limitations of the Procedure

1. The acid reaction produced by oxidative organisms is apparent at the surface and gradually spreads throughout the medium. If the oxidation is weak or slow, however, an initial alkaline reaction at the surface of the open tube may persist for several days and eventually convert to an acid reaction.
2. If an organism is unable to grow on OF Basal Medium, Cowan[10] recommends adding either 2% serum or 0.1% yeast extract to each carbohydrate tube.
3. Nonsaccharolytic organisms produce a slight alkalinity in the open tube (blue-green color), while the covered tube will not exhibit a color change (green).

References

1. **Hugh, R. and E. Leifson.** 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol. **66**:24-26.
2. **Bacteriological Analytical Manual, 8th ed.** 1995. AOAC International. Gaithersburg, MD.
3. **Vanderzant, C. and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** 1992. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. **Oberhofer, T. R.** 1985. Manual of nonfermenting gram-negative bacteria. Churchill Livingstone, New York, NY.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
7. **Knapp, J. S. and K. K. Holmes.** 1983. Modified oxidation-fermentation medium for detection of acid production from carbohydrates by *Neisseria* spp. and *Branhamella catarrhalis*. J. Clin. Microbiol. **18**:56-62.
8. **Welch, D. F., M. J. Muszynski, C. H. Pai, M. J. Marcon, M. M. Hribar, P. H. Gilligan, J. M. Matsen, P. A. Ahlin, B. C. Hilman and S. A. Chartrand.** 1987. Selective and differential medium for recovery of *Pseudomonas cepacia* from the respiratory tracts of patients with cystic fibrosis. J. Clin. Microbiol. **25**:1730-1734.
9. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
10. **Cowan, S. T.** 1974. Cowan and Steel's manual for the identification of medical bacteria, 2nd ed. Cambridge University Press, Cambridge, MA.

Packaging

OF Basal Medium	100 g	0688-15
	500 g	0688-17
Mineral Oil	30 ml	6663-30

OGYE Agar

Bacto® OGYE Agar Base · Bacto Antimicrobic Vial Oxytetracycline

Intended Use

Bacto OGYE Agar Base is for use with Bacto Antimicrobic Vial Oxytetracycline in isolating and enumerating yeasts and molds in foods.

Also Known As

OGY Agar Base[1]

OGYA (Oxytetracycline-Glucose-Yeast (Extract) Agar)[1]

Summary and Explanation

Acidified agar may be used for enumerating yeasts and molds in foods and dairy products. However, in some cases, antimicrobics better suppress bacterial growth and improve recovery of yeasts and molds.[2, 3]

Mossel et al.[4, 5] described Oxytetracycline-Glucose Yeast Extract (OGYE) Agar for selectively isolating and enumerating yeasts and molds in foods. Mossel et al. demonstrated improved recovery compared to acidified agar media.

User Quality Control

Identity Specifications

OGYE Agar Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	3.7% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, very slightly opalescent without precipitate.
Prepared Medium:	Medium amber, slightly opalescent.
Reaction of 3.7% Solution at 25°C:	pH 7.0 ± 0.2

Antimicrobic Vial Oxytetracycline

Lyophilized Appearance: Yellow cake or powder

Cultural Response

Prepare OGYE Agar Base and Antimicrobic Vial Oxytetracycline per label directions. Inoculate using pour plate technique and incubate at 22 ± 3°C for up to 5 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	good
Escherichia coli	25922*	1,000-2,000	inhibited
Saccharomyces cerevisiae	9763	100-1,000	good
Saccharomyces carlsbergensis† (cerevisiae or uvarum)	9080	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

†*S. cerevisiae* ATCC 9080 is prepared to order.

OGYE Agar is specified as a standard methods medium for use with dairy products.[2]

Principles of the Procedure

OGYE Agar Base contains Yeast Extract to supply B-complex vitamins which stimulate bacterial growth. Dextrose is the carbon energy source. Bacto Agar is the solidifying agent. Antimicrobic Vial Oxytetracycline inhibits the growth of bacteria.

Formula

OGYE Agar Base

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Dextrose	20 g
Bacto Agar	12 g

Final pH 7.0 ± 0.2 at 25°C

Antimicrobic Vial Oxytetracycline

Oxytetracycline	100 mg

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store the antimicrobic supplement at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

OGYE Agar Base
Antimicrobic Vial Oxytetracycline

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave

Method of Preparation

1. Suspend 37 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50°C.

4. Aseptically add 10 ml rehydrated Antimicrobic Vial Oxytetracycline to the medium. Mix well.

Antimicrobic Vial Oxytetracycline

1. Aseptically add 10 ml sterile distilled or deionized to the Antimicrobic Vial Oxytetracycline.
2. Shake to dissolve contents.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. p. 579-582. Williams & Wilkins, Baltimore, MD.
2. **Frank, J. F., G. L. Christen, and L. B. Bullerman**. 1993. Tests for groups of microorganisms, p. 271-286. *In* R. T. Marshall (ed.), Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **Mislivec, P. B., L. R. Beuchat, and M. A. Cousin.** 1992. Yeasts and molds, p. 239-249. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Mossel, D. A. A., A. M. C. Kleynen-Semmeling, H. M. Vincentie, H. Beerens, and M. Catsaras**. 1970. Oxytetracycline-Glucose-Yeast Extract Agar for selective enumeration of moulds and yeasts in foods and clinical material. J. Appl. Bacteriol. **33**:454-457.
5. **Mossel, D. A. A., M. Visser, and W. H. J. Mengerink**. 1962. A comparison of media for the enumeration of moulds and yeasts in food and beverages. Lab. Pract. **11**:109-112.

Packaging

OGYE Agar Base	500 g	1811-17
Antimicrobic Vial Oxytetracycline	10 ml	3267-59

Bacto® Oatmeal Agar

Intended Use

Bacto Oatmeal Agar is used for cultivating fungi, particularly for macrospore formation.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, nonhomogeneous, may be slightly lumpy.
Solution:	7.25% solution, soluble in distilled or deionized water on boiling with frequent agitation. Solution is off-white, opaque with nonhomogeneous particles.
Prepared Medium:	Off-white, opaque appearance with nonhomogeneous particles.
Reaction of 7.25% Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

Prepare Oatmeal Agar per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	good
Candida albicans	10231	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Summary and Explanation

Fungi are extremely successful organisms, as evidenced by their ubiquity in nature.[1] Of the estimated 250,000 species, fewer than 150 are known primary pathogens of humans.[1]

Identification and classification of fungi is primarily based on the morphologic differences in their reproductive structures.[2] Fungi reproduce by producing spores.[2] Large, multi-celled spores are called macroconidia, macroaleuriospores or macrospores and are produced by aerial sporulation.[2]

The detection of fungi is a great concern in the pharmaceutical, food and cosmetic industry.

Principles of the Procedure

Oatmeal is a source of nitrogen, carbon, protein and nutrients. Bacto Agar is a solidifying agent.

Formula

Oatmeal Agar

Formula Per Liter

Oatmeal	60 g
Bacto Agar	12.5 g

Final pH 6.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Oatmeal Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Sterile Petri dishes
Waterbath (optional)

Method of Preparation

1. Suspend 72.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling with constant agitation.
3. Autoclave at 121°C for 15 minutes.
4. Dispense as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

Consult to appropriate references for specific procedures on the isolation and cultivation of fungi.

Results

Refer to appropriate references and procedures for results.

References

1. **Dixon, D. M., and R. A. Fromtling.** 1995. Morphology, taxonomy, and classification of the fungi, p. 699-708. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Koneman, E. W., S. D. Allen, V. R. Dowell, Jr., W. M. Janda, H. M. Sommers, and W. C. Winn, Jr.** 1988. Color atlas and textbook of diagnostic microbiology, 3rd ed. J. B. Lippincott Company, Philadelphia, PA.

Packaging

Oatmeal Agar	500 g	0552-17

Bacto® Orange Serum Agar
Bacto Orange Serum Broth Concentrate 10X

Intended Use

Bacto Orange Serum Agar is used for cultivating aciduric microorganisms, particularly those associated with spoilage of citrus products.

Bacto Orange Serum Broth Concentrate 10X is used for cultivating and enumerating microorganisms associated with spoilage of citrus products.

Summary and Explanation

The low pH of fruit juices makes citrus fruit products susceptible to spoilage by yeasts, molds and the bacteria *Lactobacillus* and *Leuconostoc*.[1] In the 1950's, Hays investigated spoilage in frozen concentrated orange juice. He found that an agar medium containing orange serum (juice) was superior to Lindegren Agar in isolating the microorganisms responsible for spoilage causing a buttermilk off-odor.[2] In a later comparative study, Murdock, Folinazzo and Troy found Orange Serum Agar, pH 5.4 to be a suitable medium for growing *Leuconostoc*, *Lactobacillus* and yeasts.[3]

Orange Serum Agar is included in recommended methods for examining fruit beverages.[1] Orange Serum Broth Concentrate 10X is used for small samples to initiate growth of saprophytic and pathogenic fungi.[4]

Principles of the Procedure

Orange Serum Agar and Orange Serum Broth Concentrate 10X contain Tryptone as carbon and nitrogen sources for general growth requirements. Orange serum provides the acid environment favorable to recovering acid-tolerant microorganisms. Yeast Extract supplies B-complex vitamins which stimulate growth. Dextrose is the carbohydrate. Bacto Agar is the solidifying agent (Orange Serum Agar only).

Formula

Orange Serum Agar

Formula Per Liter

Orange Serum	200 ml
Bacto Yeast Extract	3 g
Bacto Tryptone	10 g
Bacto Dextrose	4 g
Dipotassium Phosphate	2.5 g
Bacto Agar	17 g

Final pH 5.5 ± 0.2 at 25°C

Orange Serum Broth Concentrate 10X

Formula Per Liter

Orange Serum Concentrate	100 ml
Bacto Yeast Extract	3 g
Bacto Tryptone	10 g
Bacto Dextrose	4 g
Dipotassium Phosphate	2.5 g

Final pH 5.6 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious material.

Storage

Store the dehydrated Orange Serum Agar and the Orange Serum Broth Concentrate 10X at 2-8°C.

The dehydrated Orange Serum Agar is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Orange Serum Agar
Orange Serum Broth Concentrate 10X

Materials Required but not Provided

For Orange Serum Agar:

Flask with closure
Distilled or deionized water
Autoclave

For Orange Serum Broth Concentrate 10X:

Sterile distilled or deionized water
Sterile test tubes
Sterile transfer pipettes, 10 ml

Method of Preparation

Orange Serum Agar

1. Suspend 45.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Avoid overheating which could cause a softer medium.

Orange Serum Broth Concentrate 10X

1. To prepare the single-strength medium, aseptically add 100 ml Orange Serum Concentrate 10X to 900 ml sterile distilled or deionized water and mix thoroughly.
2. Use aseptic technique to dispense 10 ml amounts into sterile test tubes.

User Quality Control

Identity Specifications

Orange Serum Agar

Dehydrated Appearance:	Light tan, homogeneous, free-flowing.
4.55% Solution:	Light to medium amber, very slightly to slightly opalescent, may have a slight precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 4.55% Solution at 25°C:	pH 5.5 ± 0.2

Orange Serum Broth Concentrate 10X

Concentrate Appearance:	Dark amber, clear solution.
Reaction of Solution at 25°C:	pH 5.6 ± 0.2

Cultural Response

Prepare media per label directions. Inoculate medium and incubate for 40-48 hours. *Lactobacillus* is incubated at 35 ± 2°C and the remaining organisms are incubated at 30 ± 2°C.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	100-1,000	good
Lactobacillus fermentum	9338	100-1,000	good
Leuconostoc mesenteroides subsp. *mesenteroides*	23386	100-1,000	good
Sacharomyes cerevisiae	9763	100-1,000	good

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Orange Serum Agar

1. For plate count method, prepare serial 10-fold dilutions of the test material.
2. Add 1 ml of test sample to sterile Petri dish.
3. Add 18-20 ml of sterile, molten agar (cooled to 45-50°C) and swirl plate gently to mix well.
4. Allow to solidify before incubating at 30°C for 48 hours. Plates can be held up to 5 days.

Orange Serum Broth Concentrate 10X

Orange Serum Broth Concentrate 10X is used for small samples to initiate growth.

Results

Orange Serum Agar

Record colony morphology for each type of growth.

Orange Serum Broth Concentrate 10X

Turbidity indicates growth.

Limitations of the Procedure

1. Orange Serum Agar is not a differential medium. Perform microscopic examination and biochemical tests to identify isolates to genus and species if necessary.
2. If Orange Serum Agar is divided into aliquots and allowed to solidify, remelt only once. Repeated heating may produce a softer medium.

References

1. **Vanderzant, C., and D.F. Splittstoesser (ed.)**. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
2. **Hays, G. L.** 1951. The isolation, cultivation and identification of organisms which have caused spoilage in frozen concentrated orange juice. Proc. Fla. State Hortic. Soc. **54**:135-137.
3. **Murdock, D. I., J. F. Folinazzo, and V. S. Troy**. 1952. Evaluation of plating media for citrus concentrates. Food Technol. **6**:181-185.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Orange Serum Agar	500 g	0521-17*
Orange Serum Broth Concentrate 10X	6 x 100 ml	0518-73*

*Store at 2-8°C

Bacto® Oxford Medium Base · Bacto Oxford Antimicrobic Supplement · Bacto Modified Oxford Antimicrobic Supplement

Intended Use

Bacto Oxford Medium Base is used with either Bacto Oxford Antimicrobic Supplement[1] or Bacto Modified Oxford Antimicrobic Supplement[2] for isolating and differentiating *Listeria monocytogenes*.

Summary and Explanation

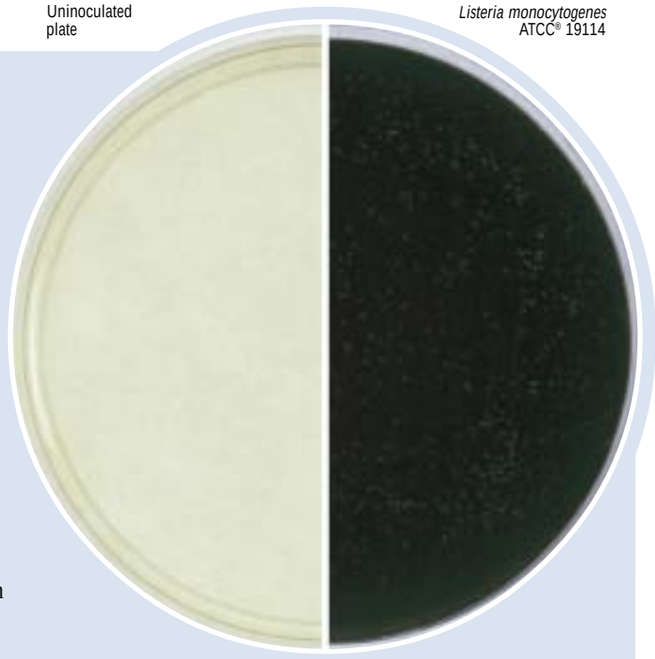
First described in 1926 by Murray, Webb and Swann,[3] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[4] The first reported food-borne outbreak of listeriosis was in 1985.[5] Since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[6]

Implicated vehicles of transmission include turkey frankfurters,[7] coleslaw, pasteurized milk, Mexican-style cheese, paté and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[8]

Listeria spp. grow over a pH range of 5.0-9.6 and survive in food products with pH levels outside these parameters.[9] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are streptococci, especially the enterococci, micrococci and *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[10]

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.



User Quality Control

Identity Specifications

Oxford Medium Base

Dehydrated Appearance:	Tan, free-flowing, homogeneous.
Solution:	5.75% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, slightly opalescent with a blue ring at the surface of the liquid.
Prepared Medium:	Light to medium amber, very slightly to slightly opalescent.
Reaction of 5.75% Solution at 25°C:	pH 7.2 ± 0.2

Oxford Antimicrobic Supplement

Appearance:	Yellow cake; yellow solution upon rehydration.

Modified Oxford Antimicrobic Supplement

Appearance:	White to off-white cake; colorless solution upon rehydration.

Cultural Response

Prepare Oxford Medium or Modified Oxford Medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY ON OXFORD MEDIUM	RECOVERY ON MODIFIED OXFORD MEDIUM
Enterococcus faecalis	29212*	1,000-2,000	marked to complete inhibition	marked to complete inhibition
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition	marked to complete inhibition
Listeria monocytogenes	19114	100-1,000	good at 40-48 hours, black colonies	good at 40-48 hours, black colonies
Saccharomyces pastorianus	9080	1,000-2,000	marked to complete inhibition	(not tested)

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information

Bacto Oxford Medium Base is prepared according to the formulation of Curtis et al.[11] who originally described the medium and its use in the selective isolation of *Listeria* from mixed cultures.

Principles of the Procedure

Columbia Blood Agar Base combines Pantone, Bitone and Tryptic Digest of Beef Heart which provide nitrogen, carbon, amino acids and vitamins. Bacto Agar is the solidifying agent. Sodium Chloride maintains the osmotic balance.

Ferric ammonium citrate aids in the differentiation of *Listeria* spp. Since all *Listeria* spp. hydrolyze esculin, the addition of ferric ions to the medium will detect the reaction. A blackening of the colony and surrounding medium in cultures containing esculin-hydrolyzing bacteria results from the formation of 6,7-dihydroxycoumarin which reacts with the ferric ions.[12]

Selectivity is provided by the presence of Lithium Chloride in the formula. The high salt tolerance of *Listeria* is used as a means to markedly inhibit growth of enterococci.

Selectivity is increased by adding various antimicrobial agents to the base. Incorporating these agents into Oxford Medium Base will completely inhibit gram-negative organisms and most gram-positive organisms after 24 hours of incubation. The most widely recognized antimicrobial agent combinations are the Oxford Medium formulation[11] and the Modified Oxford Medium formulation.[2] The Oxford Medium formulation contains cycloheximide, colistin sulfate, acriflavin, cefotetan and fosfomycin (available as Oxford Antimicrobic Supplement). The Modified Oxford Medium formulation contains moxalactam and colistin methane sulfonate or colistin sulfate (available as Modified Oxford Antimicrobic Supplement).

Modified Oxford medium is recommended for isolating and identifying *Listeria monocytogenes* from processed meat and poultry products.[2] Oxford Medium is recommended for isolating *Listeria* from enrichment broth cultures.[13]

Formula

Oxford Medium Base

Formula Per Liter

Bacto Columbia Blood Agar Base	39 g
Esculin	1 g
Ferric Ammonium Citrate	0.5 g
Lithium Chloride	15 g
Bacto Agar	2 g

Final pH 7.2 ± 0.2 at 25°C

Oxford Antimicrobic Supplement

Ingredients per 10 ml vial

Acriflavine	5 mg
Cefotetan	2 mg
Colistin Sulfate	20 mg
Cycloheximide	400 mg
Fosfomycin	10 mg

Modified Oxford Antimicrobic Supplement

Ingredients per 10 ml vial

Colistin Sulfate	10 mg
Moxalactam	20 mg

Precautions

1. For Laboratory Use.
2. **Oxford Medium Base**

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. Target Organs: Blood, Kidneys, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   Oxford Antimicrobic Supplement

   Warning! HARMFUL IF SWALLOWED OR INHALED. MAY CAUSE IRRITATION. MAY CAUSE BIRTH DEFECTS. Avoid contact with eyes, skin and clothing. Do not breathe dust.

   FIRST AID: If swallowed, induce vomiting. Call a physician. Never give anything by mouth to an unconscious person. If inhaled, remove to fresh air. If not breathing, give artificial respiration, preferably mouth-to-mouth. If breathing is difficult, give oxygen. Call a physician. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician. Flush skin with water.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

1. Store Oxford Medium Base below 30°C dehydrated powder is very hygroscopic. Keep container tightly closed.

   Store Oxford Antimicrobic Supplement and Modified Oxford Antimicrobic Supplement at 2-8°C.
2. Store prepared media at 2-8°C.

Expiration Date

The expiration date applies to the product its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Oxford Medium Base
Oxford Antimicrobic Supplement
Modified Oxford Antimicrobic Supplement

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Autoclave
Waterbath (45-50°C)
Petri dishes
Incubator (35°C)

Method of Preparation

1. Suspend 57.5 grams of Oxford Medium Base in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 10 minutes.
4. Cool medium to 45-50°C in a waterbath.
5. **To prepare Oxford Medium:** Aseptically rehydrate one vial of Oxford Antimicrobic Supplement with 5 ml ethanol and 5 ml sterile distilled water. Rotate in an end-over-end motion to dissolve completely. Add 10 ml to 1 liter sterile Oxford Medium Base.

   To prepare Modified Oxford Medium: Aseptically rehydrate one vial of Modified Oxford Antimicrobic Supplement with 10 ml sterile distilled water. Rotate in an end-over-end motion to dissolve completely. Add 10 ml to 1 liter sterile Oxford Medium Base.

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[1,2,9,13,14]
2. Clinical specimens obtained from nonsterile sites, foods and specimens obtained from the environment should be selectively enriched for *Listeria* before being plated.[14]
3. Process each specimen using procedures appropriate for that specimen or sample.[1,2,9,13,14]

Test Procedure

The USDA method[2] involves enrichment of the food sample in UVM Modified Listeria Enrichment Broth (one part sample to nine parts broth) at 30°C. After incubation, a portion of the enrichment mixture is plated onto Oxford or Modified Oxford Medium.

The FDA method[1] involves adding 25 ml of liquid or 25 grams of solid material to 225 ml Listeria Enrichment Broth and incubating at 30°C for two days. After enrichment, the broth is plated onto Oxford Medium.

For further information when testing food samples or clinical specimens for *Listeria*, consult appropriate references.[1,2,9,13,14]

Results

Select esculin-positive colonies and confirm their identity by further biochemical testing. Use macroscopic tube and rapid slide tests for definitive serological identification. For additional information, refer to appropriate references.[1,2,9,13,14]

Limitations of the Procedure

1. Since *Listeria* spp. other than *L. monocytogenes* can grow on these media, an identification of *L. monocytogenes* must be confirmed by biochemical and serological testing.[14]
2. Use freshly prepared antimicrobial agent solutions or aliquot portions and store at -20°C or below.
3. Poor growth and a weak esculin reaction may be seen after 40 hours incubation for some enterococci.

References

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2. **Lee, W. H., and D. McClain.** 1989. *Laboratory Communication No. 57* (revised May 24, 1989). U.S.D.A., F.S.I.S. Microbiology Division, Beltsville, MD.
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4. **Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low- and high-fat, frozen and refrigerated ground beef. J. Food Prot. **57**:969-974.
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11. **Curtis, G. D. W., R. G. Mitchell, A. F. King, and J. Emma.** 1989. A selective differential medium for the isolation of *Listeria monocytogenes*. Appl. Microbiol. **8:**95-98.
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14. **Swaminathan, B., J. Rocourt, and J. Bille.** 1995. *Listeria*, p. 342-343. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, R. H. Yolken (ed), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Oxford Medium Base	500 g	0225-17
	2 kg	0225-07
	10 kg	0225-08
Oxford Antimicrobic Supplement	6 x 10 ml	0214-60
Modified Oxford Antimicrobic Supplement	6 x 10 ml	0218-60

PALCAM Medium

Bacto® PALCAM Medium Base · Bacto PALCAM Antimicrobic Supplement

Intended Use

Bacto PALCAM Medium Base is used with Bacto PALCAM Antimicrobic Supplement in isolating and cultivating *Listeria* from foods.

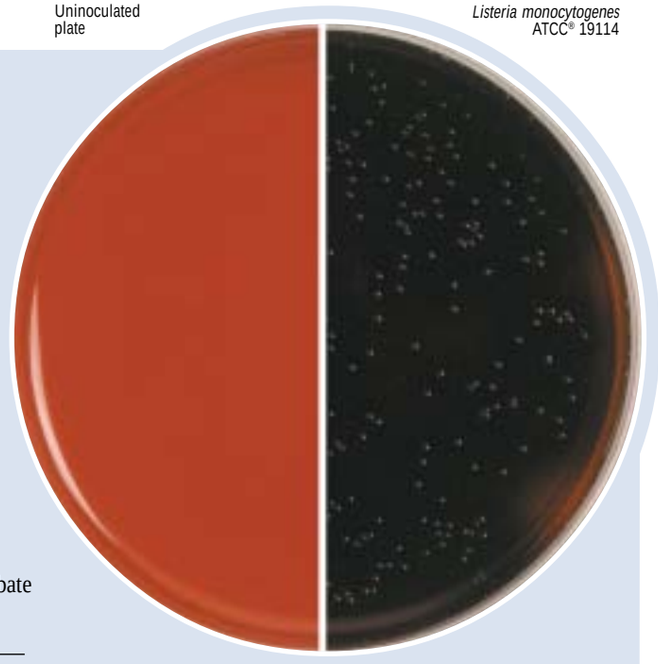
Summary and Explanation

PALCAM Medium Base and PALCAM Antimicrobic Supplement are based on the PALCAM agar formulation of van Netten et al.,[1] who developed this selective and differential medium for use in the isolation and enumeration of *Listeria* spp. from food samples. PALCAM medium is recommended by AFNOR for use in the detection of *L. monocytogenes* in foods,[2] and by the IDF as an additional plating medium for the detection of *Listeria* spp. in milk and milk products.[3] PALCAM medium is recommended by Health Canada for the detection of *L. monocytogenes* in food and environmental samples.[4]

Principles of the Procedure

Good growth of *Listeria* spp. is obtained by including Columbia Blood Agar Base in PALCAM Medium Base. Columbia Blood Agar Base provides the nutrients and cofactors required for good to excellent growth of *Listeria*. Selectivity of the complete medium is achieved through the presence of Lithium Chloride, Polymyxin B Sulfate and Acriflavine HCl, present in PALCAM Medium Base, and Ceftazidime, provided by PALCAM Antimicrobic Supplement. These agents effectively suppress growth of most commonly occurring non-*Listeria* spp. of bacteria present in foods. The ceftazidime concentration is reduced from 20 mg/l to 8 mg/l for improved growth and recovery of *Listeria*.

Differentiation on PALCAM Medium is based on esculin hydrolysis and mannitol fermentation. All *Listeria* spp. hydrolyze esculin as evidenced by a blackening of the medium. This blackening by esculin-hydrolyzing bacteria results from the formation of 6,7 dihydroxycoumarin, which reacts with ferric ions that are present in the medium as Ferric Ammonium Citrate. On occasion, organisms other than *Listeria*, such as staphylococci or enterococci, may grow on this medium. Mannitol and the pH indicator, Phenol Red, have been added to differentiate mannitol-fermenting strains of these species from *Listeria* based on mannitol fermentation. Mannitol fermentation is demonstrated by a color change in the colony and/or the surrounding medium from red or gray to yellow due to the production of acidic end products.



User Quality Control

Identity Specifications

PALCAM Medium Base

Dehydrated Appearance: Pink, free-flowing, homogeneous.

Solution: 6.8% solution, soluble in distilled or deionized water on boiling; dark red, very slightly to slightly opalescent with a slight precipitate.

Reaction of 6.8 % Solution at 25°C: pH 7.2 ± 0.2

PALCAM Antimicrobic Supplement

Lyophilized Appearance: White, free-flowing, homogeneous powder.

Rehydrated Appearance: Colorless solution.

Cultural Response

Prepare PALCAM Medium per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours under microaerophilic conditions.

ORGANISM	ATCC®	INOCULUM	GROWTH
Escherichia coli	25922*	1,000-2,000	inhibited
Listeria monocytogenes	19114	100-1,000	good growth, gray-green colonies with black precipitate
Staphylococcus aureus	25923*	1,000-2,000	inhibited
Enterococcus faecalis	29212*	1,000-2,000	inhibited

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

PALCAM Medium Base

Formula Per Liter

Bacto Columbia Blood Agar Base	39 g
Bacto Mannitol	10 g
Bacto Dextrose	0.5 g
Esculin	1 g
Ferric Ammonium Citrate	0.5 g
Lithium Chloride	15 g
Phenol Red	0.08 g
Acriflavine HCl	0.005 g
Polymyxin B Sulfate	0.01 g
Bacto Agar	2 g

Final pH 7.2 ± 0.2 at 25°C

PALCAM Antimicrobic Supplement

Formula per 10 ml vial

Ceftazidime	40 mg

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.
3. PALCAM Medium Base:

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Kidneys, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
4. PALCAM Antimicrobic Supplement:

   MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

Storage

Store PALCAM Medium Base below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store PALCAM Antimicrobic Supplement at 2-8°C.

Store the rehydrated supplement and prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

PALCAM Medium Base
PALCAM Antimicrobic Supplement

Materials Required But Not Provided

Flasks with closures
Sterile distilled or deionized water
Autoclave
Waterbath (45-50°C)
Incubator (35°C)

Depending on testing method:

Fraser Broth Base
Demi-Fraser Broth Base
Fraser Broth Supplement
Oxford Medium Base
Oxford Antimicrobic Supplement
Modified Oxford Antimicrobic Supplement
Listeria Enrichment Broth
Modified Listeria Enrichment Broth
Tryptic Soy Agar with 0.6% Yeast Extract
LPM Agar Base
Moxalactam Antimicrobic Supplement

Method of Preparation

1. Suspend 68 grams PALCAM Medium Base in 1 liter distilled or deionized water and boil to dissolve completely.
2. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
3. Aseptically add 2 ml PALCAM Antimicrobic Supplement which has been rehydrated with 10 ml sterile distilled or deionized water. Mix well.

Specimen Collection and Preparation

1. Collect food samples in sterile containers and transport immediately to the laboratory following recommended guidelines.
2. Process each food sample using procedures appropriate for that sample.

Test Procedure

A number of methods and incubation conditions may be used for detecting and isolating *Listeria* on PALCAM Medium. In their original work, van Netten et al. recommended incubation at 37°C for 48 hours under microaerophilic conditions.[1] AFNOR, HPB and IDF methods for detecting *Listeria* in foods and dairy products are listed below. Consult guidelines appropriate to your country and sample type.

AFNOR Method for Foods[2]

1. Pre-enrich the sample in Demi-Fraser Broth. Incubate at 30°C for 18-24 hours. Subculture onto Oxford Medium or PALCAM Medium.
2. Transfer 0.1 ml of the pre-enrichment culture into 10 ml of Fraser Broth and incubate at 37°C for 48 hours. Subculture onto Oxford Medium or PALCAM Medium after 18-24 and 42-48 hours of incubation.
3. After the required incubation, examine for presumptive *Listeria* colonies.

4. Confirm the identity of each presumptive *Listeria* isolate by biochemical and/or serological testing.

IDF Method for Milk and Milk Products[3]

1. Enrich the sample in Modified Listeria Enrichment Broth. Incubate at 30 ± 1°C for 48 ± 2 hours.
2. Subculture onto Oxford Medium (and onto PALCAM Medium, if desired). Incubate at 37 ± 1°C for 48 ± 2 hours.
3. After the required incubation, examine for presumptive *Listeria* colonies.
4. Subculture five presumptive colonies (or all of the colonies if there are less than five) from each isolation medium onto Tryptic Soy Agar with 0.6 % Yeast Extract.
5. Confirm the identity of each presumptive *Listeria* isolate by biochemical and/or serological testing.

Health Canada Method for Foods and Environmental Samples[4]

1. Enrich the sample in Listeria Enrichment Broth (LEB). Incubate at 30°C for 48 hours.
2. Transfer 0.1 ml of the primary enrichment broth culture into 9.9 ml of modified Fraser Broth. Incubate at 35°C for 24-48 hours. (If desired, the LEB culture may also be streaked onto Oxford Medium [OXA] and lithium chloride-phenylethanol-moxalactam agar [LPM], modified Oxford medium [MOX] or PALCAM medium [PAL]. Incubate LPM at 30°C for 24-48 hours and OXA, MOX and PAL at 35°C for 24-48 hours.)
3. Examine modified Fraser broth for reactions. Subculture all positive cultures (black, dark brown or dark green) after 24 and 48 hours of incubation onto OXA and LPM, MOX or PAL, streaking for isolation. Incubate LPM at 30°C for 24-48 hours and OXA, MOX and PAL at 35°C for 24-48 hours. If desired, all negative modified Fraser broth cultures (straw color) may be subcultured onto OXA and LPM, MOX or PAL to facilitate recovery of esculin-negative strains of *L. monocytogenes*.
4. Examine for presumptive *Listeria* colonies. Examine LPM under oblique lighting positioned at a 45° angle relative to the surface of the plate.
5. Confirm the identity of each presumptive *Listeria* isolate by biochemical and/or serological testing.

Results

On PALCAM Medium, colonies of *Listeria* appear gray-green with a black precipitate following inoculation and incubation at 35°C for 24-48 hours under aerobic or microaerophilic conditions. Confirmation of the presence of *Listeria* is made following subculture onto appropriate media and biochemical/serological identification.[2,3] Colonies of mannitol-fermenting organisms such as staphylococci, which may grow on this medium, appear yellow with a yellow halo.

References

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3. **International Dairy Federation.** 1990. Milk and milk products-Detection of *Listeria monocytogenes*. IDF Provisional International Standard no. 143. International Dairy Federation, Brussels.
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Packaging

PALCAM Medium Base	500 g	0636-17
	2 kg	0636-07
PALCAM Antimicrobic Supplement	3 x 10 ml	0637-57*

*Store at 2-8°C

Bacto® PKU Test Agar · Bacto PKU Test Agar w/o Thienylalanine Bacto Subtilis Spore Suspension No. 2

Intended Use

Bacto PKU Test Agar is used with Bacto Subtilis Spore Suspension No. 2 in estimating the phenylalanine level in blood.

Bacto PKU Test Agar w/o Thienylalanine is used with Bacto Subtilis Spore Suspension No. 2 and ß-2-thienylalanine in estimating phenylalanine levels in blood.

Also Known As

The Guthrie Modified Bacterial Inhibition Assay (BIA) for PKU

Phenylketonuria (PKU) results from an inborn error of phenylalanine metabolism. In this disease, phenylalanine hydroxylase deficiency causes accumulation of the amino acid phenylalanine with subsequent neurological damage.

In 1934, Folling[1] reported the presence of a urine phenylalanine metabolite in mentally retarded persons. Jervis[2] established that defective phenylalanine metabolism was the cause of the mental retardation. Detection and management of PKU are possible by testing infants for abnormal levels of phenylalanine or its metabolites. The Guthrie bacterial inhibition assay (BIA), which estimates the level of phenylalanine in the blood, is used for this purpose.[3,4,5,6] *Bacillus subtilis* ATCC® 6633 growth is inhibited in minimal culture medium containing ß-2-thienylalanine. Phenylalanine blocks the inhibition, allowing the organism to grow.

In the PKU Test procedure, PKU Test Agar containing thienylalanine or PKU Test Agar w/o Thienylalanine with added thienylalanine are inoculated with a suspension of *B. subtilis* ATCC® 6633. Filter paper disks saturated with infant blood and control disks impregnated with known concentrations of L-phenylalanine (2,4,6,8,12 and 20 mg%) are applied to the surface of the medium. After incubation at 35°C for 12-16 hours, the zones of growth around the test disks are compared to the zones around the control disks. A growth zone around the test disk comparable to the zone around the 4 mg% or higher disk is a presumptive positive indication of phenylketonuria. A positive result must be repeated using a duplicate test disk and a chemical or spectrofluorometric procedure.[7,8]

Principles of the Procedure

PKU Test Agar and PKU Test Agar w/o Thienylalanine are defined minimal media containing the factors necessary for *B. subtilis* growth under appropriate conditions. ß-2-thienylalanine is an inhibitor of *B. subtilis* growth. PKU Test Agar contains the inhibitor, ß-2-thienylalanine; PKU Test Agar w/o Thienylalanine does not, requiring the user to add ß-2-thienylalanine to the medium. Phenylalanine supplied from a PKU-positive patient specimen will overcome the inhibitory action of ß-2-thienylalanine.

Formula

PKU Test Agar

Formula Per Liter

L-Glutamic Acid	0.5 g
DL-Alanine	0.5 g
Bacto Asparagine	0.5 g
Bacto Dextrose	10 g
Dipotassium Phosphate	15 g
Monopotassium Phosphate	5 g
Ammonium Chloride	2.5 g
Ammonium Nitrate	0.5 g
Sodium Sulfate	0.5 g
Magnesium Sulfate	0.05 g
Manganese Chloride	0.005 g
Ferric Chloride	0.005 g
Calcium Chloride	0.0025 g
B_2 Thienylalanine	0.0033 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

PKU Test Agar w/o Thienylalanine

Formula Per Liter

L-Glutamic Acid	0.5 g
DL-Alanine	0.5 g
Bacto Asparagine	0.5 g
Bacto Dextrose	10 g
Dipotassium Phosphate	15 g
Monopotassium Phosphate	5 g
Ammonium Chloride	2.5 g
Ammonium Nitrate	0.5 g
Sodium Sulfate	0.5 g
Magnesium Sulfate	0.05 g
Manganese Chloride	0.005 g
Ferric Chloride	0.005 g
Calcium Chloride	0.0025 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Bacillus Spore Suspension No. 2

Standardized, stable suspension of *Bacillus subtilis* ATCC® 6633 containing 1.2 to 1.8 x 10^8 spores/ml.

User Quality Control

Identity Specifications

PKU Test Agar, PKU Test Agar w/o Thienylalanine

Dehydrated Appearance:	Light beige to beige, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent with a slight precipitate.
Prepared Medium:	Light amber, very slightly to slightly opalescent with a slight precipitate.
Reaction of 5.0% Solution at 25°C:	pH 7.0 ± 0.2

Subtilis Spore Suspension No. 2

Appearance:	White, opalescent, homogeneous suspension.

Cultural Response

PKU Test Agar, PKU Test Agar w/o Thienylalanine

Prepare the final medium per label directions. Apply PKU Standard Disks. Incubate at 35 + 2°C for 12-16 hours. Measure zones of growth around each PKU Standard Disk. Zones of growth should increase in size comparable to the increasing concentration of phenylalanine in the Standard Disks.

Precautions

1. For Laboratory Use.
2. **PKU Test Agar**
   PKU Test Agar w/o Thienylalanine

   HARMFUL. POSSIBLE RISK OF IRREVERSIBLE EFFECTS. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Skin, Lungs.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. **Bacillus Spore Suspension No. 2**

   CAUTION. While spore suspensions are not considered to be pathogens, they are, nevertheless, live organisms. Never use mouth pipetting. Always use some type of pipetting aid.
4. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Subtilis Spore Suspension No. 2 at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

PKU Test Agar
PKU Test Agar w/o Thienylalanine
Subtilis Spore Suspension No. 2

Materials Required but not Provided

ß-2-thienylalanine (use with PKU Test Agar w/o Thienylalanine)
PKU Standard Disks
Blood test forms with Lancet
Disk test pattern for 150 mm Petri dish
150 mm Petri dishes
Forceps
Alcohol sponges
Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 50 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. Simmer for 5 minutes.
3. PKU Test Agar w/o Thienylalanine, only: Add 1 ml of 0.33% ß-2-thienylalanine solution per liter after simmering the medium; mix thoroughly.
4. Dispense 150 ml amounts into flasks.
5. Aseptically add 1 ml Subtilis Spore Suspension No. 2 to each 150 ml aliquot at 50-55°C. Mix thoroughly to uniformly distribute the spores.

Specimen Collection and Preparation

1. Obtain the sample at least 48 hours after the first milk feeding.
2. Collect a venous blood sample by heel puncture following established collection technique.[9] Obtain sufficient blood to fill each circle by a single application of the specimen card to the drop of blood. Completely saturate the entire circle to ensure accuracy. Allow the blood sample to air dry.
3. Punch a 1/4" disk from one of the blood spots and place it into a labeled, clean, dry vial or place the entire specimen card on a wire rack in the autoclave.
4 Autoclave the patient disks for exactly three minutes at 121°C. Remove the disks promptly after the temperature has dropped below 100°C. Do not use the disks until they are dry.
5. Follow manufacturer's instructions for preparation of the control disks.

Test Procedure

1. Prepare PKU Test Agar or PKU Test Agar w/o Thienylalanine per label directions.
2. Dispense the final medium into 150 mm Petri dishes. Allow to solidify.
3. Using clean forceps, apply the autoclaved and dried test disks and the prepared PKU Standard Disks, one of each concentration, to the PKU Test Agar and press down gently.
4. Incubate at 35°C for 12-16 hours.
5. Compare zones of growth around the patient disks to those around the control disks to determine the approximate concentration of phenylalanine in the blood.

Results

Growth zone diameters around the control disks are related to the concentration of phenylalanine in the disks. A zone of growth may or may not be present around the test disks depending on the presence or absence of phenylalanine in the test specimen. The culture medium outside the zones of growth will be comparable to an inoculated and incubated plate to which no disks have been applied.

Compare the zone of growth around a test disk to the zone around the standard disk containing 4 mg% phenylalanine. If the test zone is equal to or larger than the 4 mg% control zone, the test result is a presumptive positive and should be confirmed using a second sample. If the second sample gives a similar result, determine the serum phenylalanine concentration by either a chemical[10] or a spectrofluorometric procedure.

Limitations of the Procedure

1. Collect the blood sample with care. The sample must saturate the paper. Do not allow contact between the absorbent specimen card and the collector's hands.
2. Autoclaved samples must be dry before use.
3. PKU Test Agar must not be overheated. Bring to a boil and mix gently during heating. DO NOT AUTOCLAVE.
4. Do not add spores if the temperature of the medium is above 55°C. Distribute the spores uniformly in the medium without creating bubbles.
5. Place the Petri dish on a horizontal surface while pouring the medium to ensure an even depth of agar and a uniform distribution of spores throughout the plate.
6. Test results at the 4 and 6 mg% levels are questionable and should be repeated with a second test sample and the results confirmed by a quantitative procedure.
7. Take care when opening ampules containing *B. subtilis* spores. Autoclave the emptied ampules at 121-124°C for 20 minutes.
8. Infants who are tested before 24 hours of age should have a repeat test performed by 2 weeks of age.
9. A negative test of an infant on antibiotics should be reconfirmed after antibiotic therapy is terminated. Antibiotics present in the blood sample are usually inactivated by the autoclaving procedure, but could be a source of error because some antibiotics will inhibit the growth of *B. subtilis*.[11]
10. False-negative tests can result from the submission of an inadequate sample, or if the patient has recently been exchange-transfused, or if the patient has an insufficient dietary protein load.[11]
11. False-positive results can occur.[12]

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Packaging

PKU Test Agar	500 g	0980-17
PKU Test Agar w/o Thienylalanine	500 g	0474-17
Subtilis Spore Suspension No. 2	25 x 1	0981-36
	100 x 1	0981-84

PPLO Media

Bacto® PPLO Agar · Bacto PPLO Broth w/o CV · Bacto Mycoplasma Supplement · Bacto Mycoplasma Supplement S

Intended Use

Bacto PPLO Agar when supplemented with Bacto Mycoplasma Supplement or Bacto Mycoplasma Supplement S is used for isolating and cultivating *Mycoplasma*.

Bacto PPLO Broth w/o CV when supplemented with Bacto Mycoplasma Supplement or Bacto Mycoplasma Supplement S is used for isolating and cultivating *Mycoplasma*.

Also Known As

PPLO is an abbreviation for "pleuropneumonia-like organism."

Summary and Explanation

Members of the class Mollicutes, *Mycoplasma* was first recognized from a case of pleuropneumonia in a cow.[11] The organism was designated "pleuropneumonia-like organism," or PPLO.[11] Although some species are normal human respiratory tract flora, *M. pneumoniae* is a major cause of respiratory disease (primary atypical pneumonia, sometimes called "walking pneumonia").[11] *M. hominis*, *M. genitalium*, and *Ureaplasma urealyticum* are important colonizers (and possible pathogens) of the human genital tract.[11]

PPLO Agar was described by Morton, Smith and Leberman.[1] PPLO Agar was used in a study of the growth requirements of *Mycoplasma*,[2] along with the identification and cultivation of this organism.[3,4,5]

PPLO Broth w/o CV is prepared according to the formula described by Morton and Lecci.[2] Crystal Violet is omitted from this formula due to its inhibitory action on some *Mycoplasma*. PPLO Broth w/o CV has been used for the cultivation of *Mycoplasma* for research studies.[6,7]

Mycoplasma Supplement and Mycoplasma Supplement S are sterile desiccated enrichments for use in PPLO media as described by Hayflick.[8] The supplements are prepared according to the formulations of Chanock, Hayflick and Barile[9] and Hayflick.[10]

Principles of the Procedure

Infusion from Beef Heart and Bacto Peptone provide the nitrogen, vitamins, amino acids and carbon in PPLO Agar and PPLO Broth w/o CV. Sodium Chloride maintains the osmotic balance of these formulations. Bacto Agar, a solidifying agent, is used in PPLO Agar at a concentration slightly reduced from usual to ensure formation of the largest possible colonies because the organisms grow into the agar with only slight surface growth.[12]

PPLO media are supplemented with Mycoplasma Supplement or Mycoplasma Supplement S because *Mycoplasma* spp. are fastidious in their growth requirements.[13]

Mycoplasma Supplement contains fresh Yeast Extract and Horse Serum. Yeast Extract provides the preformed nucleic acid precursors that are required by *Mycoplasma* spp.[13] Horse Serum supplies cholesterol, a growth stimulant.[13]

Mycoplasma Supplement S is a selective enrichment prepared by adding Thallium Acetate and Penicillin to Mycoplasma Supplement. Thallium Acetate and Penicillin are selective against gram-positive and gram-negative bacteria.

Formula

PPLO Agar

Formula Per Liter

Bacto Beef Heart for Infusion, Infusion from	50 g
Bacto Peptone	10 g
Sodium Chloride	5 g
Bacto Agar	14 g

Final pH 7.8 ± 0.2 at 25°C

PPLO Broth w/o CV

Formula per Liter

Bacto Beef Heart for Infusion, Infusion from	50 g
Bacto Peptone	10 g
Sodium Chloride	5 g

Final pH 7.8 ± 0.2 at 25°C

Mycoplasma Supplement

Ingredients per 30 ml vial

Bacto Yeast Extract	0.01 g
Horse Serum, Desiccated	1.6 g

Mycoplasma Supplement S

Ingredients per 30 ml vial

Bacto Yeast Extract	0.01	g
Horse Serum, Desiccated	1.6	g
Penicillin	55,000	units
Thallium acetate	50	mg

User Quality Control

Identity Specifications

PPLO Agar

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	3.5% solution, soluble in distilled or deionized water on boiling; solution is light to medium amber, slightly opalescent.
Prepared Medium:	Enriched w/30% Mycoplasma Supplement: light to medium amber, slightly opalescent.
Reaction of 3.5% Solution at 25°C:	pH 7.8 ± 0.2

PPLO Broth w/o CV

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in distilled or deionized water; solution is light amber and clear to very slightly opalescent.
Prepared Medium:	Light amber, clear.
Reaction of 2.1% Solution at 25°C:	pH 7.8 ± 0.2

Mycoplasma Supplement

Lyophilized Appearance:	Straw-colored, dried button, may be dispersed.
Rehydrated Appearance:	Light to dark straw-colored, clear to slightly opalescent, readily soluble.

Mycoplasma Supplement S

Lyophilized Appearance:	Straw-colored, dried button, may be dispersed.
Rehydrated Appearance:	Light to dark straw-colored, clear to slightly opalescent solution, readily soluble.

Cultural Response

PPLO Agar, PPLO Broth w/o CV

Prepare media enriched with 30% Mycoplasma Supplement or Mycoplasma Supplement S per label directions. Inoculate PPLO Broth w/o CV and incubate at 35 ± 2°C under 5-10% CO_2 for up to 7 days. Subculture to PPLO Agar and incubate at 35 ± 2°C under 5-10% CO_2 for up to 7 days. Examine microscopically for growth on a daily basis.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Mycoplasma bovis	25523	100-1,000	good
Mycoplasma gallinarum	19708	100-1,000	good

The organisms listed are the minimum that should be used for performance testing.

Precautions

1. For Laboratory Use.
2. **Mycoplasma Supplement S**

   HARMFUL. MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. Target Organs: Bladder, Nerves, Kidneys, Cardiovascular System.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

PPLO Agar
PPLO Broth w/o CV

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Mycoplasma Supplement
Mycoplasma Supplement S

Store the lyophilized and rehydrated supplements at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

PPLO Agar
PPLO Broth w/o CV
Mycoplasma Supplement
Mycoplasma Supplement S

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (50-60°C) (optional)
Sterile Petri dishes or tubes

Method of Preparation

PPLO Agar
PPLO Broth w/o CV

1. **PPLO Agar:** Suspend 35 grams in 700 ml distilled or deionized water and boil to dissolve completely.

   PPLO Broth w/o CV: Dissolve 21 grams in 700 mo distilled or deionized water.

2. Autoclave at 121°C for 15 minutes. Cool medium to 50-60°C.
4. Aseptically add 300 ml Mycoplasma Supplement or 300 ml Mycoplasma Supplement S to the sterile medium. Mix well.
5. Dispense as desired.

Mycoplasma Supplement
Mycoplasma Supplement S

1. Rehydrate with 30 ml sterile distilled or deionized water.
2. Rotate gently to dissolve.
3. Add 30 ml (the contents of one vial) to 70 ml sterile PPLO Agar or PPLO Broth w/o CV.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion of the isolation and identification of *Mycoplasma* spp. from clinical specimens, refer to appropriate procedures outlined in the references.[12,13,14]

Results

PPLO Agar

PPLO colonies are round with a dense center and a less dense periphery, giving a "fried egg" appearance on PPLO Agar. Vacuoles, large bodies characteristic of Mycoplasma spp., are seen in the periphery. Colonies vary in diameter from 10 to 500 microns (0.01-0.5 mm) and penetrate into the medium.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Thallium acetate can partially inhibit some mycoplasmas.[12]

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12. **Kenny, G. E.** 1985. Mycoplasmas, p. 407-411. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.). Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
13. **Taylor-Robinson, D.** 1995. *Mycoplasma* and *Ureaplasma*, p. 652-661. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
14. **Isenberg, H. D.** (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Packaging

PPLO Agar	500 g	0412-17
PPLO Broth w/o CV	500 g	0554-17
	10 kg	0554-08
Mycoplasma Supplement	6 x 30 ml	0836-68
Mycoplasma Supplement S	6 x 30 ml	0837-68

Bacto® Pagano Levin Base

Intended Use

Bacto Pagano Levin Base is used with Bacto TTC Solution 1% and neomycin in isolating and differentiating *Candida* spp.

Also Known As

Pagano Levin Base is also referred to as Pagano Levin Candida Test Medium.

Summary and Explanation

Pagano Levin Base as described by Pagano, Levin, and Trejo[1] is selective for *Candida*. *Candida* spp. reduce TTC (2,3,5-triphenyltetrazolium chloride) in the medium to produce colonies with various degrees of color. Neomycin inhibits growth of most bacteria without appreciably influencing the *Candida.* Gentamicin (50 µg/ml) may also be added to reduce bacterial populations according to Yamane and Saitoh.[2] Samaranayake, MacFarlane and Williamson[3] found that modified Pagano Levin Agar was far superior to the commonly used Sabouraud Dextrose Agar in detecting multiple yeast species in a single sample.

Principles of the Procedure

Bacto Peptone provides the carbon and nitrogen required for good growth of a wide variety of organisms. Yeast Extract provides vitamins and cofactors. Dextrose is an energy source. Bacto Agar is a solidifying agent. TTC Solution 1%, added to the basal medium, facilitates the differentiation of yeast colonies based on the color change that occurs when a microorganism reduces TTC. Neomycin added to the base inhibits the growth of most bacteria.

Formula

Pagano Levin Base

Formula Per Liter

Bacto Peptone	10 g
Bacto Yeast Extract	1 g
Bacto Dextrose	40 g
Bacto Agar	15 g

Final pH 6.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store the prepared medium at 2-8°C.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.6% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Medium:	Plain - light amber, slightly opalescent; with TTC and antibiotic - light amber, milky.
Reaction of 6.6% Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

Prepare Pagano Levin Agar per label directions. Inoculate and incubate at 25-30°C for up to 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Candida albicans	26790	100-1,000	good	cream to light pink
Candida krusei	6121	100-1,000	good	white, spreading
Candida stellatoidea	36232	100-1,000	good	light red
Escherichia coli	25922	1,000-2,000	inhibited	

The cultures listed are the minimum that should be used for performance testing.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Pagano Levin Base

Materials Required But Not Provided

Glassware
Autoclave
Incubator (25-30°C)
Waterbath (50-55°C) (optional)
TTC Solution 1%
Neomycin

Method of Preparation

1. Suspend 66 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50-55°C.
5. Aseptically add 10 ml TTC Solution 1% (100 µg TTC per ml of medium and 500 µg of neomycin per ml of medium). Mix thoroughly.

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[4-6]
2. Process each specimen, using procedures appropriate for that specimen or sample.[4-6]

Test Procedure

1. Inoculate the surface of the medium with the specimen and incubate at 25°C for 48-72 hours.

Results

C. albicans colonies appear cream-colored to light pink, smooth, round, raised, opaque and glistening. Typical *C. albicans* colonies can be confirmed on Chlamydospore Agar or Rice Extract Agar based on chlamydospore production.

References

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2. **Yamane, N., and Y. Saitoh.** 1985. Isolation and detection of multiple yeasts from a single clinical sample by use of Pagano-Levin agar medium. J. Clin. Microbiol. **21**:276-277.
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4. **Pezzlo, M. (ed.).** 1994. Aerobic bacteriology, p. 1.0.0-1.20.47. *In* H. D. Isenberg, (ed.), Clinical microbiology procedures handbook, Vol. 1. American Society for Microbiology, Washington, D.C.

5. **Baron, E. J., L. R. Peterson, S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
6. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Pagano Levin Base	500 g	0141-17

Bacto® Panthenol Assay Medium
Bacto Panthenol Supplement

Intended Use

Bacto Panthenol Assay Medium is used with Bacto Panthenol Supplement in determining panthenol concentration by the microbiological assay technique.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test.

Panthenol Assay Medium and Panthenol Supplement, modifications of the formulas of DeRitter and Ruben,[1] are used in the microbiological assay of panthenol. *Gluconobacter oxydans* subsp. *suboxydans* ATCC® 621H is the test organism used in this assay.

User Quality Control

Identity Specifications

Panthenol Assay Medium

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	1.65% (single strength) or 3.3% (double strength) solution, soluble in distilled or deionized water on boiling. Single-strength solution is light amber, clear, may have a slight precipitate.
Prepared Medium (Single-strength):	Very light amber, clear, may have a very slight precipitate.
Reaction of 1.65% Solution at 25°C:	pH 6.0 ± 0.2

Panthenol Supplement

Solution Appearance:	Colorless to very, very light amber, clear.
Reaction of Solution at 25°C:	pH 5.0-6.0

Cultural Response

Prepare Panthenol Assay Medium per label directions. Test the medium by creating a standard curve using pantoic acid reference standard at levels from 0.0 to 2.0 g per 10 ml. The medium supports the growth of *G. oxydans* subsp. *suboxydans* ATCC® 621H when prepared in single strength and supplemented with Panthenol Supplement and pantoic acid.

Principles of the Procedure

Panthenol Assay Medium with Panthenol Supplement added is a panthenol-free medium containing all other nutrients and vitamins essential for the cultivation of *G. oxydans* subsp. *suboxydans* ATCC® 621H. The addition of pantoic acid in increasing specified concentrations gives a growth response that can be measured turbidimetrically.

Formula

Panthenol Assay Medium

Formula Per Liter

Bacto Dextrose	15 g
Bacto Vitamin Assay Casamino Acids	2 g
Acid Digest of Casein	10 g
Sodium Citrate	2 g
L-Tryptophane	0.2 g
L-Cystine	0.15 g
Adenine Sulfate	10 mg
Guanine Hydrochloride	10 mg
Uracil	10 mg
ß-Alanine	2 mg
Liver Digest	0.35 mg
Nicotinic Acid	2 mg
p-Aminobenzoic Acid	2 mg
Thiamine Hydrochloride	2 mg
Riboflavin	2 mg
Pyridoxine Hydrochloride	2 mg
Folic Acid	20 µg
Biotin	16 µg
Magnesium Sulfate	0.8 g
Sodium Chloride	40 mg
Ferrous Sulfate	40 mg
Manganous Sulfate	0.16 g
Monopotassium Phosphate	2 g

Final pH 6.0 ± 0.2 at 25°C

Panthenol Supplement

Formula Per Liter

Bacto Glycerol	33 g
Sorbitan Monooleate Complex	2 g
Lactic Acid USP	0.68 g
Distilled or Deionized Water	71.5 ml

Precautions

1. For Laboratory Use.
2. Great care must be taken to avoid contamination of media and glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
3. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.
4. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store Panthenol Assay Medium and Panthenol Supplement at 2-8°C. The dehydrated Panthenol Assay Medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Panthenol Assay Medium
Panthenol Supplement

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Gluconobacter oxydans* subsp. *suboxydans* ATCC® 621H
Sterile tubes
Sterile 0.85% saline
Distilled or deionized water
Panthenol
Pantoic Acid
Lactobacilli Agar AOAC
Incubator (30 + 2°C)
Shaker (160-300 rpm)
0.1 N NaOH
0.1 N HCl
Spectrophotometer

Method of Preparation

1. Suspend 33 grams in 900 ml distilled or deionized water.
2. Boil to dissolve.
3. Dispense 4.5 ml amounts into tubes, evenly dispersing any precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 9.5 ml with distilled or deionized water.
6. Autoclave at 121°C for 10 minutes.
7. Aseptically add 0.5 ml Panthenol Supplement to each tube.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedure. For assays, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Stock cultures of *G. oxydans* subsp. *suboxydans* ATCC® 621H are grown on Lactobacilli Agar AOAC and kept in the refrigerator.

Inoculum for assay is prepared by subculturing a stock culture of G. oxydans ATCC® 621H into 10 ml of single-strength Panthenol Assay Medium supplemented with Panthenol Supplement and 4 µg/ml pantoic acid. Following incubation on a shaker (100 rpm) at 30 ± 2°C for 20-24 hours, centrifuge the culture under aseptic conditions. Decant the supernatant and wash the cells three times with sterile 0.85% saline. After the third wash, resuspend the cells in 10 ml sterile 0.85% saline and adjust to a turbidity of 65-70% transmittance when read on the spectrophotometer at 660 nm. Use one drop of this suspension to inoculate each assay flask.

A standard curve must be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve readings and cannot always be duplicated. The standard curve is obtained by using pantoic acid at levels of 0.0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.6 and 2 µg per assay flask.

The concentration of pantoic acid required for the preparation of the standard curve may be prepared by the following procedure:

1. Dissolve 69.2 mg of pure panthenol in distilled water, adjust to pH 6.0 and dilute to 1 liter (1 ml contains the equivalent of 50 µg of pantoic acid).
2. Autoclave 8 ml of this solution with 8 ml 0.1 N NaOH at 121°C for 30 minutes.
3. Cool, add distilled water, adjust to pH 6.0 with 0.1 N HCl and dilute to 100 ml. This stock solution contains 4 µg of pantoic acid per ml.

Prepare the standard solution by diluting 10 ml of the stock solution with 90 ml distilled water. This standard solution contains 0.4 µg of pantoic acid per ml. Use 0.0, 0.5, 1, 1.5, 2, 2.5, 3, 4 and 5 ml per flask (50 ml).

Following inoculation, the cultures are incubated on a suitable shaker at approximately 100-300 rpm at 30 ± 2°C for 18-24 hours. Place cultures in the refrigerator to stop growth. Measure the growth turbidimetrically using a suitable spectrophotometer.

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ± 10% from the average. Use the results only if two thirds of the values do not vary more than ± 10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on a medium recommended for this purpose.

2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. **DeRitter and Ruben.** 1949. Anal. Chem. **21**:823.

Packaging

Panthenol Assay Medium	100 g	0994-15
Panthenol Supplement	12 x 20 ml	0212-64

Bacto® Pantothenate Assay Medium

Intended Use

Bacto Pantothenate Assay Medium is used for determining the concentration of pantothenic acid and its salts by the microbiological assay technique.

Summary and Explanation

Vitamin Assay Media are used in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test.

Pantothenate Assay Medium is a modification of the formula described in the United States Pharmacopeia[1] for the microbiological assay of pantothenic acid and its salts using *Lactobacillus plantarum* ATCC® 8014 as the test organism. Pantothenate Assay Medium does not contain Tween® 80 (Sorbitan Monooleate Complex), which is included in Pantothenate Medium AOAC USP.

Principles of the Procedure

Pantothenate Assay Medium is a dehydrated medium free from pantothenic acid or pantothenate but containing all other nutrients and vitamins essential for the cultivation of *L. plantarum* ATCC® 8014. The addition of calcium pantothenate in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light beige, homogeneous with a tendency to clump.
Solution:	3.65% (single-strength) or 7.3% (double- strength) solution, soluble in distilled or deionized water on boiling 2-3 minutes. Single-strength solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	(Single strength) light amber, clear, may have a slight precipitate.
Reaction of 3.65% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Prepare Pantothenate Assay Medium per label directions. Prepare a standard curve using a pantothenic acid reference standard at levels from 0.0 to 0.10 g per 10 ml. The medium supports the growth of *L. plantarum* ATCC® 8014 when supplemented with calcium pantothenate.

Formula

Pantothenate Assay Medium

Formula Per Liter	
Bacto Vitamin Assay Casamino Acids	10 g
Bacto Dextrose	40 g
Sodium Acetate	20 g
L-Cystine	0.4 g
DL-Tryptophane	0.2 g
Adenine Sulfate	20 mg
Guanine Hydrochloride	20 mg
Uracil	20 mg
Thiamine Hydrochloride	200 µg
Riboflavin	400 µg
Niacin	1 mg
Pyridoxine	800 µg
p-Aminobenzoic Acid	200 µg
Biotin	0.8 g
Monopotassium Phosphate	1 g
Dipotassium Phosphate	1 g
Magnesium Sulfate	0.4 g
Sodium Chloride	20 mg
Ferrous Sulfate	20 mg
Manganese Sulfate	20 mg

Final pH 6.7 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Kidney, Bladder.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.

4. Take precaution to keep sterilization and cooling conditions uniform throughout the assay.
5. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Pantothenate Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus plantarum* ATCC® 8014
Sterile test tubes
Sterile 0.85% saline
Distilled or deionized water
Calcium Pantothenate
Lactobacilli Agar AOAC
Incubator (35-37°C)
Centrifuge
0.2 N Acetic acid
0.2 N Sodium acetate
Spectrophotometer

Method of Preparation

1. Suspend 7.3 grams in 100 ml distilled or deionized water.
2. Boil 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assays, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Prepare stock cultures of *L. plantarum* ATCC® 8014 in triplicate by stab inoculation of Lactobacilli Agar AOAC. Incubate cultures for 18-24 hours at 35-37°C. Store the tubes at 2-8°C. Prepare a fresh stock culture every week. Do not use a culture older than 1 week for this assay.

Inoculum

Subculture from a stock culture of *Lactobacillus plantarum* ATCC® 8014 to 10 ml of sterile single-strength Pantothenate Assay Medium supplemented with 0.02 mcg pantothenate. Incubate for 18-24 hours at 35-37°C. Centrifuge the cells under aseptic conditions and decant the supernatant. Wash the cells three times with 10 ml sterile 0.85% saline. After the third wash, resuspend the cells with sterile 0.85% saline and adjust to a turbidity of 40-45% transmittance when read on a spectrophotometer at 660 nm. Aseptically inoculate each assay tube with one drop of the cell suspension.

Standard Curve

It is essential that a standard curve be constructed each time an assay is run. Autoclave and incubation conditions can influence the standard curve readings and cannot always be duplicated. The standard curve is obtained by using calcium pantothenate solution at levels of 0.0, 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.08, and 0.1 µg per assay tube (10 ml). Turbidimetric determinations are made after 18-24 hours incubation at 35-37°C. Construct a standard curve and determine the concentration of the unknown by interpolation from the standard curve.

The concentration of pantothenic acid required for the preparation of the standard curve may be prepared by dissolving 50 mg dried calcium pantothenate in a solution containing approximately 500 ml distilled water, 10 ml 0.2N acetic acid and 100 ml 0.2N sodium acetate. Dilute to 1,150 ml with additional water to make the calcium pantothenate concentration 43.47 µg per ml; one ml equals 40 µg pantothenic acid.

This solution is diluted by adding 25 ml to a solution containing 500 ml distilled water, 10 ml 0.2N acetic acid and 100 ml 0.2N sodium acetate. Dilute to 1 liter with distilled water to make a stock solution containing 1.0 µg pantothenic acid per ml. The standard solution is made by diluting 2 ml of the stock solution to 100 ml with distilled water. This solution contains 0.02 µg pantothenic acid per ml. Use 0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 ml per assay tube. Prepare the stock solution fresh daily.

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average. Use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results to these procedures, all conditions of the assay must be followed precisely.

References

1. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Inc., Rockville, MD.

Packaging

Pantothenate Assay Medium	100 g	0604-15

Bacto® Pantothenate Medium AOAC USP

Intended Use

Bacto Pantothenate Medium AOAC USP is used for determining the concentration of pantothenic acid and pantothenate by the microbiological assay technique.

Also Known As

AOAC is an abbreviation for Association of Official Analytical Chemists. USP is an abbreviation for United States Pharmacopeia.

Summary and Explanation

Vitamin Assay Media are used in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose;
2. Inoculum Media: To condition the test culture for immediate use;
3. Assay Media: To permit quantitation of the vitamin under test.

Pantothenate Medium AOAC USP is prepared for use in the microbiological assay of pantothenic acid and pantothenate according to the procedures of Calcium Pantothenate Assay in USP[1] and Pantothenate Acid Assay in AOAC.[2] *Lactobacillus plantarum* ATCC® 8014 is the test organism used in this assay.

Principles of the Procedure

Pantothenate Medium AOAC USP is a pantothenic acid/pantothenate-free dehydrated medium containing all other nutrients and vitamins essential for the cultivation of *Lactobacillus plantarum* ATCC® 8014. The addition of calcium pantothenate in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light beige, homogeneous, tendency to clump.
Solution:	3.65% (single strength) or 7.3% (double strength) solution, soluble in distilled or deionized water on boiling 2-3 minutes. Single-strength solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	(Single strength) light amber, clear, may have a very slight precipitate.
Reaction of 3.65% Solution at 25°C:	pH 6.7 ± 0.1

Cultural Response

Prepare Pantothenate Medium AOAC USP per label directions. Test the medium by creating a standard curve using a pantothenic acid reference standard at 0.0 to 0.05 µg per 10 ml. The medium supports the growth of *Lactobacillus plantarum* ATCC® 8014 when prepared in single strength and supplemented with pantothenic acid.

Formula

Pantothenate Medium AOAC USP

Formula Per Liter		
Bacto Dextrose	40	g
Sodium Acetate	20	g
Bacto Vitamin Assay Casamino Acids	10	g
Dipotassium Phosphate	1	g
Monopotassium Phosphate	1	g
L-Cystine	0.4	g
L-Tryptophane	0.1	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20	mg
Ferrous Sulfate	20	mg
Manganese Sulfate	20	mg
Adenine Sulfate	20	mg
Guanine Hydrochloride	20	mg
Uracil	20	mg
Riboflavin	400	µg
Thiamine Hydrochloride	200	µg
Biotin	0.8	µg
p-Aminobenzoic Acid	200	µg
Nicotinic Acid	1	mg
Pyridoxine Hydrochloride	800	µg
Sorbitan Monooleate Complex	0.1	g

Final pH 6.7 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
3. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.
4. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Store in a container with calcium chloride or other desiccant. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Pantothenate Medium AOAC USP

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus plantarum* ATCC® 8014
Centrifuge
Sterile test tubes
Incubator (35-37°C)
Spectrophotometer (660 nm)
Calcium Pantothenate USP
0.2 N Acetic Acid
0.2 N Sodium Acetate
Distilled water

Method of Preparation

1. Suspend 7.3 grams in 100 ml distilled or deionized water.
2. Boil 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml.
6. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedure. The samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Follow the assay procedures as outlined in USP[1] or AOAC.[2]

Prepare stock cultures of *L. plantarum* ATCC® 8014 by stab inoculation of Lactobacilli Agar AOAC. Incubate stock cultures at 35-37°C (± 0.5°C) for 18-24 hours. Store the stock cultures at 2-8°C. Prepare fresh stab cultures every week. Do not use a culture more than one week old for preparing the inoculum.

Subculture from a stock culture of *Lactobacillus plantarum* ATCC® 8014 to a tube of sterile single-strength Pantothenate Medium AOAC USP (10 ml) supplemented with 0.2 mcg pantothenate. Incubate for 18-24 hours at 35-37°C. Centrifuge the cells under aseptic conditions and decant the supernatant. Wash the cells three times with 10 ml sterile 0.85% NaCI. After the third wash, resuspend the cells with sterile 0.85% NaCI and adjust to a turbidity of 40-45% transmittance when read on a spectrophotometer at 660 nm. Aseptically inoculate each assay tube with one drop of the cell suspension.

Prepare solutions of Calcium Pantothenate USP Reference Standard or pantothenic acid (or equivalent) according to USP[1] or AOAC.[2] Satisfactory results are obtained with the standard curve by using pantothenic acid at levels of 0.0, 0.005, 0.01, 0.015, 0.02 and 0.025 µg per assay tube (10 ml) for the AOAC procedure. Calcium pantothenate may be used at standard levels of 0.0, 0.01, 0.02, 0.03, 0.04 and 0.05 µg per assay tube for the USP procedure. Pantothenate Medium AOAC USP may be used for both turbidimetric and titrimetric analysis in the AOAC procedure, and for turbidimetric analysis only for the USP procedure. Turbidimetric readings should be made after 18-24 hours incubation at 35-37°C (± 0.5°C). Titrimetrically determinations are made following 72 hours incubation at 35-37°C (± 0.5°C).

The concentration of pantothenic acid or calcium pantothenate required for the preparation of the standard curve may be prepared as follows:

1. Dissolve 50 mg dried calcium pantothenate in 500 ml distilled water, 10 ml 0.2 N acetic acid and 100 ml 0.2 N sodium acetate.
2. Dilute with additional water to make calcium pantothenate concentration 43.47 µg per ml for the AOAC procedure or dilute to 50 µg per ml for the USP procedure. At 43.47 µg per ml, one ml should equal 40 µg pantothenic acid.

Dilute further by adding 25 ml of this solution to 500 ml distilled water, 10 ml 0.2 N acetic acid and 100 ml 0.2 N sodium acetate. Dilute this solution to 1 liter with distilled water to make a stock solution containing 1 µg pantothenic acid per ml. The standard solution is made by diluting 5 ml of the stock solution to 1000 ml distilled water to obtain a solution containing 0.005 µg pantothenic acid per ml. Use 0.0, 1, 2, 3, 4 and 5 ml per assay tube. For the USP procedure, dilute the 50 µg per ml solution with distilled water to make a standard concentration of 0.01 µg per ml. Other standard concentrations may be used provided the standard falls within the limits specified by USP[1] and AOAC.[2]

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ± 10% from the average and use the results only if two thirds of the values do not vary more than ± 10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention Inc., Rockville, MD.
2. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Pantothenate Medium AOAC USP	100 g	0816-15

Bacto® Peptamin

Intended Use

Bacto Peptamin is used in preparing microbiological culture media.

Also Known As

Peptamin is also referred to as Peptic Digest of Animal Tissue.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Golden tan, free-flowing, granules.
Solution:	1%, 2% and 10% solutions, soluble in distilled or deionized water.
	1%-Very light amber, clear to very slightly opalescent, may have a slight precipitate.
	2%-Light amber, clear to slightly opalescent, may have a slight precipitate.
	10%-Light to medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 1% Solution at 25°C:	pH 7.0-7.6

Cultural Response

Add inoculum density of organism. All solutions are prepared with pH adjusted to 7.2-7.4.

TEST	SOLUTION	ORGANISM	ATCC®	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922*	negative
Indole Production	0.1%	*Escherichia coli*	25922*	positive
Acetyle-methylcarbinol Production	0.1% w/0.5% Dextrose	*Enterobacter aerogenes*	13048*	positive
Hydrogen Sulfide	1%	*Salmonella typhi*	6539	positive
Growth Response	2% w/0.5% NaCl, 0.1% Agar, & 0.1% Dextrose	*Brucella suis*	4314	good growth
Growth Response	2% w/0.5% NaCl, 0.1% Agar, & 0.1% Dextrose	*Escherichia coli*	25922*	good growth
Growth Response	2% w/0.5% NaCl, 0.1% Agar, & 0.1%	*Staphylococcus aureus*	25923*	good growth

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Summary and Explanation

The development of Peptamin is the result of accumulated information that no single peptone is the most suitable nitrogen source for culturing fastidious bacteria. Extensive investigations were undertaken at Difco Laboratories using peptic digests of animal tissue prepared under varying digestion parameters.

Peptamin complies with the US Pharmacopeia XXIII (USP)[1] specification for peptic digest of animal tissue. Diluting and rinsing solutions, Fluid A and Fluid D, contain 0.1% Peptamin. Fluid A and Fluid D conform to the specifications of USP[1] for diluting and rinsing fluids in sterility tests.

Brucella media used for the cultivation of fastidious microorganisms contain Peptamin as the nitrogen source. Peptamin is used in Disinfectant Test Broth AOAC and Letheen Broth, media used for testing disinfectants. Media containing Peptamin are specified in standard methods for multiple applications.[2,3,4]

Principles of the Procedure

Peptamin provides nitrogen, amino acids, vitamins and carbon in microbiological culture media.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated product below 30°C. The dehydrated product is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Peptamin

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Peptamin in the formula of the medium being prepared. Add Peptamin as required.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Peptamin.

Results

Refer to appropriate references and procedures for results.

References

1. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
2. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
3. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Peptamin	500 g	0905-17

Bacto® Peptone
Bacto Peptone Bacteriological Technical

Intended Use

Bacto Peptone and Bacto Peptone Bacteriological Technical are used in preparing microbiological culture media.

Summary and Explanation

Bacto Peptone, an enzymatic digest of protein, was first introduced commercially in 1914 and became the standard Peptone for the preparation of bacteriological culture media. The importance of Peptone as a nutritive source in culture media was demonstrated by studies of Klinger.[5] The nutritive value of Peptone is largely dependent upon the amino acid content that supplies essential nitrogen.

Many studies have used Bacto Peptone in culture media preparation.[6,7,8,9] In a study by Morton, Smith and Leberman,[10] Bacto Peptone was reported to be superior to other peptones in a medium recommended for the isolation and cultivation of pleuropneumonia-like organisms. Bacto Peptone has been shown to be a satisfactory enrichment, replacing serum, for cell proliferation.[11] Peptone is routinely recommended for culture media preparation. Several media containing Peptone are specified in standard methods[1,2,3,4] for multiple applications.

Peptone Bacteriological Technical can be used as the nitrogen source in microbiological culture media when a standardized peptone is not essential. Although it has not been as carefully standardized as other peptones, certain parameters such as solubility, clarity, pH and other growth supporting properties are monitored to permit its use as a nitrogen source.

Principles of the Procedure

Bacto Peptone and Peptone Bacteriological Technical are enzymatic digests of protein. Bacto Peptone contains nitrogen in a form that is readily available for bacterial growth. Both products have a high peptone and amino acids content and only a negligible quantity of proteoses and more complex nitrogenous constituents.

Typical Analysis

Bacto Peptone

Physical Characteristics

Ash (%)	4.4	Loss on Drying (%)	3.0
Clarity, 1% Solution (NTU)	0.5	pH, 1% Solution	7.0
Filterability (g/cm^2)	0.5		

Carbohydrate (%)

Total	6.9

Nitrogen Content (%)

Total Nitrogen	15.5	AN/TN	20.0
Amino Nitrogen	3.1		

Amino Acids (%)

Alanine	8.67	Lysine	3.42
Arginine	6.76	Methionine	1.19
Aspartic Acid	5.60	Phenylalanine	1.81
Cystine	0.20	Proline	8.80
Glutamic Acid	10.21	Serine	2.87
Glycine	15.59	Threonine	1.81
Histidine	0.58	Tryptophan	0.36
Isoleucine	1.45	Tyrosine	0.64
Leucine	3.01	Valine	2.35

Inorganics (%)

Calcium	0.008	Phosphate	0.445
Chloride	1.086	Potassium	0.203
Cobalt	<0.001	Sodium	1.759
Copper	<0.001	Sulfate	0.244
Iron	0.004	Sulfur	0.410
Lead	<0.001	Tin	<0.001
Magnesium	0.007	Zinc	0.001
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.2	PABA	<0.5
Choline (as Choline Chloride)	2000.0	Pantothenic Acid	5.9
Cyanocobalamin	<0.1	Pyridoxine	1.7
Folic Acid	0.3	Riboflavin	3.9
Inositol	2400.0	Thiamine	<0.1
Nicotinic Acid	21.9	Thymidine	413.0

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	273
Salmonella	negative	Thermophile Count	13
Spore Count	90		

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Bacto Peptone

Dehydrated Appearance: Tan, free-flowing granules.

Solution: 1%, 2% and 10% solutions are soluble in distilled or deionized water:
1%- Light amber, clear, no precipitate;
2%- Light to medium amber, clear, no precipitate;
10%- Medium to dark amber, clear to very slightly opalescent, may have a very slight precipitate.

Reaction of 1% Solution at 25°C: pH 6.8-7.2

Peptone Bacteriological Technical

Dehydrated Appearance: Tan, free-flowing, granules.

Solution: 1%, 2% and 10% solutions are soluble in distilled or deionized water:
1%- Very light to light amber, clear;
2%- Light-medium amber, clear;
10%- Medium-dark amber, clear to very slightly opalescent.

Reaction of 1% Solution at 25°C: pH 6.3-7.6

Cultural Response

Bacto Peptone

All solutions are adjusted to pH 7.2-7.4.

TEST	SOLUTION	ORGANISM	ATCC®	INOCULUM	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922*		negative
Indole Production	0.1%	*Escherichia coli*	25922*		positive
Acetylmethyl-carbinol Production	0.1% with 0.5% Dextrose	*Enterobacter aerogenes*	13048*		positive
Hydrogen Sulfide Production	1%	*Salmonella typhi*	6539		positive
Growth Response	2% with 1.5% Agar and 0.5% NaCl	*Escherichia coli*	25922*	100-1,000	good growth
Growth Response	2% with 1.5% Agar and 0.5% NaCl	*Staphylococcus aureus*	25923*	100-1,000	good growth

Peptone Bacteriological Technical

Prepare 2% Peptone Bacteriological Technical in 0.5% saline and adjust to pH 7.2-7.4; add 1.5% Bacto Agar, boil and sterilize. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	RESULT
Escherichia coli	25922*	100-1,000	good growth
Staphylococcus aureus	25923*	100-1,000	good growth

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store the products below 30°C. The dehydrated product is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bacto Peptone
Peptone Bacteriological Technical

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Bacto Peptone or Peptone Bacteriological Technical in the formula of the medium being prepared. Add Bacto Peptone or Peptone Bacteriological Technical as required.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Bacto Peptone or Peptone Bacteriological Technical.

Results

Refer to appropriate references and procedures for results.

References

1. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington D.C.
2. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
3. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
4. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. **Klinger, I. J.** 1917. The effect of hydrogen ion concentration on the production of precipitates in a solution of peptone and its relation to the nutritive value of media. J. Bacteriol. **2**:351-353.
6. **Spray, R. S.** 1929-1930. J. Lab. Clin. Med. **15**:179.
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8. **Huntoon, F. M.** 1918. "Hormone" medium. A simple medium employable as a substitute for serum medium. J. Infect. Dis. **23**:169-172.

9. **Jones and Wise.** 1926. J. Bacteriol. **11**:359.
10. **Morton, H. E., P. F. Smith, and P. R. Leberman.** 1951. Venereal diseases. Am. J. Syphilis Gonorr. **35**:361.
11. **Rutzky, L. P.** 1981. Peptone growth factors for serial cell proliferation in the absence of serum. Cambridge University Press.

Packaging

Bacto Peptone	100 g	0118-15
	500 g	0118-17
	2 kg	0118-07
	10 kg	0118-08
Peptone Bacteriological, Technical	500 g	0885-17

Bacto® Peptone Iron Agar

Intended Use

Bacto Peptone Iron Agar is used for detecting hydrogen sulfide production by microorganisms.

Summary and Explanation

Levine and co-workers[1,2] described a medium containing Proteose Peptone and ferric citrate for detection of hydrogen sulfide production by coliform bacteria. They demonstrated that such a medium served to differentiate strains that were Voges-Proskauer negative, methyl-red positive and citrate positive from other members of the *Enterobacteriaceae*.

Levine reported that ferric citrate was a much more sensitive indicator of hydrogen sulfide production than lead acetate, producing a medium that gave definite reactions within 12 hours. Peptone Iron Agar is a modification of Levine's original formula in which Bacto Peptone has been included with Proteose Peptone and the more soluble ferric ammonium citrate is used in place of ferric citrate.

Tittsler and Sandholzer[3] compared Peptone Iron Agar with lead acetate agar for the detection of hydrogen sulfide and found that Peptone Iron Agar had the advantage of giving earlier reactions and clearer results.

Principles of the Procedure

Bacto Peptone and Proteose Peptone are nitrogen sources in Peptone Iron Agar. Ferric Ammonium Citrate and Sodium Thiosulfate are used to detect H_2S production. Sodium Glycerophosphate is a buffering compound. Bacto Agar is a solidifying agent.

Formula

Peptone Iron Agar

Formula Per Liter

Bacto Peptone	15 g
Bacto Proteose Peptone	5 g
Ferric Ammonium Citrate	0.5 g
Sodium Glycerophosphate	1 g
Sodium Thiosulfate	0.08 g
Bacto Agar	15 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent.
Reaction of 3.6% Solution at 25°C:	pH 6.7 ± 0.2

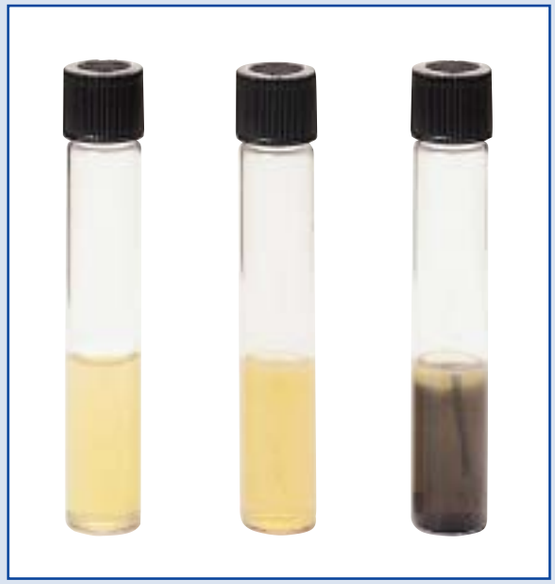
Cultural Response

Prepare Peptone Iron Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	H_2S PRODUCTION
Escherichia coli	25922*	undiluted	good	–
Salmonella enteritidis ser. *enteritidis*	13076	undiluted	good	+ (black)

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Salmonella enteritidis* ATCC® 13076

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Peptone Iron Agar

Materials Required but not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Test tubes with closures or Petri dishes
Autoclave
Waterbath (45-50°C)
Incubator (35°C)

Method of Preparation

1. Suspend 36 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. If preparing tubes, dispense the medium in 10 ml amounts. If preparing plates, leave the medium in the flask.
4. Autoclave at 121°C for 15 minutes.
5. If preparing tubes, cool in an upright position. If pouring plates, cool the medium to 45-50°C. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[4,5,6]
2. For specific information about specimen preparation and inoculation for isolation of coliform bacteria, consult appropriate references.[4,5,6]
3. Once isolated colonies of coliform bacteria are obtained on solid plated media, follow the test procedure below.

Test Procedure

1. Obtain a pure culture of a test organism. Pick the center of a single colony with an inoculating needle.
2. Inoculate a tube of Peptone Iron Agar by the stab method. Stab the needle to within 1/4 to 1/2 inch of the bottom. Withdraw the needle following the initial line of inoculation.
3. Incubate tubes at 35°C for 18-48 hours.
4. Read tubes for growth and hydrogen sulfide production.

Results

Any blackening of the medium along the line of inoculation or throughout the butt indicates hydrogen sulfide production.

For a complete discussion of the identification of coliform bacteria, refer to the appropriate references.[4,5,6]

References

1. **Levine, M., R. Vaughn, S. S. Epstein, and D. Q. Anderson.** 1932. Some differential reactions in the colon-aerogenes group of bacteria. Proc. Soc. Exp. Biol. Med. **29**:1022-1024.
2. **Levine, M., S. S. Epstein, and R. H. Vaughn.** 1934. Differential reactions in the colon group of bacteria. Am. J. Publ. Health **24:**505-510.
3. **Tittsler, R. P., and L. A. Sandholzer.** 1937. Advantages of peptone iron agar for the routine detection of hydrogen sulphide production. Am. J. Publ. Health **27:**1240-1242.
4. **Pezzlo, M. (ed.).** 1994. Aerobic bacteriology, p. 1.0.1.-1.20.47. *In* H. D. Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
5. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Peptone Iron Agar	500 g	0089-17

Bacto® Peptone Water

Intended Use

Bacto Peptone Water is used for cultivating non-fastidious organisms, for studying carbohydrate fermentation patterns, and for performing the indole test.

Summary and Explanation

The formulation of Peptone Water makes it useful for cultivating non-fastidious organisms.[1] This non-selective medium has been used as a basal medium for biochemical tests such as carbohydrate fermentation patterns and production of indole.[1,2]

Principles of the Procedure

Peptone Water contains Peptone as a source of carbon, nitrogen, vitamins and minerals. Sodium Chloride maintains the osmotic balance of the medium.

Formula

Peptone Water

Formula Per Liter

Peptone	10 g
Sodium Chloride	5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.

2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Peptone Water

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)
Tubes with closures
Fermentation tubes
Carbohydrate solutions

User Quality Control

Identity Specifications

Dehydrated Appearance:	Cream-white to light tan, free-flowing, homogeneous.
Solution:	1.5% solution, soluble in distilled or deionized water on warming with frequent agitation. Solution is light amber, clear to very slightly opalescent.
Reaction of 1.5% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Growth/Indole Reaction

Prepare Peptone Water per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours. Indole reaction is read using Indole Test Strips (1627).

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	INDOLE REACTION
Escherichia coli	25922*	undiluted	good	positive

Carbohydrate Fermentation

Prepare Peptone Water per label directions with the addition of phenol red and dextrose. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID PRODUCTION
Escherichia coli	25922*	100-1,000	good	positive
Staphylococcus aureus	25923*	100-1,000	good	positive

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

1% Phenol Red solution
Indole Test strips

Method of Preparation

1. Dissolve 15 grams in 1 liter distilled or deionized water with warming and frequent agitation.
2. Autoclave at 121°C for 15 minutes.

For Determining Carbohydrate Fermentation Patterns

1. Add 1.8 ml 1% phenol red solution to 1 liter rehydrated Peptone Water. Mix thoroughly.
2. Dispense into test tubes containing inverted Durham vials.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically add sufficient sterile carbohydrate solution to yield a 1% final concentration. Rotate each tube to thoroughly distribute the carbohydrate.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For Performing Carbohydrate Fermentation

1. Inoculate tubes with test organism.
2. Incubate tubes at 35 ± 2°C for 18-48 hours.
3. Observe for color change.

For Performing the Indole Test

1. Using aseptic technique, suspend an Indole Test Strip 10 mm above the surface of a 24 or 48 hour culture.
2. Incubate at 37°C for 5-30 minutes.

Results

For Determining Carbohydrate Fermentation Patterns

Acid is produced when carbohydrates are fermented. This is indicated by a yellow color in the medium. Gas production is indicated by the presence of gas bubbles in the fermentation tube.

For performing the Indole Test

Observe for the formation of a violet color on the strip which indicates a positive test for indole production.

Limitations of the Procedure

1. Medium is pink in color when hot but becomes colorless upon cooling.
2. *Vibrio* spp. should not be incubated longer than 18-20 hours. Longer incubation may cause the development of suppressed forms.[3]

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 610-612. Williams & Wilkins, Baltimore, MD.
2. **Balows, A., W. J. Hausler, K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.).** 1991. Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
3. **Finegold, S. M., and W. Martin.** 1982. Bailey and Scott's diagnostic microbiology, 6th ed. St. Louis

Packaging

Peptone Water	500 g	1807-17

Phenol Red Agar Media

Bacto® Phenol Red Agar Base · Bacto Phenol Red Lactose Agar Bacto Phenol Red Mannitol Agar

Intended Use

Bacto Phenol Red Agar Base is used with added carbohydrate in differentiating pure cultures of bacteria based on fermentation reactions.

Bacto Phenol Red Lactose Agar is used for differentiating pure cultures of bacteria based on lactose fermentation reactions.

Bacto Phenol Red Mannitol Agar is used for differentiating pure cultures of bacteria based on mannitol fermentation reactions.

Summary and Explanation

Phenol Red Agar Base with added carbohydrate is well suited for the study of fermentation reactions of microorganisms.[1,2,3,4] However, while liquid media are generally employed in studying fermentation reactions, many bacteriologists prefer a solid medium for this purpose. One advantage of a solid fermentation medium is that it permits observation of fermentation reactions under both aerobic and anaerobic conditions.[5,6] Deep tubes can provide sufficiently anaerobic conditions for the growth of obligate anaerobic bacilli. Any gas formation that occurs during a reaction is indicated by splitting of the agar or accumulation of gas bubbles in the base.

Phenol Red Agar Base supports excellent growth of many fastidious bacteria. It is a basal medium free of any fermentable carbohydrates that could give erroneous interpretations. With the exception of the omitted carbohydrate, it is a complete medium prepared with Phenol Red as an indicator of reaction changes. Phenol Red Agar Base permits the user to prepare any quantity of medium needed, adding to

User Quality Control

Identity Specifications

Dehydrated Appearance: Pink, homogeneous, free-flowing.

Solution:
Phenol Red Agar Base: 3.1% solution, soluble in distilled or deionized water upon boiling. Solution is orange-red to red, clear to slightly opalescent.

Phenol Red Lactose Agar: 4.1% solution, soluble in distilled or deionized water upon boiling. Solution is orange-red to red, slightly opalescent without significant precipitate.

Phenol Red Mannitol Agar: 4.1% solution, soluble in distilled or deionized water upon boiling. Solution is orange-red to red, very slightly opalescent.

Prepared Medium: Red to orange-red, slightly opalescent.

Reaction of the Solutions at 25°C: pH 7.4 ± 0.2

Uninoculated tube | Typical positive reaction with acid and gas | Typical negative reaction with positive growth

Cultural Response

Prepare media per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISMS	ATCC®	GROWTH	PHENOL RED AGAR BASE (w/o CARBOHYDRATES) ACID	GAS	PHENOL RED AGAR BASE w/1% MALTOSE ACID	GAS	PHENOL RED AGAR BASE w/1% SUCROSE ACID	GAS	PHENOL RED AGAR BASE w/1% DEXTROSE ACID	GAS
Alcaligenes faecalis	8750	good	–	–	–	–	–	–	–	–
Escherichia coli	25922*	good	–	–	+	+	–	–	+	+
Klebsiella pneumoniae	13883*	good	–	–	+	+	+	+	+	+
Shigella flexneri	12022*	good	–	–	–	–	–	–	+	–

ORGANISMS	ATCC®	GROWTH	PHENOL RED LACTOSE AGAR ACID	GAS	PHENOL RED MANNITOL AGAR ACID	GAS
Escherichia coli	25922*	good	+	+	+	+
Salmonella typhimurium	14028*	good	–	–	+	+
Staphylococcus aureus	25923*	good	+	–	+	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in the Bactrol Disks Technical Information.

different portions any fermentable substance to be tested. Usually a 1% final concentration of a test carbohydrate is added. An entire series of carbohydrate agars can be made up readily, conveniently, and economically. Phenol Red Lactose Agar and Phenol Red Mannitol Agar already contain the specified carbohydrate.

Principles of the Procedure

Proteose Peptone No. 3 and Beef Extract provide the carbon and nitrogen required for good growth in a wide variety of organisms. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is the solidifying agent. Phenol Red serves as a pH indicator, turning from red-orange to yellow when acid is produced during fermentation of the carbohydrate.

Formula

Phenol Red Agar Base

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	1 g
Sodium Chloride	5 g
Bacto Agar	15 g
Bacto Phenol Red	0.025 g

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Lactose Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	1 g
Lactose	10 g
Sodium Chloride	5 g
Bacto Agar	15 g
Bacto Phenol Red	0.025 g

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Mannitol Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	1 g
D-Mannitol	10 g
Sodium Chloride	5 g
Bacto Agar	15 g
Bacto Phenol Red	0.025 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store the prepared medium at 15-30°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Phenol Red Agar Base
Phenol Red Lactose Agar
Phenol Red Mannitol Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Choice of carbohydrates to be added to the basal medium
Tubes with closures

Method of Preparation

Phenol Red Agar Base

1. Suspend 31 grams in 1 liter distilled or deionized water and boil to dissolve completely.
2. When preparing 1% carbohydrate fermentation agars, dissolve 10 grams of the desired carbohydrate in the basal medium prior to sterilization
3. Autoclave at 121°C for 15 minutes.
4. Cool the medium to 45-50°C.

OR

1. Suspend 31 grams in 900 ml distilled or deionized water and boil to dissolve completely.
2. Autoclave at 121°C for 15 minutes.
3. Cool the medium to 45-50°C.
4. Aseptically add 100 ml of a sterile 10% carbohydrate solution (w/v).
5. Dispense into sterile tubes with closures.

Phenol Red Lactose Agar
Phenol Red Mannitol Agar

1. Suspend 41 grams of the selected medium in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Dispense into tubes. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate the sterile medium by stabbing into the butt and streaking the surface of the slant.

   If desired, inoculate obligate anaerobic bacteria into melted medium that has been cooled to 45°C. Allow the agar to solidify prior to incubation.
2. Incubate at 35 ± 2°C for 4-48 hours (or anaerobically for 24-72 hours).
3. Examine periodically for growth, acid production and gas formation.

Results

Fermentation of the carbohydrate is indicated by a change in the color of the medium from red to canary yellow. Gas formation is indicated by the collection of gas bubbles in the base or by splitting of the agar.

Limitations of the Procedure

1. The addition of some carbohydrates to the basal medium may cause an acid reaction. To restore the original pH (and color of the medium), add 0.1 N sodium hydroxide on a drop-by-drop basis. Take care not to make the medium too alkaline, which would prevent fermentation from occurring within the usual incubation period.
2. When inoculating tubes, stab gently and do not use a loop. Rough stabbing or using a loop to stab may give the false appearance of gas production when mechanical splitting of the medium is what actually occurred.

References

1. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th edition. Mosby-Year Book, Inc., St. Louis, MO.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken. (ed.).** 1995. Manual of clinical microbiology, 6th edition. American Society for Microbiology, Washington, D.C.
3. **Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams.** 1994. Bergey's manual of determinative bacteriology, 9th edition. Williams & Wilkins, Baltimore, MD.
4. **Ewing, W. H.** 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th edition. Elsevier Science Publishing Co., Inc., New York, NY.
5. **Bacteriological Analytical Manual,** 8th edition. 1995. AOAC International, Gaithersburg, MD.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Packaging

Phenol Red Agar Base	500 g	0098-17
Phenol Red Lactose Agar	500 g	0100-17
Phenol Red Mannitol Agar	500 g	0103-17

Phenol Red Carbohydrate Media

Bacto® Phenol Red Broth Base · Bacto Phenol Red Dextrose Broth · Bacto Phenol Red Lactose Broth · Bacto Phenol Red Mannitol Broth · Bacto Phenol Red Saccharose Broth

Intended Use

Phenol Red Carbohydrate Media are basal media used with added carbohydrates in differentiating pure cultures of bacteria based on fermentation reactions.

Summary and Explanation

The fermentative properties of bacteria are valuable criteria in their identification.[1,2,3,4] A basal medium for determining the fermentation reactions of microorganisms must be capable of supporting growth of test organisms and be free from fermentable carbohydrates. Vera[5] used a fermentation test medium employing the pH indicator phenol red and obtained highly accurate results.

Phenol Red Broth Base is recommended for use to determine the ability of organisms to ferment various carbohydrates.[6,7,8,9] Different fermentable substances may be added in any desired concentration. The concentration of carbohydrate generally employed for testing fermentation reactions of bacteria is 0.5 to 1%. Some investigators prefer to use 1% rather than 0.5% to ensure against reversion of the reaction due to depletion of the carbohydrate.

Phenol Red Broth Base is an excellent substrate for streptococci, as well as for other less fastidious bacteria, the growth promotion of the medium can be greatly improved for fastidious, microaerophilic, and obligately anaerobic strains by the addition of a small amount of Bacto Agar (0.1-0.2%). A medium containing this small quantity of agar may be heated it to the boiling point to drive out the dissolved air. The tubes are then cooled to below 40°C, without excessive agitation, just prior to inoculation. The fermentation reaction of gonococci may be determined by using 0.8% Bacto Agar and adding 5% sterile fresh rabbit serum to the sterile Phenol Red Broth Base containing the selected carbohydrate. Coagulase Plasma EDTA can be added to Phenol Red Mannitol Broth to prepare Coagulase Mannitol Broth. This medium is useful in determining the ability of *Staphylococcus aureus* to ferment mannitol and to coagulate plasma.[10]

Principles of the Procedure

Proteose Peptone No. 3 and Beef Extract provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Sodium Chloride maintains the osmotic balance of the medium. Phenol Red serves as an indicator, turning from red-orange to yellow when acid is produced during fermentation of the added carbohydrates.

Formula

Phenol Red Broth Base

Formula Per Liter

Bacto Beef Extract	1 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	5 g
Bacto Phenol Red	0.018 g

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Dextrose Broth

Formula Per Liter

Bacto Beef Extract	1 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	5 g
Bacto Phenol Red	0.018 g
Bacto Dextrose	5 g

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Lactose Broth

Formula Per Liter

Bacto Beef Extract	1 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	5 g
Bacto Phenol Red	0.018 g
Bacto Lactose	5 g

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Mannitol Broth

Formula Per Liter

Bacto Beef Extract	1 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	5 g
Bacto Phenol Red	0.018 g
Bacto Mannitol	5 g

Final pH 7.4 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Phenol Red Broth Base

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	1.6% solution, soluble in distilled or deionized water. Solution is orange-red to red, clear.
Prepared Media:	Orange-red to red, clear.
Reactions of 1.6% Solution at 25°C:	pH 7.4 ± 0.2

Phenol Red Dextrose Broth

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in distilled or deionized water. Solution is orange-red to red, clear.
Prepared Media:	Orange-red to red, clear.
Reactions of 2.1% Solution at 25°C:	pH 7.4 ± 0.2

Phenol Red Lactose Broth

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in distilled or deionized water. Solution is orange-red to red, clear.
Prepared Media:	Orange-red to red, clear.
Reactions of 2.1% Solution at 25°C:	pH 7.4 ± 0.2

continued on following page

Phenol Red Saccharose Broth

Formula Per Liter

Bacto Beef Extract	1 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	5 g
Bacto Phenol Red	0.018 g
Bacto Saccharose	5 g

Final pH 7.4 ± 0.2 at 25°C

Phenol Red Carbohydrate Broths contain the above ingredients with 5 g/liter of the specified carbohydrate.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated media below 30°C. The dehydrated media are very hygroscopic. Keep containers tightly closed. Store the prepared media at 2-8°C.

Expiration Date

The expiration date applies to the products in their intact containers when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Phenol Red Broth Base
Phenol Red Dextrose Broth
Phenol Red Lactose Broth
Phenol Red Mannitol Broth
Phenol Red Saccharose Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Carbohydrates (as needed)
Tubes with closures
Fermentation tubes

Method of Preparation

Phenol Red Broth Base

1. Dissolve 16 grams in 1 liter distilled or deionized water.
2. Distribute into tubes. To detect gas production, place inverted fermentation tubes (Durham tubes) in the tubes of medium.
3. Autoclave at 121°C for 15 minutes.

When preparing 0.5-1% carbohydrate fermentation broths, dissolve 5-10 grams of the desired carbohydrate in the basal medium prior to sterilization, or dissolve 16 grams of Phenol Red Broth Base in 900 ml distilled or deionized water and aseptically add 100 ml of a sterile 5-10% carbohydrate solution (w/v) after sterilizing and cooling the basal medium.

Phenol Red Dextrose Broth, Phenol Red Lactose Broth, Phenol Red Mannitol Broth, Phenol Red Saccharose Broth

1. Suspend 21 grams of the appropriate Phenol Red Carbohydrate Broth in 1 liter distilled or deionized water and stir to dissolve completely.
2. For better growth of fastidious organisms (such as streptococci, pneumococci, and gonococci) add 1 gram of Bacto Agar per liter of medium and dissolve by boiling prior to sterilizing.
3. Dispense into tubes. To detect gas production, place inverted fermentation tubes in the tubes of medium.
4. Autoclave at 121°C for 15 minutes.

If the media are not used the same day they are sterilized, prior to use, place the medium in flowing steam or a boiling water bath for a few minutes to drive off dissolved gases. Allow to cool without agitation.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate tubes with one drop of a diluted pure culture.
2. Incubate at 35 ± 2°C for 4-18 hours with caps loosened.
3. Examine tubes for growth, acid production, and gas production (if fermentation vials are used).

Results

A yellow color of the medium indicates a positive reaction for carbohydrate fermentation. If fermentation vials are used, bubbles in the inverted vials are an indication of gas production. The presence of a single bubble is recorded as positive for the production of gas.[10]

Limitations of the Procedure

1. The addition of some carbohydrates to the basal medium may result in an acid reaction. In this case, it is suggested that 0.1N sodium hydroxide be added drop by drop to restore the original color. Take care not to make the medium too alkaline for true fermentation to occur within the usual incubation period.
2. To ensure accuracy of interpretation, uninoculated control tubes and/or inoculated Phenol Red Broth Base control tubes should be run in parallel with the fermentation tests.

User Quality Control cont.

Phenol Red Mannitol Broth

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in distilled or deionized water. Solution is orange-red to red, clear.
Prepared Media:	Orange-red to red, clear.
Reactions of 2.1% Solution at 25°C:	pH 7.4 ± 0.2

Phenol Red Saccharose Broth

Dehydrated Appearance:	Pink, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in distilled or deionized water. Solution is orange-red to red, clear.
Prepared Media:	Orange-red to red, clear.
Reactions of 2.1% Solution at 25°C:	pH 7.4 ± 0.2

Uninoculated tube | Typical positive reaction with acid and gas | Typical negative reaction with positive growth

Cultural Response

Prepare media per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH	BASE A	BASE G	DEXTROSE A	DEXTROSE G	LACTOSE A	LACTOSE G	MANNITOL A	MANNITOL G	SACCHAROSE A	SACCHAROSE G
Alcaligenes faecalis	8750	good	–	–	–	–	–	–	–	–	–	–
Escherichia coli	25922*	good	–	–	+	+	+	+	+	+	–	–
Klebsiella pneumoniae	13883*	good	–	–	+	+	+	+	+	+	+	+
Shigella flexneri	12022*	good	–	–	+	–	–	–	+	–	–	–

A = Acid
G = Gas

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in the Bactrol Disks Technical Information.

References

1. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th edition. Mosby-Year Book, Inc., St. Louis, MO.
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9. **Franson, M. A. H., A. D. Eaton, L. S. Clesceri, and A. E. Greenberg.** 1995. Standard methods for the examination of water and wastewater. American Public Health Association, Washington, D.C.
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Packaging

Phenol Red Broth Base	500 g	0092-17
Phenol Red Dextrose Broth	500 g	0093-17
Phenol Red Lactose Broth	500 g	0094-17
Phenol Red Mannitol Broth	500 g	0097-17
Phenol Red Saccharose Broth	500 g	0095-17

Bacto® Phenylalanine Agar

Intended Use

Bacto Phenylalanine Agar is used for differentiating *Proteus* and *Providencia* species from other *Enterobacteriaceae* based on deamination of phenylalanine.

Also Known As

Phenylalanine Agar is also known as Phenylalanine Deaminase Medium.

Summary and Explanation

Buttiaux, Osteux, Fresnoy and Moriamez[1] developed a method to differentiate members of the *Proteus* and *Providencia* groups from

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Light amber, slightly opalescent without precipitate.
Reaction of 2.3% Solution at 25°C:	7.3 ± 0.2

Cultural Response

Prepare Phenylalanine Agar per label directions. Inoculate the medium and incubate at 35°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	REACTION
Enterobacter aerogenes	13048*	100-1,000	good	–
Proteus vulgaris	13315*	100-1,000	good	+
Providencia alcalifaciens	9886	100-1,000	good	+

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube with reagent

Proteus vulgaris ATCC® 13315

other *Enterobacteriaceae* based on the ability of *Proteus* and *Providencia* to deaminate phenylalanine to phenylpyruvic acid by enzymatic activity.[2] Bynae modified this method by incorporating phenylalanine in the medium used to grow the organisms. Ewing, Davis and Reavis[4] simplified the Bynae formulation by omitting proteose peptone. Phenylalanine Agar is prepared according their formula.

Phenylalanine Agar is used to differentiate *Proteus, Providencia* and *Morganella* (originally classified in the genus *Proteus*) from other members of the family *Enterobacteriaceae.* In addition, some strains of *Enterobacter agglomerans, Enterobacter sakazakii, Rahnella aquatilis, Tatumella ptyseos* and a few nonfermenting gram-negative bacilli are also capable of deaminating phenylalanine.[4,5]

Principles of the Procedure

Phenylalanine Agar contains DL-Phenylalanine which serves as a substrate for deamination to phenylpyruvic acid. After incubation, phenylpyruvic acid is detected by the addition of ferric chloride reagent. The ferric ions chelate the phenylpyruvic acid and form a green color.[5] Yeast Extract provides vitamins and cofactors required for growth as well as additional sources of nitrogen and carbon. Dipotassium Phosphate provides buffering capability. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

Formula

Phenylalanine Agar

Formula Per Liter	
Bacto Yeast Extract	3 g
Dipotassium Phosphate	1 g
Sodium Chloride	5 g
DL-Phenylalanine	2 g
Bacto Agar	12 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Phenylalanine Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
SpotTest™ Ferric Chloride Reagent (3557) or 8-12% ferric chloride
0.1 N HCl

Method of Preparation

1. Suspend 23 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes. Autoclave at 121°C for 15 minutes.
4. Allow medium to solidify in a slanted position.

Test Procedure

1. Inoculate the medium and incubate at 35°C for 18-24 hours.
2. After recording the growth response, add 3-5 drops of SpotTest™ Ferric Chloride Reagent to each tube.
3. Examine for color development within 1-5 minutes. A dark green color indicates a positive reaction.

Results

Positive: Dark green

Negative: No color change

Limitations of the Procedure

1. A positive phenylalanine reaction should be interpreted quickly because the green color disappears within 10 minutes after addition of ferric chloride solution. Adding additional reagent usually regenerates the color.
2. Certain species rapidly deaminate phenylalanine, allowing for a positive test result within 4 hours of incubation.[4]

References

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2. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 634-636. Williams & Wilkins, Baltimore, MD.
3. **Ewing, W. H., B. R. Davis, and R. W. Reavis.** 1957. Phenylalanine and malonate media and their use in enteric bacteriology. Public Health Lab. **15**:153.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol 1. American Society for Microbiology, Washington, D.C.
5. **Oberhofer, T. R.** 1985. Manual of nonfermenting gram-negative bacteria. Churchill Livingstone, New York, NY.

Packaging

Phenylalanine Agar	100 g	0745-15
	500 g	0745-17

Bacto® Phenylethanol Agar

Intended Use

Bacto Phenylethanol Agar is used for isolating staphylococci and streptococci from specimens containing gram-negative organisms.

Also Known As

Phenylethanol Agar is also referred to as Phenylethyl Alcohol (PEA) Agar.

Summary and Explanation

Brewer and Lilley[1,2] reported that the addition of phenylethanol to a nutritive medium will permit growth of gram-positive organisms but markedly to completely inhibit growth of gram-negative organisms found in the same specimen. Phenylethanol Agar inhibits the swarming of *Proteus* spp. and can be used to selectively isolate anaerobic bacteria from clinical specimens with mixed flora. Phenylethanol Agar is specified for use in several reference methods.[3,4,5]

Principles of the Procedure

Tryptose and Beef Extract provide the nitrogen and carbon required for good growth of a wide variety of organisms. Sodium Chloride maintains the osmotic balance. Bacto Agar is a solidifying agent. Phenylethanol is bacteriostatic for gram-negative bacteria and inhibits DNA synthesis. Optional addition of 5% defibrinated sheep blood to the basal medium can enhance microorganism recovery on the medium.

Formula

Phenylethanol Agar

Formula Per Liter

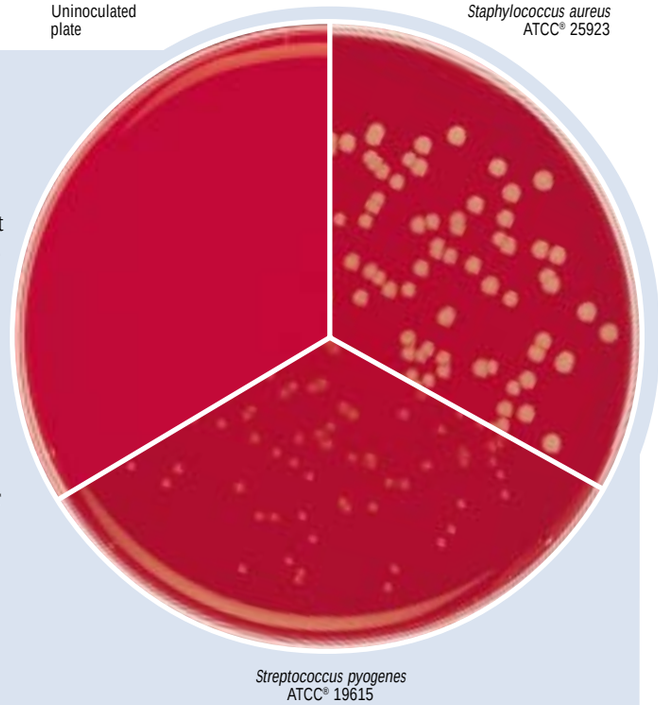
Bacto Tryptose	10 g
Bacto Beef Extract	3 g
Sodium Chloride	5 g
Bacto Agar	15 g
Phenylethanol	2.5 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Eyes, Face, Urogenital.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, homogeneous with soft clumps.
Solution:	3.55% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly to slightly opalescent.
Prepared Medium:	Without blood - light amber, slightly opalescent; With blood - cherry red, opaque.
Reaction of 3.55% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Phenylethanol Agar with 5% sterile defibrinated sheep blood per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	HEMOLYSIS
Proteus mirabilis	12453	1,000-2,000	partial inhibition	N/A
Staphylococcus aureus	25923*	100-1,000	growth	beta
Streptococcus pneumoniae	6305	100-1,000	growth	alpha
Streptococcus pyogenes	19615*	100-1,000	growth	beta

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Phenylethanol Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile defibrinated blood (optional)

Method of Preparation

1. Suspend 35.5 grams in 1 liter distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. OPTIONAL: To prepare blood agar, aseptically add 5% sterile defibrinated blood to the cooled medium at 45-50°C. Mix well.

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[3-5]
2. Process each specimen using procedures appropriate for that specimen or sample.[3-5]

Test Procedure

1. Inoculate plates with test specimens. Streak to obtain isolated colonies.
2. Incubate plates at 35 ± 2°C under 5-10% CO_2 for 18-24 hours and, if necessary, 40-48 hours.

Results

Examine plates for growth and hemolysis. Perform additional biochemical testing to identify the organism.

Limitations of the Procedure

1. Some gram-positive cocci may be slightly inhibited and may require further incubation (to 48 hours) for sufficient growth to be evident.[6]
2. Subculture gram-positive colonies onto Tryptic Soy Agar (TSA), Selenite Broth and other biochemical media for definitive identification.[6]
3. *Pseudomonas aeruginosa* is not inhibited on this medium.[7]

References

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3. **Baron, E. J., and S. M. Finegold.** 1990. Bailey & Scott's diagnostic microbiology, 8th ed. The C.V. Mosby Company, St. Louis, MO.
4. **Isenberg, H. D.** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
5. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
7. **Washington, J. A., Jr.** 1981. Laboratory procedures in clinical microbiology. Springer-Verlag, New York.

Packaging

Phenylethanol Agar	100 g	0504-15
	500 g	0504-17
	2 kg	0504-07

Phytohemagglutinins

Bacto® Phytohemagglutinin M · Bacto Phytohemagglutinin P

Intended Use

Bacto Phytohemagglutinin M and Bacto Phytohemagglutinin P are used for the isolation of lymphocytes and nucleated erythrocytes from blood and marrow. They are also used for initiating mitosis in lymphocytes for chromosomal analysis.

Also Known As

Phytohemagglutinin M is also known as PHA-M. Phytohemagglutinin P is also known as PHA-P.

Summary and Explanation

Hemagglutination

Phytohemagglutinins M or P were originally used for hemagglutination techniques.[1,2] Phytohemagglutinins were then used with dextran[3] and fibrinogen[4] to produce excellent yields of morphologically and physiologically intact lymphocytes in a suspension with no hemolysis.

Phytohemagglutinin M or P have been used to agglutinate the erythrocytes of all human blood groups, and those of many animals such as rabbit,

dog, cat, chicken, duck, mouse, rat, sheep, horse, pig, frog and guinea pig. Phytohemagglutinin has been used to obtain the plasma suspension of trypanosomes from the blood of infected rats.[5]

Mitogenic Activity

Nowell[6] discovered that phytohemagglutinin M initiates mitosis in cultures of lymphocytes isolated from peripheral blood. Later, phytohemagglutinin P was also shown to possess this property. The application of this technique is important in the characterization of chromosomes. A procedure using phytohemagglutinin-stimulated lymphoblasts has been used to cultivate human immunodeficiency virus type 1 (HIV-1) from infected individuals by cocultivation cultures.[7] Human T-lymphocytes have been activated by phytohemagglutinin to the blastic killer-cell state in preparation for in-vivo immunotherapy trials in donor cancer patients.[8]

A simplified procedure for lymphocyte mitogenesis was developed by Moorhead, Nowell, Mellman, Batipps and Hungerford,[9] in which the cultures were routinely allowed to incubate for 3 days (65-70 hours). Their method incorporated the hypotonic treatment developed by Hughes[10] and Hsu and Pomerat.[11] The flame drying of slides by Scherz[12] and the staining procedure by Rothfels and Siminovitch[13] were helpful contributions in this procedure. Staining of chromosomes by one of many methods produces characteristic bands. For more information on chromosome staining, please refer to appropriate references.[14-17]

Principles of the Procedure

Both Phytohemagglutinin M and P will agglutinate the erythrocytes of all human blood types, and those of animals. The rehydrated P-form has approximately 40 times more hemagglutinating potency than the M-form. Both forms will also stimulate the lymphocytes of peripheral blood to undergo mitosis in vitro.

Reagents

Phytohemagglutinin M is a stable, nontoxic, desiccated mucophytohemagglutinin.

Phytohemagglutinin P is a sterile, desiccated, purified, highly potent protein phytohemagglutinin from which the polysaccharide moiety has been removed.

User Quality Control

Identity Specifications

Phytohemagglutinin M or P

Lyophilized Appearance:	White, porous lyophilized cake.
Solution Appearance:	Contents of 1 vial, soluble in 5 ml sterile distilled or deionized water within 2 minutes. Solution is colorless, clear to slightly opalescent.

Performance Response

When reconstituted with 5 ml sterile distilled or deionized water, 0.1 ml Phytohemagglutinin M or 0.01 ml Phytohemagglutinin P is added to 7 ml RPMI #1640 Medium containing the lymphocytes from 5 ml heparinized human blood. The mitogenicity test is performed using the above components and procedures with 4 samples of human blood. A mitotic index of at least 75 should be obtained from the lymphocytes of each of the four samples of blood. A total of at least 400 should be obtained from the sum of all four cultures.

Precautions

1. For Laboratory Use.
2. Observe universal blood and body fluid precautions in the handling and disposing of specimens.[18,19]
3. Practice the following routine laboratory safety procedures:

   Do not pipette by mouth.

   Use aseptic technique and established laboratory procedures in handling and disposing of infectious materials.

Storage

Store desiccated Phytohemagglutinin M and Phytohemagglutinin P at 2-8°C. The rehydrated solutions are stable for at least 2 weeks at -20°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Phytohemagglutinin M
Phytohemagglutinin P

Materials Required But Not Provided

Sterile syringe
Sterile test tube
30 units sterile heparin dissolved in 0.85% sterile saline
RPMI Medium #1640
700 units of Penicillin
700 µg Streptomycin
Colchicine (10^{-5} Molar)
Hanks Balanced Salt Solution
Methanol, reagent grade
Glacial Acetic Acid, reagent grade
Deionized water
Giemsa Stain
Pipettes, 0.1 ml, 1 ml, 5 ml
Water aspirator
Pasteur pipettes
Centrifuge
Incubator, 35°C
Microscope slides
Microscope (12.5X eyepiece with 10X low power, 40X high dry, and 100X oil immersion objectives)

Reagent Preparation

Phytohemagglutinin M or the sterile Phytohemagglutinin P is rehydrated by adding 5 ml of sterile distilled or deionized water, or equivalent, and rotating gently to mix contents thoroughly. The solutions are approximately 1% in 0.85% saline. Both solutions contain approximately 50 mg protein per 5 ml.

Specimen Collection and Preparation

For each culture, a 5 ml sample of blood is adequate. Draw the blood with a sterile syringe and immediately place in a sterile screw-capped test tube containing 30 units of sterile heparin and mix thoroughly. Dissolve the heparin in 1 ml of a sterile 0.85% saline solution before collecting the specimen. Start the agglutination and mitotic procedures immediately, or they may be postponed for at least 24 hours, if the specimen is stored at 2-8°C.

Observe aseptic technique from the collection of the blood sample until the addition of the colchicine.

Test Procedure

Lymphocyte Separation and Inoculation

1. Transfer 5 ml of blood containing 30 units of heparin to a sterile screw-capped test tube under aseptic conditions.
2. Add either 0.1 ml of rehydrated Phytohemagglutinin M or 0.0025 ml of Phytohemagglutinin P to the 5 ml of heparinized blood, and mix the contents by inverting several times.
3. Let the erythrocytes agglutinate at 25°C for 15-30 minutes.
4. Centrifuge the tube at 500 rpm for 2 minutes. Excessive centrifuging must be avoided to prevent sedimentation of the lymphocytes.
5. Transfer the hazy plasma-lymphocyte suspension (about 2 ml) by means of a sterile Pasteur pipette to 7 ml of a culture medium consisting of RPMI #1640 Medium, 700 units of Penicillin, 700 µg Streptomycin, and either 0.1 ml of Phytohemagglutinin M, if the erythrocytes have been agglutinated with the M-form, or 0.01 ml of Phytohemagglutinin P, if the erythrocytes have been agglutinated with the P-form. The optimal concentration of lymphocytes in the culture is 1.0-1.2 X 10^6 per ml. If Phytohemagglutinin P and aseptic conditions are used, the antibiotics may be omitted.

Incubation of Culture

6. Incubate the culture in a vertical position at 35 ± 2°C with occasional swirling for 3-4 days. Care should be taken to maintain proper incubation temperature. A significant increase in mitotic index is often obtained by incubating 4 days instead of 3. It is very important to always maintain the proper pH range in the culture. The phenol red indicator should not become more acidic than a light amber nor more alkaline than a light pink. If the indicator becomes amber, loosen the cap for an hour or so to allow the escape of CO_2. This precaution is often most necessary at the beginning and end of the incubation.
7. End the mitosis by the addition of 1 ml of $10^{\S5}$ molar colchicine, and continue the incubation at 35 ± 2°C for another 4-6 hours. The exposure of cells to the colchicine should not be less than 4 hours or more than 6 hours.

Harvesting and Fixation of Cells

8. Transfer the entire culture to a graduated conical centrifuge tube (15 ml) and centrifuge for 6-8 minutes at 600-800 rpm.
9. Carefully aspirate off the supernatant fluid.
10. Add 5 ml of warm (35 ± 2°C) Hanks Balanced Salt Solution and resuspend the cells in the centrifuge tube with a Pasteur pipette.
11. Centrifuge at 600-800 rpm for 6-8 minutes.
12. Carefully aspirate off the supernatant with the pipette and add 1 ml of Hanks Balanced Salt Solution.
13. Resuspend the packed cells with the Pasteur pipette.
14. Add 3 ml of warm (35 ± 2°C) distilled water, in 1 ml portions, with momentary agitation after each addition to produce a hypotonic solution.
15. Incubate the suspension at 35 ± 2°C for 10 minutes only. The exposure of the cells to this hypotonic, diluted Hanks Balanced Salt Solution should not exceed 10 minutes.
16. Centrifuge the lymphocyte solution at 600-800 rpm for 6-8 minutes.
17. Carefully aspirate off the supernatant.
18. Add slowly, without disturbing the button of cells, 4 ml of freshly prepared fixative consisting of 1 part glacial acetic acid and 3 parts methanol (reagent grade only).
19. Let the cells soak in the fixative for 15-30 minutes. Cells should be treated gently during this stage of fixation. At this point, cells may be stored overnight at 2-8°C.
20. Resuspend with the Pasteur pipette.
21. Centrifuge at 600-800 rpm for 6-8 minutes, and carefully remove the supernatant by aspiration.
22. Resuspend the cells in 4 ml fresh fixative with the Pasteur pipette, and centrifuge at 600-800 rpm for 6-8 minutes. Repeat this step again if necessary to disperse clumps of cells.
23. Carefully aspirate the supernatant.
24. Add 0.5-1.0 ml of fresh fixative to the button of cells and resuspend with the Pasteur pipette to get a hazy suspension.

Preparation of Slides

25. Label clean microscope slides and place them in clean, chilled distilled water.
26. In rapid succession, shake the excess water off a chilled slide, wipe the water off its underside, add 3-4 drops of the cell suspension by means of the Pasteur pipette, tip the slide several times to spread the suspension, and ignite the fixative by bringing it momentarily in contact with a flame. When the fixative is burned off, wave the slide vigorously to hasten drying. The slide should not get hot, but drying should be accomplished as rapidly as possible.

Staining of Slides

Slides may be stained with Giemsa, orcein or other stains according to the method of Rothfels and Siminovitch.[14] The procedure using Giemsa is given below.

27. Dilute the 1 ml of stock Giemsa Stain (20X stock) with 19 ml of distilled water. The 1 ml of stock Giemsa Stain should be used the same day it is diluted 20-fold with water.
28. Place the slides in a small staining dish or Petri dish and cover them with 20 ml of the staining solution for 10-20 minutes.
29. Rinse the slides gently in distilled water and air dry.
30. Examine the slides under the microscope. The mitotic spreads may be scanned at a total magnification of 125X, examined more closely at 500X, or photographed under oil immersion at 1,000X. Slides may be protected by cover slips and made permanent by conventional procedures.

Alternatively, the chromosomes may be treated by staining procedures to show G-banding. Refer to appropriate references for alternative staining procedures.[18]

Results

A mitotic index of at least 30 may be expected from the lymphocytes from the heparinized peripheral blood of a healthy individual.

Limitations of the Procedure

1. For mitotic investigations, avoid the following:
   - Anticoagulants containing oxalates or phenols
   - Cytotoxic antibiotics, drugs or heavy metals (Penicillin and Streptomycin are acceptable.)
   - Hypertonic and hypotonic media except for the intentional swelling of the chromosomes
   - Irradiation of the patient or culture, which can produce "breaks" in the chromosomes.
   - Some plastic materials cause cytotoxic effects.

References

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2. **Takikawa, K., T. Ito, J. Kato, T. Yoshida, H. Kondo, and I. Miyata.** 1957. Studies on the isolation of granules and mitrochondria of leukocytes. Acta Haemat. **18:**179-184.
3. **Chen, H. P., and G. K. Palmer.** 1958. A method for isolating leukocytes. Am. J. Clin. Pathol. **30:**567-569.
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13. **Rothfels, K. H., and L. Siminovitch.** 1958. An air-drying technique for flattening chromosomes in mammalian cells grown in vitro. Stain Tech. **33:**73-77.
14. **Bird, B. R., and F. T. Forrester.** 1981. Basic Laboratory Techniques in Cell Culture. U. S. Department of Health and Human Services, CDC, Atlanta, GA.
15. **Freshney, R. I.** 1983. Culture of animal cells: A manual of basic technique. Alan R. Liss, Inc., New York, NY.
16. **Jones Brando, L. V.** 1995. Cell culture systems, p. 158-165. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (eds.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
17. **Gustashaw, K. M.** 1991. Chromosome stains. *In* M. J. Barch (ed.), The ACT Cytogenetics Laboratory Manual, 2nd ed. The Association of Cytogenetic Technologists, Raven Press, Ltd., New York, NY.
18. **Centers for Disease Control.** 1988. Update: universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus, and other blood borne pathogens in health-care settings. Morbidity and Mortality Weekly Reports **37:**377-382, 387-388.
19. **Occupational Safety and Health Administration, U.S. Department of Labor.** 1991. 29 CFR part 1910. Occupational exposure to blood borne pathogens; final rule. Federal Register **56:**64175-64182.

Packaging

Phytohemagglutinin M	5 ml	0528-56*
	6 x 5 ml	0528-57*
Phytohemagglutinin P	5 ml	3110-56*
	6 x 5 ml	3110-57*

*Store at 2-8°C

Bacto® Plate Count Agar
Bacto Standard Methods Agar

Intended Use

Bacto Plate Count Agar is a Standard Methods medium used for enumerating aerobic bacteria in water, wastewater, foods and dairy products.[1,2,3,4,5] This medium is also recommended as a general plating medium for determining bacterial populations.

Also Known as

Standard Methods Agar and Tryptone Glucose Yeast Agar are alternate names for Plate Count Agar.

Summary And Explanation

Plate Count Agar was developed by Buchbinder, Baris and Goldstein[6] in 1953 at the request of the American Public Health Association. Results showed that a dehydrated milk-free medium containing 0.25% Yeast Extract, 0.5% Tryptone, 0.1% Dextrose and 1.5% Agar per liter approximated the productivity of Tryptone Glucose Extract Agar with added milk. Buchbinder *et al*. recommended that a dehydrated culture medium be used in preparing the standard plate count medium rather than preparing the medium from ingredients. Bacto Plate Count Agar is prepared with the same ingredients originally suggested by Buchbinder *et al.*[7] Combinations of Yeast Extract and Tryptone have been used in media for the examination of dairy products for the presence of thermophilic organisms since 1928.[8,9] This formula is specified in *Standard Methods for the Examination of Water and Wastewater*,[1]

Standard Methods for the Examination of Dairy Products,[2] *Compendium of Methods for the Microbiological Examination of Foods*[3] and the *Association of Official Analytical Chemists (AOAC)*[4] and the *FDA Bacteriological Analytical Manual*.[5]

Principles of the Procedure

Plate Count Agar contains Tryptone and Yeast Extract which provide the carbon and nitrogen sources required for growth of a wide variety of organisms. Dextrose is a source of fermentable carbohydrate (energy source). Bacto Agar is a solidifying agent.

Formula

Plate Count Agar
Standard Methods Agar

Formula Per Liter

Bacto Tryptone	5 g
Bacto Yeast Extract	2.5 g
Bacto Dextrose (Glucose)	1 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store Plate Count Agar below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Store Standard Methods Agar at 15-30°C.

Expiration Date

The expiration date applies to the product in its intact container product when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Plate Count Agar
Standard Methods Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Waterbath (optional)

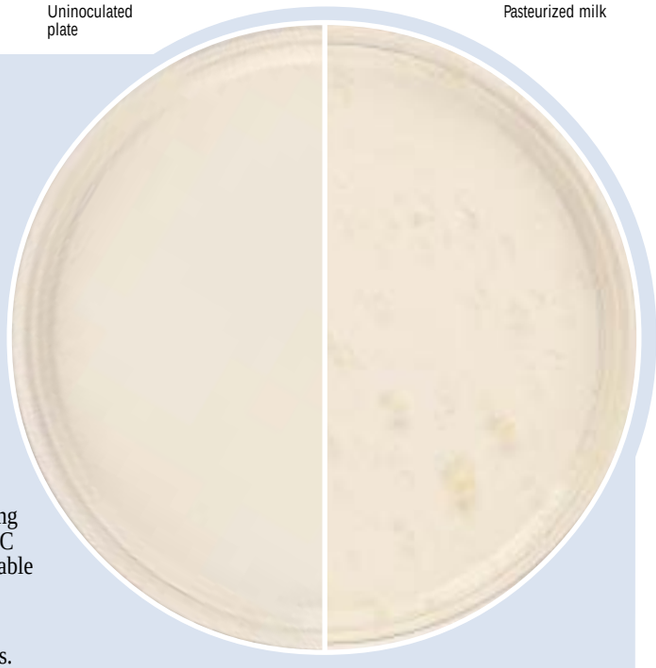
Method of Preparation

Plate Count Agar

1. Suspend 23.5 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Standard Methods Agar (prepared)

1. Loosen the caps on the bottles prior to heating.
2. Heat the medium in the autoclave for 7 minutes to melt the agar. A small solidified mass remains that can be melted by swirling the hot agar. Cycle time depends on the number of bottles in the chamber.



User Quality Control

Identity Specifications

Plate Count Agar

Dehydrated Medium:	Light beige, homogeneous, free-flowing.
Solution:	2.35% solution, soluble in distilled or deionized water on boiling; light amber, slightly opalescent, no precipitate.
Prepared Medium:	Light amber, slightly opalescent, no precipitate.
Reaction of 2.35% Solution at 25°C:	7.0 ± 0.2

Cultural Response

Plate Count Agar (dehydrated)
Prepare Plate Count Agar per label directions. Inoculate with serial dilutions (30-300 CFU/ml) of pasteurized and raw milk samples using the pour plate method (standard plate count) and incubate at 32 ± 1°C for 48 hours. Statistical analysis of data should yield counts comparable to an approved lot of medium.

Standard Methods Agar (prepared)
Melt Standard Methods Agar and aseptically dispense into Petri dishes. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus acidophilus	11506	30-300	good
Staphylococcus aureus	25923	30-300	good

The cultures listed are the minimum that should be used for performance testing.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

1. Perform serial dilutions on samples (food, water) to be tested using the heterotrophic (standard) plate count method. Select dilutions that will yield plates with counts of 30-300 colonies.
2. Dispense a portion of each test dilution (e.g., 0.1 ml, 1.0 ml) into separate sterile Petri dishes.
3. Add 10-12 ml of tempered (45°C) Plate Count Agar to Petri dishes containing test dilutions.
4. Swirl the dishes to thoroughly mix the agar and test dilution.
5. Allow plates to cool and solidify.
6. Incubate at 32 ± 1°C for 48 hours.

Results

Count colonies on all plates containing 30-300 colonies. Calculate bacterial count per milliliter of sample by multiplying the average number of colonies per plate by the reciprocal of the dilution used. Report the count as CFU/ml.

References

1. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (ed.).** 1992. Standard methods for the examination of water and wastewater, 18th ed. American Public Health Association, Washington, D.C.
2. **Marshall, R. T. (ed.).**1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3 **Vanderzant, C., and D. F. Splittstoesser (ed.).**1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **Association of Official Agricultural Chemists.** 1995. Official methods of analysis, 16th ed. Association of Official Agricultural Chemists, Washington, D.C.
5. **Bandler, R., M. E. Stack, H. A. Koch, V. H. Tournas, and P. B. Mislivec.** 1995. Yeasts, molds, and mycotoxins, p. 18.01-18.03. Bacteriological analytical manual, 8th ed. AOAC International, Arlington VA.
6. **Buchbinder, L., Y. Baris, and L. Goldstein.** 1953. Further studies on new milk-free media for the standard plate count of dairy products. Am. J. Public Health **43**:869- 872.
7. **Buchbinder, L., Y. Baris, E. Alff, E. Reynolds, E. Dillon, V. Pessin, L. Pincus, and A. Strauss.**1951. Studies to formulate new media for the standard plate count of dairy products. Pub Health Rep. **66**:327-340.
8. **Prickett, P. S.** 1928. Thermophillic and thermoduric microorganisms with special reference to species isolated from milk: V. Description of spore-forming types. Technical Bulletin. NY State Agri. Exp. Station **147**:5-58.
9. **Breed, R. S., P. A. Down, G. C. Supplee, P. S. Prickett, and G. J. Hucker.** 1932. Methods for use in the bacteriological examination of dry milk and related powders. J. Dairy Sci. **15**:383-389.

Packaging

Plate Count Agar	100 g	0479-15
	500 g	0479-17
	2 kg	0479-07
	10 kg	0479-08
Standard Methods Agar	10 x 500 ml	9081-80

Bacto® m Plate Count Broth

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige to beige, free flowing homogeneous.
Solution:	1.7% solution, soluble in distilled or deionized water; light to medium amber, clear to slightly opalescent, may have a very slight precipitate.
Reaction of 1.7% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare m Plate Count Broth per label directions. Inoculate and incubate the plates at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Escherichia coli	25922*	20-80	good to excellent
Staphylococcus aureus	25923*	20-80	good to excellent

The above cultures are the minimum used for performance testing.

*These organisms are available as Bactrol™ Disks and are to be used as directed in the Bactrol Disks Technical Information.

Intended Use

Bacto m Plate Count Broth is used for enumerating microorganisms by membrane filtration.

Also Known As

m Plate Count Broth is also referred to as m TGY Broth, m Tryptone Glucose Yeast Broth, or m Standard Methods Broth.

Summary and Explanation

m Plate Count Broth is a nonselective general-purpose medium for determining bacterial counts from food and water samples using the membrane filtration procedure. This medium has the same formulation as Plate Count Agar except that agar has been omitted and the ingredients are employed in twice the concentration as in the solid medium.[1]

Principles of the Procedure

Yeast Extract is a source of trace elements, vitamins and amino acids. Tryptone provides carbon and nitrogen for bacterial metabolism. Dextrose is a fermentable carbohydrate and carbon source.

Formula

m Plate Count Broth

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Tryptone	10 g
Bacto Dextrose	2 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

m Plate Count Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35 ± 2°C)
Pipettes
Sterile Petri dishes, 50 x 9 mm
Membrane filter equipment
Sterile 47 mm, 0.45 µm, gridded membrane filters
Sterile absorbent pads

Method of Preparation

1. Dissolve 17 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Water samples should be collected and prepared according to recommended guidelines.[2,3,4]

Test Procedure

1. Place a sterile absorbent pad in each 50 x 9 mm Petri dish.
2. Saturate the pad with approximately 2.0-2.4 ml of prepared medium.
3. Place an inoculated membrane filter, inoculated side up, on the saturated pad.
4. Incubate in a 35 ± 2°C incubator for 18-24 hours.

Results

After incubation, count the colonies on the surface of the filter. The colonies can be subcultured to appropriate media for identification, if desired.

References

1. **MacFadden, J. F.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria. vol. 1. Williams & Wilkens, Baltimore, MD.
2. **Eaton, A. D., L. S. Cleseri, and A. E. Greenberg (ed.).** 1995 Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
3. **Hitchins, A. D.** 1992. FDA Bacteriological Analytical Manual, 7th ed. AOAC International, Arlington, VA.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.)**. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

m Plate Count Broth	100 g	0751-15
	500 g	0751-17

Bacto® Potato Dextrose Agar
Bacto Potato Dextrose Broth

Intended Use

Bacto Potato Dextrose Agar is used for culturing yeasts and molds from food and dairy products. Bacto Potato Dextrose Broth is used for cultivating yeasts and molds.

Summary and Explanation

Potato Dextrose Agar is a general purpose medium for yeasts and molds that can be supplemented with acid or antibiotics to inhibit bacterial growth. It is recommended for plate count methods for foods, dairy products[1,2,3,4] and for testing cosmetics.[3] It can be used for growing clinically significant yeasts and molds.[5] The nutritionally rich base (potato infusion) encourages mold sporulation and pigment production in some dermatophytes.[6]

Potato Dextrose Broth is a general purpose broth medium for yeasts and molds formulated as is Potato Dextrose Agar, but without agar.

Principles of the Procedure

Potato Dextrose Agar and Potato Dextrose Broth contain an infusion from potatoes and Dextrose which encourage luxuriant fungal growth. Bacto Agar is added to Potato Dextrose Agar as the solidifying agent.

Many standard procedures call for lowering the pH of Potato Dextrose Agar to 3.5 ± 0.1 to inhibit bacterial growth. The label on each container of the medium specifies the amount of sterile tartaric acid (10%) to add to the sterile medium. Do not reheat the acidified medium because heating in the acid state will hydrolyze the agar.

Formula

Potato Dextrose Agar

Formula per liter

Potatoes, Infusion from	200 g
Bacto Dextrose	20 g
Bacto Agar	15 g

Final pH 5.6 ± 0.2 at 25°C

Potato Dextrose Broth

Formula per liter

Potatoes, Infusion from	200 g
Bacto Dextrose	20 g

Final pH 5.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Potato Dextrose Agar or Potato Dextrose Broth

Materials Required but not Provided

Flask with closure
Distilled or deionized water
Autoclave
Sterile tartaric acid, 10% solution (optional)

Method of Preparation

Potato Dextrose Agar

1. Suspend 39 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. To alter the pH of the medium to 3.5 ± 0.1, add the amount of sterile 10% tartaric acid specified on the label. Do not reheat the medium after adding the acid.

Potato Dextrose Broth

1. Suspend 24 grams in 1 liter distilled or deionized water and warm slightly to dissolve completely.
2. Autoclave at 121°C for 15 minutes.

User Quality Control

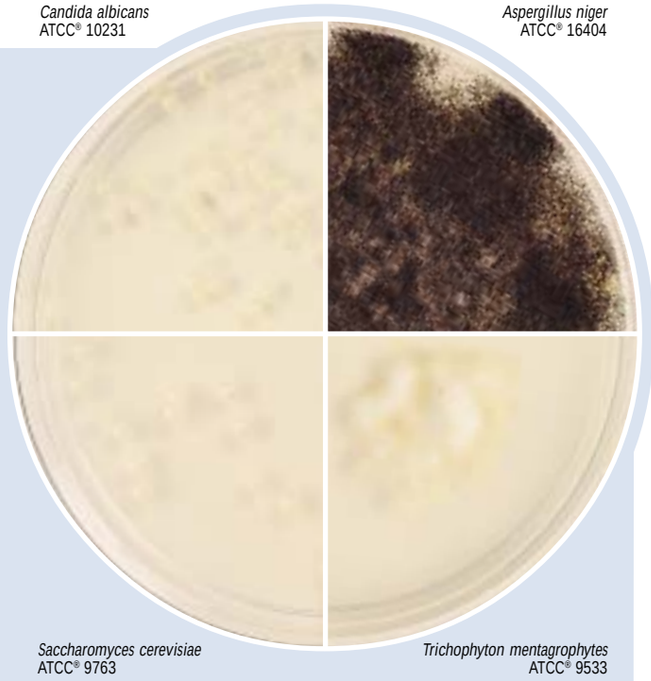
Identity Specifications

Potato Dextrose Agar

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	3.9% solution, soluble in distilled or deionized water on boiling. Solution is light amber, very slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent.
Reaction of 3.9% Solution at 25°C:	pH 5.6 ± 0.2

Potato Dextrose Broth

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	2.4% solution, soluble in distilled or deionized water upon slight warming; very light amber, clear.
Prepared Medium:	Very light amber, clear.
Reaction of 2.4% Solution at 25°C:	pH 5.1 ± 0.2



Cultural Response

Potato Dextrose Agar

Prepare Potato Dextrose Agar per label directions. Inoculate with test organisms. Incubate plates at 30 ± 2°C for up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Aspergillus niger	16404	100-1000	good
Candida albicans	10231	100-1000	good
Saccharomyces cerevisiae	9763	100-1000	good
Trichophyton mentagrophytes	9533	undiluted	good

Potato Dextrose Broth

Prepare Potato Dextrose Broth per label directions. Inoculate medium and incubate at 30 ± 2°C for 48 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Aspergillus niger	16404	100-1000	good
Candida albicans	10231	100-1000	good
Saccharomyces cerevisiae	9763	100-1000	good

The cultures listed are the minimum that should be used for performance testing.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Potato Dextrose Agar

Pour plate method[1,3]

1. Add 1 ml of test sample to a sterile Petri dish.
2. Add the specified amount (10 or 20 ml) of sterile, molten agar (cooled to 45- 50°C) and swirl gently to mix well. Allow to solidify.
3. Incubate at 22-25°C or 30-32°C (depending on the method being followed) for 5 days or longer.

Potato Dextrose Broth

For complete information, refer to Standard Methods procedures in the **References** section.

Results

Potato Dextrose Agar

Yeasts will grow as creamy to white colonies. Molds will grow as fuzzy colonies of various colors. Count the number of colonies and consider the dilution factor (if the test sample was diluted) in determining the yeast and/or mold counts per gram or milliliter of material.

Potato Dextrose Broth

Growth is indicated as turbidity.

Limitations of the Procedure

1. Heating Potato Dextrose Agar after acidifying hydrolyzes the agar and may destroy the solidifying properties.
2. Potato Dextrose Agar is not a differential medium. Perform microscopic examination and biochemical tests to identify isolates to genus and species if necessary.

References

1. **Vanderzant, C., and D. F. Splittstoesser (ed.)**. 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
2. **Frank, J. F., G. L. Christen, and L. B. Bullerman (G. H. Richardson, Tech. Comm.)** 1993. Tests for groups of microorganisms. p. 271-286. *In* **Marshall, R.T. (ed.).** Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

3 **Association of Official Analytical Chemists**. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

4. **United States Pharmacopeial Convention**. 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.
5. **Dixon, D. M., and R. A. Fromtling.** 1995. Morphology, taxonomy, and classification of the fungi, p. 699-708. *In* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol 1. Williams & Wilkins, Baltimore, MD.

Packaging

Potato Dextrose Agar	100 g	0013-15
	500 g	0013-17
	2 kg	0013-07
Potato Dextrose Broth	500 g	0549-17
	10 kg	0549-08

Bacto® Potato Infusion Agar

User Quality Control

Identity Specifications

Dehydrated Appearance:	Medium tan, free-flowing, homogeneous.
Solution:	4.9% solution, soluble in 2% glycerol solution upon boiling. Medium amber, slightly opalescent, with a slight precipitate.
Prepared Medium:	Medium amber, slightly opalescent to opalescent with a slight precipitate.
Reaction of 4.9% Solution at 25°C:	pH 6.8 ± 0.2°C

Cultural Response

Prepare Potato Infusion Agar per label directions. Inoculate prepared medium and incubate at 35 ± 2°C under approximately 5-10% CO_2 for up to 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Brucella abortus	4315	100-1,000	good
Brucella melitensis	4309	100-1,000	good
Brucella suis	4314	100-1,000	good

Intended Use

Bacto Potato Infusion Agar is used for cultivating *Brucella*, especially in mass cultivation procedures.

Summary and Explanation

Potato Infusion Agar is prepared according to the formula used by Stockman and MacFadyean for the isolation of *Brucella abortus*. Brucellosis is a zoonotic disease with a domestic-animal reservoir.[1] Transmission by milk, milk products, meat and direct contact with infected animals is the usual route of exposure.[1]

Tryptose agar w/ 5% bovine serum, with or without antibiotics, remains a standard plating medium for the isolation of brucellae.[1] Most strains of *Brucella* spp. will grow on chocolate and blood agar, and the addition of 5% heated horse or rabbit serum enhances growth on all media.[2] Potato Infusion Agar permits luxuriant growth of characteristic colonies of *B. abortus* from infected materials, and may be used with excellent results in mass cultivation of *Brucella* in the preparations of vaccines and antigens.

Principles of the Procedure

Infusion from potatoes, Beef Extract and Proteose Peptone provide the nitrogen, vitamins and amino acids in Potato Infusion Agar. Dextrose and Glycerol are used as a carbon source in this formula.

Sodium chloride maintains the osmotic balance of the medium. Bacto Agar is the solidifying agent.

Formula

Potato Infusion Agar

Formula Per Liter

Potatoes, Infusion from	200 g
Bacto Beef Extract	5 g
Bacto Proteose Peptone	10 g
Bacto Dextrose	10 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. *Brucella* spp. are classified as Biosafety Level 3 pathogens. All manipulations with live cultures and antigens must be confined to a Class II biological safety cabinet (BSC).[1]

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Material Provided

Potato Infusion Agar

Material Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Sterile Petri dishes

Method of Preparation

1. Suspend 49 grams in 1 liter distilled or deionized water containing 2% Glycerol.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense into sterile Petri dishes or as desired.

Specimen Collection and Preparation

Specimens should be collected in sterile containers or with sterile swabs and transported immediately to the laboratory in accordance with recommended guidelines.

Test Procedure

1. Incubate plates at 35 ± 2°C in 5-10% CO_2 for 10 days.[1] For a complete discussion on the inoculation and identification of *Brucella* spp., consult appropriate references.

Results

Refer to appropriate references and procedures for results.

Limitations

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Best results are obtained on freshly prepared medium with a moist surface.

References

1. **Moyer, N. P., and L. A. Holcomb.** 1995. Brucella, p. 549-555. *In* Murray, P.R., E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Baron, E. J., L. R. Peterson** and **S. M. Finegold.** 1994. Bailey & Scott's Diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Potato Infusion Agar	500 g	0051-17

Bacto® Presence-Absence Broth

Intended Use

Bacto Presence-Absence Broth is used for detecting coliforms in treated water.

Also Known As

Presence-Absence Broth is abbreviated as P-A Broth.

Summary and Explanation

The Presence-Absence (P-A) test is a presumptive detection test for coliforms in water. The test is a simple modification of the multiple-tube procedure.[1] One test sample, 100 ml, is inoculated into a single culture bottle to obtain qualitative information on the presence or absence of coliforms based on the presence or absence of lactose fermentation.[1] This test is based on the principle that coliforms and other pollution indicator organisms should not be present in a 100 ml water sample.[2-8]

Comparative studies with the membrane filter procedure indicate that the P-A test may maximize coliform detection in samples containing many organisms that could overgrow coliform colonies and cause problems in detection.[1] The P-A test is described in standard methods for water testing[1] and by US EPA.[9]

Principles of the Procedure

Beef Extract, Peptone and Tryptose provides the nitrogen, vitamins and amino acids in Presence-Absence Broth. Lactose is the carbon

source in the formula. The Potassium Phosphates provide buffering capacity; Sodium Chloride maintains the osmotic balance of the medium. Sodium Lauryl Sulfate is the selective agent, inhibiting many organisms except coliforms. Brom Cresol Purple is used as an indicator dye; lactose-fermenting organisms turn the medium from purple to yellow with or without gas production.

Formula

Presence-Absence Broth (single-strength)

Formula Per Liter	
Bacto Beef Extract	3 g
Bacto Peptone	5 g
Bacto Lactose	7.46 g
Bacto Tryptose	9.83 g
Potassium Phosphate, Dibasic	1.35 g
Potassium Phosphate, Monobasic	1.35 g
Sodium Chloride	2.46 g
Sodium Lauryl Sulfate	0.05 g
Brom Cresol Purple	0.0085 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Presence-Absence Broth

Materials Required But Not Provided

Glassware
Screw-cap dilution bottle with capacity > 150 ml
Incubator (35°C)

Method of Preparation

1. To prepare triple-strength medium, suspend 91.5 grams in 1 liter distilled or deionized water.
2. Warm gently to dissolve completely.
3. Dispense 50 ml amount into screw-cap 250 ml milk dilution bottles.
4. Autoclave at 121°C for 12 minutes, with the total autoclave time not to exceed 30 minutes.
5. Cool to room temperature.

Specimen Collection and Preparation

Collect water samples as described in recommended procedures.[1,9]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.05% solution, soluble in distilled or deionized water; purple, clear to very slightly opalescent without significant precipitate.
Prepared Medium:	Purple, clear to very slightly opalescent without significant precipitate.
Reaction of 3.05% Solution at 25°C:	pH 6.8 ± 0.2

Presence-Absence Broth

Cultural Response

Prepare Presence-Absence Broth in triple strength solution (9.15%). Sterilize in 50 ml quantities in milk dilution bottles with capacity greater than 150 ml. Add 100 ml of drinking water after medium is sterilized and cooled to room temperature. Inoculate bottles with the test organisms. Incubate bottles for 18-48 hours at 35°C.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	RESULTS
Enterococcus faecalis	29212*	100-1,000	moderate	slight yellow to purple
Escherichia coli	25922*	100-1,000	good	yellow color w/ or w/o gas production
Escherichia coli	13762	100-1,000	good	yellow color w/ or w/o gas production
Pseudomonas aeruginosa	27853*	100-1,000	poor to moderate	no color change

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Test Procedure

1. Inoculate 50 ml of the sterile triple strength P-A Broth with 100 ml of the water sample.
2. Invert the bottle a few times to achieve an even distribution of the medium throughout the test sample.
3. Incubate at 35 ± 0.5°C.
4. Inspect for acid and gas production after 24 and 48 hours of incubation.

Results

A distinct yellow color indicates lactose fermentation, an acid reaction. Gas production can be observed by a foaming reaction when the bottle is gently shaken. Any amount of gas and/or acid is a positive presumptive test requiring confirmation.[1] Report results as positive or negative for coliforms per 100 ml of sample.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. The P-A test is only a presumptive test for coliforms.
3. Confirmation and differentiation of coliforms detected by the P-A test may be achieved by use of appropriate confirmatory media, incubation times and temperatures as outlined in appropriate references.[1,9]
4. Extending the P-A test incubation period to 72 or 96 hours will allow isolation of other indicator organisms. However, indicator bacteria isolated after 48 hours incubation may not be considered for regulatory purposes.

References

1. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
2. **Weiss, J. E., and C. A. Hunter.** 1939. Simplified bacteriological examination of water. J. Am. Water Works Assoc. **31**:707-713.
3. **Clark, J. A.** 1968. A presence absence (P-A) test providing sensitive and inexpensive detection of coliforms, fecal coliforms, and fecal streptococci in municipal drinking water supplies. Can. J. Microbiol. **14**:13-18.
4. **Clark, J. A.** 1969. The detection of various bacteria indicative of water pollution by a presence-absence (P-A) procedure. Can. J. Microbiol. **15**:771-780.
5. **Clark, J. A., and L. T. Vlassoff.** 1973. Relationships among pollution indicator bacteria isolated from raw water and distribution systems by the presence-absence (P-A) test. Health Lab. Sci. **10**:163-172.
6. **Clark, J. A., and J. E. Pagel.** 1977. Pollution indicator bacteria associated with municipal raw and drinking water supplies. Can. J. Microbiol. **23**:465-470.
7. **Clark, J. A.** 1980. The influence of increasing numbers of nonindicator organisms upon the detection of indicator organisms by the membrane filter and presence-absence tests. Can. J. Microbiol. **26**:827-832.
8. **Clark, J. A., C. A. Burger, and L. E. Sabatinos.** 1982. Characterization of indicator bacteria in municipal raw water, drinking water and new main water samples. Can J. Microbiol. **28**:1002-1013.
9. **Federal Register.** 1989. National primary drinking water regulations; total coliforms (including fecal coliforms and *E. coli*). Fed regist. **54**:27544-27568.

Packaging

Presence-Absence Broth	500 g	0019-17
	2 kg	0019-07

Bacto® Proteose No. 3 Agar

Intended Use

Bacto Proteose No. 3 Agar is used with added enrichment in isolating and cultivating *Neisseria* and *Haemophilus.*

Summary and Explanation

Proteose No. 3 Agar, introduced in 1938, is used for isolating *Neisseria gonorrhoeae.* When enriched with Hemoglobin and Supplement B,[2,3] Proteose No. 3 Agar recovers gonococci in a manner comparable to more complex media, ranking only slightly lower than GC Medium at 24 hours.

Chocolate agar may be prepared from Proteose No. 3 Agar with the addition of 2% Hemoglobin. Hemoglobin provides X factor (hemin), required for growth of *Haemophilus* and enhanced growth of *Neisseria*.

The growth rate of *Neisseria* and *Haemophilus* spp. may be improved with the addition of 1% Supplement B or VX, which provide the growth factors glutamine and cocarboxylase.

Principles of the Procedure

Proteose Peptone No. 3 provides nitrogen, vitamins and amino acids. Dextrose is a carbon source. Sodium Chloride maintains the osmotic balance in the medium, which is buffered by Disodium Phosphate. Bacto Agar is the solidifying agent.

Proteose Peptone No. 3 Agar is intended for use with supplementation by 2% hemoglobin and Supplement B or Supplement VX.

Formula

Proteose No. 3 Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	20 g
Bacto Dextrose	0.5 g
Sodium Chloride	5 g
Disodium Phosphate	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.

2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provide

Proteose No. 3 Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Hemoglobin (2%)
Supplement B or Supplement VX
Sterile Petri dishes

Method of Preparation

1. Suspend 45 grams in 500 ml liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50-60°C.
4. Aseptically add 500 ml sterile 2% Hemoglobin solution. Mix well.
5. Add 10 ml of Supplement B or Supplement VX. Mix thoroughly.
6. Dispense into sterile Petri dishes or as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion of the isolation and identification of *Haemophilus* or *Neisseria* spp., refer to the procedures outlined in the references.[4,5,6]

Results

Refer to appropriate references and procedures for results.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	9% (double strength) solution, soluble in distilled or deionized water upon boiling with frequent agitation. Light to medium amber in color, opalescent with a slight flocculent precipitate.
Prepared Medium (Single-strength):	Light amber, opalescent with a slight flocculent precipitate, firmly solid.
Reaction of 9% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Proteose Agar No. 3 per label directions. Inoculate and incubate at 35 ± 2°C under approximately 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Haemophilus influenzae	10211	100-1,000	good
Neisseria gonorrhoeae	43070	100-1,000	good
Neisseria meningitidis	13102	100-1,000	good
Neisseria sicca	9913*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium
2. Proteose No. 3 Agar is intended for use with supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.

References

1. **Carpenter, C. M., M. A. Bucca, T. C. Buck, E. P. Casman, C. W. Christensen, E. Crowe, R. Drew, J. Hill, C. E. Lankford, H. E. Morton, L. R. Peizer, C. S. Shaw, and J. D. Thayer.** 1949. Evaluation of twelve media for the isolation of the gonococcus. Am. J. Syphil. Gonorrh. Vener. Dis. **33**:164
2. **Lankford, C. E., V. Scott, M. F. Cox, and W. R. Cooke.** 1943. Some aspects of nutritional variation of the gonococcus. J. Bacteriol. **45**:321.
3. **Lankford, C. E., and E. E. Snell.** 1943. Glutamine as growth factor for certain strains of *Neisseria gonorrhoeae*. J. Bacteriol. **45**:410.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol.1. American Society for Microbiology, Washington, D.C.
5. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **Baron, E. J., L. R. Petersons, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Proteose No. 3 Agar	500 g	0065-17
Hemoglobin 2% Solution	6 x 100 ml	3248-73
Supplement B w/Reconstituting Fluid	6 x 10 ml	0276-60
	100 ml	0276-72
Supplement VX w/Reconstituting Fluid	6 x 10 ml	3354-60
	100 ml	3354-72

Proteose Peptones

Bacto® Proteose Peptone · Bacto Proteose Peptone No. 2 Bacto Proteose Peptone No. 3

Intended Use

Bacto Proteose Peptone is used in preparing microbiological culture media and in producing bacterial toxins.

Bacto Proteose Peptone No. 2 is used in preparing microbiological culture media.

Bacto Proteose Peptone No. 3 is used in preparing microbiological culture media.

User Quality Control

Identity Specifications

Proteose Peptone

Dehydrated Appearance:	Tan, free-flowing granules.
Solution:	1%, 2% and 10% solutions are soluble in distilled or deionized water: 1%-Light amber, clear to very slightly opalescent, may have a slight precipitate; 2%-Light to medium amber, clear to slightly opalescent, may have a slight precipitate; 10%-Medium to dark amber, clear to slightly opalescent, may have a slight precipitate.
Nitrogen (Kjeldahl Method):	12.4-14.5%
Amino Nitrogen (Modified Sorensen Method):	2.0-3.75%
Reaction of 1% Solution at 25°C:	pH 6.6-7.6

Proteose Peptone No. 2

Dehydrated Appearance:	Tan, free-flowing granules.
Solution:	1%, 2% and 10% solutions are soluble in distilled or deionized water: 1%-Light to medium amber, clear, no precipitate; 2%-Medium amber, clear, no precipitate; 10%-Medium to dark amber, slightly opalescent to opalescent with precipitate.
Nitrogen (Kjeldahl Method):	11.2-12.8%
Amino Nitrogen (Modified Sorensen Method):	4.1-5.3%
Reaction of 1% Solution at 25°C:	pH 7.2-7.6

continued on following page

Summary and Explanation

Difco Laboratories conducted extensive investigations to optimize peptone production. Studies of peptic digests of animal tissue prepared under varying digestion parameters led to the development of Proteose Peptone, Proteose Peptone No. 2 and Proteose Peptone No. 3. Data accumulated during these studies demonstrated that no one peptone is the most suitable nitrogen source for every microbiological application.

Proteose Peptone was originally developed to produce a diphtheria toxin of high and uniform potency. Its suitability for this purpose was quickly established. Proteose Peptone is used in preparing toxin for diphtheria antitoxin, toxin-antitoxin mixtures, and for toxoid. Many studies support use of Proteose Peptone in culture media for diphtheria toxin production.[1,2,3,4]

Proteose Peptone is exceptionally valuable in the production of bacterial toxins, including toxins of *Corynebacterium diphtheriae*, *Clostridium botulinum*, *Pneumococcus*, *Salmonella pullorum* and scarlet fever toxin.[5,6,7,8] Proteose Peptone has many properties that account for its suitability in culturing fastidious pathogens, including its nitrogenous components, buffering range and high proteose content. These elements create an environment suitable for the maintenance of virulence and the elaboration of bacterial by-products. For this reason, stock cultures are well preserved on media containing Proteose Peptone.

Proteose Peptone No. 2 was originally developed for use in media intended for producing diphtheria toxin. Interest was renewed by Bunney and Thomas[9] through their study of diphtheria toxin production in a semisynthetic medium. Proteose Peptone No. 2 is used in media for producing bacterial toxins and for cultivating a wide range of bacterial species.

Proteose Peptone No. 3, a modification of Proteose Peptone, is used in preparing chocolate agar for propagating *Neisseria* species and chocolate tellurite agar for propagating *Corynebacterium diphtheriae*. While investigating the nutritional values of the Proteose Peptones, Proteose Peptone No. 3 was found to provide superior nutrition for fastidious microorganisms. It can replace the meat infusion-peptone combination in infusion media. Proteose Peptone No. 3 supports growth of streptococci, staphylococci, meningococci, pneumococci, gonococci and other microorganisms requiring a highly nutritious substrate. Proteose No. 3 Agar, prepared with Proteose Peptone No. 3 as its major source of nitrogen, vitamins and amino acids, is used with added enrichments for isolating and cultivating *Neisseria* and *Haemophilus*.

Principles of the Procedure

Proteose Peptone is an enzymatic digest of protein high in proteoses. Proteose Peptone No. 2 and Proteose Peptone No. 3 are enzymatic digests of protein.

Typical Analysis

	PROTEOSE PEPTONE	PROTEOSE PEPTONE NO. 2	PROTEOSE PEPTONE NO. 3
Physical Characteristics			
Ash (%)	11.1	12.7	11.4
Clarity, 1% Solution (NTU)	1.4	1.5	2.2
Filterability (g/cm^2)	0.9	0.6	0.5
Loss on Drying (%)	3.1	3.5	4.0
pH, 1% Solution	7.2	7.2	7.2
Carbohydrate (%)			
Total	<0.1	1.3	1.4
Nitrogen Content (%)			
Total Nitrogen	14.0	12.6	13.2
Amino Nitrogen	2.9	5.0	3.5
AN/TN	20.7	39.7	26.5
Amino Acids (%)			
Alanine	6.50	6.08	5.99
Arginine	5.12	5.47	5.49
Aspartic Acid	7.28	7.45	6.92
Cystine	0.87	0.40	1.12
Glutamic Acid	11.95	10.57	12.38
Glycine	9.68	10.84	9.26
Histidine	2.01	<0.01	1.74
Isoleucine	3.04	1.00	2.65
Leucine	5.66	3.57	5.70
Lysine	5.33	5.22	5.02
Methionine	1.97	1.51	1.86
Phenylalanine	2.86	7.94	2.72
Proline	5.93	5.31	4.94
Serine	3.49	4.64	3.65
Threonine	3.14	3.90	3.32
Tryptophan	0.60	0.94	0.59
Tyrosine	2.35	1.92	1.96
Valine	3.76	4.73	3.62

	PROTEOSE PEPTONE	PROTEOSE PEPTONE NO. 2	PROTEOSE PEPTONE NO. 3
Inorganics (%)			
Calcium	0.021	0.024	0.023
Chloride	4.510	3.644	3.581
Cobalt	<0.001	<0.001	<0.001
Copper	<0.001	<0.001	<0.001
Iron	0.002	<0.001	0.002
Lead	<0.001	<0.001	<0.001
Magnesium	0.027	0.024	0.027
Manganese	<0.001	<0.001	<0.001
Phosphate	0.872	1.674	1.447
Potassium	0.685	0.815	0.982
Sodium	3.677	3.956	3.815
Sulfate	0.162	0.232	0.232
Sulfur	0.812	0.698	0.975
Tin	<0.001	<0.001	<0.001
Zinc	0.002	0.003	0.007
Vitamins (µg/g)			
Biotin	0.1	0.3	0.4
Choline (as Choline Chloride)	2300.0	4500.0	3700.0
Cyanocobalamin	<0.1	<0.1	<0.1
Folic Acid	0.4	0.5	0.3
Inositol	5000.0	4700.0	8900.0
Nicotinic Acid	79.9	157.1	124.2
PABA	4.2	1.2	<0.5
Pantothenic Acid	20.0	47.0	20.0
Pyridoxine	1.1	4.0	1.3
Riboflavin	<0.1	6.4	6.8
Thiamine	1.2	1.6	0.1
Thymidine	99.7	1319.0	659.6
Biological Testing (CFU/g)			
Coliform	negative	negative	negative
Salmonella	negative	negative	negative
Spore Count	393	75	890
Standard Plate Count	443	1450	915
Thermophile Count	73	<50	25

The values presented above are "typical". This information is for broad comparison use only and is not indicative of the makeup of any particular lot of material. No guarantee is made, either expressed or implied, that any specific lot of product will match the values presented.

User Quality Control cont.

Proteose Peptone No. 3

Dehydrated Appearance:	Golden tan, free-flowing granules.
Solution:	1%, 2% and 10% solutions are soluble in distilled or deionized water: 1%-Very light amber, clear to very slightly opalescent, may have a slight precipitate; 2%-Light amber, clear to slightly opalescent, may have a slight precipitate; 10%-Light to medium amber, clear to slightly opalescent, may have a slight precipitate.
Nitrogen (Kjeldahl Method):	11.5-13.3%
Amino Nitrogen (Modified Sorensen Method):	2.25-4.85%
Reaction of 1% Solution at 25°C:	pH 7.0-7.6

continued on following page

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store below 30°C. The dehydrated ingredient is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Proteose Peptone
Proteose Peptone No. 2
Proteose Peptone No. 3
H_2S Test Strips
Indole Test Strips
KL Antitoxin Strips
KL Virulence Enrichment

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Proteose Peptone, Proteose Peptone No. 2 or Proteose Peptone No. 3 in the formula of the medium being prepared. Add as required.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Proteose Peptone, Proteose Peptone No. 2 or Proteose Peptone No. 3.

Results

Refer to appropriate references and procedures for results.

References

1. **Hewitt.** 1930. Biochem. J. **24**:984.
2. **Bunney.** 1930. J. Immunol. **20**:71.
3. **Kirkbride, Berthelsen and Clark.** 1931. J. Immunol. **21**:1.
4. **Hazen and Heller.** 1932. J. Bacteriol. **23**:195.
5. **Kirkbride and Wheeler.** 1926. J. Immunol. **11**:477.
6. **Nelson.** 1927. J. Infect. Dis. **41**:9.
7. **Kneeland and Dawes.** 1932. J. Exp. Med. **55**:735.
8. **Hanks and Rettger.** 1932. J. Immunol. **22**:283.
9. **Bunney and Thomas.** 1936. J. Immunol. **31**:95.

Packaging

Proteose Peptone	500 g	0120-17
	10 kg	0120-08
Proteose Peptone No. 2	500 g	0121-17
	10 kg	0121-08
Proteose Peptone No. 3	500 g	0122-17
	2 kg	0122-07
	10 kg	0122-08

User Quality Control cont.

Cultural Response

Proteose Peptone, Proteose Peptone No. 2 and Proteose Peptone No. 3

For each Test specified, prepare a Test Solution of the desired Proteose Peptone and, if necessary, adjust to pH 7.2-7.4; sterilize, inoculate and incubate according to standard test procedure.

TEST	TEST SOLUTION	ORGANISM	ATCC®	INOCULUM	RESULT
Fermentable Carbohydrate	2%	*Escherichia coli*	25922*	1 drop, undiluted	negative; red color
Indole Production	0.1%	*Escherichia coli*	25922*	1 drop, undiluted	positive; pink color on Indole Test Strip
Acetylmethylcarbinol Production (AMC)	0.1% w/ 0.5% dextrose	*Enterobacter aerogenes*	13048*	1 drop, undiluted	positive; pink color upon adding reagents
Hydrogen Sulfide Production	1%	*Salmonella typhi*	6539	1 drop, undiluted	positive; brownish blackening of H_2S Test Strip
Growth Response	2% w/ 0.1% agar, 0.5% NaCl and 0.1% dextrose	*Brucella suis*	4314	undiluted	good growth
Growth Response	2% w/ 0.1% agar, 0.5% NaCl and 0.1% dextrose	*Staphylococcus aureus*	25923*	100-1,000 CFU	good growth
Growth Response	2% w/ 0.1% agar, 0.5% NaCl and 0.1% dextrose	*Escherichia coli*	25922*	100-1,000 CFU	good growth

Proteose Peptone

Prepare KL Virulence Agar from individual ingredients using 2 grams of the test Proteose Peptone; sterilize, add KL Virulence Enrichment and dispense into Petri dishes containing KL Antitoxin Strips. Inoculate with a loopful of surface growth and incubate at 35 ± 2°C for 72 hours. Examine at 24, 48 and 72 hours.

TEST	ORGANISM	ATCC®	RESULT
Toxin Production	*Corynebacterium diphtheriae* Type intermedius	8032	precipitin line
Toxin Production	*Corynebacterium diphtheriae* Type gravis	8028	precipitin line
Toxin Production	*Corynebacterium diphtheriae* Type mitis	8024	precipitin line

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Pseudomonas Agar Media

Bacto® Pseudomonas Agar F · Bacto Pseudomonas Agar P

Intended Use

Pseudomonas Agar F is used with Bacto Glycerol for detecting and differentiating *Pseudomonas aeruginosa* from other pseudomonads based on fluorescein production.

Pseudomonas Agar P is used with Bacto Glycerol for detecting and differentiating *Pseudomonas aeruginosa* from other pseudomonads based on pyocyanin production.

Also Known As

Pseudomonas Agar F is known as Pseudomonas Agar Medium for Detection of Fluorescein.

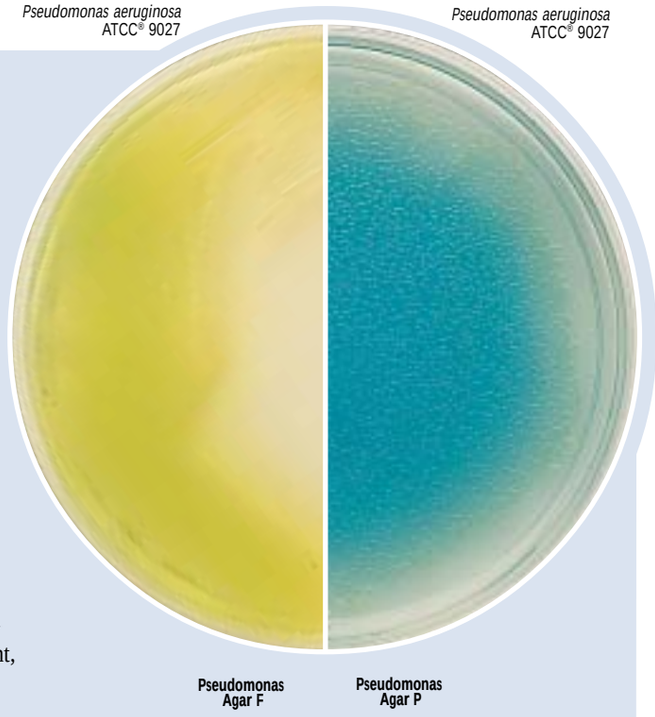
Pseudomonas Agar P is also known as Pseudomonas Agar Medium for Detection of Pyocyanin.

Summary and Explanation

Pseudomonas Agar F and Pseudomonas Agar P, patterned after the formulations described by King, Ward and Raney,[1] are modified to USP specifications.[2]

Pseudomonas Agar F enhances the production of fluorescein by *Pseudomonas* and inhibits the formation of pyocyanin. Pseudomonas Agar P, in contrast, enhances the production of pyocyanin and inhibits the formation of fluorescein. Both pigments diffuse from *Pseudomonas* colonies into the medium in which they grow. Fluorescein elaborated on Pseudomonas Agar F is a fluorescent yellow color, while pyocyanin elaborated on Pseudomonas Agar P is a blue color.

Some *Pseudomonas* strains elaborate both pigments, while others



User Quality Control

Identity Specifications

Pseudomonas Agar F

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.8% solution with 1% Glycerol, soluble in distilled or deionized water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent, without precipitate.
Reaction of 3.8% Solution at 25°C:	pH 7.0 ± 0.2

Pseudomonas Agar P

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.64% solution with 1% Glycerol, soluble in distilled or deionized water upon boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent, without precipitate.
Reaction of 4.64% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	GROWTH	PIGMENT PRODUCTION PSEUDOMONAS AGAR F	PSEUDOMONAS AGAR P
Pseudomonas aeruginosa	9027	good	greenish yellow	blue
Pseudomonas aeruginosa	27853*	good	greenish yellow	blue
Pseudomonas cepacia	25609	good	no pigment	no pigment

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as a Bactrol™ Disk and should be used as directed in Bactrol Disks Technical Information.

elaborate only one of the two. When Pseudomonas Agar F and Pseudomonas Agar P are used together, they provide for easy and rapid identification of most *Pseudomonas* strains as specified in the FDA Bacteriological Analytical Manual.[3]

Principles of the Procedure

Pseudomonas Agar F

Tryptone and Proteose Peptone No. 3 provide carbon and nitrogen sources required for good growth and also aid in fluorescein production. Phosphate stimulates fluorescein production and has an inhibitory effect on pyocyanin. Dipotassium Phosphate increases the phosphorus content over that supplied by the peptones. Magnesium Sulfate provides necessary cations for the activation of fluorescein production. Bacto Agar is a solidifying agent. Glycerol, added during preparation of the medium, is a carbon source.

Pseudomonas Agar P

Bacto Peptone provides the carbon and nitrogen sources required for good growth. Glycerol is a carbon source. Magnesium Chloride and Potassium Sulfate stimulate pyocyanin production. Bacto Agar is a solidifying agent.

Formula

Pseudomonas Agar F

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Proteose Peptone No. 3	10 g
Dipotassium Phosphate	1.5 g
Magnesium Sulfate	1.5 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Pseudomonas Agar P

Formula Per Liter	
Bacto Peptone	20 g
Magnesium Chloride	1.4 g
Potassium Sulfate	10 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store the prepared media at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Pseudomonas Agar F
Pseudomonas Agar P

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Sterile Petri dishes
Tubes with closures
Bacto Glycerol

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water containing 10 grams of Glycerol:

   Pseudomonas Agar F - 38 grams;

   Pseudomonas Agar P - 46.4 grams.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Obtain the inoculum from a pure 18-24 hour culture of *Pseudomonas*.
2. Inoculate plates or agar slants by streaking the surface.
3. Incubate at 35 ± 2°C for 18-24 hours.

Results

Examine colonies under ultraviolet light (Wood's lamp).[4] Take care when using UV illumination because it may have a bactericidal effect. Be sure there is good growth before placing the culture under UV light.

Pseudomonas Agar F: Positive result is indicated by a light, bright greenish-yellow color diffusing into the agar with a fluorescent zone surrounding the growth.

Pseudomonas Agar P: Positive result is indicated by a blue pigment that diffuses into the agar.

Limitations of the Procedure

1. Occasionally, a *Pseudomonas* culture is encountered that will produce small amounts of pigment in the medium. When this happens, a yellow-green color will appear on Pseudomonas Agar F or a blue-green color on Pseudomonas Agar P. If a blue-green color occurs on Pseudomonas Agar P, confirmation of the presence of pyocyanin can be made by extraction with chloroform ($CHCl_3$).[4]
2. The formation of nonpigmented colonies does not completely rule out a *Pseudomonas aeruginosa* isolate.
3. A pyocyanin-producing *Pseudomonas* strain will usually also produce fluorescein. It must, therefore, be differentiated from other simple fluorescent pseudomonads by other means. Temperature can be a determining factor as most other fluorescent strains will not grow at 35°C. Rather, they grow at 25-30°C.[4]

References

1. **King, E. O., M. K. Ward, and D. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. **44**:301.
2. **The United States Pharmacopeia.** 1995. The United States pharmacopeia, 23rd ed. United States Pharmacopeial Convention, Rockville, MD.

3. **Bacteriological Analytical Manual, 8th edition.** 1995. AOAC International, Gaithersburg, MD.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Pseudomonas Agar F	100 g	0448-15
	500 g	0448-17
Pseudomonas Agar P	500 g	0449-17

Bacto® Pseudomonas Isolation Agar

Intended Use

Bacto Pseudomonas Isolation Agar is used with added glycerol in isolating *Pseudomonas* and differentiating *Pseudomonas aeruginosa* from other pseudomonads based on pigment formation.

Summary and Explanation

Pseudomonas aeruginosa is an opportunistic pathogen that can infect eyes, ears, burns and wounds.[2,4] It is also a leading cause of hospital acquired infections. Patients undergoing antibiotic therapy are especially susceptible to infection by *Pseudomonas aeruginosa*.

Pseudomonas Isolation Agar is prepared according to a slight modification of the Medium A formulation of King, Ward and Raney.[1] It is especially useful for isolating *Pseudomonas* from clinical specimens such as stools, wounds and urine.[2] Pseudomonas Isolation Agar includes Irgasan®, a potent broad spectrum antimicrobial that is not active against *Pseudomonas*.[3] As well as being selective, Pseudomonas Isolation Agar is formulated to enhance the formation of the blue or blue-green pyocyanin pigment by *Pseudomonas aeruginosa*. The pigment diffuses into the medium surrounding growth.

Principles of the Procedure

Bacto Peptone provides the carbon and nitrogen necessary for bacterial growth. Magnesium Chloride and Potassium Sulfate promote production of pyocyanin. Irgasan, an antimicrobial agent, selectively inhibits gram-positive and gram-negative bacteria other than *Pseudomonas* spp. Bacto Agar is a solidifying agent. Glycerol serves as an energy source and also helps to promote pyocyanin production.

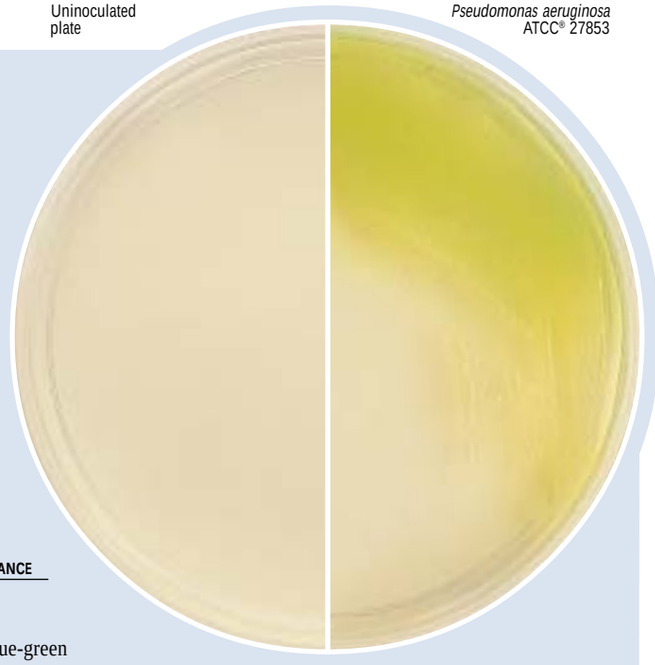
Formula

Pseudomonas Isolation Agar

Formula Per Liter

Bacto Peptone	20 g
Magnesium Chloride	1.4 g
Potassium Sulfate	10 g
Irgasan®	0.025 g
Bacto Agar	13.6 g

Final pH 7.0 ± 0.2 at 25°C



User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light beige, homogeneous, free-flowing.
Solution:	4.5% solution, soluble on boiling in distilled or deionized water containing 2% glycerol. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent, firm.
Reaction of 4.5% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Pseudomonas Isolation Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition	
Pseudomonas aeruginosa	10145	100-1,000	good	green to blue-green
Pseudomonas aeruginosa	27853*	100-1,000	good	green to blue-green

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Pseudomonas Isolation Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Distilled or deionized water
Glycerol

Method of Preparation

1. Suspend 45 grams in 980 ml distilled or deionized water.
2. Add 20 ml of Glycerol.
3. Boil to dissolve completely.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[2,4,5]
2. Process each specimen, using procedures appropriate for that specimen or sample.[2,4,5]

Test Procedure

1. Inoculate the medium using the streak plate method to obtain isolated colonies.
2. Incubate for 18-48 hours at 35 ± 2°C.

Results

Examine for the presence of good growth. *Pseudomonas aeruginosa* colonies will be green to blue-green with pigment that diffuses into the medium.

Limitations of the Procedure

1. Some strains of *Pseudomonas aeruginosa* may fail to produce pyocyanin.[6]
2. Non-*Pseudomonas aeruginosa* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Pseudomonas aeruginosa*. Consult appropriate references.[2,5]

References

1. **King, E. O., M. K. Ward, and D. E. Raney.** 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. & Clin. Med. **44(2)**:301-307.
2. **Baron, E. J., and S. M. Finegold.** 1990. Bailey & Scott's Diagnostic Microbiology, 8th ed. C.V. Mosby Company, St. Louis, MO.
3. **Furia and Schenkel.** 1968. Soap and Chemical specialties. January.
4. **Gilligan, P. H.** 1995. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
5. **Pezzlo, M. (ed.).** 1992. Aerobic bacteriology, p. 1.0.0-1.20.47. *In* H. D. Isenberg, (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
6. **Gaby, W. L., and E. Free.** 1931. J. Bacteriol. **22:**349.

Packaging

Pseudomonas Isolation Agar	500 g	0927-17
Glycerol	100 g	0282-15
	500 g	0282-17

Bacto® Purple Broth Base
Bacto Purple Agar Base

Intended Use

Bacto Purple Broth Base and Purple Agar Base are used with added carbohydrate in differentiating pure cultures of bacteria, particularly of enteric organisms, based on fermentation reactions.

Summary and Explanation

Purple Broth Base and Purple Agar Base are carbohydrate-free fermentation media that are preferred by some bacteriologists because of their slightly acid reaction (pH 6.8). When supplemented with carbohydrates, these media are useful in obtaining accurate fermentation reactions in the identification of *Enterobacteriaceae* and other microorganisms. The concentration of carbohydrate generally employed for testing the fermentation reactions of bacteria is 0.5 or 1%. Some investigators prefer to use 1% rather than 0.5% to insure against reversion of the reaction due to depletion of the carbohydrate by some microorganisms. Purple Broth Base with added carbohydrates is specified in several standard methods.[1,2,3,4]

Principles of the Procedure

Proteose Peptone No. 3 and Beef Extract provide the carbon and nitrogen sources required for good growth of a wide variety of organisms. Sodium Chloride maintains the osmotic balance of the medium. Brom Cresol Purple serves as an indicator, assuming a yellow color when

acid is produced during the fermentation of the added carbohydrate. In Purple Agar Base, the Bacto Agar serves as a solidifying agent.

Formula

Purple Broth Base

Formulas Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	1 g
Sodium Chloride	5 g
Bacto Brom Cresol Purple	0.02 g

Final pH at 25°C 6.8 ± 0.2

User Quality Control

Identity Specifications

Purple Broth Base

Dehydrated Appearance:	Light tan with grayish-green cast, free-flowing, homogeneous.
Solution:	1.6% solution soluble in distilled or deionized water. Solution is purple, clear to very slightly opalescent.
Prepared Tubes:	Purple, clear to very slightly opalescent.
Reaction of 1.6% Solution at 25°C:	pH 6.8 ± 0.2

Purple Agar Base

Dehydrated Appearance:	Light tan with grayish-green cast, free-flowing, homogeneous.
Solution:	3.1% solution soluble in distilled or deionized water upon boiling. Solution is purple, very slightly to slightly opalescent.
Prepared Medium:	Purple, slightly opalescent.
Reaction of 3.1% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Purple Broth Base

Prepare Purple Broth Base per label directions with 1.0% Dextrose. Inoculate and incubate the tubes at 35 ± 2°C for 18-48 hours. A color change to yellow indicates acid production, and the appearance of bubbles in the inverted fermentation vial indicates gas production.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	REACTION w/1% DEXTROSE ACID	GAS
Alcaligenes faecalis	8750	100-1,000	good	–	–
Escherichia coli	25922*	100-1,000	good	+	+
Salmonella typhimurium	14028*	100-1,000	good	+	+

Purple Agar Base

Prepare Purple Agar Base per label directions with 1.0% Dextrose. Inoculate tubes with test organisms by stabbing the butt of the tube and streaking the slant. Incubate at 35 ± 2°C for 18-48 hours. A color change to yellow indicates acid production, and the appearance of bubbles indicates gas production.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	REACTION w/1% DEXTROSE ACID	GAS
Alcaligenes faecalis	8750	1,000-2,000	good	–	–
Escherichia coli	25922*	1,000-2,000	good	+	+
Salmonella typhimurium	14028*	1,000-2,000	good	+	+

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube | Typical positive growth with acid and gas reaction | Typical negative growth with acid and gas reaction

Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Escherichia coli* ATCC® 25922 + Dextrose

Purple Agar Base

Purple Agar Base

Formulas Per Liter

Bacto Proteose Peptone No. 3	10 g
Bacto Beef Extract	1 g
Sodium Chloride	5 g
Bacto Brom Cresol Purple	0.02 g
Bacto Agar	15 g

Final pH at 25°C 6.8 ± 0.2

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated media below 30°C. The dehydrated media are very hygroscopic. Keep containers tightly closed.

Expiration Date

The expiration date applies to the products in their intact containers when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bacto Purple Broth Base
Bacto Purple Agar Base

Materials Required But Not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)
Choice of carbohydrates
Fermentation vials (Purple Broth Base)

Method of Preparation

Purple Broth Base

1. Suspend 16 grams in 1 liter distilled or deionized water and heat to boiling to dissolve completely.
2. Autoclave at 121°C for 15 minutes.

   To prepare fermentation broths, add 0.5-1% carbohydrate before or after sterilization, depending on heat lability. Dispense into tubes containing inverted fermentation vials.

Purple Agar Base

1. Suspend 31 grams in 1 liter distilled or deionized water and boil to dissolve completely.
2. To prepare 0.5-1% carbohydrate fermentation agars, dissolve 5-10 grams of the desired carbohydrate in the basal medium prior to sterilization.
3. Autoclave at 121°C for 15 minutes.

OR

1. Dissolve 31 grams in 900 ml distilled or deionized water and boil to dissolve completely.
2. Autoclave at 121°C for 15 minutes.
3. Cool the basal medium to 45-50°C
4. Aseptically add 100 ml sterile 5-10% carbohydrate solution (w/v).

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate tubes using a light inoculum from an 18-24 hour pure culture. To inoculate Purple Broth Base tubes, use a loopful of inoculum. For Purple Agar Base tubes, stab with an inoculating needle to within 1/4 inch from the bottom of the tube.
2. Incubate tubes for 24-72 hours at 35 ± 2°C in an aerobic or anaerobic atmosphere, depending on the organisms being tested.
3. Examine tubes daily for acid production and gas formation. Hold negative tubes for a total of 30 days.

Results

A yellow color is a positive reaction for fermentation of the carbohydrate. Bubbles in the inverted fermentation vials are an indication of gas production. Even the presence of a single bubble is significant to record as positive.[5]

Limitations of the Procedure

1. The addition of some carbohydrates to the media may result in an acid reaction. In this case, it is suggested that the proper pH be restored by adding sterile 0.1N sodium hydroxide dropwise.
2. Avoid excessive heating or prolonged heat exposure of media to avoid hydrolysis of the carbohydrates.
3. Tubes should be tightly stoppered during the incubation period for fermentation studies of the enteric group to avoid reversion caused by rapid depletion of the carbohydrate(s).[5]

References

1. **Bacteriological Analytical Manual,** 8th edition. 1995. AOAC International, Gaithersburg, MD.
2. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products. American Public Health Assoc., Washington, D.C.
3. **Vanderzant, C., and D. F. Splittstoesser.** 1992. Compendium of methods for the microbiological examination of foods. American Public Health Assoc., Washington, D.C.
4. **Association of Official Analytical Chemists.** 1995 Official methods of analysis of AOAC International. AOAC International, Arlington, VA.
5. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Packaging

Purple Broth Base	500 g	0227-17
Purple Agar Base	500 g	0228-17

Bacto® Purple Lactose Agar

Intended Use

Bacto Purple Lactose Agar is used for cultivating coliform organisms; for differentiating lactose-fermenting from lactose-nonfermenting organisms.

Summary and Explanation

Purple Lactose Agar is a modification of Litmus Lactose Agar, described by Wurtz.[1] In Purple Lactose Agar, brom cresol purple replaces litmus, which is less selective and less stable.

Purple Lactose Agar is used for detecting coliforms and in differential studies based on the fermentation of lactose. Tests used to differentiate *Enterobacteriaceae* determine the organism's ability to use a carbohydrate with the production of acid metabolic end products.[2] Colonies of lactose-fermenting organisms are differentiated from lactose non-fermenters by a color change of the indicator from blue-purple (alkaline) to yellow (acid). If gas is produced during fermentation of the carbohydrate, bubbles will appear in the medium.[2]

Principles of the Procedure

Beef Extract and Bacto Peptone provide the nitrogen, vitamins and amino acids in Purple Lactose Agar. Bacto Lactose is the carbohydrate used in the fermentation reaction. Bacto Agar is the solidifying agent. Bacto Brom Cresol Purple is the pH indicator.

Formula

Purple Lactose Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Peptone	5 g
Bacto Lactose	10 g
Bacto Agar	10 g
Bacto Brom Cresol Purple	0.025 g

Final pH 6.8 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Purple Lactose Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige with greenish cast, free-flowing and homogeneous.
2.8% Solution:	Soluble in distilled or deionized water on boiling. Solution is purple, clear to very slightly opalescent.
Reaction of 2.8% Solution:	pH 6.8 ± 0.1 at 25°C

Cultural Response

Inoculate the agar slant by stabbing the butt and streaking with an inoculating needle. Incubate tubes at 35 ± 2°C for 18-48 hours. Acid production is indicated by a yellow color.

ORGANISM	ATCC®	GROWTH	ACID (YELLOW)	GAS
Enterobacter aerogenes	13048*	good	+	+
Escherichia coli	25922*	good	+	+
Salmonella typhi	19430	good	–	–
Staphylococcus aureus	25923*	good	+	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Salmonella typhi* ATCC® 19430

Method of Preparation

1. Suspend 28 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense into sterile tubes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

For a complete discussion on the expected reactions of specific Enterobacteriaceae species, refer to Manual of Clinical Microbiology,[3] Clinical Microbiology Procedures Handbook [4] and Bailey & Scott's Diagnostic Microbiology.[2]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Medium is slightly acid (pH 6.8) and positive reactions may be slower than with phenol red carbohydrate medium.[5]

References

1. **Wurtz.** 1897. Technique Bacteriologique, Masson, Paris.
2. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, MO.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol 1. American Society for Microbiology, Washington, D.C.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol 1. Williams & Wilkins, Baltimore, MD.

Packaging

Purple Lactose Agar	500 g	0082-17

Bacto® Pyridoxine Y Medium

Intended Use

Bacto Pyridoxine Y Medium is used for determining pyridoxine concentration by the microbiological assay technique.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose.
2. Inoculum Media: To condition the test culture for immediate use.
3. Assay Media: To permit quantitation of the vitamin under test.

Pyridoxine Y Medium is patterned after the formulation of Campling and Nixon,[1] and modified by Hurley[2] and Parrish, Loy and Kline.[3] This medium is used in the microbiological assay of pyridoxine using *Saccharomyces cerevisiae* ATCC® 9080 (*Saccharomyces uvarum)* as the test organism.

User Quality Control

Identity Specifications

Dehydrated Appearance:	White to off-white, fine, free-flowing, homogeneous.
Solution:	2.65% (single strength) 5.3% (double strength) solution, soluble in distilled or deionized water upon boiling for 2-3 minutes. Solution is almost colorless to very light amber, clear, may have a slight precipitate.
Prepared Medium:	Single strength solution is colorless to very light amber, clear, may have a slight precipitate.
Reaction of 2.65% Solution at 25°C:	pH 4.4 ± 0.2

Cultural Response

Prepare Pyridoxine Y Medium per label directions. This medium should support the growth of *Saccharomyces cerevisiae* ATCC® 9080 when prepared in single strength and supplemented with a mixture containing 1 ng per ml each of pyridoxal hydrochloride, pyridoxamine hydrochloride and pyridoxine hydrochloride.

Principles of the Procedure

Pyridoxine Y Medium is free from pyridoxine, but contains all other nutrients and vitamins essential for the growth of *S. cerevisiae* ATCC® 9080. The addition of pyridoxine in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Pyridoxine Y Medium

Formula Per Liter

L-Asparagine	4 g
L-Histidine Hydrochloride	20 mg
DL-Methionine	40 mg
DL-Tryptophane	40 mg
DL-Isoleucine	40 mg
DL-Valine	40 mg
Bacto Dextrose	40 g
Thiamine Hydrochloride	400 µg
Calcium Pantothenate	400 µg
Nicotinic Acid	400 µg
Biotin Salt	8 mg

Riboflavin	20	µg
Inositol	5	mg
Boric Acid	200	µg
Monopotassium Phosphate	3	g
Magnesium Sulfate	1	g
Ammonium Sulfate	4	g
Calcium Chloride	0.49	g
Potassium Iodide	200	µg
Ammonium Molybdate	40	µg
Manganese Sulfate	80	µg
Copper Sulfate	90	µg
Zinc Sulfate	80	µg
Ferrous Sulfate	500	µg

Final pH 4.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware is heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
3. Take precautions to keep sterilizing and cooling conditions uniform throughout assay.
4. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
5. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Pyridoxine Y Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Saccharomyces cerevisiae* ATCC® 9080
Sterile tubes
Distilled or deionized water
Pyridoxal HCl
Pyridoxamine 2 HCl
Pyridoxine HCl
Lactobacilli Agar AOAC
Shaker (100 rpm)
Incubator (25-30°C)
Centrifuge
Ethyl alcohol
Spectrophotometer

Method of Preparation

1. Dissolve 5.3 grams in 100 ml distilled or deionized water.
2. Boil 2- 3 minutes to dissolve completely.
3. Dispense 5 ml amounts into flasks, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust flask volume to 10 ml with distilled or deionized water.
6. Steam at 100°C for 10 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to specific assay procedures. For assays, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Stock cultures of *S. cerevisiae* ATCC® 9080 are carried on Lactobacilli Agar AOAC. Following incubation at 25-30°C (held constant within ±0.5°C) for 18-24 hours, store the cultures in the dark at 2-8°C. Prepare fresh slant cultures every week. Do not use stock cultures for preparing the inoculum if more than one week old. Inoculum for assay is prepared by subculturing a stock culture of *S. cerevisiae* ATCC 9080 into a tube (10 ml) of single strength Pyridoxine Y Medium containing 1 ng per ml each of pyridoxal hydrochloride, pyridoxamine dihydrochloride and pyridoxine hydrochloride. After 18-24 hours incubation at 25-30°C (held constant within ±0.5°C), centrifuge the cells under aseptic conditions and decant the liquid supernatant. Wash the cells 3x with 10 ml sterile 0.85% saline. After the third wash, resuspend in 10 ml sterile single strength medium and adjust to a turbidity of 45-50% transmittance when read on the spectrophotometer at 660 nm.

It is essential that a standard curve be set up for each separate assay. Conditions of steaming and temperature of incubation which influence the standard curve readings cannot always be duplicated. Obtain the standard curve by using pyridoxine hydrochloride at levels of 0, 1, 2, 4, 6, 8 and 10 ng per flask (10 ml).

The concentrations of pyridoxine hydrochloride required for the preparation of the standard curve may be prepared as follows:

A. Dissolve 50 mg dried pyridoxine hydrochloride in about 100 ml in HCL solution.
B. Dilute 500 ml with in HCL.
C. Further dilute by adding 2 ml to 998 ml distilled water to make a stock solution containing 200 ng pyridoxine hydrochloride per ml. Prepare the stock solution fresh daily.

To make the standard solution, dilute 1 ml of stock solution with 99 ml distilled water, to make a solution containing 2 ng pyridoxine hydrochloride per ml. Use 0.0, 0.05, 1, 2, 3, 4 and 5 ml per assay tube.

Following inoculation, incubate the tubes on a shaker (about 100 rpm) at 25-30°C for 22 hours. Steam in the autoclave for 5 minutes to stop growth. Measure the growth turbidimetrically using a spectrophotometer at any specific wavelength between 540 and 660 nm.

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average and use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be grown and maintained on a medium recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. **Campling, and Nixon.** 1954. J. Physiol. **126**:71.
2. **Hurley.** 1960. J. AOAC. **43**:43.
3. **Parrish, Loy, and Kline.** 1956. J. AOAC. **39**:157.

Packaging

Pyridoxine Y Medium	100 g	0951-15*

*Store at 2-8°C

Bacto® R2A Agar

Intended Use

Bacto R2A Agar is used for enumerating heterotrophic organisms in treated potable water.

Summary and Explanation

R2A Agar was developed by Reasoner and Geldreich[1] for bacteriological plate counts of treated potable water. A low nutrient medium, such as R2A Agar, in combination with a lower incubation temperature and longer incubation time stimulates the growth of stressed and chlorine-tolerant bacteria.[1] Nutritionally rich media, such as Tryptone Glucose Yeast Extract Agar (TGEA) or Plate Count Agar (PCA), support the growth of fast-growing bacteria but may suppress slow growing or stressed bacteria found in treated water. When compared with TGEA and PCA, R2A Agar has been reported to improve the recovery of stressed and chlorine-tolerant bacteria from drinking water systems.[2,3,4]

R2A Agar is recommended in *Standard Methods for the Examination of Water and Wastewater*[5] for pour plate, spread plate and membrane filter methods for heterotrophic plate counts.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1.82% solution, soluble in distilled or deionized water on boiling. Solution is light amber in color, slightly opalescent, with a slight precipitate.
Prepared Plates:	Light amber in color, slightly opalescent, with a slight precipitate.
Reaction of 1.82% Solution at 25°C:	7.2 ± 0.2.

Cultural Response

Prepare R2A Agar per label directions. Inoculate with tap water samples using the streak plate method and/or the membrane filter method. Incubate at 35 ± 2°C for 40-72 hours. Recovery is typical compared to an approved control lot and greater than parallel plates of Plate Count Agar.

Principles of the Procedure

Yeast Extract provides a source of trace elements and vitamins. Proteose Peptone No. 3 and Casamino Acids provide nitrogen, vitamins, amino acids, carbon and minerals. Dextrose serves as a carbon source. Soluble Starch aids in the recovery of injured organisms by absorbing toxic metabolic by-products. Sodium Pyruvate increases the recovery of stressed cells. Potassium Phosphate is used to balance the pH and provide phosphate. Magnesium Sulfate is a source of divalent cations and sulfate. Bacto Agar is the solidifying agent.

Formula

R2A Agar

Formula Per Liter	
Bacto Yeast Extract	0.5 g
Bacto Proteose Peptone No. 3	0.5 g
Bacto Casamino Acids	0.5 g
Bacto Dextrose	0.5 g
Soluble Starch	0.5 g
Sodium Pyruvate	0.3 g
Potassium Phosphate, Dibasic	0.3 g
Magnesium Sulfate	0.05 g
Bacto Agar	15 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

R2A Agar

Materials Required But Not Provided

Autoclave
Petri dishes
Membrane filter equipment and filters
Dilution blanks
Pipettes or glass rods
Incubator (20, 28 or 35°C)
Colony counter

Method of Preparation

1. Suspend 18.2 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Water samples should be collected as described in Standard *Methods for the Examination of Water and Wastewater*, Section 9060A.[5]

To minimize changes in bacterial population, water samples should be tested as soon as possible, but at least within six hours of collection if the sample has not been refrigerated or within 30 hours if refrigerated.

Test Procedure

1. Prepare test dilutions for heterotrophic plate count.
2. Plate the test sample and dilutions by the spread plate, pour plate or membrane filter method. Do not exceed 1 ml of sample or dilution per spread or pour plate. The volume of test sample to be filtered for the membrane filter technique will vary.
3. Maintain proper humidity during prolonged incubation:

INCUBATION TEMPERATURE	MINIMUM INCUBATION TIME[3]	OPTIMAL INCUBATION TIME[3]
35°C	72 hours	5-7 days
20 or 28°C	5 days	7 days

Results

Count colonies on spread or pour plates demonstrating 30-300 colonies per plate or 20-200 colonies when using the membrane filter method. Compute bacterial count per ml of sample by multiplying the average number of colonies per plate by the reciprocal of the appropriate dilution.

Report counts as colony forming units (CFU) per ml and report variables of incubation such as temperature and length of time.

Limitations of the Procedure

1. R2A Agar is intended for use only with treated potable water since it is recommended for compromised bacteria.
2. Use of the pour plate method is discouraged because recovery of stressed bacteria may be compromised by the heat shock (44-46°C) and low oxygen tension that are part of the procedure.[6,7]
3. Incubation time longer than indicated above may be necessary to recover additional slow-growing bacteria.
4. R2A Agar performs best with the spread plate technique; however, that procedure is limited to a small sample volume.
5. Fast-growing bacteria may produce smaller size colonies on R2A Agar than on nutritionally rich media.
6. R2A Agar is a low nutrient medium intended for culturing compromised microorganisms. Good growth of standard, healthy control organisms does not necessarily reflect the ability of the medium to recover stressed organisms. Each new lot of medium should be performance tested against a previous lot of R2A Agar using tap water.

References

1. **Reasoner, D. J., and E. E. Geldreich.** 1979. A new medium for the enumeration and subculture of bacteria from potable water. Abstracts of the Annual Meeting of the American Society for Microbiology 79th Meeting, Paper No. N7.
2. **Fiksdal, L., E. A. Vik, A. Mills, and T. Staley**. 1982. Non-standard methods for enumerating bacteria in drinking water. Journal AWWA. **74**:313-318.
3. **Kelly, A. J., C. A. Justice, and L. A. Nagy**. 1983. Predominance of chlorine tolerant bacteria in drinking water systems. Abstracts of the Annual Meeting of the American Society for Microbiology 79th Meeting, Paper No. Q122.
4. **Means, E. G., L. Hanami, H. F. Ridgway, and B. H. Olson**. 1981. Evaluating mediums and plating techniques for enumerating bacteria in water distribution systems. Journal AWWA. **53**:585-590.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.)**. 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
6. **Van Soestberger, A. A., and C. H. Lee**. 1969. Pour plates or streak plates? Appl. Microbiol. **18**:1092.
7. **Klein, D. A., and S. Wu**. 1974. Stress: a factor to be considered in heterotrophic microorganism enumeration from aquatic environments. Appl. Microbiol. **27**:429.

Packaging

R2A Agar	100 g	1826-15
	500 g	1826-17
	2 kg	1826-07

Bacto® Raka-Ray No. 3 Broth
Bacto Raka-Ray No. 3 Medium

Intended Use

Bacto Raka-Ray No. 3 Broth and Medium are recommended for the isolation of lactic acid bacteria encountered in beer and the brewing process.

Summary and Explanation

Spoilage organisms are often seriously detrimental to beer flavor. Lactic acid bacteria including lactobacilli and pediococci which can cause spoilage are physiologically very diverse.

Raka-Ray No. 3 Broth and Medium were developed from a formulation suggested by Saha, Sondag, and Middlekauff[1] who tested a range of ingredients for their ability to stimulate growth of lactic acid bacteria. Tween® 80, liver extract, maltose, N-acetyl glucosamine and yeast extract were found to stimulate growth. Tomato juice, free fatty acids and lyophilized beer solids (all of which are found in several media formulations for lactic acid bacteria) were inhibitory.

In comparative studies using in-process beer samples, Raka-Ray media gave higher colony counts for lactobacilli than Tomato Juice Agar, W-L Differential Agar and Universal Beer Agar, with larger colonies developing after 2-4 days of anaerobic incubation.[1,2]

Raka-Ray No. 3 Medium yields larger lactic acid bacterial colonies than Universal Beer Agar.[3] Raka-Ray No. 3 Medium also suppressed the growth of non-lactic acid, facultative bacteria such as *Aerobacter aerogenes* and *Flavobacterium proteus* that are often associated with lactic beer spoilage organisms.[3]

Raka-Ray No. 3 Medium is also recommended by the 'European Brewing Congress Analytical Microbiologica' for enumeration of lactobacilli and pediococci[4]. The broth and agar may be made more selective by the addition of 3 grams of 2-phenylethanol and 3 mg of cycloheximide (Actidione®) dissolved in a small quantity of acetone per liter of medium before autoclaving. Yeasts and gram-negative bacteria are suppressed, facilitating enumeration of the lactic bacterial flora.

User Quality Control

Identity Specifications

Raka-Ray No. 3 Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.89% solution, soluble in distilled or deionized water with 1% Tween® 80. Solution is medium to dark amber, clear.
Reaction of 5.89% Solution at 25°C:	pH 5.4 ± 0.2

Raka-Ray No. 3 Medium

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	7.49% solution, soluble in distilled or deionized water with 1% Tween® 80 upon boiling. Solution is medium to dark amber, clear to very slightly opalescent.
Reaction of 7.49% Solution at 25°C:	pH 5.4 ± 0.2

Cultural Response

Prepare Raka-Ray No. 3 Broth or Medium with selective agents per label directions. Inoculate and incubate anaerobically at 27-30°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	1,000-2,000	none to poor
Lactobacillus brevis	367	30-300	good
Lactobacillus buchneri	11307	30-300	good
Pediococcus acidilactici	8042	30-300	good

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as a Bactrol™ Disk and should be used as directed in Bactrol Disks Technical Information.

Principles of Procedure

Polysorbate 80, Liver Digest, Maltose and other sugars, N-Acetyl Glucosamine and Yeast Extract stimulate the growth of lactobacilli. The optional addition of cycloheximide provides increased selectivity against yeasts and gram-negative bacteria.

Formula

Raka-Ray No. 3 Broth

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Tryptone	20 g
Liver Digest	1 g
Maltose Reagent	10 g
Fructose	5 g
Dextrose	5 g
Betaine Hydrochloride	2 g
Di-ammonium Citrate	2 g
L-Aspartic Acid	2.5 g
Magnesium Sulphate	0.98 g
Manganese Sulphate	0.42 g
Dipotassium Phosphate	2 g
N-Acetyl Glucosamine	0.5 g
Potassium Glutamate	2.5 g

Final pH 5.4 ± 0.2 at 25°C

Raka-Ray No. 3 Medium

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Tryptone	20 g
Liver Digest	1 g
Maltose Reagent	10 g
Fructose	5 g
Dextrose	5 g
Betaine Hydrochloride	2 g
Di-ammonium Citrate	2 g
Potassium Aspartate	2.5 g
Magnesium Sulphate	0.98 g
Manganese Sulphate	0.42 g
Dipotassium Phosphate	2 g
N-Acetyl Glucosamine	0.5 g
Potassium Glutamate	2.5 g
Bacto Agar	16 g

Final pH 5.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Raka-Ray No. 3 Broth
Raka-Ray No. 3 Medium

Materials Required but not Provided

Flasks with closures
Distilled or deionized water
Tween® 80
2-phenylethanol
Actidione®
Acetone
Autoclave
Waterbath (50°C)
Petri dishes
Sterile tubes
Anaerobic chamber

Method of Preparation

Raka-Ray No. 3 Broth

1. Suspend 58.9 grams in 1 liter of distilled or deionized water containing 10 ml Tween® 80. Dispense into tubes with closures.
2. Autoclave at 121°C for 15 minutes.

Raka-Ray No. 3 Medium

1. Suspend 74.9 grams in 1 liter of distilled or deionized water containing 10 ml Tween® 80.
2. Heat to boiling to dissolve.
3. To increase the selectivity of the medium, add 3 grams of 2-phenylethanol and 3 mg cycloheximide (Actidione®) per liter before autoclaving. Do not overheat.
4. Autoclave at 121°C for 15 minutes.
5. Pour 15-20 ml of Raka-Ray Medium into each Petri dish and allow to solidify.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Overlay Technique for Enumeration of Lactic Acid Bacteria

1. Inoculate 0.1 ml of the beer sample onto well-dried plates containing 15-20 ml Raka-Ray No. 3 Medium. Five replicates of each sample are recommended.
2. Spread over the surface of the medium using a sterile glass rod.
3. Overlay the surface with 4 ml of the molten sterilized medium cooled to 50°C.
4. Incubate plates at 27-30°C in an anaerobic (H_2/CO_2) atmosphere.

Results

Lactobacilli are visible after 48 hours incubation as smooth, moist colonies that are 1 mm in diameter. Incubate the medium for a total of 7 days to allow development of slow-growing *Pediococcus* strains.

If the number of colonies on each plate exceeds 300, the sample should be diluted 1:10 in sterile physiological saline and retested.

References

1. **Saha, R. B., R. J. Sondag, and J. E. Middlekauff.** 1974. An improved medium for the selective culturing of lactic acid bacteria. Proceedings of the American Society of Brewing Chemists. 9th Congress, 9-10.
2. **VanKeer, C., L. Van Melkebeke, W. Vertriest, G. Hoozee, and E. Van Schoonenberghe.** 1983. Growth of *Lactobacillus* species on different media. J. Inst. of Brewing **89**:360-363.
3. **Report of the Technical Subcommittee.** 1976. Microbiological Controls. J. Am. Soc. of Brewing Chemists **34**:93-94.
4. **European Brewing Congress Analytica Microbiologica.** 1981. J. Inst. of Brewing **87**:314.

Packaging

Raka-Ray No. 3 Broth	500 g	1865-17
Raka-Ray No. 3 Medium	500 g	1867-17

Rappaport-Vassiliadis Medium Semisolid

Bacto® Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification · Novobiocin Antimicrobic Supplement

Intended Use

Bacto Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification is is used with Bacto Novobiocin Antimicrobic Supplement in rapidly detecting motile *Salmonella* in feces and food products.

Summary and Explanation

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification is a modification of Rappaport-Vassiliadis enrichment broth for detecting motile *Salmonella* in feces and food products. The original work on

MSRV medium showed that a semi-solid medium in Petri dishes could be used as a rapid and sensitive means of isolating motile *Salmonella* from food products following pre-enrichment or selective enrichment.[1,2] The semisolid medium allows motility to be detected as halos of growth around the original point of inoculation.

The medium is recommended by the European Chocolate Manufacturer's Association. A collaborative study performed with support of the American Cocoa Research Institute (ACRI) and the Canadian Chocolate Manufacturer's Association (CCMA) resulted in first action adoption of the MSRV method by AOAC International.[3]

MSRV Medium may be used as a plating medium for isolating *Salmonella* spp. (other than *S. typhi* and *S. paratyphi* type A) from stool specimens with high sensitivity and specificity.[4,5]

Principles of the Procedure

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification contains Tryptose and Casein Hydrolysate as carbon and nitrogen sources for general growth requirements. Magnesium Chloride raises the osmotic pressure in the medium. Novobiocin (Novobiocin Antimicrobic Supplement) and Malachite Green inhibit organisms other than *Salmonella*. The low pH of the medium combined with the Novobiocin, Malachite Green and Magnesium Chloride select for highly resistant *Salmonella* spp. Bacto Agar is the solidifying agent.

Formula

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification

Formula per Liter

Bacto Tryptose	4.59 g
Casein Hydrolysate (Acid)	4.59 g
Sodium Chloride	7.34 g
Potassium Dihydrogen Phosphate	1.47 g
Magnesium Chloride Anhydrous	10.93 g
Malachite Green Oxalate	0.037 g
Bacto Agar	2.7 g

Final pH 5.2 ± 0.2 at 25°C

Novobiocin Antimicrobic Supplement

Formula per 10 ml

Sodium Novobiocin	20 mg

Precautions

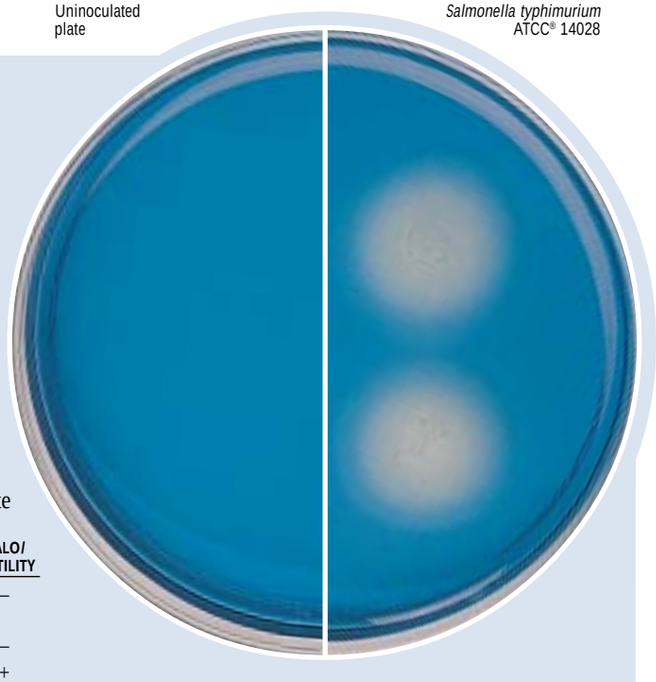
1. For Laboratory Use.
2. **Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification**

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Nerves, Kidneys.

   Novobiocin Antimicrobic Supplement

   HARMFUL. HARMFUL BY INHALATION AND IF SWALLOWED. (EC) MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pale green, homogeneous, free-flowing.
Solution:	3.16% solution, soluble in distilled or deionized water upon boiling. Blue, clear to slightly opalescent.
Prepared Medium:	Blue, slightly opalescent, no significant precipitate, semisolid.
Reaction of 3.16% Solution at 25°C:	pH 5.2 ± 0.2

Cultural Response

Prepare Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification per label directions. Inoculate using three drops (approximately 0.1 ml) at discreet locations on the plate and incubate at 42 ± 0.5°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HALO/ MOTILITY
Citrobacter freundii	8090	1,000-2,000	marked to complete inhibition	–
Pseudomonas aeruginosa	27853*	1,000-2,000	none	–
Salmonella typhimurium	14028*	100-1,000	good	+
Salmonella senftenberg (NCTC)	10384	100-1,000	good	+

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Store Novobiocin Antimicrobic Supplement at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification

Novobiocin Antimicrobic Supplement

Materials Required but not Provided

Flask with closure

Distilled or deionized water

Autoclave

Incubator (35°C)

Waterbath

Method of Preparation

1. Suspend 31.6 grams of Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. Do not autoclave.
3. Cool to 50°C.
4. Aseptically add 10 ml Novobiocin Antimicrobic Supplement, rehydrated per label instructions with sterile distilled or deionized water. Mix well.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure[3,6]

Pre-enrichment

1. Add 25 grams of cocoa or chocolate to 225 ml of sterile reconstituted nonfat dry milk with 0.45 ml of a 1% aqueous brilliant green dye solution; mix well.[6]
2. Incubate at 35°C for 20 ± 2 hours.[3]

Selective Enrichment[3]

3. Inoculate 10 ml Tetrathionate Broth (prewarmed to 35°C) with 1 ml of the pre-enrichment culture.
4. Incubate at 35°C for 8 ± 0.5 hours.

Motility Enrichment on MSRV[3]

5. After selective enrichment incubation, mix the broth culture. Inoculate 3 drops at separate spots on an MSRV plate.
6. Incubate at 42 ± 0.5°C for 16 ± 0.5 hours.

Results

Positive: Growth of migrated cells is visible as a gray-white, turbid zone extending out from the inoculated drop. Test sample is considered presumptively positive for motile *Salmonella*.

Negative: Medium remains blue-green around the drops, with no gray-white, turbid zone extending out from the drop. Test sample is considered negative for motile *Salmonella*.

To confirm a presumptive identification of *Salmonella*:[3]

Rapid serologic confirmation

1. Inoculate M Broth with growth from migration edge on MSRV plate.
2. Incubate at 35°C for 4 to 6 hours (until turbid). M-broth culture can be held for up to 24 hours at 35°C.
3. Test with *Salmonella* O and H antisera.

Culture confirmation

1. Transfer a loopful of growth from the migration edge on MSRV plate onto Hektoen Enteric Agar and streak for isolation.
2. Incubate at 35°C for 24 ± 2 hours.
3. From colonies of Hektoen agar that show colony appearance typical of *Salmonella* (green colonies with black centers), perform biochemical tests to confirm the identification.

Limitations of the Procedure

The combination of malachite green, magnesium chloride and a low pH may inhibit certain *Salmonella*, such as *S. typhi* and *S. choleraesuis*. Isolation techniques should include a variety of enrichment broths and isolation media.

References

1. **DeSmedt, J. M., R. Bolderdijk, H. Rappold, and D. Lautenschlaeger.** 1986. Rapid *Salmonella* detection in foods by motility enrichment on a modified semi-solid Rappaport-Vassiliadis medium. J. Food Prot. **49**:510-514.
2. **DeSmedt, J. M., and R. Bolderdijk**. 1987. Dynamics of *Salmonella* isolation with modified semi-solid Rappaport-Vassiliadis medium. J. Food Prot. **50**:658- 661.
3. **DeSmedt, J. M., R. Bolderdijk, and J. Milas**. 1994. *Salmonella* detection in cocoa and chocolate by motility enrichment on modified semi-solid Rappaport-Vassiliadis medium: a collaborative study. J. AOAC Int. **77**:365-373.
4. **Dusch, H., and M. Altwegg**. 1995. Evaluation of five new plating media for isolation of *Salmonella* sp. J. Clin. Micro. **33**:802-804.
5. **Aspinall, S. T., M. A. Hindle, and D. N. Hutchinson**. 1992. Improved isolation of Salmonellae from faeces using a semi-solid Rappaport-Vassiliadis Medium. Eur. J. Clin. Microbiol. Infect. Dis. **11**:936-939.
6. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella.* p. 5.01-5.20. *In* FDA bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification	500 g	1868-17
Novobiocin Antimicrobic Supplement	6 x 10 ml	3197-60*

*Store at 2-8%C

Bacto® Rappaport-Vassiliadis R10 Broth

Intended Use

Bacto Rappaport-Vassiliadis R10 Broth is used for selectively enriching *Salmonella* from meat and dairy products, feces and sewage polluted water.

Also Known As

Rappaport-Vassiliadis R10 Broth is also known as RV Enrichment Broth or R10 Broth.

Summary and Explanation

Rappaport et al[1] formulated an enrichment medium for *Salmonella* that was modified by Vassiliadis et al.[2] The Rappaport formulation, designated R25/37°C, recommended incubation at 37°C; the Vassiliadis modification, designated R10/43°C, had a reduced level of malachite green and recommended incubation at 43°C. Later work by Peterz showed that incubation at 41.5° ± 0.5°C for 24 hours improved recovery of *Salmonella* spp.[3]

Rappaport-Vassiliadis R10 Broth is a selective enrichment medium that is used following pre-enrichment of the specimen in a suitable pre-enrichment medium. It has gained approval for use in analyzing milk and milk products,[4] raw flesh foods, highly contaminated foods and animal feeds.[5,6]

This medium selectively enriches for salmonellae because bacteria, including other intestinal bacteria, are typically resistant to or inhibited by malachite green, high osmotic pressure and/or low pH. *S. typhi* and *S. choleraesuis* are sensitive to malachite green and may be inhibited.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Pale green to green, free-flowing, homogeneous.
Solution:	2.66% solution, soluble in distilled or deionized water upon gentle heating; blue, clear.
Reaction of 2.66% Solution at 25°C:	pH 5.1 ± 0.2

Cultural Response

Prepare Rappaport-Vassiliadis R10 Broth per label directions. Inoculate and incubate at 41.5 ± 0.5°C for 18-48 hours. Subculture to Brilliant Green Agar and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Escherichia coli	25922*	1,000-2,000	markedly inhibited
Salmonella enteritidis	13076	100-1,000	good
Salmonella typhimurium	14028*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Rappaport-Vassiliadis R10 Broth contains Tryptone as carbon and nitrogen sources for general growth requirements. Magnesium Chloride raises the osmotic pressure in the medium. Malachite Green is inhibitory to organisms other than salmonellae. The low pH of the medium (5.1 ± 0.2 at 25°C), combined with the presence of malachite green and magnesium chloride, select for the highly resistant *Salmonella* spp.

Formula

Rappaport-Vassiliadis R10 Broth

Formula Per Liter

Bacto Tryptone	4.54 g
Sodium Chloride	7.2 g
Potassium Dihydrogen Phosphate	1.45 g
Magnesium Chloride Anhydrous	13.4 g
Malachite Green Oxalate	0.036 g

Final pH 5.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Nerves, Kidneys.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious material.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Rappaport-Vassiliadis R10 Broth

Materials Required but not Provided

Flask with closure
Containers suitable for 10 ml aliquots
Distilled or deionized water
Autoclave

Method of Preparation

1. Suspend 26.6 grams in 1 liter distilled or deionized water. Heat gently to dissolve.
2. Dispense 10 ml amounts into suitable containers. Sterilize at 115-116°C for 15 minutes.

Specimen Preparation

Consult an appropriate reference for specific instructions related to the type of product being tested.[4,5,6]

Test Procedure

Water and Sewage Samples

For isolating *Salmonella* (other than *S. typhi*) from water and associated materials such as sewage liquor, sewage sludge, digested sludge and pressed sludge cake.

1. Concentrate the sample by filtering it through a plug of sterile absorbent cottonwool inserted in the neck of a large sterile funnel or through a Whatman No. 17 absorbent pad.

Pre-enrichment

2. Using aseptic technique, transfer the cottonwool plug or the pad to 100 ml of a suitable pre-enrichment medium such as Buffered Peptone Water.
3. Incubate at 37 ± 0.5°C for 18-24 hours.

Selective Enrichment

4. Inoculate 10 ml of Rappaport-Vassiliadis R10 Broth with 0.1 ml of the pre-enrichment culture. Inoculate 10 ml of Muller-Kauffman Tetrathionate Broth with 1 ml of the pre-enrichment culture.
5. Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C. Incubate Muller- Kauffman Tetrathionate Broth at 42 ± 1°C for 48 hours.

Results

6. After incubation, subculture both selective enrichment broths to Brilliant Green Agar and XLD Agar. Incubate at 35 ± 2°C for 18-24 hours.
7. Examine for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and serologic tests.

Milk and Foods

For isolating *Salmonella* (other than *S. typhi*) from milk and milk products,[4] raw flesh foods, highly contaminated foods and animal feeds.[5,6]

Pre-enrichment

1. Add 25 grams or a 25 ml sample of the specimen to 225 ml of pre-enrichment medium. Consult appropriate references for the type of product being tested.[4,5,6]
2. Incubate at 35°C for 24 ± 2 hours[5,6] or at 37°C for 16-20 hours,[4] depending on the referenced procedure being followed.

Selective Enrichment

1. Inoculate 10 ml of Rappaport-Vassiliadis R10 Broth with 0.1 ml of pre-enrichment culture. Inoculate 10 ml of another selective enrichment medium such as Tetrathionate Broth or Selenite Cystine Broth with 1 ml of the pre-enrichment culture.[4,5,6]
2. Incubate Rappaport-Vassiliadis R10 Broth at 41.5 ± 0.5°C[4] for 24 ± 2 hours. Incubate the other selective enrichment broths appropriately.

Results

1. After incubation, subculture Rappaport-Vassiliadis R10 Broth and the other selective enrichment broths to selective agar media and incubate at 35 ± 2°C for 24 ± 2 hours.[4,5]
2. Examine for typical *Salmonella* colonies. Confirm identification of isolates by biochemical and serologic tests.

Limitations of the Procedure

The combined inhibitory factors of this medium (malachite green, magnesium chloride, low pH) may inhibit certain *Salmonella*, such as *S. typhi* and *S. choleraesuis*. Isolation techniques should include a variety of enrichment broths and isolation media.

References

1. **Rappaport, F., N. Konforti, and B. Navon**. 1956. A new enrichment medium for certain salmonellae. J. Clin. Pathol. **9**:261-266.
2. **Vassiliadis, P., D. Trichopoulos, A. Kalandidi, and E. Xirouchaki**. 1978. Isolation of salmonellae from sewage with a new procedure of enrichment. J. Appl. Bacteriol. **44**:233-239.
3. **Peterz, M., C. Wiberg, and P. Norberg**. 1989. The effect of incubation temperature and magnesium chloride concentration on growth of salmonella in home-made and commercially available dehydrated Rappaport-Vassiliadis broths. J. Appl. Bacteriol. **66**:523-528.
4. **International Dairy Federation**. 1995. Milk and milk products: detection of *Salmonella*. IDF Standard **93B**:1005. Brussels, Belgium.
5. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella.* p. 5.01-5.20. *In* FDA bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **Andrews, W. H.** (ed.). 1995. Microbial methods, p.1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Rappaport-Vassiliadis R10 Broth	500 g	1858-17

Bacto® Reinforced Clostridial Medium

Intended Use

Bacto Reinforced Clostridial Medium is used for cultivating and enumerating clostridia, other anaerobes, and other species of bacteria from foods and clinical specimens.

Summary and Explanation

Reinforced Clostridial Medium is a semisolid medium formulated by Hirsch and Grinstead.[1] Their work demonstrated that the medium outperformed other media in supporting growth of clostridia from small inocula and produced higher viable cell counts.[1] Barnes and Ingram[2]

used the medium to dilute vegetative cells of *Clostridium perfringens*. Barnes et al[3] used a solid (agar) version of the medium to enumerate clostridia in food. The medium is a non-selective enrichment medium and grows various anaerobic and facultative bacteria when incubated anaerobically.[4]

Principles of the Procedure

Reinforced Clostridial Agar contains Tryptose and Beef Extract as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. Sodium Chloride maintains the osmotic balance. In low concentrations, Soluble Starch detoxifies metabolic by-products. Cysteine Hydrochloride is the reducing agent. Sodium Acetate acts as a buffer. The small amount of Bacto Agar makes the medium semisolid.

Formula

Reinforced Clostridial Medium

Formula Per Liter		
Bacto Tryptose	10	g
Bacto Beef Extract	10	g
Bacto Yeast Extract	3	g
Bacto Dextrose	5	g
Sodium Chloride	5	g
Soluble Starch	1	g
Cysteine Hydrochloride	0.5	g
Sodium Acetate	3	g
Bacto Agar	0.5	g

Final pH 6.8 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, slightly opalescent. Upon cooling medium becomes more opalescent.
Reaction of 3.8% Solutionat 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Reinforced Clostridial Medium per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacteroides fragilis	23745	100-1,000	good
Clostridium botulinum	25763	100-1,000	good
Clostridium perfringens	13124*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Reinforced Clostridial Medium

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C, anaerobic conditions)

Method of Preparation

1. Suspend 38 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Hirsch, A., and E. Grinstead**. 1954. Methods for the growth and enumeration of anaerobic spore formers from cheese, with observations on the effect of nisin. J. Dairy Res. **21**:101-110.
2. **Barnes, E. M., and M. Ingram**. 1956. The effect of redox potential on the grown *Clostridium welchii* strain isolated from horse muscle. J. Appl. Bacteriol. **19**:117- 128.
3. **Barnes, E. M., J. E. Despaul, and M. Ingram**. 1963. The behavior of a food poisoning strain of *Clostridium welchii* in beef. J. Appl. Bacteriol. **26**:415.
4. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 660-668. Williams & Wilkins, Baltimore, MD.

Packaging

Reinforced Clostridial Medium	500 g	1808-17

Bacto® Riboflavin Assay Medium

Intended Use

Riboflavin Assay Medium is used for determining riboflavin concentration by the microbiological assay technique.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For maintaining the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose.
2. Inoculum Media: To condition the test culture for immediate use.
3. Assay Media: To permit quantitation of the vitamin under test.

Riboflavin Assay Medium is a modification of the medium described by Snell and Strong.[1] It is recommended for use in the microbiological assay of riboflavin following the methodology outlined by the U.S. Food and Drug Administration[2] using *Lactobacillus casei* subsp. *rhamnosus* ATCC® 7469 as the test organism.

Principles of the Procedure

Riboflavin Assay Medium is free from riboflavin but contains all other nutrients and vitamins essential for the growth of *Lactobacillus casei* subsp. *rhamnosus* ATCC® 7469. The addition of riboflavin in specified increasing concentrations gives a growth response that can be measured turbidimetrically or titrimetrically.

Formula

Riboflavin Assay Medium

Formula Per Liter	
Bacto Dextrose	20 g
Sodium Acetate	15 g
Bacto Vitamin Assay Casamino Acids	10 g
Dipotassium Phosphate	1 g
Monopotassium Phosphate	1 g
L-Asparagine	0.6 g
DL-Tryptophane	0.2 g
L-Cystine	0.2 g
Adenine Sulfate	20 mg
Guanine Hydrochloride	20 mg
Uracil	20 mg
Xanthine	20 mg
Magnesium Sulfate USP	0.4 g
Ferrous Sulfate	20 mg
Manganese Sulfate Monohydrate	20 mg
Sodium Chloride USP	20 mg
Pyridoxine Hydrochloride	4 mg
Pyridoxal Hydrochloride	4 mg
p-Aminobenzoic Acid	2 mg
Calcium Pantothenate	800 µg
Folic Acid	800 µg
Nicotinic Acid	800 µg
Thiamine Hydrochloride	400 µg
Biotin	1 µg

Final pH 6.8 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.4% solution (single strength) and 4.8% (double strength), soluble in distilled or deionized water on boiling. Light to medium amber, clear, may have a slight precipitate.
Prepared Medium:	Light amber, clear, may have a very slight precipitate.
Reaction of 2.4% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Riboflavin Assay Medium per label directions. The medium supports the growth of *L. casei* subsp. *rhamnosus* ATCC® 7469 when prepared in single strength and supplemented with riboflavin. Measure growth response turbidimetrically with increasing concentrations of riboflavin to produce a standard curve.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. Take great care to avoid contamination of media or glassware for microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware, free from detergents and other chemicals, must be used.
4. Take precautions to keep sterilizing and cooling conditions uniform throughout assay. Glassware is heated to 250°C for at least 1 hour to burn off any organic residues that might be present.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Riboflavin Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus casei* subsp. *rhamnosus* ATCC® 7469
Sterile tubes
Sterile 0.85% saline
Distilled or deionized water
Lactobacilli Agar AOAC or Micro Assay Culture Agar
Lactobacilli Broth AOAC or Micro Inoculum Broth
Riboflavin USP
Spectrophotometer

Method of Preparation

1. Suspend 4.8 grams in 100 ml of distilled or deionized water.
2. Boil 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assays, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Follow assay procedures as outlined in AOAC.[1] Levels of riboflavin used in the determination of the standard curve should be prepared according to this reference or according to the following procedure.

Stock Cultures

Stock cultures of *L. casei* subsp. *rhamnosus* ATCC® 7469 are prepared by stab inoculation into 10 ml of Lactobacilli Agar AOAC. After 24-48 hours incubation at 35-37°C, the stock cultures are kept in the refrigerator. Transfers are made at monthly intervals in triplicate.

Inoculum

Inoculum for assay is prepared by subculturing a stock culture of *L. casei* subsp. *rhamnosus* ATCC® 7469 into 10 ml of Lactobacilli Broth AOAC or Micro Inoculum Broth. Following incubation for 16-24 hours at 35-37°C, the culture is centrifuged under aseptic conditions and the supernatant liquid decanted. After washing 3 times with 10 ml sterile 0.85% saline, the cells are resuspended in 10 ml sterile 0.85% saline. The cell suspension is then diluted with sterile 0.85% saline, to a turbidity of 35-40% transmittance when read on the spectrophotometer at 660 nm. One drop of this latter suspension is then used to inoculate each of the assay tubes.

Riboflavin Assay Medium may be used for both turbidimetric and titrimetric determinations. Turbidimetric readings should be made after 18-24 hours incubation at 35-37°C, where as titrimetric determinations are best made after 72 hours incubation at 35-37°C. Using Riboflavin Assay Medium, the most effective assay range is between 0.025 and 0.15 µg riboflavin.

Standard Curve

It is essential that a standard curve be constructed each time an assay is run. Conditions of autoclaving and temperature of incubation, which influence the standard curve readings, cannot be duplicated exactly from assay to assay. The standard curve is obtained by using Riboflavin USP Reference Standard or equivalent at levels of 0.0, 0.025, 0.05, 0.075, 0.1, 0.15, 0.2 and 0.3 µg riboflavin per assay tube (10 ml).

The concentration of riboflavin required for the preparation of the standard curve may be prepared by dissolving 0.1 g of Riboflavin USP Reference Standard or equivalent in 1,000 ml of distilled water by heating, giving a stock solution of 100 µg per ml. Dilute the stock solution by adding 1 ml to 999 ml distilled water. Use 0.0, 0.25, 0.5, 0.75, 1, 1.5, 2 and 3 ml of the diluted stock solution per tube. Prepare the stock solution fresh daily.

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average and use the results only if two thirds of the values do not vary by more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.
5. Maintain pH below 7.0 to prevent loss of riboflavin.

References

1. **Snell and Strong.** 1939. Ind. and Eng. Chem. **11**:346.
2. **Association of Analytical Chemists.** 1996. U.S. Food and Drug Administration methods or the microbiological analysis of selected nutrients. AOAC Internationl, Gaithersburg, MD.

Packaging

Riboflavin Assay Medium	100 g	0325-15*

*Store at 2-8°C

Bacto® Rice Extract Agar

Intended Use

Bacto Rice Extract Agar is used for differentiating *Candida albicans* and other *Candida* spp. based on chlamydospore formation.

Summary and Explanation

Rice Extract Agar is prepared according to the formulation of Taschdjian.[1] Chlamydospores were observed consistently and in abundance upon this medium 17-24 hours after inoculation with *Candida albicans*. The morphology of both the pathogenic and nonpathogenic *Candida* agreed in every respect with that of the cultures grown on corn meal agar; corn meal agar was the medium most routinely used in laboratories at that time, but was time-consuming and laborious to prepare. In later studies Taschdjian[2] and Kelly and Funigiello[3] showed that the addition of Tween® 80 (polysorbate 80) to Rice Extract Agar enhanced chlamydospore formation by *C. albicans*. The addition of 2% dextrose enhanced pigment production by *Trichophyton rubrum* permitting the differentiation between this dermatophyte and *Trichophyton mentagrophytes*.

Taubert and Smith[4] recommended Rice Extract Agar for use in the diagnosis of vulvovaginal candidiasis. A cotton-tipped applicator was

used for obtaining a specimen and then rolled on the surface of a rice extract agar plate; a cover glass was then applied to the agar, covering most of the inoculum.

Principles of the Procedure

The Rice Extract provides the sole source of nutrients in the medium. This lack of nutrients together with the oxygen-deficient culture conditions (covering the inoculum with a cover glass) creates a deficient environment that induces the formation of specific morphological forms (chlamydospores and pseudomycelia in particular) in some yeasts. The addition of Tween 80 further stimulates chlamydospore formation due to its content of oleic acids. Bacto Agar is incorporated into the medium as a solidifying agent.

Formula

Rice Extract Agar

Formula Per Liter	
White Rice, Extract from	20 g
Bacto Agar	20 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.5% solution, soluble in distilled or deionized water upon boiling. Solution is light amber, opalescent with precipitation.
Prepared Medium:	Colorless to light amber, opaque, precipitate.
Reaction of 2.5% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Prepare Rice Extract Agar per label directions. Inoculate and incubate at 23-25°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	CHLAMYDOSPORES
Candida albicans	10231	30-300	good	+
Candida albicans	26790	30-300	good	+

The cultures listed are the minimum that should be used for performance testing.

Procedure

Materials Provided

Rice Extract Agar

Materials Required But Not Provided

Glassware
Autoclave
Distilled or deionized water

Method of Preparation

1. Suspend 25 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically dispense medium into sterile Petri dishes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Inoculate the plates by cutting through the surface of the agar with an inoculating wire.
2. Cover the inoculated area with a sterile cover slip.
3. Invert plates and incubate at 23-25°C for 18-72 hours.
4. Examine for chlamydospores microscopically using approximately 100X magnification and by focusing upon the line of inoculation.

Results

After 24 to 48 hours most strains of *C. albicans* and *C. stellatoidea* will have formed typical chlamydospores.[5]

Limitations of the Procedure

1. Further studies should be performed to confirm the results obtained.
2. Tween 80 enhances chlamydospore production in many species of *Candida*. It is therefore necessary to use additional media for species identification.[6]
3. High temperatures for incubation should be avoided as chlamydospores are not formed at 37°C.

References

1. **Taschdjian, C. L.** 1953. A simple prepared identification medium for *Candida albicans*. Mycologia **45**:474.
2. **Taschdjian, C. L.** 1957. Routine identification of *Candida albicans*: Current methods and a new medium. Mycologia **49**:332.
3. **Kelly, J. P., and F. Funigiello.** 1959. *Candida albicans*: A study of media designed to promote chlamydospore production. J. Lab. Clin. Med. **53**:807-809.
4. **Taubert, H. D., and A. G. Smith.** 1960. The clinical use of Taschdjian's medium in the diagnosis of vulvovaginal candidiasis. J. Lab. Clin. Med. **55**:820- 828.
5. **Cooper, and Silva-Hutner**. 1985. *In* Lennette, Balows, Hausler, and Shadomy (ed.), Manual of clinical microbiology, 4th ed. ASM, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Rice Extract Agar	500 g	0899-17

Bacto® Rogosa SL Agar
Bacto Rogosa SL Broth

Intended Use

Bacto Rogosa SL Agar and Bacto Rogosa SL Broth are used for cultivating oral, vaginal and fecal lactobacilli.

Also Known As

Rogosa SL Agar is also known as RMW Agar.

Summary and Explanation

Rogosa SL Agar and Broth are a modification of media described by Rogosa, Mitchell and Wiseman.[1,2] These media are used for isolation, enumeration and identification of lactobacilli in oral bacteriology, feces, vaginal specimens and foodstuffs.[3,4] The low pH and high acetate concentrations effectively suppress other bacterial flora allowing lactobacilli to flourish.

User Quality Control

Identity Specifications

Rogosa SL Agar

Dehydrated Appearance:	Beige, homogeneous with soft clumps.
Solution:	7.5% solution, soluble in distilled or deionized water upon boiling. Solution is light amber, slightly opalescent and may have a slight precipitate.
Reaction of 7.5% Solution at 25°C:	pH 5.4 ± 0.2

Rogosa SL Broth

Dehydrated Appearance:	Beige, appears moist, slightly lumpy.
Solution:	6.0% solution, soluble in distilled or deionized water upon boiling. Solution is light amber, clear to slightly opalescent.
Reaction of 6.0% Solution at 25°C:	pH 5.4 ± 0.2

Cultural Response

Rogosa SL Agar

Prepare Rogosa SL Agar per label directions. Inoculate and incubate at 35 ± 2°C for 40-48 hours.

Rogosa SL Broth

Prepare Rogosa SL Broth per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus casei	9595	100-1,000	good
Lactobacillus delbrueckii	4797	100-1,000	good
Staphylococcus aureus	25923*	1,000-2,000	marked to complete inhibition

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Tryptone provides carbon and nitrogen. Yeast Extract is a source of trace elements, vitamins and amino acids. Dextrose, Arabinose and Saccharose are carbohydrate sources that provide carbon. Sodium Acetate and Ammonium Citrate inhibit streptococci, molds and other oral microbial flora and restrict swarming. Monopotassium Phosphate provides buffering capability. Magnesium Sulfate, Manganese Sulfate and Ferrous Sulfate are sources of inorganic ions. Sorbitan Monooleate (Polysorbate 80) acts as a surfactant. Bacto Agar is a solidifying agent.

Formula

Rogosa SL Agar

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Bacto Dextrose	10 g
Bacto Arabinose	5 g
Bacto Saccharose	5 g
Sodium Acetate	15 g
Ammonium Citrate	2 g
Monopotassium Phosphate	6 g
Magnesium Sulfate	0.57 g
Manganese Sulfate	0.12 g
Ferrous Sulfate	0.03 g
Sorbitan Monooleate	1 g
Bacto Agar	15 g

Final pH 5.4 ± 0.2 at 25°C

Rogosa SL Broth

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Bacto Dextrose	10 g
Bacto Arabinose	5 g
Bacto Saccharose	5 g
Sodium Acetate	15 g
Ammonium Citrate	2 g
Monopotassium Phosphate	6 g
Magnesium Sulfate	0.57 g
Manganese Sulfate	0.12 g
Ferrous Sulfate	0.03 g
Sorbitan Monooleate	1 g

Final pH 5.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated media at 2-8°C. The dehydrated media are very hygroscopic. Keep containers tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Rogosa SL Agar

Rogosa SL Broth

Materials Required but not Provided

Glassware

Distilled or deionized water

Glacial acetic acid

Incubator (35°C)

Method of Preparation

Rogosa SL Agar

1. Suspend 75 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Add 1.32 ml glacial acetic acid and mix well.
4. Boil 2-3 minutes. DO NOT AUTOCLAVE.

Rogosa SL Broth

1. Suspend 60 grams in 1 liter distilled or deionized water.
2. Add 1.32 ml glacial acetic acid and mix well.
3. Boil 2-3 minutes. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

The salt in the formulation makes the media not suitable for isolation of dairy lactobacilli; e.g., *L. lactis, L. bulgaricus and L. helveticus*.[4]

References

1. **Rogosa, M., J. A. Mitchell, and R. F. Wiseman.** 1951. A selective medium for the isolation and enumeration of oral and fecal lactobacilli. J. Bacteriol. **62**:132.
2. **Rogosa, M., J. A. Mitchell, and R. F. Wiseman.** 1951. A selective medium for the isolation and enumeration of oral and fecal lactobacilli. J. Dental Res. **30**:682.
3. **Vedamuthu, E. R., M. Raccach, B. A. Glatz, E. W. Seitz, and M. S. Reddy.** 1992. Acid-producing Microorganisms. *In* C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Assoc., Washington, D.C.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 678-680. Williams & Wilkins, Baltimore, MD.

Packaging

Rogosa SL Agar	500 g	0480-17
	10 kg	0480-08
Rogosa SL Broth	500 g	0478-17

Rose Bengal Agar

Bacto® Rose Bengal Agar Base · Bacto Rose Bengal Antimicrobic Supplement C

Intended Use

Bacto Rose Bengal Agar Base is used with Bacto Rose Bengal Antimicrobic Supplement C in isolating and enumerating yeasts and molds.

Also Known As

Rose Bengal Agar is also known as Rose Bengal Chloramphenicol Agar and Rose Bengal-Malt Extract Agar.

Summary and Explanation

A number of methods have been described for the selective isolation of fungi from environmental materials and foodstuffs containing mixed populations of fungi and bacteria. The use of media with an acid pH that selectively inhibits the growth of bacteria and thereby promotes the growth of fungi has been widely employed.[1,2,3] A number of investigators have reported, however, that acidified media may actually inhibit fungal growth,[4,5] fail to completely inhibit bacterial growth,[5] and have little effect in restricting the size of mold colonies.[6] Smith and Dawson[7] used Rose Bengal in a neutral pH medium for the selective isolation of fungi from soil samples. Chloramphenicol, streptomycin, oxytetracycline and chlortetracycline have been used for the improved, selective isolation and enumeration of yeasts and molds from soil, sewage and foodstuffs.[4,8,9,10,11]

Rose Bengal Agar Base supplemented with Rose Bengal Antimicrobic Supplement C is a modification of the Rose Bengal Chlortetracycline Agar formula of Jarvis.[11] Instead of chlortetracycline, chloramphenicol is employed in this medium as a selective supplement. Of the antibiotics most frequently employed in media of neutral pH, chloramphenicol is recommended because of its heat stability and broad antibacterial spectrum.[12] Rose Bengal Agar is recommended in standard methods for the enumeration of yeasts and molds from foodstuffs and water.[12,13,14,15]

Principles of the Procedure

Soytone provides the carbon and nitrogen sources required for good growth of a wide variety of organisms. Dextrose is an energy source.

Monopotassium Phosphate provides buffering capability. Magnesium Sulfate provides necessary trace elements. Rose Bengal is included as a selective agent that inhibits bacterial growth and restricts the size and height of colonies of the more rapidly growing molds. The restriction in growth of molds aids in the isolation of slow-growing fungi by preventing overgrowth by more rapidly growing species. Rose Bengal is taken up by yeast and mold colonies, thereby facilitating their recognition and enumeration. Rose Bengal Antimicrobic Supplement C is a lyophilized antimicrobic supplement containing chloramphenicol which inhibits bacteria. Bacto Agar is the solidifying agent.

Formula

Rose Bengal Agar Base

Formula Per Liter

Bacto Soytone	5 g
Bacto Dextrose	10 g
Monopotassium Phosphate	1 g
Magnesium Sulfate	0.5 g
Rose Bengal	0.05 g
Bacto Agar	15 g

Final pH 7.2 ± 0.2 at 25°C

Rose Bengal Antimicrobic Supplement C

Formula Per 2 ml Vial

Chloramphenicol	0.05 g

Precautions

1. For Laboratory Use.
2. **Rose Bengal Antimicrobic Supplement C**

   TOXIC. MAY CAUSE CANCER. MAY CAUSE HERITABLE GENETIC DAMAGE. POSSIBLE RISK OF HARM TO THE UNBORN CHILD. MAY CAUSE SENSITIZATION BY INHALATION AND SKIN CONTACT. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately. (Show label where possible.) Do not breathe dust. Keep container tightly closed. Target Organs: Blood, Bone Marrow.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If swallowed seek medical advice immediately and show this container or label. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store **Rose Bengal Agar Base** dehydrated below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store the prepared medium at 2-8°C.

Store **Rose Bengal Antimicrobic Supplement C** at 2-8°C. Do not open or rehydrate vials until ready to use. Store rehydrated vials at 2-8°C and use within 24 hours.

User Quality Control

Identity Specifications

Rose Bengal Agar Base

Dehydrated Appearance:	Beige to faint pink, free-flowing, homogeneous.
Solution:	3.2% solution, soluble in distilled or deionized water on boiling. Solution is reddish pink, very slightly to slightly opalescent.
Complete Prepared Medium:	Bright pink, very slightly to slightly opalescent.
Reaction of 3.2% Solution at 25°C:	pH 7.2 ± 0.2

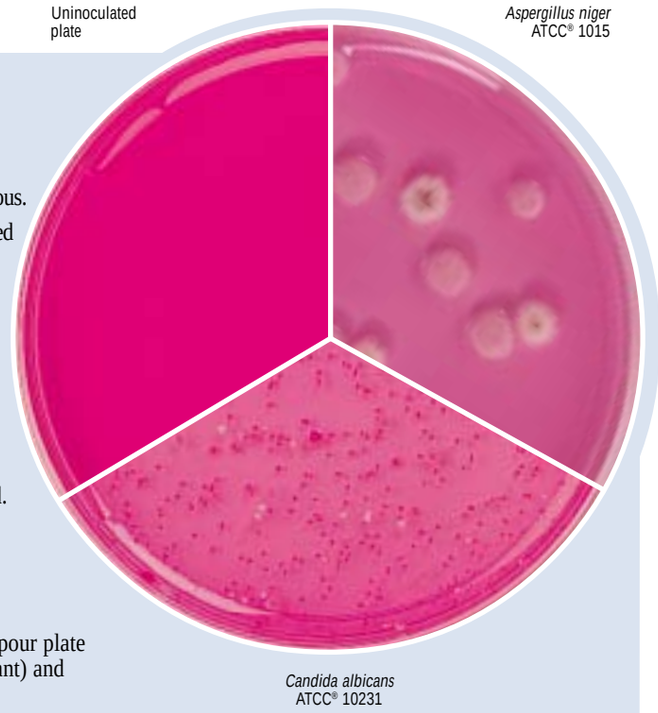
Rose Bengal Antimicrobic Supplement C

Lyophilized Appearance:	Lyophilized white cake, may be dispersed.
Rehydrated Appearance:	Colorless, clear.
Solubility:	Soluble in 2 ml ethanol.

Cultural Response

Prepare Rose Bengal Agar per label directions. Inoculate using the pour plate technique (for *Aspergillus niger*, inoculate the surface of an agar slant) and incubate aerobically at 25-30°C for up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	COLONY COLOR
Aspergillus niger	1015	100-300	good	white
Candida albicans	10231	100-300	good	pink
Escherichia coli	25922*	1,000-2,000	inhibited	–
Micrococcus luteus	10240	1,000-2,000	inhibited	–



The cultures listed are the minimum that should be used for performance testing.

*This culture is available as a Bactrol™ Disk and should be used as directed in Bactrol Disks Technical Information.

Expiration Date

The expiration date applies to the products in their intact containers when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Rose Bengal Agar Base
Rose Bengal Antimicrobic Supplement C

Materials Required But Not Provided

Glassware
Autoclave
Incubator (25°C)
Sterile Petri dishes
Ethanol (reagent grade)
Bent glass rods

Method of Preparation

1. **Rose Bengal Antimicrobic Supplement C:** To rehydrate, aseptically add 2 ml of ethanol per vial of dehydrated supplement and invert several times to dissolve the powder.
2. **Rose Bengal Agar Base:** To rehydrate, suspend 16 grams in 500 ml distilled or deionized water.
3. Heat to boiling to dissolve completely.
4. Sterilize the basal medium at 121°C for 15 minutes and then cool to 45-50°C.
5. Aseptically add 2 ml of the rehydrated Rose Bengal Antimicrobic Supplement C to 500 ml of cooled agar base. Mix thoroughly.
6. Dispense into sterile Petri dishes and allow to dry overnight at room temperature (21-25°C).

Specimen Collection and Preparation

Collect specimens in sterile containers and transport immediately to the laboratory in accordance with recommended guidelines.[12,13] Prepare samples for dilution plating inoculation. It is recommended that yeast and molds be enumerated by a surface spread-plate technique rather than with pour plates.[12] The spread-plate technique provides maximal exposure of cells to atmospheric oxygen and eliminates heat stress from molten agar.[12]

Test Procedure

1. Inoculate 0.1 ml of appropriate dilutions in duplicate on the solidified agar. Spread over the entire surface using a sterile bent glass rod.
2. Incubate plates at 25-30°C for up to 7 days.

Results

Colonies of yeast appear pink due to the uptake of rose bengal. Count plates containing 15 to 150 colonies and report the counts as colony forming units (CFU) per gram or ml of sample.

Limitations of the Procedure

1. Although this medium is selective primarily for fungi, microscopic examination is recommended for presumptive identification. Biochemical testing using pure cultures is required for complete identification.
2. Due to the selective properties of this medium and the type of specimen being cultured, some strains of fungi may be encountered that fail to grow or grow poorly on the complete medium; similarly, some strains of bacteria may be encountered that are not inhibited or only partially inhibited.
3. Care should be taken not to expose this medium to light since photodegradation of rose bengal yields compounds that are toxic to fungi.

References

1. **Waksman, S. A.** 1922. A method for counting the number of fungi in the soil. J. Bacteriol. **7**:339-341.
2. **Koburger, J. A.** 1976. Yeasts and molds, p. 225-229. *In* M. L. Speck (ed.), Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
3. **Mossel, D. A. A., M. Visser, and W. H. J. Mengerink.** 1962. A comparison of media for the enumeration of moulds and yeasts in foods and beverages. Lab Practice **11**:109-112.
4. **Martin, J. P.** 1950. Use of acid, rose bengal and streptomycin in the plate method for estimating soil fungi. Soil Sci. **69**:215-232.
5. **Koburger, J. A.** 1972. Fungi in foods. IV. Effect of plating medium pH on counts. J. Milk Food Technol. **35**:659-660.
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7. **Smith, N. R., and V. T. Dawson.** 1944. The bacteriostatic action of rose bengal in media used for the plate counts of soil fungi. Soil Sci. **58**:467-471.
8. **Cooke, W. B.** 1954. The use of antibiotics in media for the isolation of fungi from polluted water. Antibiotics and Chemotherapy **4**:657-662.
9. **Papavizas, G. C., and C. B. Davey.** 1959. Evaluation of various media and antimicrobial agents for isolation of soil fungi. Soil Sci. **88**:112-117.
10. **Overcast, W. W., and D. J. Weakley.** 1969. An aureomycin-rose bengal agar for enumeration of yeast and mold in cottage cheese. J. Milk Technol. **32**:442-445.
11. **Jarvis, B.** 1973. Comparison of an improved rose bengal-chlortetracycline agar with other media for the selective isolation and enumeration of molds and yeasts in foods. J. Appl. Bact. **36**:723-727.
12. **Mislivec, P. B., L. R. Beuchat, and M. A. Cousin.** 1992. Yeasts and Molds. *In* C. Vanderzant and D. F. Splittstoesser (eds.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Assoc., Washington, D.C.
13. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Assoc., Washington, D.C.
14. **Eaton, A.D., L.S. Clesceri, and A.E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
15. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Packaging

Rose Bengal Agar Base	500 g	1831-17
	10 kg	1831-08
Rose Bengal Antimicrobic Supplement C	6 x 2 ml	3352-54

Bacto® SABHI Agar Base

Intended Use

Bacto SABHI Agar Base is for use with chloromycetin and blood (optional) in isolating and cultivating pathogenic fungi.

Summary and Explanation

Sabouraud[1] formulated Sabouraud Dextrose Agar as a general purpose medium for the recovery of dermatophytes. Brain Heart Infusion is a highly nutritive medium used for cultivating a variety of fastidious organisms and medically important fungi.[2] SABHI Agar Base, prepared according to the formulation of Gorman[3], combines the ingredients from both Sabouraud Dextose Agar and Brain Heart Infusion. It is particularly useful for maximum recovery of *Blastomyces dermatitidis* and *Histoplasma capsulatum* from body tissues and fluids and as a primary recovery medium for saprophytic and pathogenic fungi.[4]

Gorman reported that the addition of blood to this medium increased recovery and conversion to the yeast phase of *H. capsulatum* and *B. dermatitidis*.[3,5] Selectivity can be obtained by adding chloromycetin or other antimicrobics to the medium.[5]

Principles of the Procedure

Infusions from Calf Brains and Beef Heart are sources of carbon, protein and nutrients. Proteose Peptone and Neopeptone are sources of nitrogen, amino acids and carbon. Dextrose is an additional carbon source. Sodium Chloride provides essential ions while maintaining osmotic balance. Disodium Phosphate provides buffering capacity. Bacto Agar is a solidifying agent. Chloromycetin, when added, is a broad spectrum antibiotic that inhibits a wide variety of gram-negative bacteria.

Formula

SABHI Agar Base

Formula Per Liter

Calf Brains, Infusion from	100 g
Beef Heart, Infusion from	125 g
Bacto Proteose Peptone	5 g
Bacto Neopeptone	5 g
Bacto Dextrose	21 g
Sodium Chloride	2.5 g
Disodium Phosphate	1.25 g
Bacto Agar	15 g

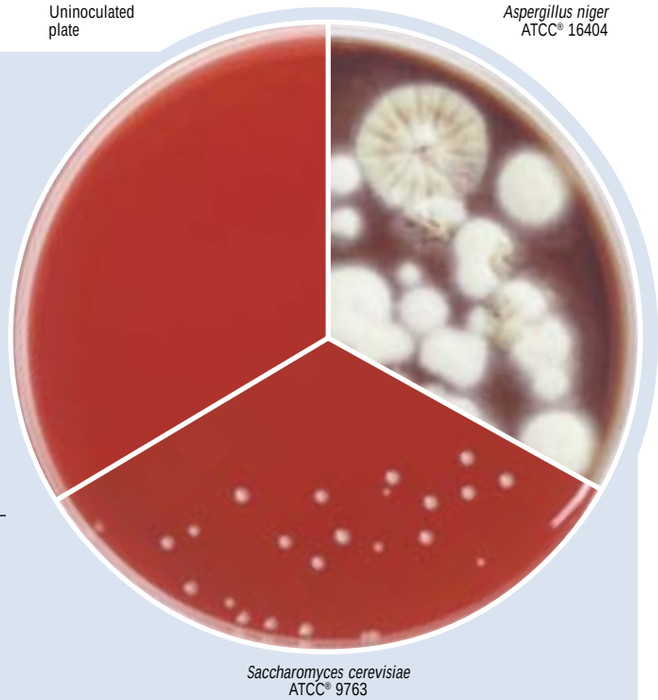
Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	5.9% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, slightly opalescent without significant precipitate.
Reaction of 5.9% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare SABHI Agar Base according to the label directions and 100 mg/ml of chloromycetin, with and without 10% sheep blood. Inoculate and incubate tubes at 30 ± 2°C for up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH WITH AND WITHOUT BLOOD
Aspergillus niger	16404	100-1,000	good
Candida albicans	10231	100-1,000	good
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition
Saccharomyces cerevisiae	9763	100-1,000	good
Staphylococcus aureus	25923*	1,000-2,000	marked to complete inhibition
Trichophyton mentagrophytes	9533	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

SABHI Agar Base

Materials Required but not Provided

Glassware
Autoclave
OPTIONAL: Chloromycetin or other sterile antimicrobics
OPTIONAL: Defibrinated sheep blood

Method of Preparation

1. Suspend 59 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool medium to 50-55°C.
5. OPTIONAL: To prepare selective medium, aseptically add 1 mL chloromycetin solution (100 mg/ml) to 1 liter of sterile medium.
6. OPTIONAL: To prepare blood agar, aseptically add sterile sheep blood at a concentration of 10% (e.g. 100 ml blood to 900 ml of sterile medium).

Specimen Collection and Preparation

1. Specimens should be collected in sterile containers or with sterile swabs and transported immediately to the laboratory according to recommended guidelines.[6]

Test Procedure

1. Inoculate SABHI tubes/plates with specimen.
2. Incubate SABHI tubes/plates at 30 ± 2°C for up to 7 days.

Results

Observe SABHI tubes/plates for growth and record colony morphology.

Limitations of the Procedure

1. Non-selective fungal media should be used concurrently with selective media when isolating fungi due to the sensitivity of some strains to antibiotics.[5]

References

1. **Sabouraud, R.** 1892. Ann. Dermatol. Syphilol. **3:**1061.
2. **Dixon, D. M., and R. A Fromtling.** 1995. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F .C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Gorman, J. W.** 1967. Sabhi, a new culture medium for pathogenic fungi. Am. J. Med. Technol. **33**:151.
4. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's Diagnostic Microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 687-691. Williams & Wilkins, Baltimore, MD.
6. **Miller, J. M., and H. T. Holmes.** 1995. Specimen collection and handling, p. 19-32. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken, (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

SABHI Agar Base	500 g	0797-17
	2 kg	0797-07

Bacto® SF Medium

Intended Use

Bacto SF Medium is used for isolating and cultivating fecal streptococci from milk, water, sewage and feces.

Also Known As

Streptococcus Faecalis Medium

Summary and Explanation

Hajna and Perry[1] specified the formulation of SF Broth, a medium that is selective for fecal streptococci when incubated at 45.5°C. SF Broth has been used for testing water and other materials for fecal contamination.[2,3,4] Detection of fecal streptococci is used as an indicator of pollution.

SF medium is used to differentiate Group D enterococci from Group D non-enterococci and other *Streptococcus* spp. that are not Group D. SF Medium is differential in two ways. First, it differentiates based on whether an organism has the ability to grow in the presence of the inhibitor, sodium azide. Second, it detects whether an organism can ferment the carbohydrate, dextrose, producing a pH color change.

Principles of the Procedure

Tryptone is a source of carbon, nitrogen, vitamins and minerals. Dextrose is a fermentable carbohydrate. Sodium Chloride maintains the osmotic balance of the medium. Sodium Azide inhibits cytochrome oxidase of gram-negative bacteria. Brom Cresol Purple is a pH indicator. Phosphates buffer the medium.

Group D enterococci will grow in the presence of azide and ferment glucose. This produces an acid pH that changes the color of the medium from purple to yellow.

Formula

SF Medium

Formula Per Liter

Bacto Tryptone	20 g
Bacto Dextrose	5 g
Dipotassium Phosphate	4 g
Monopotassium Phosphate	1.5 g

Sodium Chloride	5 g
Sodium Azide	0.5 g
Bacto Brom Cresol Purple	0.032 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Cardiovascular, Lungs, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige to gray, may have a light greenish tint, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water. Solution is purple, clear with no precipitate.
Prepared Tubes:	Purple, clear with no precipitate.
Reaction of 3.6% Solution at 25°C:	pH 6.9 ± 0.2

Cultural Response

Prepare SF Medium per label directions. Inoculate and incubate at 45 ± 0.5°C 18-24 and 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID REACTION
Enterococcus faecalis	19433*	1,000-2,000	good	yellow (acid)
Enterococcus faecium	27270	1,000-2,000	good	yellow (acid)
Escherichia coli	25922*	1,000-2,000	inhibited	no change
Streptococcus bovis	33317	1,000-2,000	none to poor	no change

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Procedure

Materials Provided

SF Medium

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (45 ± 0.5°)

Method of Preparation

1. Suspend 36 grams in 1 liter distilled or deionized water. Rehydrate with proportionally less water when liquid inocula will exceed 1 ml.
2. Dispense into tubes with closures.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure[1]

1. Inoculate SF Medium with a heavy inoculum from a pure 18-24 hour culture of the test organism.
2. Incubate at 45 ± 0.5°C for 18-48 hours.
3. Read tubes for growth and acid production at 18-24 hours and 40-48 hours.

Results

A positive result is indicated by growth (turbidity) in the medium with the production of a yellowish-brown color (acid production). A negative reaction is indicated by poor or no growth and no color change in the medium.

Limitations of the Procedure

1. Pure cultures of *Streptococcus* spp. should be inoculated into SF Broth.
2. Group D streptococci include both enterococcal and non-enterococcal strains. Consult appropriate references for further identification of Group D streptococci.[5,6,7,8]

References

1. **Hajna, A. A., and C. A. Perry**. 1943. Comparative study of presumptive and confirmative media for bacteria of the coliform group and fecal streptococci. Am. J. Public Health **33**:550-556.
2. **Facklam, R. R.** 1972. Recognition of group D streptococcal species of human origin by biochemical and physiological tests. Appl. Microbiol. **23:**1131.
3. **Kenner, B. A., H. F. Clark, and P. W. Kabler.** 1961. Fecal streptococci. I. Cultivation and enumeration of streptococci in surface water. Appl. Microbiol. **9:**15.
4. **Shattock, P. M. F.** *Enterococci*, p. 303-319. *In* J. C. Ayers, A. A. Kraft, H. E. Snyder, and H. W. Walker (eds.), Clinical and biological hazards in food. University Press, Ames, Iowa.
5. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

6. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
7. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall, (ed.), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
8. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (eds.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Packaging

SF Medium	500 g	0315-17

SFP Agar

Bacto SFP Agar Base · Bacto Egg Yolk Enrichment 50% Bacto Antimicrobic Vial K · Bacto Antimicrobic Vial P

Intended Use

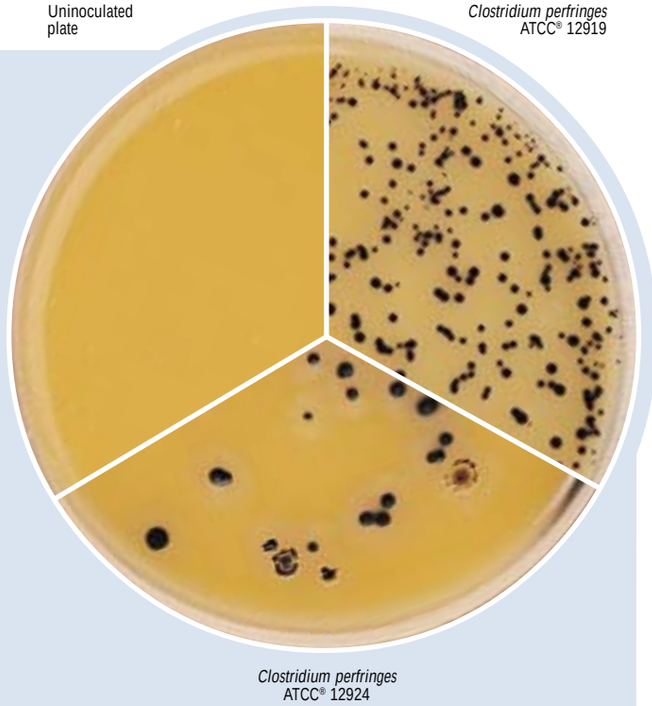
Bacto SFP Agar Base is used with Bacto Egg Yolk Enrichment 50%, Bacto Antimicrobic Vial P and Bacto Antimicrobic Vial K in detecting and enumerating *Clostridium perfringens* in foods.

Also Known As

Tryptose Sulfite Cycloserine (TSC) Agar

Summary and Explanation

Shahidi Ferguson Perfringens (SFP) Agar Base is prepared according to the formulation of Shahidi and Ferguson.[1] With the addition of 50% egg yolk emulsion, both the lecithinase reaction and the sulfite reaction can identify *Clostridium perfringens*. The selectivity of the medium is due to the added kanamycin and polymixin B.



User Quality Control

Identity Specifications

SFP Agar Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.7% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, slightly opalescent.
Prepared Medium (Final):	Canary yellow, opaque.
Reaction of 4.7% Solution at 25°C:	pH 7.6 ± 0.2

Egg Yolk Enrichment 50%

Appearance:	Canary yellow, opaque solution with a resuspendable precipitate.

Antimicrobic Vial K

Dehydrated Appearance:	White cake or powder.
Rehydrated Appearance:	Colorless, clear solution.

Antimicrobic Vial P

Dehydrated Appearance:	White cake or powder.
Rehydrated Appearance:	Colorless, clear solution.

Cultural Response

SFP Agar

Prepare the SFP Agar base layer and cover layer per label directions, inoculating the base layer. Incubate at 35 ± 2°C under anaerobic conditions for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLOR OF COLONIES
Clostridium perfringens	12919	30-300	good	black with halo
Clostridium perfringens	12924	30-300	good	black with halo

The cultures listed are the minimum that should be used for performance testing.

C. perfringens is found in raw meats, poultry, dehydrated soups and sauces, raw vegetables and other foods and food ingredients, but occurrences of food borne illness are usually associated with cooked meat or poultry products.[2] Spores of some strains that may resist heat during cooking germinate and grow in foods that are not adequately refrigerated.[3] Enumerating the microorganism in food samples plays a role in the epidemiological investigation of outbreaks of food borne illness.[2]

SFP Agar (with added kanamycin and polymixin B) is comparable to Tryptose Sulfite Cycloserine (TSC) Agar, which uses cycloserine as the inhibitory component.[2, 4]

Principles of the Procedure

SFP Agar Base contains Tryptose and Soytone as sources of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Ferric Ammonium Citrate and Sodium Sulfite are H_2S indicators. Clostridia reduce sulfite to sulfide, which reacts with iron to form a black iron sulfide precipitate. Antimicrobic Vial P contains Polymyxin B and Antimicrobic Vial K contains Kanamycin; both are inhibitors to organisms other than *Clostridium* spp. Egg Yolk Enrichment 50% provides egg yolk lecithin which some clostridia hydrolyze. Bacto Agar is the solidifying agent.

Formula

SFP Agar Base

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Tryptose	15 g
Bacto Soytone	5 g
Ferric Ammonium Citrate	1 g
Sodium Bisulfite	1 g
Bacto Agar	20 g

Final pH 7.6 ± 0.2 at 25°C

Egg Yolk Enrichment 50%

Sterile concentrated egg yolk emulsion

Antimicrobic Vial K

25,000 mcg Kanamycin per 10 ml vial

Antimicrobic Vial P

30,000 units Polymyxin B per 10 ml vial

Precautions

1. For Laboratory Use.
2. **Antimicrobic Vial K**

   HARMFUL. MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   Antimicrobic Vial P

   MAY BE HARMFUL IF ABSORBED OR INTRODUCED THROUGH SKIN. (US) MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store SFP Agar Base below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Egg Yolk Enrichment 50%, Antimicrobic Vial K and Antimicrobic Vial P at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided (one of the following)

SFP Agar Base
Egg Yolk Enrichment 50%
Antimicrobic Vial K
Antimicrobic Vial P

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator, anaerobic (35°C)

Method of Preparation

SFP Agar Base

Base Layer:

1. Suspend 47 grams in 900 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50°C.
4. Add 100 ml Egg Yolk Enrichment 50%, 10 ml of rehydrated Antimicrobic Vial P (30,000 units polymyxin B sulfate) and 4.8 ml rehydrated Antimicrobic Vial K (12 mg kanamycin).
5. Mix thoroughly.

Cover Layer:

1. Suspend 47 grams in 1 liter distilled or deionized water.
2. Prepare as above, except omit Egg Yolk Enrichment 50%.

Egg Yolk Enrichment 50%

1. Ready for use.
2. Shake gently to resuspend precipitate.

Antimicrobic Vial K

1. Aseptically add 10 ml sterile distilled or deionized water to the Antimicrobic Vial K.
2. Shake to dissolve contents.

Antimicrobic Vial P

1. Aseptically add 10 ml sterile distilled or deionized water to the Antimicrobic Vial P.
2. Rotate in an end-over-end motion to dissolve contents.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Shahidi, S. A., and A. R. Ferguson.** 1971. New quantitative, qualitative, and confirmatory media for rapid analysis of food for *Clostridium perfringens*. Appl. Microbiol. 21:500-506.
2. **Labbe, R. G., and S. M. Harmon.** 1992. *Clostridium perfringens*, p. 623-635. *In* C. Vanderzant, and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Rhodehamel, E. J., and S. M. Harmon.** 1995. *Clostridium perfringens*, p. 16.01- 16.06. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4. **Andrews, W.** 1995. Microbial methods, p. 1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

SFP Agar Base	500 g	0811-17
Antimicrobic Vial K	6 x 10 ml	3339-60
Antimicrobic Vial P	6 x 10 ml	3268-60
Egg Yolk Enrichment 50%	12 x 10 ml	3347-61
	6 x 100 ml	3347-73

Bacto® SIM Medium

Intended Use

SIM Medium is used for differentiating *Salmonella* and *Shigella* species based on hydrogen sulfide production, indole formation and motility.

Also Known As

Sulfide Indole Motility Medium

Summary and Explanation

Semisolid media have been used extensively in the determination of bacterial motility throughout the history of bacteriology.[1] The production of hydrogen sulfide, indole formation and motility are useful diagnostic tests in the identification of *Enterobacteriaceae*, especially *Salmonella* and *Shigella*. In 1940, Sulkin and Willett[2] showed motility, hydrogen sulfide production and carbohydrate fermentation by members of the *Salmonella* and *Shigella* groups. They called attention to the "brush- like growth" or motility of the typhoid organisms. Green and co-workers[3] used SIM medium to detect motility in a large series of cultures of typhoid organisms.

Principles of the Procedure

Bacto Peptone provides nitrogen, amino acids and additional carbon. Beef Extract is a source of carbon, protein and nutrients. Peptonized Iron and Sodium Thiosulfate are indicators of hydrogen sulfide production. Bacto Agar is a solidifying agent.

Formula

Nitrate Broth

Formula Per Liter

Bacto Peptone	30 g
Bacto Beef Extract	3 g
Peptonized Iron	0.2 g
Sodium Thiosulfate	0.02 g
Bacto Agar	3 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

SIM Medium

Materials Required but not Provided

Glassware

Autoclave
Inoculating needle
SpotTest Indole Reagent Kovacs

Method of Preparation

1. Suspend 36 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense the medium into tubes to an approximate depth of 3 inches.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

1. Using an inoculum from the growth of a pure culture at 18-24 hours, stab with an inoculating needle two-thirds into the medium. Carefully ensure the needle is withdrawn through the same stab line.
2. Incubate aerobically at 35 ± 2°C for 18-24 hours.
3. Observe for motility, H_2S and Indole production.
4. Add 3-4 drops of SpotTest Indole Reagent Kovacs.

Results

Motility and H_2S production should be determined before the addition of reagents for determination of indole production. Motility is observed as a diffuse growth outward from the stab line or turbidity of the medium. H_2S production is shown by a blackening along the stab line. Indole production is seen as the production of a red color after the addition of 3-4 drops of SpotTest Indole Reagent Kovac's.

Limitations of the Procedure

1. Do not take inoculum from liquid or broth suspensions because growth initiation will be delayed.[4]
2. Reactions are not sufficient to speciate organisms. Additional biochemical and serological tests are required for confirmation.[5]
3. When using Ehrlich's reagent for indole test, 1 ml. of chloroform must be added prior to adding the reagent.[6]

References

1. **Tittsler, R. P., and L. A. Sandholzer.** 1936. The use of semi-solid agar for the detection of bacterial motility. Journal of Bacteriology. **31**:575.
2. **Sulkin and Willett.** 1940. J. Lab. Clin. Med. **25**:649.
3. **Greene, R. A., E. F. Blum, C. T. DeCoro, R. B. Fairchild, M. T. Kaplan, J. L. Landau, and T. R. Sharp.** 1951. Rapid methods for the detection of motility. J. Bact. **62**:347.
4. **Sosa.** 1943. Dr. Carlos G. Malbran. Rev. Inst. Bact. **11**:286.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, p.275-284. vol. 1. Williams & Wilkins, Baltimore, MD.
6. **Koneman, E. W., S. D. Allen, V. R. Dowell, Jr., W. M. Janda, H. M. Sommers, and W. C. Winn, Jr.** 1988. Color Atlas and textbook of diagnostic microbiology, p. 147. 3rd ed. J. B. Lippincott Company, Philadelphia.

Packaging

SIM Medium	500 g	0271-17

User Quality Control

Identity Specifications

Dehydrated Media Appearance:	Beige, homogeneous, free-flowing.
Solution:	3.6% solution, soluble in distilled or deionized water upon boiling. Solution is medium amber, clear to slightly opalescent.
Reaction of 3.6% Solution at 25° C:	pH 7.3 ± 0.2

Cultural Response

Prepare SIM Medium per label instructions. Dispense 15 ml of medium into standard size tubes. Inoculate using a straight needle with a single stab to the center through two-thirds of the medium. Incubate tubes at 35 ± 2°C for 18-24 hours and read for growth, H_2S production and motility. Add 3-4 drops of SpotTest™ Indole Reagent Kovacs. Indole production is indicated by a red color after reagent addition.

ORGANISM	ATCC®	GROWTH	H_2S	MOTILITY	INDOLE
Escherichia coli	25922*	good	–	+	+
Salmonella typhimurium	14028*	good	+	+	–
Salmonella typhi	6539	good	+	+	–
Shigella flexneri	12022*	good	–	–	–

Uninoculated tube | *Escherichia coli* ATCC® 25922 with indole reagent | *Salmonella typhimurium* ATCC® 14028 with indole reagent

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Bacto® SOB Medium

Intended Use

Bacto SOB Medium is used for cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

SOB Medium was developed by Hanahan[1] as a nutritionally rich growth medium for preparation and transformation of competent cells. Transformation requires making perforations in the bacterium (i.e., making the cells "competent") to allow the introduction of foreign DNA into the cell. To survive this process, competent cells need a rich, isotonic environment.

SOC Medium, used in the final stage of transformation, may be prepared by aseptically adding 20 ml of a filter-sterilized 20% solution of glucose (dextrose) to the sterile SOB Medium. This addition provides a readily available source of carbon and energy in a form *E. coli* can use in mending the perforations and for replication.[2]

Principles of the Procedure

Tryptone and Yeast Extract provide sources of nitrogen and growth factors which allow the bacteria to recover from the stress of transformation and grow well. Sodium Chloride and Potassium Chloride provide a suitable osmotic environment. Magnesium Sulfate is a source of magnesium ions required in a variety of enzymatic reactions, including DNA replication.

Formula

SOB Medium

Formula Per Liter

Bacto Tryptone	20 g
Bacto Yeast Extract	5 g
Sodium Chloride	0.5 g
Magnesium Sulfate, Anhydrous	2.4 g
Potassium Chloride	0.186 g

Final pH 7.0 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.8% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear.
Reaction of 2.8% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare SOB Medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli (DH-5)	53868	100-300	Good

The culture listed is the minimum that should be used for performance testing.

Precautions

1. For Laboratory Use.
2. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

SOB Medium

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Autoclave
Incubator 35°C
Waterbath 45-50°C (optional)
Filter-sterilized 20% solution of glucose (dextrose) (optional)

Method of Preparation

1. Dissolve 28 grams in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. If desired, SOC Medium can be prepared by adding 20 ml of a filter-sterilized 20% glucose solution cooled to 45-50°C.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references for recommended test procedures.[2]

Results

Growth is evident in the form of turbidity.

References

1. **Hanahan, D.** 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol. **166:**557.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis**. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Packaging

SOB Medium	500 g	0443-17

Bacto® SPS Agar

Intended Use

Bacto SPS Agar is used for detecting and enumerating *Clostridium perfringens* in food.

Also Known As

SPS Agar is also known as Sulfite Polymixin Sulfadiazine Agar or Perfringens Selective Agar.

Summary and Explanation

In the 1950's, Mossel[1] and Mossel et al.[2] proposed media for enumerating anaerobic sulfite-reducing clostridia in foods. Angelotti et al.[3] modified the formula as Sulfite Polymixin Sulfadiazine (SPS) Agar and used it to quantitate *C. perfringens* in foods.

C. perfringens is found in raw meats, poultry, dehydrated soups and sauces, raw vegetables and other foods and food ingredients. Occurrences of food borne illness from *C. perfringens* are usually associated with cooked meat or poultry products.[4] Spores of some strains that may resist heat during cooking germinate and grow in foods that are not adequately refrigerated.[5] Enumerating the microorganism in food samples plays a role in epidemiological investigation of outbreaks of food borne illness.[4]

Principles of the Procedure

SPS Agar contains Tryptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Ferric Citrate and Sodium Sulfite are H_2S indicators. Clostridia reduce the sulfite to sulfide which reacts with the iron from ferric citrate to form a black iron sulfide precipitate. Tween® 80 is a dispersing agent. Polymyxin B Sulfate and Sulfadiazine are inhibitors to organisms other than *Clostridium* spp. Sodium Thioglycollate is a reducing agent. Bacto Agar is the solidifying agent.

Formula

SPS Agar

Formula Per Liter

Bacto Tryptone	15 g
Bacto Yeast Extract	10 g
Ferric Citrate	0.5 g
Sodium Sulfite	0.5 g
Sodium Thioglycollate	0.1 g
Tween® 80	0.05 g
Sulfadiazine	0.12 g
Polymyxin B Sulfate	0.01 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.1% solution, soluble in distilled or deionized water on boiling. Solution light to medium amber, slightly opalescent.
Reaction of 4.1% Solution at 25°C:	pH 7.0 ± 0.2

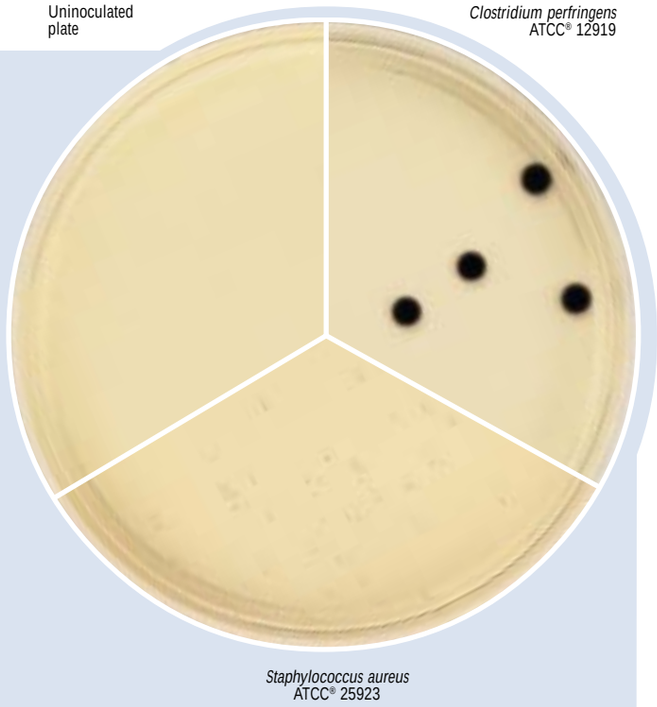
Cultural Response

Prepare SPS Agar per label directions. Inoculate and incubate the plates at 35 ± 2°C anaerobically for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Clostridium perfringens	12919	100-1,000	good	black colonies
Clostridium sporogenes	11437	100-1,000	none to fair	black colonies
Escherichia coli	25922*	100-1,000	marked to complete inhibition	–
Salmonella typhimurium	14028*	100-1,000	marked to complete inhibition	–
Staphylococcus aureus	25923*	100-1,000	fair to good	white colonies

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

SPS Agar

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator, anaerobic (35°C)

Method of Preparation

1. Suspend 41 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Consult appropriate standard methods.[4, 5.]

Test Procedure

1. Dispense inoculum into sterile Petri dish.
2. Pour medium cooled to 50-55°C over the inoculum.
3. Gently but thoroughly mix the inoculum and medium. Allow to solidify on a flat surface.
4. Incubate anaerobically at 35 ± 2°C for 24-48 hours.

Results

Clostridium perfringens will grow as black colonies with good growth.

Limitations of the Procedure

The high degree of selectivity of SPS Agar may inhibit some strains of *C. perfringens* while other strains that grow may fail to produce distinguishing black colonies.[4]

References

1. **Mossel, R. S**. 1959. Enumeration of sulfite-reducing clostridia occurring in foods. J. Sci. Food Agric. **19**:662.
2. **Mossel, D. A. A., A. S. DeBruin, H. M. J. van Diepen, C. M. A. Vendrig, and G. Zoutewelle.** 1956. The enumeration of anaerobic bacteria, and of *Clostridium* species in particular, in foods. J. Appl. Microbiol. **19**:142.
3. **Angelotti, R., H. E. Hall, M. J. Foster, and K. M. Lewis**. 1962. Quantitation of *Clostridium perfringens* in foods. Appl. Microbiol. **10**:193.
4. **Labbe, R. G., and S. M. Harmon.** 1992. *Clostridium perfringens*, p. 623-635. *In* C. Vanderzant, and D. F. Splittstoesser (ed.),. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Rhodehamel, E. J., and S. M. Harmon**. 1995. *Clostridium perfringens*, p. 16.01- 16.06. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

SPS Agar	100 g	0845-15*
	500 g	0845-17*

*Store at 2-8°C

Bacto® SS Agar

Intended Use

Bacto SS Agar is used for isolating *Salmonella* and some *Shigella*.

Also Known As

SS Agar is also known as *Salmonella-Shigella* Agar.

Summary and Explanation

Salmonellosis continues to be an important public health problem worldwide, despite efforts to control the prevalence of *Salmonella* in domesticated animals. Infection with non-typhi *Salmonella* often causes mild, self-limiting illness. Typhoid fever, caused by *S. typhi*, is characterized by fever, headache, diarrhea, and abdominal pain, and can produce fatal respiratory, hepatic, splenic, and/or neurological damage.[1] These illnesses result from the consumption of raw, undercooked or improperly processed foods contaminated with *Salmonella*.

Shigella spp. cause classic bacillary dysentery (shigellosis), which is a descending intestinal illness characterized by abdominal pain, fever, and watery diarrhea. *Shigella dysenteriae* can cause a severe form of dysentery that has been reported to have fatality rates of up to 20%. Most cases of shigellosis are individual cases due to person-to-person transmission. When associated with outbreaks, the disease usually is transmitted by contaminated food and/or water.[1]

SS Agar is a modification of the Desoxycholate Citrate Agar described by Leifson.[2] SS Agar was found to be superior to other media for the isolation of *Salmonella* and *Shigella* spp.[3] Ewing and Bruner found SS Agar to have the advantage that large amounts of inoculum could be used when isolating *Salmonella* or *Shigella* from clinical samples.[4] Caudill[5] reported on the satisfactory use of SS Agar in isolation of *Shigella* organisms. Hormaeche and his co-workers[6] used SS Agar with other media for isolation of *Shigella* as the causative agent of infantile summer diarrhea.

The use of SS Agar is recommended for testing clinical specimens for the presence of *Salmonella* and some *Shigella* spp.[1,7] For food testing, consult appropriate references on the use of SS Agar.[8]

Principles of the Procedure

In SS Agar, Bacto Bile Salts No. 3 and Brilliant Green are complementary in inhibiting gram-positive bacteria, most coliform bacteria, and the swarming phenomenon of *Proteus* spp., while allowing

Salmonella spp. to grow. Sodium thiosulfate and ferric citrate allow the detection of hydrogen sulfide by the production of colonies with black centers. Lactose is the carbohydrate present in SS Agar. Neutral red and brilliant green are present as pH indicators.

Formula

SS Agar

Formula Per Liter

Bacto Beef Extract	5 g
Bacto Proteose Peptone	5 g
Bacto Lactose	10 g
Bacto Bile Salts No. 3	8.5 g
Sodium Citrate	8.5 g
Sodium Thiosulfate	8.5 g
Ferric Citrate	1 g
Bacto Agar	13.5 g
Brilliant Green	0.33 mg
Neutral Red	0.025 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared plates at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

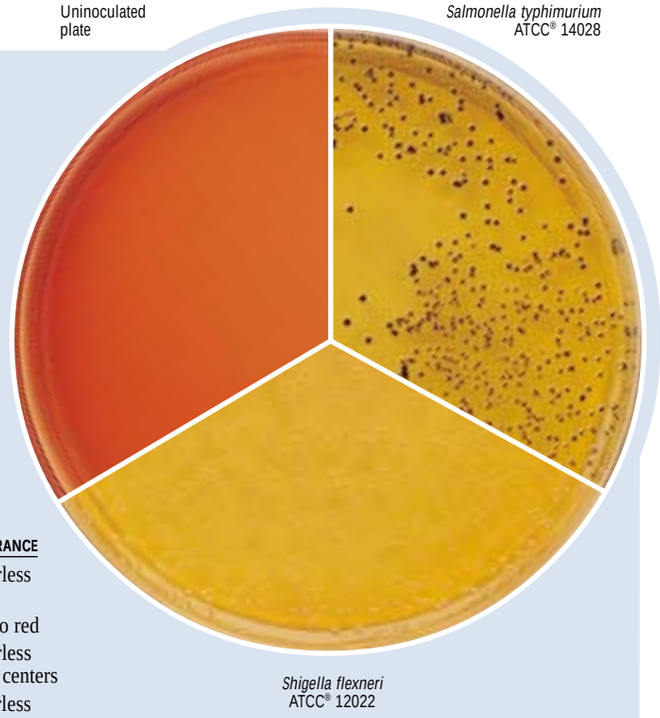
SS Agar

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Waterbath (45-50°C)
Petri dishes
Incubator (35°C)

Method of Preparation

1. Suspend 60 grams in 1 liter distilled or deionized water.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light buff to pink, free flowing, homogeneous.
Solution:	6.0% solution, soluble in distilled or deionized water on boiling. Solution is red-orange, very slightly to slightly opalescent.
Prepared Plates:	Red-orange, slightly opalescent.
Reaction of 6.0% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare SS Agar per label directions. Inoculate and incubate plates at 35 ± 2°C for 18-24 hours and 48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	1,000-2,000	partial inhibition	colorless
Escherichia coli	25922*	1,000-2,000	partial inhibition	pink to red
Salmonella typhimurium	14028*	100-1,000	good	colorless w/black centers
Shigella flexneri	12022*	100-1,000	fair to good	colorless

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

2. Heat to boiling for no more than 2-3 minutes to dissolve completely. Avoid overheating. DO NOT AUTOCLAVE.
3. Cool to 45-50°C in a waterbath.
4. Dispense into sterile Petri dishes. Allow the surface of the medium to air dry for two hours by leaving the lids ajar.

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[1,7,8]
2. Process each specimen, using procedures appropriate for that specimen or sample.[1,7,8]

Test Procedure

For isolation of *Salmonella* and *Shigella* spp. from clinical specimens, inoculate fecal samples and rectal swabs onto one quadrant of a SS Agar plate and streak for isolation. This will permit the development of discreet colonies. Incubate plates at 35°C. Examine at 24 hours and again at 48 hours for colonies resembling *Salmonella* or *Shigella* spp. Note: SS Agar is inhibitory to some strains of *Shigella* spp. For additional information about specimen preparation and inoculation of clinical specimens, consult appropriate references.[1,7]

For testing food samples, consult appropriate references.[8]

Results

Enteric organisms are differentiated by their ability to ferment lactose. *Salmonella* and *Shigella* spp. are lactose non-fermenters and form colorless colonies on SS Agar. *Salmonella* spp. that are H_2S positive produce colonies with black centers. Some *Shigella* spp. are inhibited on SS Agar.

Coliforms are partially inhibited on SS Agar. *E. coli* produces pink to red colonies and may have some bile precipitation. Colonies of *Enterobacter aerogenes* appear cream to pink in color. *Citrobacter* and *Proteus* spp. may grow on SS Agar and produce colonies with gray to black centers due to H_2S production. *Enterococcus faecalis* is partially inhibited on SS Agar; colonies of *E. faecalis* are colorless.

Limitations of the Procedure

1. SS Agar is a highly selective medium. For this reason, it is not recommended as the sole medium for primary isolation of *Shigella*.[1,2,9] Some strains of *Shigella* may not grow.
2. A few nonpathogenic organisms may grow on SS Agar. These organisms can be differentiated by their ability to ferment lactose.[10]

References

1. **Gray, L. D.** 1995. *Escherichia*, *Salmonella*, *Shigella* and *Yersinia*, p. 450-456. *In* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Leifson, E.** 1935. New culture media based on sodium desoxycholate for the isolation of intestinal pathogens and for the enumeration of colon bacilli in milk and water. J. Pathol. Bacteriol. **40:**581.
3. **Rose, H. M., and M. H. Kolodny.** 1942. The use of SS (*Shigella-Salmonella*) Agar for the isolation of Flexner Dysentery bacilli from the feces. J. Lab. Clin. Med. **27:**1081-1083.
4. **Ewing, W. H., and D. W. Bruner.** 1947. Selection of *Salmonella* and *Shigella* cultures for serologic classification. Am. J. Clin. Pathol. **17:**1-12.
5. **Caudill, F. W., R. E. Teague, and J. T. Duncan.** 1942. A rural shiga dysentery epidemic. JAMA **119:**1402-1406.
6. **Hormaeche, E., N. L. Surraco, C. A. Peluffo, and P. L. Aleppo.** 1943. Causes of infantile summer diarrhea. Am. J. Dis. Child. **66:**539-551.
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8. **Vanderzant, C., and D. F. Splittstoesser (ed.)** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
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Packaging

SS Agar	100 g	0074-15
	500 g	0074-17
	2 kg	0074-07
	10 kg	0074-08

Sabouraud Media

Bacto® Sabouraud Agar Modified · Bacto Sabouraud Dextrose Agar Sabouraud Dextrose Broth · Bacto Sabouraud Maltose Agar Bacto Sabouraud Maltose Broth · Bacto Fluid Sabouraud Medium

Intended Use

Bacto Sabouraud Agar Modified is used for cultivating fungi at a neutral pH.

Bacto Sabouraud Dextrose Agar and Broth and Bacto Sabouraud Maltose Agar and Broth are used for culturing yeasts, molds and aciduric microorganisms.

Bacto Fluid Sabouraud Medium is used for cultivating yeasts, molds and aciduric microorganisms and for detecting yeasts and molds in normally sterile materials.

User Quality Control

Identity Specification

Sabouraud Agar Modified

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, slightly opalescent without significant precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent without significant precipitate.
Reaction of 5.0% Solution at 25°C:	pH 7.0 ± 0.2

Sabouraud Dextrose Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent without precipitate.
Reaction of 6.5% Solution 25°C:	pH 5.6 ± 0.2

Sabouraud Dextrose Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in distilled or deionized water. Solution is light amber, clear.
Prepared Medium:	Light amber, clear.
Reaction of 3.0% Solution at 25°C:	pH 5.6 ± 0.2

Sabouraud Maltose Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in distilled or deionized water on boiling. Solution is light amber, slightly opalescent, may have a slight precipitate.
Prepared Medium:	Very light amber, slightly opalescent without significant precipitate.
Reaction of 6.5% Solution at 25°C:	pH 5.6 ± 0.2

Sabouraud Maltose Broth

Dehydrated Appearance:	White, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in distilled or deionized water. Solution is light amber, clear to slightly opalescent.
Prepared Medium:	Light amber, clear to slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 5.6 ± 0.2

continued on following page

Summary and Explanation

Sabouraud Agar Modified is a modification of the Sabouraud Dextrose Agar formulation devised by Raymond Sabouraud for his dermatophyte studies.[1] Sabouraud Agar Modified, used for the recovery of dermatophytes[2], contains reduced dextrose (2%) and has a neutral pH (7.0). The selectivity of the medium can be improved with the addition of antibiotics, such as chloramphenicol to inhibit bacterial growth and cycloheximide to inhibit saprophytic fungi.[3,4]

Sabouraud Dextrose Agar and Sabouraud Dextrose Broth are modifications of the Dextrose Agar described by Sabouraud.[1] They are used for cultivating pathogenic fungi, particularly those associated with skin infections. The high dextrose concentration and acidic pH make these media selective for fungi.[5] Georg[6] demonstrated that the addition of cycloheximide, streptomycin and penicillin to Sabouraud Dextrose Agar produces an excellent medium for the primary isolation of dermatophytes. Sabouraud Dextrose Agar is also used for determining the microbial content of cosmetics[7] and for the mycological evaluation of food.[8] Sabouraud Dextrose Agar is available in the dehydrated form and prepared in 200 ml amounts. In the prepared form, Sabouraud Dextrose Agar is used for pouring plates.

Sabouraud Maltose Agar is a modification of Sabouraud Dextrose Agar with maltose substituted for dextrose. It is a selective medium due to the acid pH. Davidson, Dawding and Buller[9] reported that Sabouraud Maltose Agar was a satisfactory medium in their studies of the infections caused by *Microsporon audouini*, *M. lanosum* and *Trichophyton gypseum*. Davidson and Dawding[10] also used this medium in isolating *T. gypseum* from a case of tinea barbae.

Sabouraud Maltose Broth is a modification of Sabouraud Dextrose Broth in which maltose is substituted for dextrose. It is selective due to its acid pH and is used for the detection of fungi.

Fluid Sabouraud Medium is employed in sterility test procedures for determining the presence of molds, yeasts and aciduric microorganisms. The acid reaction of the final medium is inhibitive to a large number of bacteria and makes the medium particularly well suited for cultivating fungi and acidophilic microorganisms.

Principles of the Procedure

Sabouraud Agar Modified, Sabouraud Dextrose Agar**,** and Sabouraud Dextrose Broth contain Neopeptone which provides the carbon and nitrogen required for growth of a wide variety of organisms. Dextrose is included as an energy source. Bacto Agar is incorporated into the agar media as a solidifying agent.

Sabouraud Maltose Agar and Sabouraud Maltose Broth contain Neopeptone which provides the carbon and nitrogen sources required for growth of a wide variety of organisms. Maltose is included in the medium as an energy source. Sabouraud Maltose Agar contains Bacto Agar as the solidifying agent.

Fluid Sabouraud Medium contains Casitone and Peptamin which provide nitrogen, vitamins, minerals and amino acids. Dextrose is an energy source.

Formula

Sabouraud Agar Modified

Formula Per Liter	
Bacto Neopeptone	10 g
Bacto Dextrose	20 g
Bacto Agar	20 g

Final pH 7.0 ± 0.2 at 25°C

Sabouraud Dextrose Agar

Formula Per Liter

Bacto Neopeptone	10 g
Bacto Dextrose	40 g
Bacto Agar	15 g

Final pH 5.6 ± 0.2 at 25°C

Sabouraud Dextrose Broth

Formula Per Liter

Bacto Neopeptone	10 g
Bacto Dextrose	20 g

Final pH 5.6 ± 0.2 at 25°C

Sabouraud Maltose Agar

Formula Per Liter

Bacto Neopeptone	10 g
Bacto Dextrose	40 g
Bacto Agar	15 g

Final pH 5.6 ± 0.2 at 25°C

Sabouraud Maltose Broth

Formula Per Liter

Bacto Neopeptone	10 g
Bacto Maltose	40 g

Final pH 5.6 ± 0.2 at 25°C

Fluid Sabouraud Medium

Formula Per Liter

Bacto Casitone	5 g
Bacto Peptamin	5 g
Bacto Dextrose	20 g

Final pH 5.7 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

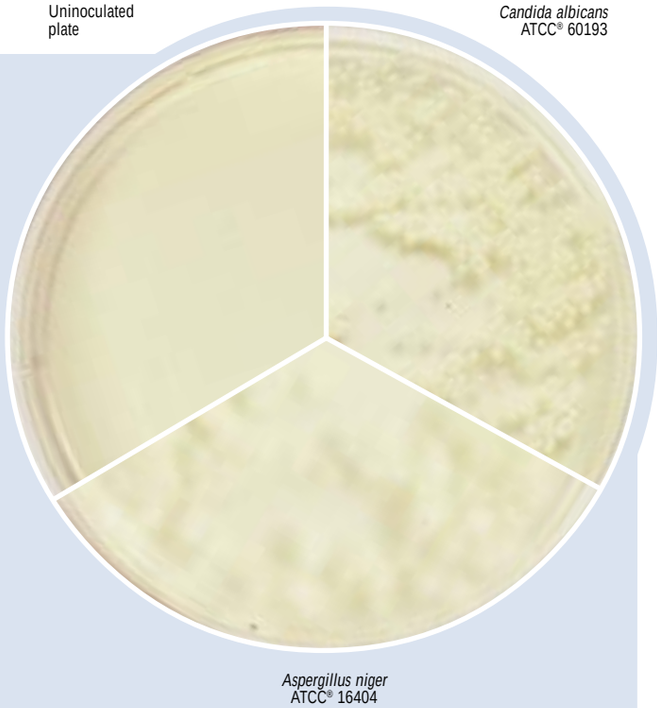
Storage

Store dehydrated Sabouraud media below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store prepared Sabouraud Dextrose Agar at 15-30°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.



User Quality Control cont.

Fluid Sabouraud Medium

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in distilled or deionized water. Solution is light amber, clear to very slightly opalescent.
Prepared Medium:	Light amber, clear to very slightly opalescent without precipitate.
Reaction of 3.0% Solution at 25°C:	pH 5.7 ± 0.2

Cultural Response

Sabouraud Agar Modified, Sabouraud Maltose Agar, Sabouraud Maltose Broth, Fluid Sabouraud Mediumm Sabouraud Dextrose Agar, Sabouraud Dextrose Broth

Prepare dehydrated medium per label directions. Inoculate and incubate at 30 ± 2°C for 18-48 hours or up to 7 days if necessary.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Aspergillus niger	16404	100-1,000	good
Candida albicans	10231	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good

Sabouraud Dextrose Agar (prepared)

Melt medium and aseptically dispense into plates. Inoculate and incubate at 30 ± 2°C for 18-48 hours, except *Aspergillus niger* which is incubated at room temperature for 3-5 days.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Aspergillus niger	16404	100-1,000	good
Candida albicans	10231	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good

The cultures listed are the minimum that should be used for perfoemance testing.

Procedure

Materials Provided

Sabouraud Agar Modified
Sabouraud Dextrose Agar (dehydrated or prepared)
Sabouraud Dextrose Broth
Sabouraud Maltose Broth
Sabouraud Maltose Agar
Fluid Sabouraud Medium. (For Laboratory Use)

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Sterile Petri dishes or tubes with closures
Waterbath (optional)

Method of Preparation

Dehydrated Media

1. Suspend the indicated amount of dehydrated medium in 1 liter of distilled or deionized water and boil to dissolve completely. Avoid overheating which could cause a softer medium.

   Sabouraud Agar Modified - 50 grams

   Sabouraud Dextrose Agar - 65 grams

   Sabouraud Maltose Agar - 65 grams

   Dissolve the indicated amount of dehydrated medium in 1 liter of distilled or deionized water.

   Sabouraud Dextrose Broth - 30 grams

   Sabouraud Maltose Broth - 50 grams

   Fluid Sabouraud Medium - 30 grams

2. Autoclave at 121°C for 15 minutes.

Prepared Sabouraud Dextrose Agar

Melt the agar to pour into plates by one of the following methods.

- Loosen the bottle caps, then autoclave bottles at 121°C for 3 minutes to melt the agar. A small solidified mass may remain that can be melted by swirling the hot agar. Autoclave time depends on the number of bottles in the chamber.

  NOTES: Autoclave small batches to limit darkening of the medium.

Long cycles have a tendency to shrink the clear label material.

- Heat bottles in boiling water. Time will vary; it may take up to 40 minutes to melt the agar.
- Microwave the bottles to melt the agar. Time will vary with the microwave and the number of bottles to be melted. When microwaving, boiling over is a significant problem with smaller bottles.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

Consult appropriate references for recommended test procedures.[2]

Results

Growth is evident in the form of turbidity.

Limitations of the Procedure

1. Antimicrobial agents incorporated into a medium to inhibit bacteria may also inhibit certain pathogenic fungi.
2. Avoid overheating a medium with an acidic pH because this often causes a soft medium.

References

1. **Sabouraud**, **R.** 1892. Ann. Dermatol. Syphilol. **3**:1061.
2. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
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8. **Beuchat, L. R., J. E. Corry, A. D. King, Jr., and J. I. Pitt (ed.).** 1986. Methods for the mycological examination of food. Plenum Press, New York.
9. **Davidson, A. M., E. S. Dowding, and A. H. R. Buller.** 1932. Hyphal fusions in dermatophytes. Can. J. Res. **6**:1.
10. **Davidson, A. M., and E. S. Dowding.** 1932. Tinea barbae of the upper lip. Arch. Dermatol. Syphilol. **26**:660.
11. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
12. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
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Packaging

Sabouraud Agar Modified	500 g	0747-17
	2 kg	0747-07
Sabouraud Dextrose Agar	50 g	0109-17
	100 g	0109-15
	10 kg	0109-08
	2 kg	0109-07
	10 x 200 ml	9074-76
Sabouraud Dextrose Broth	500 g	0382-17
	100 g	0382-15
	2 kg	0382-07
Sabouraud Maltose Agar	500 g	0110-17
	2 kg	0110-07
Sabouraud Maltose Broth	500 g	0429-17
Fluid Sabouraud Medium	500 g	0642-17

Bacto® Schaedler Agar
Bacto Schaedler Broth

Intended Use

Bacto Schaedler Agar is used with or without blood in cultivating and enumerating anaerobic and aerobic microorganisms.

Bacto Schaedler Broth is used for cultivating anaerobic and aerobic microorganisms with or without added blood or enrichment.

Summary and Explanation

Schaedler Agar and Schaedler Broth are prepared according to the formulation described by Schaedler, Dubos and Costello[1] and modified by Mata, Carrillo and Villatoro.[2] Modifications include reduced dextrose to avoid interference with hemolytic reactions and reduced yeast extract to avoid darkening of the medium[3] as well as adjusted sodium chloride and peptone concentrations. Schaedler Broth is the same formulation as Schaedler Agar but with the agar omitted.

While studying the gastrointestinal flora of mice, Schaedler et al.[1] formulated a medium to recover both aerobic and anaerobic microorganisms. Mata et al.[2] used a modification of the Schaedler formula to study human fecal microflora.

Stalons, Thornsberry and Dowell[4] evaluated nine broth media in varied carbon dioxide atmospheres for their ability to support growth of anaerobic bacteria. Schaedler Broth in an atmosphere of 5% CO_2, 10% hydrogen and 85% nitrogen exhibited the fastest and highest growth response.

Anaerobic bacteria cause a variety of human infections including endocarditis, meningitis, wound infections following bowel surgery or trauma, and bacteremia.[5,6] Since anaerobes vary in their sensitivity to oxygen and nutritional requirements[7], appropriate collection, culture medium and incubation are vital to recovery.[7] Schaedler media are suitable for standard procedures used in cultivating anaerobic bacteria.[7,8,9]

User Quality Control

Identity Specifications

Schaedler Agar

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.19% solution, soluble in distilled or deionized water on boiling. Light to medium amber, clear to slightly opalescent, may have a fine precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent, may have a fine precipitate.
Reaction of 4.19% Solution at 25°C	pH 7.6 ± 0.2

Schaedler Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.84% solution, soluble in distilled or deionized water on boiling 1-2 minutes. Light to medium amber, clear to slightly opalescent, may have a very slight black precipitate.
Prepared Medium:	Light to medium amber, clear to very slightly opalescent, may have a very slight black precipitate.
Reaction of 2.84% Solution at 25°C:	pH 7.6 ± 0.2

Cultural Response

Prepare Schaedler Agar or Schaedler Broth per label directions. Prereduce Schaedler Broth prior to inoculation with anaerobic organisms. Inoculate medium; incubate at 35 ± 2°C for 18-48 hours under aerobic or anaerobic conditions, depending on the requirements of the inoculum.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacteroides fragilis†	25285*	100-1,000	good
Bacteroides vulgatus†	8482	100-1,000	good
Clostridium novyi B†	27606	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

†Incubate anaerobically.

Principles of the Procedure

Tryptic Soy Broth, Proteose Peptone No.3 and Yeast Extract provide the vitamins, nitrogen and amino acids in Schaedler media. Dextrose is a carbon source, and Tris (Hydroxymethyl) Amino Methane is used to buffer the medium. Hemin (X factor) stimulates growth. Bacto Agar is the solidifying agent in Schaedler Agar.

The following supplements can be added to Schaedler media.

- Sheep, horse or rabbit blood (5%) - for enrichment and for detecting hemolysis and pigment production.[9]
- Vitamin K_1 (1%) - to promote growth of some pigmented *Prevotella* and *Porphyromonas* spp. (formerly known as *Bacteroides*).[9]
- Colistin and nalidixic acid (0.01 grams/liter, each) (Schaedler CNA agar) - for selectively isolating anaerobic gram-positive cocci.[10]
- Kanamycin (0.01 grams/liter) and vancomycin (7.5 mg/liter) (Schaedler KV Agar) - for selectively isolating anaerobic gram-negative bacteria.[10]

Formula

Schaedler Agar

Formula Per Liter

Bacto Tryptic Soy Broth	10 g
Bacto Proteose Peptone No.3	5 g
Bacto Yeast Extract	5 g
Bacto Dextrose	5 g
Tris (Hydroxymethyl) Amino Methane	3 g
L-Cystine	0.4 g
Hemin	0.01 g
Bacto Agar	13.5 g

Final pH 7.6 ± 0.2 at 25°C

Schaedler Broth

Formula Per Liter

Bacto Tryptic Soy Broth	10 g
Bacto Proteose Peptone No.3	5 g
Bacto Yeast Extract	5 g

Bacto Dextrose	5	g
Tris (Hydroxymethyl) Amino Methane	3	g
L-Cystine	0.4	g
Hemin	0.01	g

Final pH 7.6 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Schaedler Agar

Schaedler Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes
Sterile defibrinated sheep, horse or rabbit blood (optional)

Method of Preparation

1. Suspend the appropriate amount of medium in 1 liter distilled or deionized water:
   Schaedler Agar - 41.9 grams/liter;
   Schaedler Broth - 28.4 grams/liter.
2. OPTIONAL: Add 1 ml of 1% vitamin K_1 in absolute ethanol.
3. Heat to boiling for 1-2 minutes to dissolve completely.
4. Autoclave at 121°C for 15 minutes. Cool to room temperature.
5. OPTIONAL: To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.

Specimen Collection and Preparation

Anaerobic bacteria are overlooked or missed unless the specimen is properly collected and transported to the laboratory.[7] Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion of aerobic and anaerobic bacteria from clinical specimens, refer to the appropriate procedures outlined in the references.[7,8,9] For the examination of bacteria in food, refer to standard methods.[11,12,13]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Clinical specimens must be obtained properly and transported to the laboratory in a suitable anaerobic transport container.[7]
3. The microbiologist must be able to verify quality control of the medium and determine whether the environment is anaerobic.[7]
4. The microbiologist must perform aerotolerance testing on each isolate recovered to ensure that the organism is an anaerobe.[7]
5. Because of the high dextrose concentration in Schaedler Agar when it is supplemented with 5% blood, beta-hemolytic streptococci may produce a hemolytic reaction that is similar to alpha hemolysis.

References

1. **Schaedler, R. W., R. Dubos, and R. Costello.** 1965. The development of the bacterial flora in the gastrointestinal tract of mice. J. Exp. Med. **122**:59.
2. **Mata, L. J. , C. Carrillo, and E. Villatoro.** 1969. Fecal microflora in healthy persons in the preindustrial region. Appl. Microbiol. **17**:596.
3. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 695-699, vol. 1. Williams & Wilkins, Baltimore, MD.
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5. **Balows, A., W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadmony (ed.).** 1991. Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
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Packaging

Schaedler Agar	500 g	0403-17
Schaedler Broth	500 g	0534-17

Bacto® Selenite Broth

Intended Use

Bacto Selenite Broth is used for enriching *Salmonella* spp. during isolation procedures and for isolating *Salmonella* in foods.

Also Known As

Selenite Broth is also referred to as Selenite F (Fecal) Broth.

Summary and Explanation

Selenite Broth is used as a selective enrichment for the cultivation of *Salmonella* spp. that may be present in small numbers and competing with intestinal flora. *Salmonella* organisms are also injured in food-processing procedures, including exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives or sanitizers.[1] Although injured cells may not form colonies on selective media, they can cause infection if ingested.[2] *Salmonella* spp. cause many types of infections, from mild self-limiting gastroenteritis to life-threatening typhoid fever.[3] The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhea lasting less than 7 days.[3]

The formula of Selenite Broth is described by Leifson[4] as Selenite F Broth. Guth,[5] according to Handel and Theodorascu, observed that *Escherichia coli* was more susceptible to the toxicity of sodium selenite than *S. typhi*. Guth employed sodium selenite as a selective agent in agar medium and enrichment broth for the isolation of *S. typhi* from feces. Leifson[4] extended Guth's observations and developed a selenite agar and selenite broth for the isolation of typhoid and paratyphoid bacilli from clinical specimens.

Leifson[4] found the selenite broth was not sufficiently toxic to completely inhibit fecal coliforms and enterococci. These organisms were inhibited during the first 8-12 hours but increased rapidly after this time period. *Salmonella* spp. multiply fairly rapidly after inoculation. It is suggested that selenium toxicity may be a reaction with sulphur and sulphydryl groups in certain strains of bacteria.[6,7]

There have been many modifications of Selenite Broth from the original formula described by Leifson. Selenite Cystine Broth is used as a selective enrichment broth recommended by AOAC[8] and USP[9] for detecting *Salmonella* in food, dairy products and other materials of sanitary importance. Selenite Brilliant Green Sulfa (SBS) Enrichment Broth and Selenite Brilliant Green Mannitol (SBM) Enrichment Broth have also been used for the cultivation of *Salmonella*.[10]

Selenite Broth conforms with APHA[11] and is specified in Clinical Microbiology Procedures Handbook[12] and Manual of Clinical Microbiology.[13]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in distilled or deionized water on boiling; very light amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Medium:	Very light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 2.3% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Selenite Broth per label directions. Incubate inoculated medium at 35 ± 2°C for 18-24 hours. After incubation, subculture onto MacConkey Agar plates and incubate plated media at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	CFU	GROWTH	MACCONKEY AGAR
Escherichia coli	25922*	100-1,000	partial to marked inhibition	pink w/bile ppt
Salmonella typhimurium	14028*	100-1,000	good	colorless

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Bacto Tryptone provides the nitrogen, vitamins and amino acids in Selenite Broth. Bacto Lactose is a fermentable carbohydrate. Selenite is reduced by organism growth. A rise in pH decreases the selective activity of the selenite. The acid produced by lactose fermentation helps to maintain a neutral pH. Sodium selenite inhibits the growth of gram-positive bacteria and many gram-negative bacteria. Sodium phosphate is a buffering agent.

Formula

Selenite Broth

Formula Per Liter

Bacto Tryptone	5 g
Bacto Lactose	4 g
Sodium Selenite	4 g
Sodium Phosphate	10 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Very TOXIC.** FATAL IF INHALED OR SWALLOWED.(US) VERY TOXIC BY INHALATION AND IF SWALLOWED.(EC) DANGER OF CUMULATIVE EFFECTS.(EC) IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Lungs, Kidneys, Spleen, Liver.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If skin irritation persists, seek medical advice. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed, induce vomiting; seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Material Provided

Selenite Broth

Materials Required But Not Provided

Glassware
Distilled or deionized water
Incubator
Waterbath (45-50°C) (optional)
Sterile tubes

Method of Preparation

1. Dissolve 23 grams in 1 liter distilled or deionized water.
2. Heat to boiling to pasteurize.
3. Avoid overheating. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

For a complete discussion on the isolation and identification of *Salmonella* species refer to the appropriate procedures outlined in the references.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Hartman, P. A., and S. A. Minnich.** 1981. Automation for rapid identification of salmonellae in foods. J. Food Prot. **44**:385-386.
2. **Sorrells, K. M., M. L. Speck, and J. A. Warren.** 1970. Pathogenicity of *Salmonella gallinarum* after metabolic injury by freezing. Appl. Microbiol. **19**:39- 43.
3. **Gray, L. D.** 1995. *Escherichia*, *Salmonella*, *Shigella* and *Yersinia*, p. 450-456. *In* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Leifson, E.** 1936. New selenite selective enrichment medium for the isolation of typhoid and paratyphoid (*Salmonella*) bacilli. Am. J. Hyg. **24**:423.
5. **Guth, F.** 1916. Centr. Bakt. I Abt. Orig. **77**:487.
6. **Weiss, K. F., J. C. Ayres, and A. A. Kraft.** 1965. Inhibitory action of selenite on *Escherichia coli*, *Proteus vulgaris*, and *Salmonella thompson*. J. Bacteriol. **90**:857-862.
7. **Rose, M. J., N. K. Enriki, and J. A. Alford.** 1971. Growth and survival of enterobacteria in selenite-cystine broth containing thiosulfate. J. Food Sci. **36**:590-593.
8. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
9. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.
10. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, p. 701-705, vol 1, Williams & Wilkins, Baltimore, MD.
11. **Russell, S. F., J.-Y. D'Aoust, W. H. Andrews, and J. S. Bailey.** 1992. *Salmonella*, p. 371-422. *In* Vanderzant, C., and D. F. Splittstoesser (ed.) Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
12. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1, American Society for Microbiology, Washington. D.C.
13. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.)** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.

Packaging

Selenite Broth	100 g	0275-15
	500 g	0275-17
	10 kg	0275-08

Bacto® Selenite Cystine Broth

Intended Use

Bacto Selenite Cystine Broth is used for selectively enriching *Salmonella* in food and water.

Summary and Explanation

Selenite Cystine Broth is the formulation by Leifson[1] with cystine added. Leifson determined that Selenite Broth favored the growth of *Salmonella* while reducing growth of fecal coliforms and enterococci.[1]

The growth and recovery of *Salmonella* in food samples can be hindered by non-*Salmonella* bacteria, substances indigenous to the food sample, and in dried, processed food, the *Salmonella* may be present in low numbers and in a injured condition.[2] Using protocols that involve preenrichment, selective enrichment and selective plating increase the likelihood of recovering *Salmonella*. In most standard method procedures Selenite Cystine Broth is recommended in the selective enrichment step.[2,3,4,5,6] As a selective enrichment medium, Selenite

Cystine Broth is formulated to allow the proliferation of *Salmonella* and while inhibiting the growth of competing non-*Salmonella* bacteria.[2]

Principles of the Procedure

Selenite Cystine Broth contains Tryptone as a source of carbon, nitrogen, vitamins and minerals. Lactose is the carbohydrate. Sodium Acid Selenite inhibits gram-positive bacteria and most enteric gram-negative bacteria except *Salmonella*. L-cystine is a reducing agent.

Formula

Selenite Cystine Broth

Formula Per Liter

Bacto Tryptone	5 g
Bacto Lactose	4 g
Disodium Phosphate	10 g
Sodium Acid Selenite	4 g
L-Cystine	0.01 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **VERY TOXIC.** FATAL IF INHALED OR SWALLOWED. (US) VERY TOXIC BY INHALATION AND IF SWALLOWED. (EC) DANGER OF CUMULATIVE EFFECTS. (EC) IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	2.3% solution, soluble in distilled or deionized water on boiling.
Prepared Medium:	Very light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 2.3% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Selenite Cystine Broth per label directions. Inoculate and incubate the tubes at 35 ± 2°C for 24 ± 2 hours and subculture on MacConkey Agar plates.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Escherichia coli	25922*	100-1,000	partial to complete inhibition	pink with bile precipitate
Salmonella typhimurium	14028*	100-1,000	good	colorless
Shigella sonnei	9290*	100-1,000	fair to good	colorless

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

TARGET ORGAN(S): Lungs, Kidneys, Spleen, Liver.

FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If skin irritation persists, seek medical advice. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed, induce vomiting; seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Selenite Cystine Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)
Tetrathionate Broth
Bismuth Sulfite Agar
XLD Agar
Hektoen Enteric Agar
MacConkey Agar

Method of Preparation

1. Suspend 23 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes to a depth of 60 mm.
4. DO NOT AUTOCLAVE. Use immediately.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure[4,5]

1. Prepare sample according to food type.
2. Inoculate into recommended pre-enrichment broth.
3. Transfer 1 ml of mixture to 10 ml Selenite Cystine Broth and to 10 ml Tetrathionate Broth.
4. Incubate at 35°C for 24 ± 2 hours.
5. Mix and streak 3 mm loopful (10 µl) of sample from both broths onto Bismuth Sulfite Agar, Xylose Lysine Desoxycholate Agar, Hektoen Enteric Agar or MacConkey Agar.
6. Incubate plates at 35°C for 24 ± 2 hours.
7. Examine plates for the presence of colonies that are typical for *Salmonella* spp.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. A brick red precipitate may appear if Selenite Cystine Broth is overheated during preparation or exposed to excessive moisture during storage.

References

1. **Leifson, E**. 1936. New selenite selective enrichment medium for the isolation of typhoid and paratyphoid (*Salmonella*) bacilli. Am J. Hyg. **24**:423-432.
2. **Flowers, R. S., J-Y. D'Aoust, W. H. Andrews, and J. S. Bailey.** 1992. *Salmonella*, p. 371-422. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Flowers, R. S., W. H. Andrews, E. W. Donnely, and E. Koenig.** 1992. Pathogens in milk and milk products, p. 103-124. *In* R. T. Marshall (ed.), Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
4. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana**. 1995. *Salmonella*, p. 5.01-5.20. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
5. **Andrews, W.** 1995. Microbial methods, p. 1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International. Arlington, VA.
6. **United States Pharmacopeial Convention**. 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.

Packaging

Selenite Cystine Broth	100 g	0687-15
	500 g	0687-17
	2 kg	0687-07
	10 kg	0687-08

Bacto® Simmons Citrate Agar

Intended Use

Bacto Simmons Citrate Agar is used for differentiating *Enterobacteriaceae* based on citrate utilization.

Summary and Explanation

Koser[1] first developed a liquid medium for differentiating coliforms from fecal coliforms. Fecal coliforms were unable to use citrate as the sole source of carbon and inorganic ammonium salt as a sole source of nitrogen. Non-fecal coliforms, such as *Enterobacter aerogenes* or *Salmonella enteritidis* could use citrate in such a medium with resultant alkalinity. The liquid medium had the disadvantage of appearing turbid when large inocula were used although no growth had taken place. This observation led Simmons[2] to devise a solid medium that eliminated the problem with turbidity.

Simmons Citrate Agar is a modification of Koser's medium to which brom thymol blue and 1.5% agar have been added. Organisms able to metabolize the citrate grow luxuriantly. The medium is alkalinized and changes from its initial green to deep blue in 24-48 hours. *E. coli* either do not grow at all on this medium, or grow so sparsely that no change in reaction is apparent.

Simmons Citrate Agar is recommended for differentiation of enteric gram-negative bacilli from clinical specimens,[3,4] water samples,[5] and food samples.[6-9]

Principles of the Procedure

The ammonium dihydrogen phosphate is the sole source of nitrogen in Simmons Citrate Agar. Magnesium is a cofactor for a variety of metabolic reactions. Phosphate acts as a buffer. Sodium citrate is the sole source of carbon in this medium. Sodium chloride maintains the osmotic balance of the medium. Agar is the solidifying agent. Brom thymol blue is the pH indicator. Organisms that can utilize ammonium dihydrogen phosphate and sodium citrate as their sole sources of nitrogen and carbon will grow on this medium and produce a color change from green (neutral) to blue (alkaline).

Formula

Simmons Citrate Agar

Formula Per Liter

Magnesium Sulfate	0.2 g
Ammonium Dihydrogen Phosphate	1 g
Dipotassium Phosphate	1 g
Sodium Citrate	2 g
Sodium Chloride	5 g
Bacto Agar	15 g
Bacto Brom Thymol Blue	0.08 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared tubes at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Simmons Citrate Agar

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Tubes with closures
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 24.2 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes with closures.
4. Autoclave at 121°C for 15 minutes. Cool in a slanted position with long slant and short butt.

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[3-9]
2. Process each specimen, using procedures appropriate for that specimen or sample.[3-9]

Test Procedure

1. Obtain a pure culture of the organism to be tested.
2. With an inoculating needle or loop, pick the center of a well-isolated colonies obtained from solid culture media.
3. Streak only the surface of the slant with a light inoculum.
4. Loosen the closure on the tube.
5. Incubate at 35 ± 2°C for 18-48 hours.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Mustard yellow to yellow-green, free flowing, homogeneous.
Solution:	2.42% solution; soluble in distilled or deionized water on boiling. Solution is forest green, slightly opalescent, may have a slight precipitate.
Prepared Tubes:	Forest green, slightly opalescent, may have a slight precipitate.
Reaction of 2.42% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Prepare Simmons Citrate Agar per label directions. Inoculate with 1 µl of a dilution equivalent to a 0.5 McFarland Standard and incubate the tubes at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH	COLONY COLOR
Enterobacter aerogenes	13048*	good	blue
Escherichia coli	25922*	none to poor	green
Salmonella typhimurium	14028*	good	blue

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Results

A positive reaction is indicated by growth on the slant with an intense blue color (alkaline reaction). A negative reaction is indicated by no growth to poor growth without change in color (medium remains green).

Limitations of the Procedure

1. When inoculating a variety of biochemicals, flame the inoculating loop or needle before streaking Simmons Citrate Agar or inoculate Simmons Citrate Agar first to avoid a false positive result.[10]
2. Some citrate positive organisms require 48 hours or longer incubation for a pH change to occur.[10]

References

1. **Koser, S. A.** 1923. Utilization of the salts of organic acids by the colon-aerogenes group. J. Bacteriol. **8:**493.
2. **Simmons, J. S.** 1926. A culture medium for differentiating organisms of typhoid- colon aerogenes groups and for isolation of certain fungi. J. Infect. Dis. **39:**209.
3. **Pezzlo, M. (ed.).** 1992. Aerobic bacteriology, p. 1.0.0-1.20.47. *In* Isenberg, H.D. (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
4. **Baron, E. J., L. R. Peterson, S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
5. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Uninoculated tube | *Enterobacter aerogenes* ATCC® 13048 | *Escherichia coli* ATCC® 25922

6. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
7. **FDA Bacteriological Analytical Manual**, 8th ed. AOAC International, Gaithersburg, MD.
8. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
9. **Federal Register.** 1996. Pathogen reduction; hazard analysis and critical point (HACCP) systems; final rule. Fed. Regis. **61:**38917-38925.
10. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Simmons Citrate Agar	100 g	0091-15
	500 g	0091-17

Bacto® Skim Milk

Intended Use

Bacto Skim Milk is used for preparing microbiological culture media and for differentiating organisms based on coagulation and proteolysis of casein.

Summary and Explanation

Skim Milk is soluble, spray-dried skim milk. When prepared in a 10% solution, it is equivalent to fresh skim milk.

Skim Milk can be used to prepare skim milk agar for detecting proteolytic microorganisms in foods[1], including dairy products.[2] It can also be used to prepare litmus milk, a differential test medium for determining lactose fermentation and for detecting proteolytic enzymes that hydrolyze casein (milk protein) and cause coagulation (clot formation).[3]

User Quality Control

Identity Specifications

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous.
Solution:	10% solution, soluble in distilled or deionized water on warming. Solution is white, opalescent. After autoclaving, solution is off-white to beige, opaque.
Reaction of 10% Solution at 25°C:	pH 6.3 ± 0.2
Chemical Test:	Positive reaction with 3,5-dinitro salicylic acid.*

* Place 2 drops of a 2% solution of Skim Milk on filter paper and air dry. Dispense 3 drops of 0.5% 3,5-dinitro salicylic acid in 4% sodium hydroxide over the spot. Heat to 105°C for 5 minutes and note color development. A positive test is indicated by development of a brown color.

Cultural Response

Prepare Skim Milk per label directions. Inoculate with a drop or loopful of undiluted culture and incubate the tubes at 35 ± 2°C for 1-7 days.

ORGANISM	ATCC®	GROWTH	APPEARANCE
Lactobacillus casei	9595	good	acid, reduction, curd
Escherichia coli	25922*	good	acid, reduction, curd
Clostridium perfringens	12919	good	stormy fermentation

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Skim Milk is a source of lactose and casein. In the differential test medium, Litmus Milk, lactose fermentation is detected by the pH indicator, litmus. Hydrolysis of casein is detected by visible formation of a clot.

Formula

Skim Milk

Formula Per Liter

Skim Milk ..... 100 g

Final pH 6.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Skim Milk

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Dissolve 100 grams in 1 liter distilled or deionized water (with warming, if necessary).
2. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure
See appropriate references for specific procedures.

Results
Refer to appropriate references and procedures for results.

Limitations of the Procedure
Skim Milk supports growth of many microorganisms. Perform microscopic examination and other biochemical tests to identify isolates to the genus and species level, if necessary.

References

1. **Lee, J. S., and A. A. Kraft.** 1992. Proteolytic microorganisms, p. 193-198. *In* C. Vanderzant and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
2. **Frank, J. F., G. L. Christen, and L. B. Bullerman.** 1993. Tests for groups of microorganisms, p. 271-286. *In* Marshall, R. T. (ed.) Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **MacFaddin, J. F**. 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 440-445. Williams & Wilkins, Baltimore, MD.

Packaging
Skim Milk	500 g	0032-17

Bacto® Snyder Test Agar

Intended Use
Bacto Snyder Test Agar is used for estimating the relative number of lactobacilli in saliva based on acid production.

Also Known As
BCG Dextrose Agar[1]

Summary and Explanation
Tooth decay (dental caries) is a localized, progressive demineralization of the hard tissues of the crown and root surfaces of teeth. *Streptococcus mutans* and possibly lactobacilli ferment dietary carbohydrates that produce acids that cause the de- mineralization. The organisms reside in dental plaque, which is a gelatinous material that adheres to the surfaces of teeth. Demineralization of the tooth alternates with periods of remineralization. If demineralization exceeds remineralization, a subsurface carious lesion becomes a clinical cavity with extension of the decay into the dentine.[2]

Snyder [3,4] described a test procedure for determining, by colorimetric analysis, the rate and amount of acid produced by microorganisms in saliva. The procedure uses an agar medium that is known as Snyder Test Agar. Alban[5] simplified the procedure, used it extensively and reported it to be more accurate than Snyder's original procedure.

Principles of the Procedure
Snyder Test Agar contains Tryptose as a source of carbon, nitrogen, vitamins and minerals. Dextrose is the carbohydrate. Brom Cresol Green is the pH indicator. Bacto Agar is the solidifying agent.

Microorganisms that use the dextrose in the medium acidify the medium and the pH indicator, brom cresol green, changes color from blue-green to yellow.

User Quality Control

Identity Specifications
Dehydrated Appearance:	Light green, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in distilled or deionized water on boiling. Solution is dark emerald green, slightly opalescent.
Prepared Medium:	Dark emerald green, slightly opalescent.
Reaction of 6.5% Solution at 25°C:	pH 4.8 ± 0.2

Cultural Response
Prepare Snyder Test Agar per label directions. Inoculate and incubate the tubes at 35 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID PRODUCTION
Lactobacillus casei	9595	100-1,000	good	+
Lactobacillus fermentum	9338	100-1,000	good	+

The cultures listed are the minimum that should be used for performance testing.

Uninoculated tube — *Lactobacillus fermentum* ATCC® 9338

Formula

Snyder Test Agar

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	20 g
Sodium Chloride	5 g
Bacto Agar	20 g
Brom Cresol Green	0.02 g

Final pH 4.8 ± 0. 2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Snyder Test Agar

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)
Waterbath (45°C)
Cotton Swab
Paraffin

Method of Preparation

1. Suspend 65 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Specimens should be collected preferably before breakfast, lunch, or dinner, and before the teeth are brushed. This procedure can be done just before lunch or dinner.

Test Procedure

Snyder Procedure [3,4]

1. Collect specimens of saliva in a sterile container while patient is chewing paraffin for 3 minutes.
2. Shake specimens thoroughly and transfer 0.2 ml to a tube of sterile Snyder Test Agar melted and cooled to 45°C. (Prepared medium in tubes is heated in a boiling water bath for 10 minutes and cooled to 45°C.
3. Rotate the inoculated tubes to mix the inoculum uniformly with the medium and allow to solidify in an upright position.
4. Incubate at 35°C. Observe color at 24, 48 and 72 hours.

Alban Modication

1. Collect enough unstimulated saliva to just cover the medium in the tube. When specimen collection is difficult, dip a sterile cotton swab into the saliva under the tongue or rub on tooth surfaces and place the swab just below the surface of the medium.
2. Incubate the inoculated tubes and an uninoculated control at 35°C.
3. Examine tubes daily for four days.
4. Observe daily color change compared to control tube.

Results

Snyder Procedure

Observe tubes for a change in color of the medium from bluish-green (control) to yellow. A positive reaction is a change in color so that green is no longer dominant. Record as ++ to ++++. A negative reaction is no change in color or only a slight change. Green is still dominant. Record as 0 to +.

Interpretation:

CARIES ACTIVITY	HOURS INCUBATION 24	48	72
Marked	Positive	–	–
Moderate	Negative	Positive	–
Slight	Negative	Negative	Positive
Negative	Negative	Negative	Negative

Data summarizing the correlation between the Snyder colorimetric test and *Lactobacillus* counts on specimens of saliva collected routinely are tabulated.

Alban Modification

a. No color change
b. Color beginning to change to yellow from top of medium down (+)
c. One half of medium yellow (++)
d. Three fourths of medium yellow (+++)
e. The entire medium is yellow (++++)

The final report is a composite of the daily readings, for example; – + ++ +++. The readings indicate the rapidity and amount of acid production.

Limitations of the Procedure

1. The data indicate only what is happening at the time the specimen was collected.
2. At least two specimens collected with 2-4 days must be obtained to establish a base-line or reference point.
3. Only when two or more specimens have been cultured can any reliability or prediction be obtained.
4. The clinician must study enough cases by use of periodic laboratory data to establish the value of significance for the purpose intended.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 713-715. vol. 1. Williams & Wilkins, Baltimore, MD.

2. **Lewis, D. W., and A. I. Ismail.** 1995. Periodic health examination, 1995 update: 2. Prevention of dental caries. Canadian Medical Association Journal **152**:836- 846.
3. **Snyder**. 1941. J. Dent. Res. **20**:189.
4. **Snyder**. 1941. J. Am. Dent. Assoc. **28**:44.
5. **Alban**. 1970. J. Dent. Res. **49**:641.

Packaging

Snyder Test Agar	500 g	0247-17

Bacto® Soytone

User Quality Control

Identity Specifications

Soytone

Dehydrated Appearance:	Light to medium tan, free-flowing, homogenous.
Solution:	2% solution, soluble in distilled or deionized water. Light to medium amber, clear to very slightly opalescent.
Reaction of 1% Solution at 25°C:	pH 7.0 ± 0.5

Cultural Response

TEST	SOLUTION OF SOYTONE	ORGANISM	ATCC®	SOYTONE RESULT
Fermentable Carbohydrate	2%	*Escherichia coli*	25922*	positive
Indole Production	0.1%	*Escherichia coli*	25922*	positive
Acetylmethyl-carbinol Production	1% w/0.5% NaCl and 0.5% dextrose	*Enterobacter aerogenes*	13048*	positive
Hydrogen Sulfide Production	1%	*Salmonella typhimurium*	14028*	positive

TEST	SOLUTION OF SOYTONE	ORGANISM	ATCC®	RESULT
Growth Response	2% w/0.5% NaCl and 1.5% agar	*Brucella suis*	4314	good growth
Growth Response	2% w/0.5% NaCl and 1.5% agar	*Escherichia coli*	25922*	good growth
Growth Response 1.5% agar	2% w/0.5% NaCl and	*Staphylococccus aureus*	25923*	good growth

The cultures listed are the minimum that should be used for performance testing.

*These culture are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Intended Use

Bacto Soytone is an enzymatic digest of soybean meal.

Also Known As

Soytone is also known as Peptone S and Peptone Soya.

Summary and Explanation

Soytone is an enzymatic hydrolysate of soybean meal prepared under controlled conditions for use in microbiological procedures. Soytone is recommended for use in media for the cultivation of a large variety of organisms, including fungi and microbiological assay media. The nitrogen source in Soytone contains the naturally occurring high concentrations of vitamins and carbohydrates of soybean. Media supplemented with blood produce typical bacterial hemolytic patterns with Soytone as the main source of nitrogen.

Principles of the Procedure

Soytone is an enzymatic digest of soybean meal.

Typical Analysis

Soytone

Physical Characteristics

Ash (%)	12.0	Loss on Drying (%)	4.6
Clarity, 1% Solution (NTU)	1.0	pH, 1% Solution	7.2
Filterability (g/cm^2)	1.2		

Carbohydrate (%)

Total	24.0

Nitrogen Content (%)

Total Nitrogen	9.4	AN/TN	33.0
Amino Nitrogen	3.1		

Amino Acids (%)

Alanine	2.46	Lysine	3.45
Arginine	3.82	Methionine	0.86
Aspartic Acid	7.27	Phenylalanine	2.46
Cystine	1.45	Proline	2.92
Glutamic Acid	12.76	Serine	2.87
Glycine	2.51	Threonine	2.17
Histidine	1.24	Tryptophan	0.47
Isoleucine	2.37	Tyrosine	1.93
Leucine	4.03	Valine	2.65

Inorganics (%)

Calcium	0.055	Phosphate	0.820
Chloride	0.165	Potassium	2.220
Cobalt	<0.001	Sodium	3.404
Copper	<0.001	Sulfate	2.334
Iron	0.008	Sulfur	1.660
Lead	<0.001	Tin	<0.001
Magnesium	0.161	Zinc	0.001
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.2	PABA	9.0
Choline (as Choline Chloride)	2200.0	Pantothenic Acid	13.0
Cyanocobalamin	<0.1	Pyridoxine	11.0
Folic Acid	3.0	Riboflavin	<0.1
Inositol	2100.0	Thiamine	1.2
Nicotinic Acid	19.1	Thymidine	113.2

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	38
Salmonella	negative	Thermophile Count	<3
Spore Count	10		

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store Soytone below 30°C. The product is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Soytone

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Soytone in the formula of the medium being prepared. Add Soytone as required.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Soytone.

Results

Refer to appropriate references and procedures for results.

Packaging

Soytone	500 g	0436-17
	10 kg	0436-08

Bacto® Spirit Blue Agar Lipase Reagent

Intended Use

Bacto Spirit Blue Agar is for use with Bacto Lipase Reagent or other lipid source for detecting and enumerating lipolytic microorganisms.

Summary and Explanation

In 1941, Starr[1] described a lipid emulsion medium for detecting lipolytic (lipase-producing) microorganisms to which he added the dye, spirit blue. Other dyes as indicators of lipolysis were toxic to many microorganisms. Spirit blue did not have toxic effects. When testing samples of dairy products, air and sewage on Spirit Blue Agar, Starr obtained accurate counts of lipolytic microorganisms and total microbial counts on the same medium.

Lipolytic microorganisms, such as psychrotrophic bacteria, molds or yeasts, can adversely affect the flavor of milk and high fat dairy products. Spirit Blue Agar is a recommended medium for testing milk and dairy products.[2]

Lipase Reagent, a mixture of tributyrin and Polysorbate 80, is recommended as the lipid source. Other lipoidal emulsions may be prepared from cottonseed meal, cream, Wesson® oil and olive oil. A satisfactory emulsion can be prepared by dissolving 10 grams gum acacia or 1 ml Tween 80® in 400 ml warm distilled water, adding 100 ml cottonseed or olive oil and agitating vigorously to emulsify.

Principles of the Procedure

Spirit Blue Agar contains Tryptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Spirit Blue is the indicator of lipolysis. Bacto Agar is the solidifying agent.

Lipase Reagent contains tributyrin, a true fat and the simplest triglyceride occurring in natural fats and oils. It is a good substrate when testing for lipolytic microorganisms because some microorganisms that hydrolyze tributyrin will not hydrolyze other triglycerides or fats containing longer chain fatty acids.[2]

Formula

Spirit Blue Agar

Formula Per Liter

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Bacto Agar	20 g
Spirit Blue	0.15 g

Final pH 6.8 ± 0.2 at 25°C

Lipase Reagent

A ready-to-use lipid suspension, containing a mixture of tributyrin and Polysorbate 80.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store the Lipase Reagent at 15-30°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Spirit Blue Agar
Lipase Reagent

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 35 grams of Spirit Blue Agar in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50-55°C.
4. Aseptically add 30 ml Lipase Reagent or other lipid source and mix thoroughly.

Specimen Collection and Preparation

Collect specimens in sterile containers and transport immediately to the laboratory following recommended guidelines.

User Quality Control

Identity Specifications

Spirit Blue Agar

Dehydrated Appearance:	Grayish-beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water on boiling. Solution is royal blue, slightly opalescent.
Prepared Medium:	plain - royal blue, opalescent plain + 3% Lipase reagent - pale blue, opalescent
Reaction of 3.5% Solution at 25°C:	pH 6.8 ± 0.2

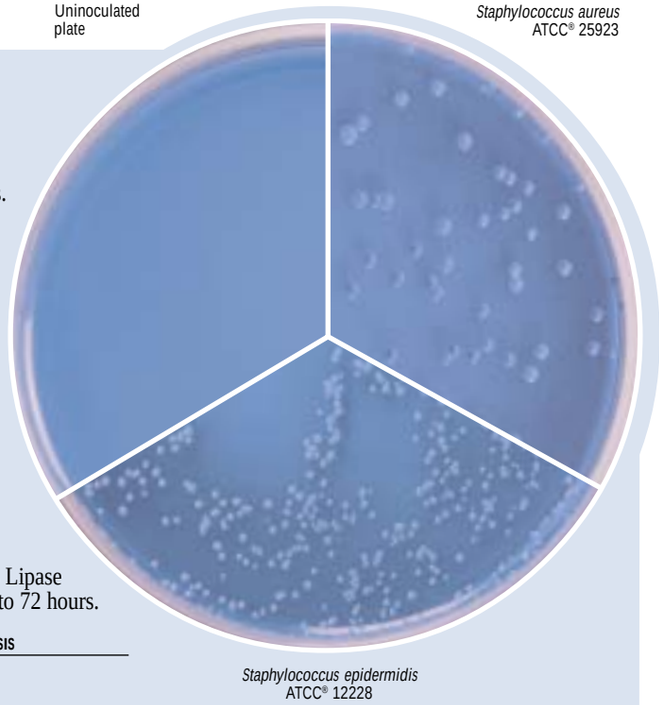
Lipase Reagent

Appearance:	White, opaque emulsion

Cultural Response

Prepare Spirit Blue Agar per label directions, with the addition of 3% Lipase Reagent after sterilization. Inoculate and incubate at 35 ± 2°C for up to 72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HALO/LIPOLYSIS
Proteus mirabilis	25933	100-1,000	good	no halo
Staphylococcus aureus	25923*	100-1,000	good	halo
Staphylococcus aureus	6538	100-1,000	good	halo
Staphylococcus epidermidis	12228*	100-1,000	good	halo



The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Test Procedure

1. Inoculate organism onto medium.
2. Incubate plates at 35 ± 2°C for up to 72 hours.

Results

Lipolytic microorganisms metabolize the lipid in the medium and form colonies with halos indicating lipolysis.

Limitations of the Procedure

1. Because the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Starr, M. P**. 1941. Spirit blue agar: a medium for the detection of lipolytic microorganisms. Science **93**:333-334.
2. **Frank. J. F., G. L. Christen, and L. B. Bullerman.** 1993. Tests for groups of microorganisms, p. 276-277. *In* R. T. Marshall (ed.), Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Packaging

Spirit Blue Agar	100 g	0950-15
	500 g	0950-17
Lipase Reagent	6 x 20 ml	0431-63

Bacto® m Staphylococcus Broth

Intended Use

Bacto m Staphylococcus Broth is used for isolating staphylococci by the membrane filtration technique.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	10.4% solution, soluble in distilled or deionized water on warming. Solution is light amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Medium:	Light amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 10.4% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare m Staphylococcus Broth per label directions. Use the membrane filtration technique with the test organisms. Inoculate and incubate at 35 ± 2°C under humid conditions for 40-48 hours. Plates are read for recovery and pigment production. Mannitol fermentation is detected by adding a drop of Brom Thymol Blue to the site where a colony was removed. Yellow color indicates a positive result for Mannitol fermentation.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	MANNITOL FERMENTATION	PIGMENT PRODUCTION
Escherichia coli	25922*	20-200	inhibited	N/A	–
Staphylococcus aureus	25923*	20-200	good	+	+
Staphylococcus epidermidis	12228*	20-200	good	–	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Summary and Explanation

Staphylococci, along with other bacteria, are indicators of recreational water quality.[4] Indicators of health risk include normal skin flora that are likely to be shed, such as *Pseudomonas*, *Streptococcus*, and *Staphylococcus*.[5] These organisms account for a large percentage of swimming pool-associated illness.[4]

The coagulase-positive species, *Staphylococcus aureus*, is well documented as a human opportunistic pathogen.[3] Coagulase-negative *Staphylococcus* spp. are a major component of the normal microflora of humans.[3] Staphylococci are widespread in nature, though they are mainly found living on the skin, skin glands, and mucous membranes of mammals and birds.[3]

Chapman[1] added 7.5% NaCl to Phenol Red Mannitol Agar to achieve a selective medium for staphylococci. While studying this medium formulation, Chapman[2] developed Staphylococcus Medium 110. m Staphylococcus Broth is patterned after the formula of Staphylococcus Medium 110.

m Staphylococcus Broth, with the addition of sodium azide, is specified for Recreational Waters in Standard Methods for the Examination of Water and Wastewater.[4]

Principles of the Procedure

Tryptone provides the nitrogen, amino acids and minerals in m Staphylococcus Broth. Yeast Extract is the vitamin source in this formula. Lactose and Mannitol are the carbohydrates for bacterial growth. Dipotassium Phosphate is the buffering agent. The high concentration of Sodium Chloride permits this medium to be selective for staphylococci.

Formula

m Staphylococcus Broth

Formula Per Liter

Bacto Tryptone	10 g
Bacto Yeast Extract	2.5 g
Bacto Lactose	2 g
Bacto Mannitol	10 g
Dipotassium Phosphate	5 g
Sodium Chloride	75 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

m Staphylococcus Broth

Materials Required But Not Provided

Membrane filtration equipment
Membrane filter
Autoclave
Glassware
Incubator (35°C)
Sterile tubes
Distilled or deionized water
Paper pads

Method of Preparation

1. Suspend 104 grams in 1 liter distilled or deionized water.
2. Warm to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

NOTE: When autoclave sterilization is not practical, boil medium for 5 minutes.

Specimen Collection and Preparation

Collect water samples as described in Standard Methods, Section 9213[4] or as specified by laboratory procedures.

Test Procedure

1. Follow the membrane filtration procedure described in Standard Methods, Section 9213,[4] or as described by laboratory procedures.
2. Use 2.0-2.5 ml of medium to saturate the paper pads on which the inoculated membrane is placed.
3. Incubate at 35 ± 2°C for 40-48 hours.

Results

Observe tubes for growth, indicating a positive reaction. Inoculate tubes showing turbidity to the appropriate medium for confirmation of *Staphylococcus*.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. m Staphylococcus Broth is used in sequence with an additional medium for confirmation. If necessary, confirm positive isolates using biochemical reactions.

References

1. **Chapman.** 1945. J. Bacteriol. **50**:201.
2. **Chapman.** 1946. J. Bacteriol. **51**:409.
3. **Kloos, W. E., and T. L. Bannerman.** 1995. *Staphylococcus* and *Micrococcus*, p. 282-298. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
5. **Seyfried, P. L., R. S. Tobin, N. E. Brown, and P. F. Ness.** 1985. A prospective study of swimming-related illness. II. Morbidity and the microbiological quality of water. Amer. J. Public Health **75**:1071.

Packaging

m Staphylococcus Broth	100 g	0649-15
	500 g	0649-17

Bacto® Staphylococcus Medium 110

Intended Use

Bacto Staphylococcus Medium 110 is used for isolating and differentiating staphylococci based on mannitol fermentation, pigment formation and gelatinase activity.

Also Known As

Staphylococcus Medium 110 is also known as Staphylococcus Agar No. 110 (Staphy-110, S-110) and Stone Gelatin Agar.[1]

Summary and Explanation

Stone[2] described a culture medium on which food-poisoning staphylococci gave a positive gelatinase test. Chapman, Lieb and Curcio[3] later reported that pathogenic staphylococci strains typically ferment mannitol, form pigment and produce gelatinase. Chapman[4] suggested adding 7.5% NaCl to Phenol Red Mannitol Agar to make a selective isolation medium for staphylococci using a high salt content. Further studies by Chapman[5] led to the development of Staphylococcus Medium 110. This medium is included in standard methods procedures for selectively isolating pathogenic staphylococci from foods.[6]

Principles of the Procedure

Staphylococcus Medium 110 contains Tryptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Sodium Chloride, in high concentration, inhibits most bacteria other than staphylococci. Lactose and D-Mannitol are the carbohydrates. Gelatin is included for testing liquefaction. Bacto Agar is the solidifying agent.

Pathogenic staphylococci (coagulase-positive staphylococci) typically resist the high salt concentration and form colonies with a yellow-orange pigment. These organisms typically ferment mannitol and produce acid, and liquefy gelatin, producing zones of clearing around the colonies.

Formula

Staphylococcus Medium 110

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Yeast Extract	2.5 g
Bacto Gelatin	30 g
Bacto Lactose	2 g
Bacto D-Mannitol	10 g
Sodium Chloride	75 g
Dipotassium Phosphate	5 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light beige to beige, free-flowing, homogeneous.
Solution:	14.9% solution, soluble in distilled or deionized water on boiling. Solution is light amber, slightly opalescent to opalescent, with heavy precipitate.
Prepared Medium:	Light amber, slightly opalescent to opalescent.
Reaction of 14.9% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Prepare Staphylococcus Medium 110 per label directions. Inoculate the plates and incubate the plates at 35 ± 2°C for 18-48 hours.

To test for mannitol fermentation, remove a colony from the medium, add a drop of 0.04% brom thymol blue to the plate, and observe for the formation of a yellow color (positive reaction).

To test for gelatinase reaction, flood the plate with 5 ml of saturated ammonium sulfate solution and incubate at 35 ± 2°C for 10 minutes. Observe for a zone of clearing around the colonies (positive reaction).

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	PIGMENT**	Gelatinase	Mannitol
Escherichia coli	25922*	100-300	marked to complete inhibition	–	N/A	N/A
Staphylococcus aureus	25923*	100-300	good	+	+	+
Staphylococcus epidermidis	12228*	100-300	good	–	+	–

**Pigment is seen as a yellow to orange color.

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Staphylococcus Medium 110

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)
0.04% Bromthymol blue
Saturated ammonium sulfate solution

Method of Preparation

1. Suspend 149 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 10 minutes.
4. Evenly disperse the precipitate when dispensing.

Specimen Collection and Preparation

Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[6]

Test Procedure

Consult appropriate references for procedures concerning selection and enumeration of staphylococci.[6]

Results

Growth of pathogenic staphylococci produces colonies with yellow-orange pigment.

Limitations of the Procedure

1. *Enterococcus faecalis* may grow on Staphylococcus Medium 110 as tiny colonies with mannitol fermentation. Differentiate these organisms from staphylococci with the Gram stain and catalase test.
2. Suspected staphylococci must be subcultured to Nutrient Broth, Blood Agar, BHI Broth, or Tryptose Phosphate Broth for coagulase testing as the high salt content of Staphylococcus Medium 110 may interfere with results.
3. Pigment production is not a reliable criterion for differentiation of staphylococcal spp.

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. p. 722-726. Williams & Wilkins, Baltimore, MD.
2. **Stone, R. V.** 1935. A cultural method for classifying staphylococci as of the "food poisoning" type. Proc. Soc. Exptl. Biol. Med. **33**:185-187.
3. **Chapman, G. H., C. W. Lieb, and L. G. Curcio.** 1937. Isolation and cultural differentiation of food-poisoning staphylococci. Food Research. **2**:349.
4. **Chapman, G. H.** 1945. The significance of sodium chloride in studies of staphylococci. J. Bacteriol. **50**:201.
5. **Chapman, G. H.** 1946. A single culture medium for selective isolation of plasma-coagulating staphylococci and for improved testing of chromogenesis, plasma coagulation, mannitol fermentation and the Stone reaction. J. Bacteriol. **51**:409.
6. **Association of Official Analytical Chemists**. 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

Staphylococcus Medium 110	500 g	0297-17
	2 kg	0297-07
	10 kg	0297-08

Bacto® Starch Agar

Intended Use

Bacto Starch Agar is used for cultivating microorganisms being tested for starch hydrolysis.

Summary and Explanation

In 1915,[1] Vedder formulated Starch Agar for cultivating *Neisseria*. Since then, other media have been developed that are superior to Starch Agar for the isolation of *Neisseria* spp, including enriched GC Medium Base. Starch Agar is used in differentiating microorganisms based on the starch hydrolysis test.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.5% solution, soluble in distilled or deionized water on boiling. Light amber, slightly opalescent without precipitate.
Prepared Medium:	Light amber, slightly opalescent without significant precipitate.
Reaction of 2.5% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

Inoculate with a single streak of undiluted test organism and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	RECOVERY	STARCH HYDROLYSIS
Bacillus subtilis	6633	good	positive
Escherichia coli	25922*	good	negative
Staphylococcus aureus	25923*	good	negative
Streptococcus pyogenes	19615*	good	negative

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Starch Agar Medium for *Pseudomonas*[2] and Starch Agar with Bromcresol Purple[3] are modifications of Starch Agar used for the differentiation of *Gardnerella vaginalis*.

Principles of the Procedure

Beef Extract provides the nitrogen, vitamins, carbon and amino acids in Starch Agar. Starch reacts with Gram's Iodine to give a blue color. Organisms hydrolyzing starch through amylase production will produce a clearing around the isolate while the remaining medium is blue. Bacto Agar is a solidifying agent.

Formula

Starch Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Soluble Starch	10 g
Bacto Agar	12 g

Final pH 7.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The powders are very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Starch Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Gram Iodine
Sterile Petri dishes

Method of Preparation

1. Dissolve 25 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 45-50°C.
5. Dispense into sterile Petri dishes or as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

Starch Hydrolysis Test

Flood the surface of a 48-hour culture on Starch Agar with Gram Iodine.

For a complete discussion of the collection, isolation and identification of microorganisms, refer to appropriate references.[4,5]

Results

Starch hydrolysis (+) is indicated by a colorless zone surrounding colonies. A blue or purple zone indicates that starch has not been hydrolyzed (-).

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Vedder.** 1915. J. Infect. Dis. **16**:385.
2. **Atlas, R. M.** 1993. Handbook of microbiological media, p. 844-845, CRC Press, Boca Raton, FL.
3. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 727-729, Williams & Wilkins, Baltimore, MD.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Packaging

Starch Agar	500 g	0072-17

Bacto® Stock Culture Agar

Intended Use

Bacto Stock Culture Agar is used for maintaining stock cultures of bacteria, particularly streptococci.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in distilled or deionized water on boiling. Solution is medium amber, opalescent.
Prepared Medium:	Medium amber, opalescent.
Reaction of 5% Solution at 25°C:	pH 7.5 ± 0.2

Cultural Response

Prepare Stock Culture Agar per label directions. Inoculate undiluted broth cultures of the test organisms by stabbing the medium with an inoculating needle. Incubate at 35°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH
Staphylococcus aureus	25923*	good
Streptococcus pneumoniae	6305	good
Streptococcus pyogenes	19615*	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Summary and Explanation

Ayers and Johnson[1] reported a medium that gave luxuriant growth and extended viability of streptococci and other organisms. The success of their medium can be attributed to its semisolid consistency, added casein, buffered environment and dextrose, which serves as a readily available source of energy. This study reported that pathogenic streptococci remained viable for at least four months at room temperature (24°C) in the medium. Organisms such as *Streptococcus pneumoniae*, *Mycobacterium* spp. and others, grew well on their medium. Stock Culture Agar is prepared to duplicate the medium described by Ayers and Johnson.[1]

Stock Culture Agar may also be prepared with L-asparagine (1 gram/liter) for the maintenance of pathogenic and non-pathogenic bacteria, especially streptococci.[2]

Principles of the Procedure

Infusion from Beef Heart, Proteose Peptone, Gelatin and Isoelectric Casein provide the nitrogen, vitamins and amino acids in Stock Culture Agar. Dextrose is a carbon source. Disodium phosphate is a buffering agent. Sodium citrate acts as a preservative. Bacto Agar is a solidifying agent.

Formula

Stock Culture Agar

Formula Per Liter

Beef Heart, Infusion from	500 g
Bacto Proteose Peptone	10 g
Bacto Gelatin	10 g
Isoelectric Casein	5 g
Bacto Dextrose	0.5 g
Disodium Phosphate	4 g
Sodium Citrate	3 g
Bacto Agar	7.5 g

Final pH 7.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Stock Culture Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Sterile Petri dishes
L-aspargine (optional)

Method of Preparation

1. Suspend 50 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Dispense as desired.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Ayers, S. H., and W. T. Johnson.** 1924. Studies of the streptococci. J. Bacteriol. **9**:111-114.
2. **Atlas, R. M.** 1993. Handbook of microbiological media. CRC Press, Boca Raton, FL.

Packaging

Stock Culture Agar	500 g	0054-17

Bacto® Sulfite Agar

Intended Use

Bacto Sulfite Agar is used for detecting thermophilic, H_2S-producing anaerobes, particularly in foods.

Summary and Explanation

Sulfide spoilage of foods is due to three factors: high spore counts, the heat resistance of the spores, and subjecting the finished product to elevated temperatures. The last factor may occur if the processed food is not cooled adequately.[3]

Clark and Tanner[1] described the thermophilic organisms that cause spoilage in canned foods as flat-sour spoilage organisms, thermophilic anaerobes and sulfide-spoilage organisms. They used Sulfite Agar to study sulfide-spoilage organisms in sugar and starch.

Both beet and cane sugar can carry spores of the thermophilic bacteria that are spoilage agents.[2] *Desulfotomaculum nigrificans*, first classified as *Clostridium nigrificans*, causes spoilage in non-acid canned foods such as vegetables and infant formula.[3] The growth of *D. nigrificans* occurs in the range of pH 6.2-7.8, with the best growth occurring at pH 6.8-7.3. Scanty growth can be observed at pH 5.6. The reaction of most vegetables, except corn and peas, falls below pH 5.8, so sulfide spoilage is rare.[3]

Sulfite Agar is a recommended Standard Methods medium for isolating *D. nigrificans*.[2, 3]

Principles of the Procedure

Sulfite Agar contains Tryptone as a source of carbon, nitrogen, vitamins and minerals. Sodium Sulfite, upon reduction, produces hydrogen sulfide. Bacto Agar is the solidifying agent.

Iron nails or iron strips will combine with any dissolved oxygen in the medium and provide an anaerobic environment.

Formula

Sulfite Agar

Formula Per Liter

Bacto Tryptone	10 g
Sodium Sulfite	1 g
Bacto Agar	20 g

Final pH 7.6 ± 0. 2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Sulfite Agar

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)
Sterile tubes with closures
Iron nails or strips

Method of Preparation

1. Suspend 31 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation[3]

Dry Sugar

1. Place 20 grams of dry sugar in a dry, sterile, graduated 250 ml Erlenmeyer flask closed with a rubber stopper.
2. Add sterile water to the 100 ml mark and shake to dissolve.
3. Replace the stopper with a sterile cotton plug, bring the solution rapidly to a boil, and continue boiling for 5 minutes.
4. Replace evaporated liquid with sterile water.
5. Cool immediately in cold water.

Liquid Sugar

Prepare as for dry sugar except determine the amount of liquid sugar needed on the basis of %Brix in order to be equivalent to 20 grams of dry sugar.[2]

Starch and Flour

1. Place 20 grams of starch or flour in a dry, sterile, graduated 250 ml Erlenmeyer flask.
2. Add sterile water to the 100 ml mark, swirling occasionally.
3. Close the flask with a sterile rubber stopper.
4. Shake well to obtain a uniform, lump-free suspension. Add sterile glass beads to the sample mixture to aid in thoroughly mixing during shaking.

Nonfat Dry Milk

1. Place 10 grams of nonfat dry milk in a sterile, graduated 250 ml Erlenmeyer flask.
2. Add .02N sodium hydroxide to the 100 ml mark.
3. Shake to completely dissolve.
4. Autoclave at 5 pounds pressure for 10 minutes.
5. Cool immediately.

Cream

1. Mix 2 grams of gum tragacanth and 1 gram of gum arabic in 100 ml of water in an Erlenmeyer flask.
2. Sterilize at 121°C for 20 minutes.
3. Transfer 20 ml of cream sample to a sterile, graduated 250 ml Erlenmeyer flask.
4. Add sterilized gum mixture to the 100 ml mark.
5. Shake carefully using a sterile rubber stopper.
6. Loosen the stopper. Autoclave at 5 pounds pressure for 5 minutes.

Soy Protein Isolates

1. Prepare a 10% suspension of soy protein isolate in sterile 0.1% peptone water in milk dilution or similar bottles.

User Quality Control

Identity Specifications

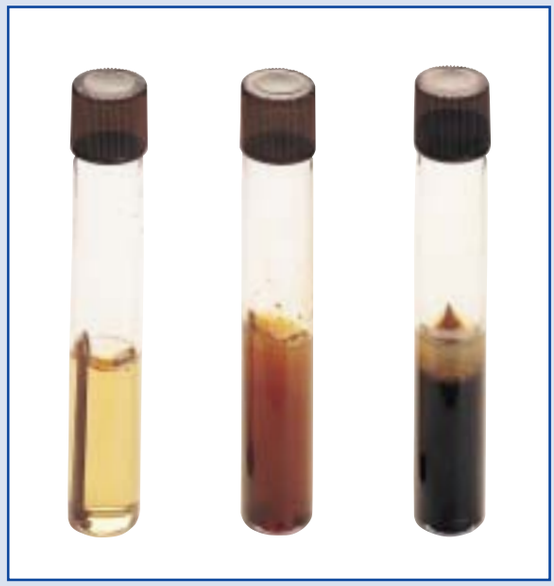
Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	3.1% solution, soluble in distilled or deionized water upon boiling. Light amber, very slightly to slightly opalescent.
Prepared Medium:	Light amber, very slightly to slightly opalescent.
Reaction of 3.1% Solution at 25°C:	pH 7.6 ± 0.2

Cultural Response

Prepare Sulfite Agar per label directions. Inoculate molten medium, solidify, and incubate aerobically at 55 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	SULFITE REDUCTION
Bacillus stearothermophilus	10149	30-100	good	–
Clostridium thermosaccharolyticum	7956	30-100	good	+
Desulfotomaculum nigrificans	19858	30-100	good	+

The cultures listed are the minimum that should be used for performance testing.
*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Uninoculated tube | *Bacillus stearothermophilus* ATCC® 10149 | *Desulfotomaculum nigrificans* ATCC® 19858

2. Adjust to pH 7.0 ± 0.1.
3. Autoclave at 5 pounds pressure for 20 minutes.

Test Procedure

Sugar

1. Divide 20 ml of heated sugar solution among 6 screw-cap tubes (20 x 150 mm) containing approximately 10 ml of freshly autoclaved, still molten Sulfite Agar and a nail.
2. Cool and solidify immediately in cold water.
3. Preheat the tubes to 50-55°C.
4. Incubate at 50-55°C for 24-48 hours.

Starch and Flour

1. Divide 20 ml of the starch or flour suspension among 6 screw-cap tubes (20 x 150 mm) containing approximately 10 ml of freshly autoclaved, still molten Sulfite Agar and a nail.
2. Swirl the tubes several times to ensure even dispersion of the starch or flour in the medium. Heat in a boiling water bath for 15 minutes, continuing to swirl the tubes.
3. Cool and solidify immediately in cold water.
4. Preheat the tubes to 50-55°C.
5. Incubate at 50-55°C for 24-48 hours.

Nonfat Dry Milk

1. Transfer 2 ml of nonfat dry milk solution to each of 2 screw cap tubes (20 X 150 mm) containing freshly autoclaved, still molten Sulfite Agar and a nail.
2. Gently swirl several times.
3. Cool and solidify immediately in cold water.
4. Preheat the tubes to 50-55°C.
5. Incubate at 50-55°C for 24-48 ± 3 hours.
6. Count colonies of *D. nigrificans* and report on the basis of a 10 gram sample.

Soy Protein Isolates

1. Add 1 ml of soy protein isolate suspension to each of 10 tubes containing freshly autoclaved, still molten Sulfite Agar and a nail. If using already prepared medium, heat the tubes immediately before inoculation to eliminate oxygen.
2. Mix tubes.
3. Solidify in an ice water bath.
4. Overlay with Vaspar.
5. Preheat the tubes to 55°C.
6. Incubate at 55°C for 14 days. Take preliminary counts at 48 hours, 7 days and 14 days in case tubes become completely blackened.
7. Count the blackened areas for each tube and report as the number of spores per gram of soy isolate.

Results

Hydrogen sulfide production from the reduction of sulfite causes a blackening of the medium.

Sulfide spoilage spores should be present in not more than 2 of 5 samples tested (40%) with not more than 5 spores per 10 gram in any one sample.[4]

Limitations of the Procedure

1. Nails or iron strips should be cleaned in hydrochloric acid and rinsed well to remove any rust before being placed into tubes of medium.
2. If iron nails or iron strips are not available, substitute 10 ml of 5% ferric citrate solution.
3. Spoiled peas may not show discoloration but will show blackening with a dark- colored brine.
4. Spangling of the enamel may occur as a result of the interaction of dissolved hydrogen sulfide with the iron of the container.

References

1. **Clark, F. M., and F. W. Tanner.** 1937. Thermophilic canned-food spoilage organisms in sugar and starch. Food Res. **2**:27-39.
2. **Andrews, W.** 1995. Microbial methods, p. 1-119. *In* Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.
3. **Donnelly, L. S., and R. R. Graves.** 1992. Sulfide spoilage sporeformers, p. 317- 323. *In* C. Vanderzant and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
4. **NCA Research Laboratories.** 1968. Laboratory Manual for Food Canners and Processors, vol. 1, p. 104. Natl. Canners Assn. (Now, Natl. Food Processors Assn.) AVI Inc., Westport, CT.

Packaging

Sulfite Agar	500 g	0972-17

Bacto® Synthetic Broth AOAC

Intended Use

Bacto Synthetic Broth AOAC is used for maintaining disinfectant test cultures.

Summary and Explanation

Synthetic Broth AOAC is a chemically defined broth recommended by the Association of Official Analytical Chemists (AOAC).[1] It contains all the nutrients essential for growth of the test cultures used in determining the phenol coefficients of disinfectants.

Principles Of The Procedure

The chemically-defined ingredients in Synthetic Broth AOAC provide nitrogen, carbon, vitamins and minerals required for bacterial growth.

Formula

Synthetic Broth AOAC

Formula Per Liter

L-Cystine	0.05 g
DL-Methionine	0.37 g
L-Arginine HCl	0.4 g

DL-Histidine HCl	0.3 g
L-Lysine HCl	0.85 g
L-Tyrosine	0.21 g
DL-Threonine	0.5 g
DL-Valine	1 g
L-Leucine	0.8 g
DL-Isoleucine	0.44 g
Glycine	0.06 g
DL-Serine	0.6 g
DL-Alanine	0.43 g
L-Glutamic Acid HCl	1.3 g
L-Aspartic Acid	0.45 g
DL-Phenylalanine	0.26 g
DL-Tryptophan	0.05 g
L-Proline	0.05 g
Sodium Chloride	3 g
Potassium Chloride	0.2 g
Magnesium Sulfate Anhydrous Reagent	0.05 g
Potassium Phosphate	1.5 g
Disodium Phosphate	4 g
Thiamine HCl	0.01 g
Nicotinamide	0.01 g

Final pH 7.1 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	White, homogeneous, free-flowing.
Solution:	1.7% solution, soluble in distilled or deionized water on boiling. Solution is colorless and clear with no precipitate.
Prepared Medium:	Colorless and clear with no precipitate.
Reaction of 1.7% Solution at 25°C:	pH 7.1 ± 0.1

Cultural Response

Prepare Synthetic Broth AOAC per label directions. Inoculate and incubate the tubes at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	APPROXIMATE INOCULUM CFU	GROWTH
Pseudomonas aeruginosa	15442	100	good
Salmonella choleraesuis	10708	100	good
Salmonella typhi	6539	100	good
Staphylococcus aureus	6538	100	good

The cultures listed are the minimum that should be used for performance testing.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Synthetic Broth AOAC

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°)
20 x 150 mm tubes with closures
Sterile 10% dextrose solution

Method of Preparation

1. Suspend 17 grams in 1 liter distilled or deionized water.
2. Boil for 1-2 minutes.
3. Dispense 10 ml amounts into 20 x 150 mm culture tubes.
4. Autoclave at 121°C for 20 minutes.
5. Before inoculating, aseptically add 0.1 ml sterile 10% dextrose solution to each tube.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

Not applicable

References

1. **Association of Official Analytical Chemists**. 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Synthetic Broth AOAC	500 g	0352-17
	10 kg	0352-08

Bacto® m T7 Agar

Intended Use

Bacto mT7 Agar is used for recovering injured coliforms from treated water by membrane filtration.

Summary and Explanation

Selective media used with the membrane filter method do not adequately recover injured coliforms.[1,2,3,4] McFeters et al. studied the influences of diluents, media and procedures in recovering injured coliform bacteria and found improved recovery using Tergitol 7 Agar.[5] LeChevallier et al. modified Tergitol 7 Agar and developed a new medium, m T7 Agar, for improved recovery of injured coliforms from drinking water.[6] In a later study, LeChevallier et al.[7] evaluated mT7 Agar as a fecal coliform medium and found optimum recovery using preincubation at 37°C for 8 hours followed by incubation at 44.5°C for 12 hours.[7] The authors found that incorporation of 0.1 µg of penicillin G per ml, aseptically added to the medium after autoclaving, prevented growth of gram-positive cocci that may break through. Later, they found that 1.0 µg/ml of penicillin G provided far better inhibition of gram-positive organisms without interfering with the recovery of coliforms. LeChevallier and McFeters reported the work of five collaborating laboratories testing coliform recovery from contaminated surface water and sewage samples.[8] They found m T7 Agar to be more effective than m Endo Agar in recovering coliforms.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Yellow-green to blue-green, free-flowing, homogeneous, may have a slightly moist appearance and/or tendency to form soft lumps.
Solution:	4.86% solution, soluble in distilled or deionized water upon boiling. Reddish purple, slightly opalescent without significant precipitate.
Prepared Medium:	Reddish purple, slightly opalescent without significant precipitate.
Reaction of 4.86% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare mT7 Agar per label directions. Inoculate with test organisms diluted in 10 ml of water. Incubate at 35 ± 2°C for 8 hours and then at 44.5°C for an additional 12 hours.

ORGANISM	ATCC®	INOCULUM CFU (approx.)	GROWTH	COLONY COLOR
Escherichia coli	25922*	100	good	yellow
Escherichia coli	13762	100	good	yellow
Enterococcus faecalis	19433	100	poor to fair	–
Pseudomonas aeruginosa	27853*	100	poor to fair	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Standard Methods procedures to recover injured total coliform bacteria from treated water specify m T7 Agar.[9] Stressed organisms can be present in treated drinking water and wastewater, saline waters and relatively clean surface waters.[9]

Principles of the Procedure

The ingredients of m T7 Agar support growth of injured coliforms. Proteose Peptone No. 3 provides nitrogen and amino acids. Yeast Extract is a vitamin source and Lactose provides carbon. Tergitol 7 and Polyoxyethylene Ether W-1 are selective agents at optimal concentrations that will not affect recovery of injured coliforms. Brom Cresol Purple and Brom Thymol Blue are indicators of lactose fermentation. The combination of dyes provides a good differential reaction as well as additional inhibition to noncoliform bacteria. Bacto Agar is a solidifying agent.

Penicillin G (1.0 µg/ml), aseptically added to the medium after autoclaving, prevents growth of gram-positive cocci without interfering with recovery of coliforms.[8]

Formula

m T7 Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Bacto Lactose	20 g
Tergitol 7	0.4 ml
Polyoxyethylene Ether W-1	5 g
Bacto Brom Thymol Blue	0.1 g
Bacto Brom Cresol Purple	0.1 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store prepared plates containing penicillin G at 2-8°C and use within 1 week after preparation.[6]

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

m T7 Agar

Materials Required But Not Provided

Glassware
Autoclave
Distilled or deionized water

Membrane filter equipment
Sterile 47 mm 0.45 µm gridded membrane filters
Sterile Petri dishes 50 x 9 mm
Pipettes
Stereoscopic microscope
Dilution bottles
Incubator or waterbath (37°C and 45°C)
Penicillin G (1.0 µg/ml)

Method of Preparation

1. Suspend 48.6 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. To prepare a more selective medium, aseptically add 1.0 µg penicillin G per ml to the sterile medium cooled to 45°C.
5. Dispense 4-5 ml amounts into 50 x 9 mm Petri dishes.

Note: Stock solutions of 0.1 mg/ml of penicillin G (sodium salt) can be filter sterilized, frozen in aliquots, and stored for up to 6 months. (One international or USP penicillin unit is equivalent to 0.6 µg of benzylpenicillin sodium).

Specimen Collection and Preparation

Water samples should be collected as described in Standard Methods for the Examination of Water and Wastewater.[9]

Test Procedure

For a complete discussion of stressed organisms in water testing, refer to the membrane filter procedure for the coliform group as described in Standard Methods for the Examination of Water and Wastewater.[9]

Incubate inoculated plates at 37°C for 8 hours and then at 44.5°C for an additional 12 hours. This procedure has been found to produce consistently higher fecal coliform counts with mT 7 Agar.[7]

Results

After incubation, count all yellow, smooth, convex colonies as coliforms with the aid of a stereoscopic microscope.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. The procedure for enumerating fecal coliforms with m T7 Agar requires two incubation temperatures.
3. The addition of penicillin G is required for better inhibition of gram-positive bacteria.
4. m T7 Agar may recover other coliforms in addition to *E. coli*. Some drinking water samples contain so many non-coliform bacteria that confluent growth may occur. Care must be taken to distinguish yellow colonies from background growth.[9]

References

1. **Maxcy, R. B.** 1970. Non-lethal injury and limitations of recovery of coliform organisms on selective media. J. Milk Food Technol. **33**:445-448.
2. **Scheusner, D. L., F. F. Busta, and M. L. Speck.** 1971. Inhibition of injured *Escherichia coli* by several selective agents. Appl. Microbiol. **21**:46-49.
3. **Grabow, W. O. K., and M. du Preez.** 1979. Comparison of mEndo LES, MacConkey and Teepol media for membrane filtration counting of total coliform bacteria in water. Appl. Environ. Microbiol. **38**:351-358.
4. **Hoadley, A. W., and C. M. Cheng.** 1974. Recovery of indicator bacteria on selective media. J. Appl. Bacteriol. **37**:45-57.
5. **McFeters, G. A. , S. C. Cameron, and M. W. LeChevallier.** 1982. Influence of diluents, media and membrane filters on detection of injured waterborne coliform bacteria. Appl. Environ. Microbiol. **43**:97-103.
6. **LeChevallier, M. W., S. C. Cameron, and G. A. McFeters.** 1983. New medium for improved recovery of coliform bacteria from drinking water. Appl. Environ. Microbiol. **45**:484-492.
7. **LeChevallier, M. W., P. E. Jajanoski, A. K. Camper, and G. A. McFeters.** 1984. Evaluation of m-T7 agar as a fecal coliform bacteria from drinking water. Appl. Environ. Microbiol. **48**:371-375.
8. **LeChevallier, M. W., and G. A. McFeters.** 1985. Enumerating injured coliforms in drinking water. Research and Technology. J. AWWA. **77**:81-87.
9. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

Packaging

mT7 Agar	100 g	0018-15

Bacto® TAT Broth Base
Bacto TAT Broth

Intended Use

Bacto TAT Broth Base with added Tween® 20 and Bacto TAT Broth are used for cultivating microorganisms from highly viscous or gelatinous materials.

Also Known As

TAT (Tryptone-Azolectin-Tween) Broth Base is also referred to as Fluid Casein Digest-Soy Lecithin Polysorbate 20 Medium.

Summary and Explanation

TAT Broth Base with the addition of Tween® 20 is recommended for sterility testing of viscous materials, such as salves or ointments. It is especially adapted to the sterility testing of cosmetics. Cosmetics and pharmaceutical products are subject to contamination during manufacturing and use by consumers.[1] Preservatives are used in aqueous products to make them self-sterilizing for vegetative bacteria, yeasts and molds.[1]

TAT Broth Base is an enrichment medium developed to isolate and cultivate microorganisms. TAT Broth Base conforms to the formula specified by US Pharmacopeia for use in Microbial Limit Tests.[2]

Principles of the Procedure

Tryptone provides the nitrogen, vitamins, amino acids and carbon in TAT Broth Base. Azolectin and Tween® 20 neutralize preservatives in the cosmetics or pharmaceutical products, allowing bacteria to grow.

Formula

TAT Broth Base

Formula Per Liter

Bacto Tryptone	20 g
Azolectin	5 g

Final pH 7.2 ± 0.2 at 25°C

TAT Broth

Formula Per Liter

Bacto Tryptone	20 g
Azolectin	5 g
Tween® 20	40 ml

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store the prepared medium at 15-30°C.

User Quality Control

Identity Specifications

TAT Broth Base

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	2.5% solution with 4% Tween® 20; solution is light amber, clear to very slightly opalescent with a very slight precipitate.
Prepared Medium:	Light amber, clear to very slightly opalescent.
Reaction of 2.5% Solution w/ 4% Tween® 20 at 25°C:	pH 7.2 ± 0.2

Cultural Response

TAT Broth

Prepare TAT Broth Base per label directions or use prepared TAT Broth. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Pseudomonas aeruginosa	27853*	100-1,000	good
Salmonella typhi	6539	100-1,000	good
Staphylococcus aureus	25923*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disk Technical Information.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

TAT Broth Base (dehydrated)
TAT Broth (prepared)

Materials Required But Not Provided

Tween® 20 (for dehydrated TAT Broth Base)
Glassware
Autoclave
Waterbath (50-60°C)
Sterile test tubes

Method of Preparation

TAT Broth Base (dehydrated)

1. Suspend 25 grams in 960 ml distilled or deionized water.
2. Add 40 ml Tween® 20.
3. Heat to 50-60°C.
4. Let stand 15-30 minutes with occasional agitation to dissolve completely.
5. Autoclave at 121°C for 15 minutes.
6. Dispense as desired.

TAT Broth (prepared)

1. In an area adjacent to the clean room, remove bottles from their boxes.
2. Follow careful aseptic technique when uncapping bottles for testing.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

1. Add one gram or one ml of an undiluted sample to 40 ml of complete medium and agitate to obtain an even suspension.
2. Incubate tubes at 35 ± 2°C for 18-48 hours.

For a complete discussion on sterility testing refer to appropriate procedures in USP.[2]

Results

Tubes or bottles exhibiting growth should be subcultured for identification.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Orth, D. S.** 1993. Handbook of cosmetic microbiology. Marcel Dekker, Inc., New York, N.Y.
2. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. Microbial limits tests, p. 1681-1686. The United States Pharmacopeial Convention Inc., Rockville, MD.

Packaging

TAT Broth Base	500 g	0984-17
TAT Broth	10 x 90 ml	9072-73

Bacto® TB Hydrolysis Reagent

Intended Use

Bacto TB Hydrolysis Reagent is used for differentiating mycobacteria on their ability to hydrolyze Polysorbate 80. TB Hydrolysis Reagent is also used for differentiating *Moraxella catarrhalis* from *Neisseria* spp.

Also Known As

"Tween hydrolysis" is a common term for the TB Hydrolysis Reagent test.

Summary and Explanation

Wayne, Doubek and Russell[1] differentiated various species and subgroups of acid-fast bacilli using the Tween 80 hydrolysis test. Kubica and Dye[2] used the test to differentiate clinically significant from clinically insignificant mycobacteria. In 1970, Runyan, Kubica, Morse, Smith and Wayne[3] defined a positive reaction as one that occurs in less than five days. A doubtful reaction was defined as one that occurs in five to ten days. A negative reaction was defined as one occurring after ten days.

In 1973, Kubica[4] recognized the greater reliability of a 10-day Tween hydrolysis test for separation of clinically insignificant from clinically significant members of both the scotochromagenic and non-photochromogenic mycobacteria. These observations were later confirmed by Wayne *et al*.[5] citing that a 10-day reading was regarded as a better end point for the Tween hydrolysis test.

In 1990, Weiner and Penha[6] described the differentiation of *Moraxella catarrhalis* from other *Moraxella* and *Neisseria* spp. using TB Hydrolysis Reagent.

Principles of the Procedure

Polysorbate 80 binds to the neutral red indicator, causing the solution to be amber colored. If the mycobacterial lipase splits the Polysorbate 80, it can no longer complex with the neutral red indicator which then exhibits its normal red color at pH 7. The intensity of the red depends upon how much Polysorbate 80 is split.

Some mycobacteria possess a lipase capable of splitting Polysorbate 80 into oleic acid and polyoxyethylated sorbitol, modifying the solution from yellow to pink. The differential criterion of this test is based on the relative time necessary for a particular species or subgroup to hydrolyze the compound. Most *M. kansasii*[2] strains and clinically insignificant species are positive in five days[4,5] or less, while clinically significant species may be negative even after three weeks. *Mycobacterium tuberculosis* generally yields a positive reaction in 10-20 days.

Moraxella catarrhalis hydrolyzes Tween 80 after 24 hours of incubation, producing a clear change in color from amber to pink-red. Other *Moraxella* and *Neisseria* spp. remain negative after an additional 24 hours of incubation.[6]

User Quality Control

Identity Specifications

Reagent Appearance: Reddish-amber solution

Cultural Response

The TB Hydrolysis Test is performed on cultures which are inoculated and incubated at 35 ± 2°C for 5-10 days for *Mycobacterium* sp. and 18-48 hours for *Moraxella* and *Neisseria* spp.

ORGANISM	ATCC®	TIME TO COLOR CHANGE	REACTION
Mycobacterium gordonae	14470	5 days	positive
Mycobacterium kansasii	12478	5 days	positive
Mycobacterium scrofulaceum	19981	10 days	negative
Moraxella (Branhamella) catarrhalis	25238	24 hours	positive
Neisseria sicca	9913	48 hours	negative

Positive - any change in reagent color from the original color

Negative - no change in color

The cultures listed are the minimum that should be used for performance testing.

Formula

TB Hydrolysis Reagent is a sterile, phosphate-buffered solution of Tween 80 and neutral red.

Precautions

1. CAUTION: Laboratory acquired infection is always a distinct possibility when handling and processing specimens containing *Mycobacterium tuberculosis*. Laboratory procedures with specimens containing *M. tuberculosis* should be performed in a properly equipped laboratory (*i.e.*, under a Class 1 negative pressure or Class 2 laminar flow biological safety cabinet) and by personnel thoroughly familiar with proper techniques. For detailed information, consult the appropriate references.[7,8,9]
2. For In Vitro Diagnostic Use.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store TB Hydrolysis Reagent at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

TB Hydrolysis Reagent

Materials Required But Not Provided

13 x 75 mm screw cap test tubes
Bacteriological loop
Incubator (35°C)
White paper or porcelain

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory according to recommended guidelines.[7]

2. Process each specimen, using procedures appropriate for that sample.[7]
3. Test actively metabolizing 3-4 week old pure cultures of *Mycobacterium*, or an 18-24 hour isolate of *Moraxella* spp. Carefully exclude underlying culture medium.

Test Procedure

1. Prepare and sterilize 13 x 75 mm screw cap test tubes containing 1 ml distilled or deionized water. Cool to room temperature.
2. Add two drops TB Hydrolysis Reagent, taking care not to touch the glass dropper, which could contaminate the reagent and cause aberrant test results.
3. Transfer one loopful of test culture to the tube. Thoroughly emulsify the culture in the reagent.
4. When testing *Mycobacterium* spp.:
   a. use a known positive (*M. kansasii* ATCC® 12478) and negative (uninoculated tube and *M. scrofulaceum*) control in parallel with the test culture to ascertain the validity of test results.
   b. incubate at 35 ± 2°C in the dark with caps tight for 5-10 days.
   c. read tubes at 5 and 10 days for any change in color in a strong light against a white background.
5. When testing *Moraxella* and *Neisseria* spp.:
   a. use *Moraxella catarrhalis* ATCC® 25238 for a positive control, and *Neisseria sicca* ATCC® 9913 as a negative control.
   b. incubate at 35 ± 2°C in the dark with caps tight for 18-48 hours.
   c. read tubes at 24 and 48 hours.
6. Do not shake the tubes. Examine the liquid, not the sedimented cells. Compare the color of the liquid with the control tube color.
7. Record results.
8. Upon completion of the test, follow proper established laboratory procedures in disposing of infectious materials.

Results

For *Mycobacterium* spp. - A positive reaction is indicated by a color change of the solution from amber to pink or red in 5 days or less. A doubtful reaction is a color change in 5 to 10 days. A negative reaction is no color change after 10 days.

For *Moraxella* and *Neisseria* spp. - A positive reaction is a color change of the solution from amber to red or pink after 24 hours of incubation. A negative reaction is no color change after 48 hours of incubation.

References

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Packaging

TB Hydrolysis Reagent	5 ml	3192-56*

*Store at 2-8°C

Bacto® TCBS Agar

Intended Use

Bacto TCBS Agar is used for isolating and cultivating *Vibrio cholerae* and other enteropathogenic vibrios.

Also Known As

TCBS Agar is an abbreviation for Thiosulfate-Citrate-Bile-Sucrose Agar. TCBS is also called Vibrio Selective Agar.

Summary and Explanation

TCBS Agar, prepared according to the formula of Kobayashi *et al.*[1], is a modification of the selective medium from Nakanishi.[2] All *Vibrio* spp. that are pathogenic to humans, except *V. hollisae*, will grow on TCBS Agar. This medium is recommended for isolating *Vibrio* spp. from stool specimens[3] and is specified in Standard Methods as Thiosulfate-Citrate-Bile-Sucrose Agar for food testing.[4,5]

TCBS Agar is highly selective, meets the nutritional requirements of *Vibrio* spp., and allows vibrios to compete with intestinal flora. All members of the genus are able to grow in media containing increased salt concentrations and some species are halophilic.[6] Vibrios are natural inhabitants of sea water.[6] Human disease has been associated with ingestion of contaminated water and consumption of contaminated shellfish or seafood.

V. cholerae is the etiologic agent of a secretory diarrhea spread by the fecal-oral route.[3] Infections may be asymptomatic, mild, or severe.[3] If not treated, patients with severe cholera may die within 5 hours as a result of massive fluid and electrolyte loss.[3]

Seven cholera pandemics have been reported since 1817.[7] In 1993, the first reports of epidemic cholera due to a new serogroup,

non-01 cholerae, appeared.[8,9] This strain was designated *V. cholerae* 0139 and given the synonym Bengal.[3]

Principles of the Procedure

Yeast Extract and Proteose Peptone No. 3 provide the nitrogen, vitamins, and amino acids in TCBS Agar. Sodium Citrate, Sodium Thiosulfate and Oxgall are selective agents which provide an alkaline pH to inhibit gram-positive organisms and suppress coliforms. The pH of the medium is increased to enhance growth of *Vibrio cholerae* because this organism is sensitive to acid environments. Saccharose is a fermentable carbohydrate, and Sodium Chloride stimulates growth. Sodium Thiosulfate is a sulfur source and acts with Ferric Citrate as an indicator to detect hydrogen sulfide production. Brom Thymol Blue and Thymol Blue are pH indicators. Bacto Agar is a solidifying agent.

Formula

TCBS Agar

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Proteose Peptone No. 3	10 g
Sodium Citrate	10 g
Sodium Thiosulfate	10 g
Bacto Oxgall	8 g
Bacto Saccharose	20 g
Sodium Chloride	10 g
Ferric Citrate	1 g
Bacto Brom Thymol Blue	0.04 g
Thymol Blue	0.04 g
Bacto Agar	15 g

Final pH 8.6 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

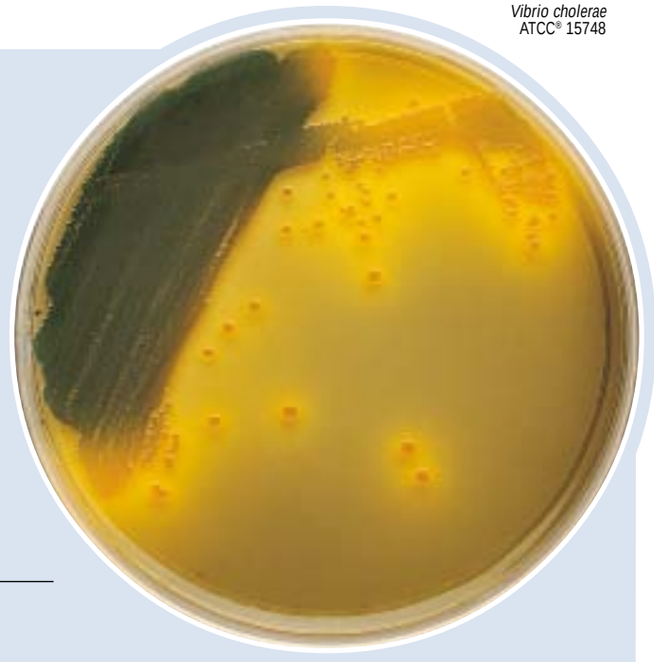
The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

TCBS Agar

Vibrio cholerae
ATCC® 15748



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan with greenish cast, free-flowing, homogeneous.
Solution:	8.9% solution, soluble on boiling in distilled or deionized water. Solution is forest green and very slightly opalescent.
Prepared Medium:	Green, slightly opalescent.
Reaction of 8.9% Solution at 25°C:	pH 8.6 ± 0.2

Cultural Response

Prepare TCBS Agar per label directions. Inoculate the medium with 10 microliters (l) of a heavy suspension and incubate at 35°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM (HEAVY SUSPENSION)	GROWTH	COLONY COLOR
Escherichia coli	25922*	10 µl	inhibited	–
Vibrio cholerae El Tor	15748	10 µl	good	yellow
Vibrio parahaemolyticus		10 µl	good	blue green

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Materials Required But Not Provided

Glassware
Incubator (35°C)
Waterbath (45-50°)
Sterile Petri dishes

Method of Preparation

1. Suspend 89 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. DO NOT AUTOCLAVE.
3. Cool to 45-50°C.
4. Dispense into sterile Petri dishes or as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy. If any delay in culturing is anticipated, addition of the specimen to Cary-Blair transport medium is essential because *Vibrio* spp. are particularly susceptible to drying.[3] Inoculation into alkaline peptone water is acceptable if subculture will be within 6-8 hours.[10]

Test Procedure

For a complete discussion of the isolation and identification of *Vibrio cholerae* and other enteropathogenic vibrios, refer to the procedures outlined in the references.

Results

After 18-24 hours of incubation at 35°C, sucrose-fermenting vibrios (*V. cholerae*, *V. alginolyticus*, *V. harveyi*, *V. cincinnatiensis*, *V. fluvialis*, *V. furnissii*, *V. metschnikovii*, some *V. vulnificus*) appear as medium-sized, smooth, opaque, thin-edged yellow colonies on TCBS Agar.[6] The other clinically important vibrios and most *V. vulnificus* do not ferment sucrose and appear green.[6]

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Further tests are necessary for identification and confirmation of *Vibrio* spp.[11]
3. On initial isolation, *V. parahaemolyticus* may be confused with *Aeromonas hydrophila*, *Plesiomonas shigelloides* and *Pseudomonas* species.[12]
4. Sucrose-fermenting *Proteus* species produce yellow colonies which may resemble those of *Vibrio*.[11]
5. TCBS is an unsatisfactory medium for oxidase testing of *Vibrio* spp.[13]
6. A few strains of *V. cholerae* may appear green or colorless on TCBS due to delayed sucrose fermentation.[11]

References

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11. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol 1, p. 763-767. Williams & Wilkins, Baltimore, MD.
12. **Bottone, E. J., and T. Robin.** 1978. *Vibrio parahaemolyticus*; Suspicion of presence based on aberrant biochemical and morphological features. J. Clin. Microbiol. **8**:760.
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Packaging

TCBS Agar	100 g	0650-15
	500 g	0650-17

Bacto® m TEC Agar

Intended Use

Bacto m TEC Agar is used for isolating, differentiating and rapidly enumerating thermotolerant *Escherichia coli* from water by membrane filtration and an *in situ* urease test.

Also Known As

m TEC is an abbreviation for membrane (medium) Thermotolerant *E. coli*.

Summary and Explanation

Escherichia coli is widely used as an indicator of fecal pollution in water. There are many procedures for enumerating *E. coli* based on its

ability to grow at elevated temperatures and produce indole from tryptophane.[1,2] The determination of indole production in conjunction with the most-probable-number procedure often requires the use of another medium and additional incubation time.

The membrane filter procedure has been recognized by *Standard Methods for the Examination of Water and Wastewater* as an alternate test procedure.[3] In 1981, Dufour et al. developed a simple, accurate, nonlethal membrane filter technique for the rapid enumeration of *E. coli*.[4] This medium, m TEC Agar, quantifies *E. coli* within 24 hours without requiring subculture and identification of isolates. The authors reported that they were able to recover *E. coli* from marine, estuarine and fresh water samples.

Principles of the Procedure

m TEC Agar contains sufficient nutrients to support the growth of *E. coli*. Proteose peptone is a source of nitrogen, amino acids, carbon and amino acids. Yeast Extract provides trace elements, vitamins and amino acids. Potassium Phosphate Monobasic and Potassium Phosphate Dibasic offer buffering capabilities. Lactose is a fermentable carbohydrate and carbon source. Sodium Lauryl Sulfate and Sodium Desoxycholate are selective against gram-positive bacteria. Brom cresol purple and brom phenol red are indicator components and Bacto Agar solidifies the medium.

Formula

m TEC Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Bacto Lactose	10 g
Sodium Chloride	7.5 g
Potassium Phosphate Monobasic	1 g
Potassium Phosphate Dibasic	3.3 g
Sodium Lauryl Sulfate	0.2 g
Sodium Desoxycholate	0.1 g
Brom Cresol Purple	0.08 g
Brom Phenol Red	0.08 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product is its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

m TEC Agar

Materials Required But Not Provided

Autoclave
Sterile Petri dishes, 50 x 10 mm
Membrane filter equipment
Sterile 47 mm, 0.45 µm, gridded membrane filters
Pipettes
Stereoscopic microscope
Dilution blanks
35°C incubator
44.5°C waterbath or incubator
Waterproof plastic bags if water bath is used
Sterile absorbent pads
Urea
Phenol Red

User Quality Control

Identity Specifications

Dehydrated Appearance: Green to grayish tan, free-flowing and homogeneous.

Solution: 4.53% solution, soluble in distilled or deionized water on boiling. Solution is deep purple with red cast, slightly opalescent.

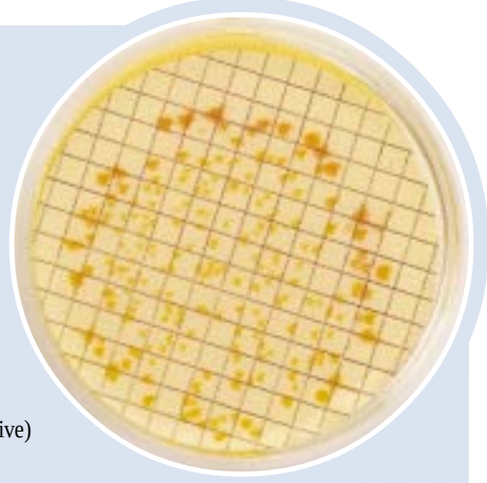
Reaction of 4.53% Solution at 25°C: pH 7.3 ± 0.2

Cultural Response

Prepare m TEC Agar per label directions. Inoculate and incubate the plates at 35°C for two hours. Transfer plates and incubate at 44.5 ± 0.5°C for approximately 22 ± 2 hours. After incubation, filters are removed and placed over pads, saturated with approximately 2 ml of urease substrate. Yellow to yellow-brown colonies (urease negative) are counted after 15-20 minutes.

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY	APPEARANCE
Escherichia coli	8739	20-80	good	yellow to yellow-brown colonies

The culture listed is the minimum that should be used for performance testing.



Escherichia coli ATCC® 8739

Method of Preparation

m TEC Agar

1. Suspend 45.3 g in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense 4-5 mls amounts into 50 x 10 mm Petri dishes and allow to solidify.

Urea Substrate

1. Combine 2 g urea and 10 mg phenol red in 100 ml distilled water.
2. Adjust pH to 5.0 ± 0.2.
3. Store at 2-8°C. Use within one week.

Note: Other methods may recommend an alternative pH.[3,6] Prepare substrate according to recommended guidelines.

Specimen Collection and Preparation

Water samples should be collected and prepared in accordance to recommended guidelines.[3,6]

Test Procedure

1. Follow the membrane filter procedure described in *Standard Methods for the Examination of Water and Wastewater*.[3]
2. Incubate inoculated plates for 2 hours at 35°C to resuscitate injured cells.
3. Transfer the plates to a 44.5 ± 0.5°C waterbath or incubator and incubate for 22 ± 2 hours.
4. Transfer countable filters to pads saturated with urea substrate.
5. After 15-20 minutes, count all yellow to yellow-brown colonies with the aid of a stereoscopic microscope.

Results

Yellow to yellow-brown colonies (urease negative) may be presumptively identified as *E. coli*.

Limitations of the Procedure

1. The 35°C incubation step is required to resuscitate stressed organisms. The 44.5°C incubation temperature is required to inhibit non-thermotolerant organisms.
2. The urease test is required to presumptively identify *E. coli*.
3. Choose a water sample size that will result in 20-80 colonies per filter. Plates containing more than 80 colonies are not recommended because high counts may not provide accurate urease test results.
4. Do not trap air bubbles underneath the filter.

References

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Packaging

m TEC Agar	100 g	0334-15

Bacto® TPEY Agar Base

Intended Use

Bacto TPEY Agar Base is used with Bacto EY Tellurite Enrichment and Bacto Antimicrobic Vial P in detecting and enumerating coagulase-positive staphylococci.

Also Known As

TPEY Agar Base conforms with Tellurite-Polymyxin-Egg Yolk Agar Base.

Summary and Explanation

TPEY Agar Base is prepared according to the formulation of Crisley.[1,2] The complete medium is prepared by aseptically adding EY Tellurite Enrichment and Antimicrobic Vial P (polymyxin B) to the sterile TPEY Agar Base. This medium permits the isolation and enumeration of coagulase-positive staphylococci from a variety of specimens such as food products, air, dust and soil. Coagulase-negative staphylococci and other organisms are markedly to completely inhibited.

Principles of the Procedure

Lithium Chloride, Potassium Tellurite and Polymyxin B inhibit a wide variety of microorganisms, including coagulase-negative staphylococci. Yeast Extract provides vitamins and cofactors required for growth, as well as additional sources of nitrogen and carbon. Tryptone provides nitrogen, vitamins and amino acids. Mannitol is an energy source. Sodium Chloride maintains the osmotic balance. Bacto Agar is incorporated into the medium as a solidifying agent.

Formula

TPEY Agar Base

Formula Per Liter	
Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Bacto Mannitol	5 g
Sodium Chloride	20 g
Lithium Chloride	2 g
Bacto Agar	18 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.

2. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) MAY CAUSE HARM TO THE UNBORN CHILD. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Kidneys, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

TPEY Agar Base
EY Tellurite Enrichment
Antimicrobic Vial P (Polymyxin B)

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	60 grams per 900 ml solution, soluble in distilled or deionized water on boiling. Prior to adding enrichment, solution is light to medium amber, opalescent.
Prepared Medium:	Yellowish-beige, opaque.
Reaction of 6.0% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare TPEY Agar Base per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY APPEARANCE	HALO†
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition	–	–
Staphylococcus aureus	25923*	100-1,000	good	black	+
Staphylococcus epidermidis	14990	100-1,000	poor to fair	black	–

†Zone of precipitation/clearing around the colony.

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Materials Required But Not Provided

Glassware
Autoclave
Waterbath (50-55°C)
Incubator (35°C)

Method of Preparation

1. Suspend 60 grams of TPEY Agar Base in 900 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 50-55°C.
4. Aseptically add 100 ml of EY Tellurite Enrichment warmed to room temperature and 10 ml of rehydrated Antimicrobic Vial P. Mix thoroughly.

   Alternatively, use 100 ml of a 30% egg yolk emulsion, 10 ml of Chapman Tellurite Solution 1% and 0.4 ml of filter sterilized 1% polymyxin B solution.
5. Pour 15-17 ml amounts into sterile Petri dishes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references.[3]

Results

Coagulase-positive staphylococci form black or dark-gray colonies due to the reduction of colorless tellurite to free tellurium. Three types of egg yolk precipitation reactions are produced by coagulase-positive staphylococci:

1. a discrete zone of precipitated egg yolk around and beneath the colonies;
2. a clear zone or halo surrounding the colonies with a possible zone of precipitate beneath the colonies; and,
3. no zone or halo around the colonies, but a precipitate beneath the colonies.

Limitations of the Procedure

1. Mannitol-positive and/or tellurite-positive staphylococcal strains that are coagulase-negative are occasionally found. Definitive identification of *S. aureus*, therefore, should be based primarily on the coagulase reaction, with mannitol fermentation and tellurite reduction being used only for confirmation.[3,5]
2. The prepared medium becomes less inhibitory to coagulase-negative strains of staphylococci if it is stored for longer than one week.[2]
3. Graves and Frazier[4] showed that *Bacillus* spp. able to grow on TPEY Agar produce an antibiotic that inhibits growth of staphylococci.

References

1. **Crisley, F. D., R. Angelotti, and M. J. Foter.** 1964. Multiplication of *Staphylococcus aureus* in synthetic cream fillings and pies. Public Health Rep. **79**:369.
2. **Crisley, F. D., J. T. Peeler, and R. Angelotti.** 1965. Comparative evaluation of five selective and differential media for the detection and enumeration of coagulase-positive staphylococci in foods. Appl. Microbiol. **13**:140.

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4. **Graves, R. R., and W. C. Frazier.** 1963. Food microorganisms influencing the growth of *Staphylococcus aureus.* Appl. Microbiol. **11**:513.
5. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, Vol. 1. Williams & Wilkins, Baltimore, M.D.

Packaging

TPEY Agar Base	500 g	0556-17

Bacto® TSA Blood Agar Base · Bacto Tryptic Soy Blood Agar Base No. 2 · Bacto Tryptic Soy Blood Agar Base EH

Intended Use

Bacto TSA Blood Agar Base is used with blood in isolating and cultivating fastidious microorganisms where clear and distinct hemolytic reactions are of prime importance.

Bacto Tryptic Soy Blood Agar Base No. 2 is used with blood in isolating and cultivating fastidious microorganisms from specimens where clear and distinct hemolytic reactions are of prime importance.

Bacto Tryptic Soy Blood Agar Base EH is used with blood in isolating and cultivating fastidious microorganisms from specimens where clear and distinct hemolytic reactions are of prime importance.

User Quality Control

Identity Specifications

TSA Blood Agar Base

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 4.0% solution, soluble in distilled or deionized water upon boiling, medium amber, slightly opalescent without significant precipitate.

Prepared Medium: With 5% sheep blood - bright medium red, opaque, firm.

Reaction of 4.0% Solution at 25°C: pH 7.3 ± 0.2

Tryptic Soy Blood Agar Base No. 2

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 4.0% solution, soluble in distilled or deionized water on boiling, light to medium amber, slightly opalescent without significant precipitate.

Prepared Medium: With 5% sheep blood - bright cherry red, opaque.

Reaction of 4.0% Solution at 25°C: pH 7.3 ± 0.2

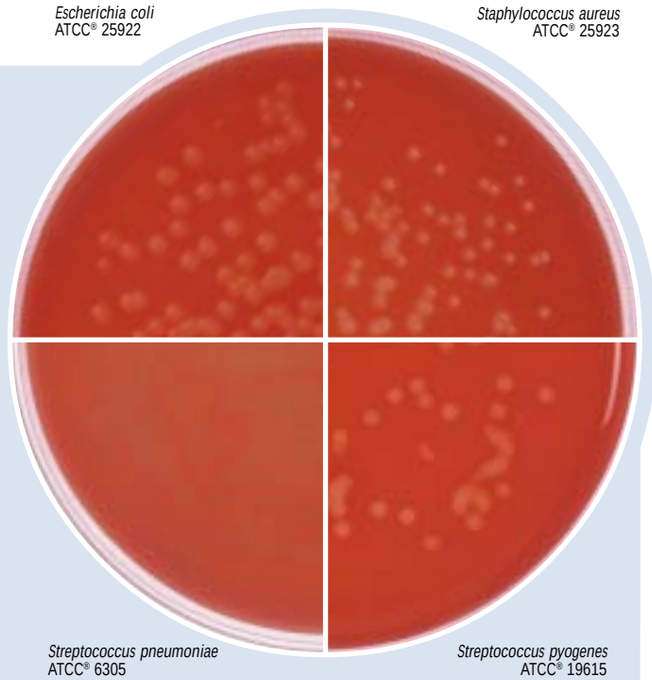
Tryptic Soy Blood Agar Base EH

Dehydrated Appearance: Light beige, free-flowing, homogeneous.

Solution: 4.0% solution, soluble in distilled or deionized water on boiling, light to medium amber, clear to slightly opalescent without significant precipitate.

Prepared Medium: With 5% sheep blood - bright cherry red, opaque.

Reaction of 4.0% Solution at 25°C: pH 7.3 ± 0.2



Cultural Response

Prepare TSA Blood Agar per label directions. Inoculate and incubate at 35°C under 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HEMOLYSIS
Escherichia coli	25922*	100-1,000	good	–
Staphylococcus aureus	25923*	100-1,000	good	beta
Streptococcus pneumoniae	6305	100-1,000	good	alpha
Streptococcus pyogenes	19615*	100-1,000	good	beta

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Also Known As

Blood Agar Base is abbreviated as BAB. Tryptic Soy Agar is abbreviated as TSA, and is referred to as Soybean-Casein Digest Agar Medium, USP.

Summary and Explanation

Blood Agar Bases are typically supplemented with 5-10% sheep, rabbit or horse blood for use in isolating, cultivating and determining hemolytic reactions of fastidious pathogenic microorganisms.

In 1919, Brown[1] experimented with blood agar formulations to determine their affects on colony formation and hemolysis. Growth of pneumococci was noticeably influenced when a medium contained peptone manufactured by Difco.

Tryptic Soy Agar is based on the Soybean-Casein Digest formula specified by US Pharmacopeia.[2] Tryptic Soy Agar is a general purpose medium used for multiple applications, e.g., maintaining culture collections, performing colony counts[3] and testing bacterial contaminants in cosmetics.[4] TSA Blood Agar Base was developed to achieve good growth and to improve hemolytic reactions of pathogenic microorganisms.

Tryptic Soy Blood Agar Base No. 2 and Tryptic Soy Blood Agar Base EH represent further improvements to TSA Blood Agar Base. TSA Blood Agar Base No. 2 provides clearer hemolytic reactions with group A streptococci while TSA Blood Agar Base EH provides dramatic, enhanced hemolysis.

Blood Agar Base media are specified in Standard Methods[5,6] for food testing.

Principles of the Procedure

Blood Agar Base media formulations have been prepared using specially selected raw materials to support good growth of a wide variety of fastidious microorganisms. TSA Blood Agar Base contains two peptones, Pancreatic Digest of Casein and Papaic Digest of Soybean Meal, which provide nitrogen, carbon, amino acids and vitamins. Agar is a solidifying agent; Sodium Chloride maintains osmotic balance.

Tryptic Soy Blood Agar Base No. 2 and Tryptic Soy Blood Agar Base EH are similar in composition to TSA Blood Agar Base. The formulations have been modified through the use of peptones (Tryptone H and Tryptone H Plus) developed at Difco Laboratories to improve and enhance hemolysin production while minimizing antagonism or loss in activity of streptococcal hemolysins. Both basal media contain Soytone for additional nitrogen, Agar as a solidifying agent, and Sodium Chloride to maintain osmotic balance.

Supplementation with blood (5-10%) provides additional growth factors for fastidious microorganisms and is the basis for determining hemolytic reactions. Hemolytic patterns may vary with the source of animal blood or type of basal medium used.[7]

Blood agar bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of beta-hemolytic streptococci.[8]

Formula

TSA Blood Agar Base

Formula Per Liter

Pancreatic Digest of Casein	15 g
Papaic Digest of Soybean Meal	5 g
Sodium Chloride	5 g
Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Tryptic Soy Blood Agar Base No. 2

Formula Per Liter

Bacto Tryptone H	15 g
Bacto Soytone	5 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Tryptic Soy Blood Agar Base EH

Formula Per Liter

Bacto Tryptone H Plus	15 g
Bacto Soytone	5 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

TSA Blood Agar Base
Tryptic Soy Blood Agar Base No. 2
Tryptic Soy Blood Agar Base EH

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile defibrinated blood
Sterile Petri dishes

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water:
   TSA Blood Agar Base - 40 grams;
   Tryptic Soy Blood Agar Base No. 2 - 40 grams;
   Tryptic Soy Blood Agar Base EH - 40 grams.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory in accordance with recommended guidelines outlined in the references.

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions owing to the activity of both oxygen-stable and oxygen-labile streptolysins.[7]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.
3. Examine the medium for growth and hemolytic reactions after 18-24 and 48 hours incubation.
4. Four types of hemolysis on blood agar media have been described:[9]
   a. Alpha hemolysis (α) is the reduction of hemoglobin to meth emoglobin in the medium surrounding the colony, causing a greenish discoloration of the medium.
   b. Beta hemolysis (β) is the lysis of red blood cells, producing a clear zone surrounding the colony.
   c. Gamma hemolysis (γ) indicates no hemolysis. No destruction of red blood cells occurs and there is no change in the medium.
   d. Alpha-prime hemolysis (ὰ) is a small zone of complete hemoly sis that is surrounded by an area of partial lysis.

Limitations of the Procedure

1. TSA Blood Agar Base media are intended for use with blood supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immuno-logical testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.
2. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
3. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-hemolytic on horse, human and rabbit blood agar and alpha-hemolytic on sheep blood agar.[7]
4. Colonies of *Haemophilus haemolyticus* are beta-hemolytic on horse and rabbit blood agar and must be distinguished from colonies of beta-hemolytic streptococci using other criteria. The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of *H. haemolyticus*.[10]
5. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[7] For optimal performance, incubate blood agar base media under increased CO_2 or anaerobic conditions.

References

1. **Brown, J. H.** 1919. The use of blood agar for the study of streptococci, NY Monograph No. 9. The Rockefeller Institute for Medical Research.
2. **The United States Pharmacopeia (USP XXIII) and The National Formulary (NF 18).** 1995. Sterility tests, p. 1686-1690. United States Pharmacopeial Convention Inc., Rockville, MD.
3. **Swanson, K. J., F. F. Busta, E. H. Peterson, and M. G. Johnson.** 1992. Colony count methods, p. 75-95. *In* Vanderzant, C., and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
4. **Curry, A. S., G. G. Joyce, and G. N. McEwen Jr.** 1993. CTFA Microbiology guidelines. The Cosmetic, Toiletry and Fragrance Association, Inc. Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. App. 3.08-3.09, Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
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7. **Ruoff, K. L.** 1995. Streptococcus, p. 299-305. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
8. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. **17:**281-289.
9. **Isenberg, H. D. (ed.).** 1992. Interpretation of aerobic bacterial growth on primary culture media, p. 1.6.1-1.6.7, Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington D.C.
10. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. p. 415. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

TSA Blood Agar Base	500 g	0026-17
	2 kg	0026-07
	10 kg	0026-08
Tryptic Soy Blood Agar Base No. 2	500 g	0027-17
	2 kg	0027-07
	10 kg	0027-08
Tryptic Soy Blood Agar Base EH	500 g	0028-17
	2 kg	0028-07
	10 kg	0028-08

Bacto® TT Broth Base Hajna

Intended Use

Bacto TT Broth Base Hajna is used for enriching *Salmonella* from food and dairy products prior to isolation procedures.

Also Known As

TT Broth Base Hajna is also referred to as Tetrathionate Broth Base Hajna.

Summary and Explanation

TT Broth Base Hajna is used as a selective enrichment for the cultivation of *Salmonella* spp. *Salmonella* organisms can be injured in food-processing procedures. These procedures include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives and sanitizers.[1] Although injured cells may not form colonies on selective media, they can cause disease if ingested.[2] *Salmonella* spp., in particular, cause many types of infections from mild self-limiting gastroenteritis

to life-threatening typhoid fever.[3] The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than 2 days and diarrhea lasting less than 7 days.[3]

TT Broth Base Hajna conforms to the formulation of Hajna and Damon.[4] The medium is a modification of the enrichment described by Kauffmann[5] and Knox.[6] Hajna and Damon[4] developed a new broth containing yeast extract, peptone, carbon sources and the selective agents, sodium desoxycholate and brilliant green (replacing Bile Salts).

TT Broth Base Hajna is used in testing *Salmonella* in egg processing plants.[7] It is specified in the Compendium of Methods for the Microbiological Examination of Foods.[8]

Principles of the Procedure

Tryptose provides nitrogen and amino acids. Yeast Extract supplies growth factors and vitamins. Dextrose and Mannitol are fermentable carbohydrates. Selectivity is accomplished by the combination of Sodium Thiosulfate and tetrathionate, suppressing coliform organisms.[6] Tetrathionate is formed in the medium by the addition of a solution containing iodine and potassium iodide. Organisms containing the enzyme tetrathionate reductase will proliferate in this medium.

Sodium Desoxycholate and Brilliant Green are selective agents that suppress coliform bacteria and inhibit gram-positive organisms. Sodium Chloride maintains the osmotic balance of the medium. Calcium Carbonate is a neutralizer that absorbs toxic metabolites.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige to very light green, free-flowing, homogeneous.
Solution:	9.15% solution, insoluble in distilled or deionized water on boiling; light green, slightly opalescent with a heavy white precipitate.
Prepared Medium:	Light green, slightly opalescent with a heavy white precipitate.
Reaction of 9.15% Solution at 25°C:	pH 7.6 ± 0.2 (after addition of the iodine solution)

Cultural Response

Prepare TT Broth Base Hajna with 4% iodine solution per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours. After incubation, plate the inoculated broth onto MacConkey Agar and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR ON MACCONKEY AGAR
Escherichia coli	25922*	100-1,000	none to poor	pink with bile ppt.
Salmonella typhimurium	14028*	100-1,000	good	colorless

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used a directed in Bactrol Disks Technical Information.

Formula

TT Broth Base Hajna

Formula Per Liter

Bacto Yeast Extract	2 g
Bacto Tryptose	18 g
Bacto Dextrose	0.5 g
Bacto D-Mannitol	2.5 g
Sodium Desoxycholate	0.5 g
Sodium Chloride	5 g
Sodium Thiosulfate	38 g
Calcium Carbonate	25 g
Brilliant Green	0.01 g

Final pH 7.6 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID:

   In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

TT Broth Base Hajna

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Iodine crystals
Potassium iodide
MacConkey Agar

Method of Preparation

1. Suspend 91.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Cool below 50°C.
4. Add 40 ml iodine solution (5 grams iodine crystals and 8 grams potassium iodide dissolved in 40 ml distilled or deionized water) and mix well.

5. Dispense into sterile tubes while keeping suspension well mixed.
6. Do not heat the medium after adding iodine.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy. For a complete discussion on the collection, isolation and identification of *Salmonella*, refer to the appropriate procedures outlined in the references.

Results

Refer to appropriate references and procedures for results.

References

1. **Hartman, P. A., and S. A. Minnich.** 1981. Automation for rapid identification of salmonellae in foods. J. Food Prot. **44**:385-386.
2. **Sorrells, K. M., M. L. Speck, and J. A. Warren.** 1970. Pathogenicity of *Salmonella gallinarum* after metabolic injury by freezing. Appl. Microbiol. **19**:39- 43.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Hajna, A. A., and S. R. Damon.** 1956. New enrichment and plating medium for the isolation of *Salmonella* and *Shigella* organisms. Appl. Microbiol. **4**:341.
5. **Kauffman, F.** 1930. Ein kombiniertes Anreicherungsverfahren fur Typhus-und-Paratyphusbazillen. Zentralb. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abr. I Orig. **113**:148.
6. **Knox, R., P. H. Gell, and M. R. Pollack.** 1942. Selective media for organisms of the *Salmonella group*. J. Pathol. Bacteriol. **54**:469-483.
7. **Catalano, C. R., and S. J. Knable.** 1994. Incidence of *Salmonella* in Pennsylvania egg processing plants and destruction by high pH. J. Food Prot. **57**:587-591.
8. **Russell, S. F., J.-Y. D'Aoust, W. H. Andrews, and J. S. Bailey.** 1992. *Salmonella*, p. 371-422. *In* C. Vanderzant, and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

TT Broth Base Hajna	500 g	0491-17
	2 kg	0491-07

Bacto® Tellurite Blood Solution

Intended Use

Bacto Tellurite Blood Solution is used with Bacto Proteose No. 3 Agar and Bacto Dextrose in isolating *Corynebacterium diphtheriae*.

Summary and Explanation

Tellurite Blood Solution is used in the cultural diagnosis of diphtheria, an acute infectious disease primarily of the upper respiratory tract but occasionally of the skin.[1] Diphtheria is caused by toxigenic strains of *C. diphtheriae*, of which there are three biotypes; mitis, intermedius and gravis.[1] The symptoms of the disease are development of a pharyngeal membrane, sore throat, malaise, headache and nausea.[2] Death can result from respiratory obstruction by the membrane or from myocarditis caused by the toxin.[2]

Tellurite Blood Solution is a mixture of defibrinated bloods with added potassium tellurite. This solution is used to markedly reduce growth of commensal organisms encountered when isolating *Corynebacterium diphtheriae*.

User Quality Control

Identity Specifications

Solution Appearance: Dark, red-brown liquid.

Cultural Response

Prepare Proteose No. 3 Agar with Dextrose (1.5 grams per liter) and 5% Tellurite Blood Solution. Heat to 70-80°C to chocolatize the medium. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLOR
Corynebacterium diptheriae type *gravis*	8028	100-1,000	good	black
Corynebacterium diptheriae type *intermedius*	8032	100-1,000	good	black
Corynebacterium diptheriae type *mitis*	8024	100-1,000	good	black
Escherichia coli	25922*	1,000-2,000	inhibited	–
Streptococcus pyogenes	19616*	1,000-2,000	inhibited	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Tellurite Blood Solution contains defibrinated blood for essential nutrients to enhance the growth of *C. diphtheriae*. Potassium Tellurite is a selective agent that inhibits the growth of commensal organisms.

Reagent

Tellurite Blood Solution is a combination of approximately 95% defibrinated, lysed horse and bovine blood from which most of the cellular debris has been removed. One-percent (1%) Potassium Tellurite is added.

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store Tellurite Blood Solution at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Tellurite Blood Solution

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

1. Shake Tellurite Blood Solution before use.
2. Refer to the final concentration of Tellurite Blood Solution in the formula of the medium being prepared. Add Tellurite Blood Solution as required.

Specimen Collection and Preparation[1]

Both throat and nasopharyngeal specimens are necessary in cases of respiratory illness. If cutaneous diphtheria is suspected, collect skin, throat and nasopharynx specimens. Use sterile silica gel for shipping clinical specimens when cultures are not taken locally.

Test Procedure

For a complete discussion of the collection, isolation and identification of *C. diphtheriae* and other *Corynebacterium* spp., refer to appropriate procedures in the references.[1,2]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Definitive identification of a strain of *C. diphtheriae* as a true pathogen requires demonstration of toxin production.[3]

References

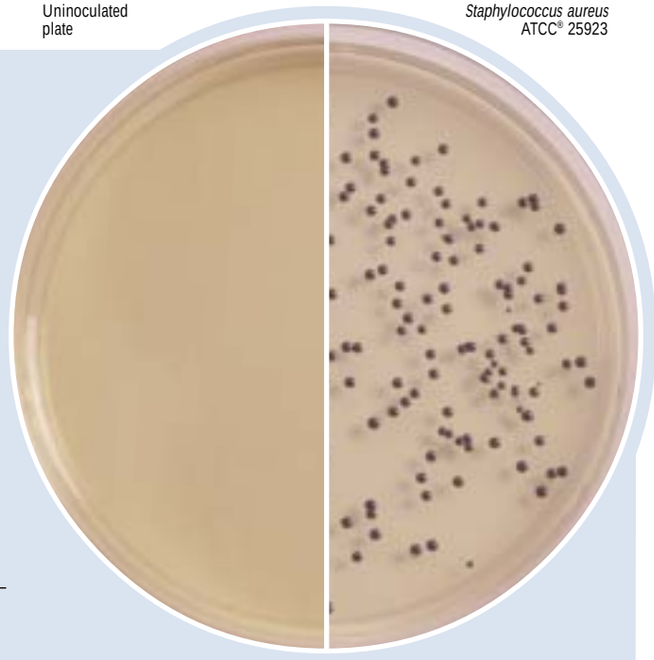
1. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
2. **Clarridge, J. E., and C. A. Spiegel.** 1995. *Corynebacterium* and miscellaneous irregular gram-positive rods, Erysipelothrix, and *Gardnerella*, p. 357-377. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Tellurite Blood Solution	6 x 25 ml	0139-66

Tellurite Glycine Agar

Bacto® Tellurite Glycine Agar · Bacto Chapman Tellurite Solution 1%



User Quality Control

Identity Specifications

Tellurite Glycine Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.25% solution; soluble in distilled or deionized water upon boiling. Medium amber, opalescent with precipitation.
Prepared Medium:	Medium amber, opalescent with precipitation.
Reaction of 6.25% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Tellurite Glycine Agar per label directions and enrich with Chapman Tellurite Solution 1%. Incubate inoculated medium at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Escherichia coli	25922*	1,000-2,000	inhibited	–
Staphylococcus aureus	25923*	100-1,000	good	black
Staphylococcus epidermidis	12228*	100-1,000	good	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Intended Use

Bacto Tellurite Glycine Agar is used with Bacto Chapman Tellurite Solution 1% for isolating coagulase-positive staphylococci.

Summary and Explanation

The coagulase-positive species *Staphylococcus aureus* is well documented as a human opportunistic pathogen.[3] Foods are examined for the presence of *S. aureus* and/or its enterotoxins to confirm that *S. aureus* is the causative agent of foodborne illness, to determine whether a food is the source of "staph" food poisoning, and to determine post-processing contamination.[4]

Ludlam[2] described a selective medium for the isolation of staphylococci. This medium was alkaline in reaction, contained mannitol, and lithium chloride with potassium tellurite as the selective agents. Zebovitz, Evans and Niven[1] modified Ludlam's medium by adding glycine as a selective agent and adjusting the reaction of the basal medium to pH 7.2 instead of pH 9.6.

Tellurite Glycine Agar is prepared according to the formula of Zebovitz, Evans and Niven.[1] The medium permits the isolation of coagulase-positive staphylococci from food, air, dust, soil and clinical specimens. Coagulase-negative staphylococci and other bacteria are markedly to completely inhibited.

Principles of the Procedure

Tryptone and Soytone are sources of nitrogen and amino acids in Tellurite Glycine Agar. Yeast Extract is a vitamin source in this formulation. D-Mannitol is a source of fermentable carbohydrate for coagulase-positive staphylococci. Lithium chloride, glycine and potassium tellurite are the selective agents. Dipotassium phosphate is used to buffer the medium. Bacto Agar is the solidifying agent.

Chapman Tellurite Solution is a sterile 1% solution of potassium tellurite, a differential agent. Coagulase-positive staphylococci reduce tellurite and produce black colonies.[5]

Formula

Tellurite Glycine Agar

Formula Per Liter

Bacto Yeast Extract	6.5 g
Bacto Soytone	3.5 g
Bacto Tryptone	10 g
Glycine	10 g
Bacto D-Manniol	5 g
Dipotassium Phosphate	5 g
Lithium Chloride	5 g
Bacto Agar	17.5 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Tellurite Glycine Agar: HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Kidneys, Nerves.

   Chapman Tellurite Solution 1%: MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.(US) Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store Tellurite Glycine Agar dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Chapman Tellurite Solution 1% at 15-30°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bacto Tellurite Glycine Agar
Bacto Chapman Tellurite Solution 1%

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (50-55°C) (optional)
Sterile Petri dishes

Method of Preparation

1. Suspend 62.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Aseptically add 10 ml Chapman Tellurite Solution 1% to the medium at 50-55°C. Mix well.
5. Dispense as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion on the isolation and identification of coagulase-positive staphylococci from clinical specimens refer to appropriate procedures.[3,6] For the examination of staphylococci in foods refer to standard methods.[4,7]

Results

Coagulase-positive staphylococci produce black colonies within 24 hours of incubation at 35°C.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. An occasional coagulase-negative staphylococci may produce small gray colonies, not readily confused with black coagulase-positive colonies.

References

1. **Zebovitz, E., J. B. Evans, and C. F. Niven Jr.** 1955. Tellurite glycine agar: A selective plating medium for the quantitative detection of coagulase-positive staphylococci. J. Bacteriol. **70**:686-690.
2. **Ludlam.** 1949. Monthly Bull. Ministry of Health. **8**:15.
3. **Kloos, W. E., and T. L. Bannerman.** 1995. *Staphylococcus* and *Micrococcus*, p. 282 - 298. *In* Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, M.D.
5. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, Williams & Wilkins, Baltimore, M.D.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.
7. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Tellurite Glycine Agar	500 g	0617-17
Chapman Tellurite Solution 1%	6 x 1 ml	0299-51
	6 x 25 ml	0299-66

Bacto® Tergitol 7 Agar
Bacto Tergitol 7 Broth

Intended Use

Bacto Tergitol 7 Agar and Bacto Tergitol 7 Broth are selective media used for enumerating and differentiating coliform bacteria.

Also Known As

Bacto Tergitol 7 Agar and Bacto Tergitol 7 Broth are also know as T7 Agar and T7 Broth, respectively.

Summary and Explanation

Tergitol 7 Agar and Broth, prepared according to the formula published by Chapman, are selective for *Escherichia coli* and members of the coliform group.[1] Chapman reported that the addition of Tergitol 7 to an agar medium consisting of Proteose Peptone No. 3, Yeast Extract, lactose, and brom thymol blue permitted unrestricted development of all coliform bacteria and inhibited development of gram-negative spore formers as well as gram-positive microorganisms. Counts of coliform organisms on Tergitol 7 Agar plates were found to be 30% higher than on some other selective media.

Chapman[2] modified his original Tergitol 7 Agar formula by adding 40 mg of triphenyltetrazolium chloride (TTC) per liter. This medium was found to be helpful in the early recognition and identification of *Escherichia coli*. Confirmation of the presence of *E. coli* was possible after only 10 hours incubation at 35°C. Chapman also reported that Tergitol 7 Agar with added TTC gave a selective medium suitable for the isolation of *Candida* and other fungi. *Candida* growing on this medium produce white, circular, convex, entire colonies about 1 mm in diameter in 24 hours. *Candida* colonies may appear pale blue because of the color of the medium, while yeasts produce red colonies.

Tergitol 7 Agar with TTC was shown to be useful in routine water analysis and the examination of foods.[3,4] The medium conforms with the recommendations of the APHA.[5]

Principles of the Procedure

Tergitol 7 (sodium heptadecyl sulfate) inhibits growth of gram-positive microorganisms and spore-forming gram-negative microorganisms, as well as the swarming of *Proteus*, while allowing for superior recovery of coliforms. Lactose fermentation is indicated by a color change of the pH indicator, brom thymol blue. Lactose-fermenting microorganisms produce yellow colonies. *Escherichia coli* produces yellow colonies with yellow zones, while *Enterobacter* and *Klebsiella* colonies are greenish-yellow. Nonfermenting organisms, such as *Salmonella* and *Shigella*, produce colonies surrounded by blue zones.

When TTC is added to the medium, it serves as an indicator of bacterial growth. TTC is rapidly reduced to insoluble red formazan by most lactose-fermenting organisms except *Escherichia coli*, *Enterobacter* and *Klebsiella* species. In the presence of TTC, lactose fermenters, which includes the coliforms, produce greenish-yellow colonies with yellow zones, while lactose nonfermenters produce red colonies surrounded by blue zones.

Proteose Peptone No. 3 provides the carbon and nitrogen sources required for good growth of a wide variety of organisms. Vitamins and cofactors required for growth, as well as additional sources of nitrogen and carbon, are provided by yeast extract. The Agar incorporated into Tergitol 7 Agar serves as a solidifying agent.

Formula

Tergitol 7 Agar

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Bacto Lactose	10 g
Bacto Agar	15 g
Tergitol 7	0.1 g
Bacto Brom Thymol Blue	0.025 g

Final pH 6.9 ± 0.2 at 25°C

Tergitol 7 Broth

Formula Per Liter

Bacto Proteose Peptone No. 3	5 g
Bacto Yeast Extract	3 g
Bacto Lactose	10 g
Tergitol 7	0.1 g
Bacto Brom Thymol Blue	0.025 g

Final pH 6.9 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated media below 30°C. The dehydrated media are very hygroscopic. Keep containers tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

User Quality Control

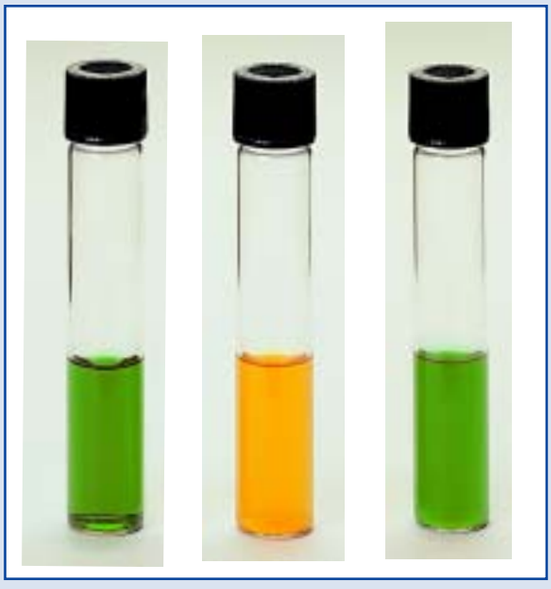
Identity Specifications

Tergitol 7 Agar

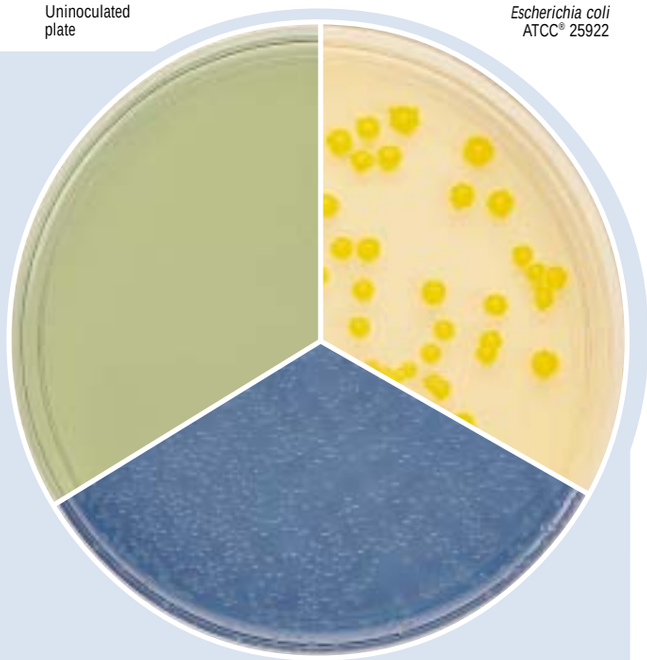
Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.3% solution, soluble in distilled or deionized water on boiling. Solution is green, slightly opalescent.
Prepared Plates:	Green, slightly opalescent without precipitate.
Reaction of 3.3% Solution at 25°C:	pH 6.9 ± 0.2

Tergitol 7 Broth

Dehydrated Appearance:	Beige, may have slight greenish tint, free-flowing, homogeneous.
Solution:	1.8% solution, soluble in distilled or deionized water on boiling.
Prepared Tubes:	Green, slightly opalescent.
Reaction of 1.8% Solution at 25°C:	pH 6.9 ± 0.2



Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Salmonella typhimurium* ATCC® 14028



Uninoculated plate | *Escherichia coli* ATCC® 25922 | *Salmonella typhimurium* ATCC® 14028

Cultural Response

Prepare medium per label directions. Inoculate Tergitol 7 Agar plates with test organisms. Inoculate Tergitol 7 Broth tubes and leave caps loosened. Incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID PRODUCTION
Enterococcus faecalis	19433	100-1,000	none to poor	N/A
Escherichia coli	25922*	100-1,000	good	+
Salmonella typhimurium	14028*	100-1,000	good	–

+ = positive, yellow colony or medium
– = negative, blue colony or medium as directed.

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Procedure

Materials Provided

Tergitol 7 Agar
Tergitol 7 Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
OPTIONAL: Bacto TTC Solution 1% or Bacto TTC

Method of Preparation

1. Suspend medium in 1 liter distilled or deionized water:
   Tergitol 7 Agar-33 grams;
   Tergitol 7 Broth-18 grams.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. OPTION: Cool Tergitol 7 Agar to 50°C. Add 4 ml of either TTC Solution 1% or a filter-sterilized 1% solution of TTC.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the medium with TTC permits growth of coliform organisms, this fact must be taken into consideration in the isolation of *Candida* from specimens.
2. Pour plates do not give satisfactory results.
3. Allow plates to dry with lids slightly ajar for 1-2 hours after dispensing.[6]
4. Reduction of TTC is an irreversible reaction that produces an insoluble formazan compound.

References

1. **Chapman, G. H.** 1947. A superior culture medium for the enumeration and differentiation of coliforms. J. Bacteriol. **53**:504.
2. **Chapman, G. H.** 1951. A culture medium for detecting and confirming *Escherichia coli* in ten hours. Am. J. Public Health **41**:1381.
3. **Kulp, W., C. Mascoli, and O. Tavshanjian.** 1953. Use of tergitol-7 triphenyl tetrazolium chloride agar as the coliform confirmatory medium in routine sanitary water analysis. Am. J. Public Health **43**:1111.
4. **Mossel, D. A. A.** 1962. An ecological investigation on the usefulness of two specific modifications of Eijkman's test as an element of the methods for the detecting of faecal contamination of foods. J. Appl. Bacteriol. **25**:20.
5. **Speck, Marvin L.** (ed.). 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Tergitol 7 Agar	500 g	0455-17
Tergitol 7 Broth	500 g	0912-17
TTC Solution 1%	30 ml	3112-67*

*Store at 2-8°C

Bacto® Terrific Broth

Intended Use

Bacto Terrific Broth is used with Bacto Glycerol in cultivating recombinant strains of *Escherichia coli*.

Summary and Explanation

Terrific Broth is a highly enriched medium developed by Tartoff and Hobbs to improve yield in plasmid bearing *E. coli*.[1] Recombinant strains have an extended growth phase in the medium. The addition of

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.76% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear.
Prepared Medium:	Light to medium amber, clear.
Reaction of 4.76% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Terrific Broth per label directions. Inoculate and incubate the tubes at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli (C600)	23724	100-1,000	good
Escherichia coli (HB101)	33694	100-1,000	good
Escherichia coli (DH-1)	33849	100-1,000	good
Escherichia coli (JM103)	39403	100-1,000	good
Escherichia coli (JM107)	47014	100-1,000	good
Escherichia coli (DH-5)	53868	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

extra Tryptone and Yeast Extract in the medium allows higher plasmid yield per volume. Glycerol is used as the carbohydrate source in this formulation. Unlike glucose, glycerol is not fermented to acetic acid.

Principles of the Procedure

Tryptone and Yeast Extract provide necessary nutrients and cofactors for excellent growth of recombinant strains of *E. coli*. The Yeast Extract concentration is increased to allow for elevated cell yields. Potassium Phosphates are added to provide potassium for cellular systems and prevent cell death due to a drop in pH. Glycerol is added as a carbon and energy source.

Formula

Terrific Broth

Formula Per Liter

Bacto Tryptone	12 g
Bacto Yeast Extract	24 g
Potassium Phosphate, Dibasic	9.4 g
Potassium Phosphate, Monobasic	2.2 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Terrific Broth

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Autoclave
Incubator (35°C)
Glycerol

Method of Preparation

1. Dissolve 47.6 grams in 1 liter of distilled or deionized water. Add 4 ml of Glycerol to the medium.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references for recommended test procedures.[1,2]

Results

Growth is evident in the form of turbidity.

References

1. **Tartoff, K. D., and C. A. Hobbs**. 1987. Improved media for growing plasmid and cosmid clones. Bethesda Research Laboratories Focus **9:**12.
2. **Sambrook, J., E. F. Fritsch, and T. Maniatis**. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Packaging

Terrific Broth	500 g	0438-17
Glycerol	100 g	0282-15
	500 g	0282-17

Bacto® Tetrathionate Broth Base

Intended Use

Bacto Tetrathionate Broth Base is used for enriching *Salmonella* species during isolation procedures.

Also Known As

Tetrathionate Broth (Base) can be abbreviated as TT Broth (Base).

Summary and Explanation

Tetrathionate Broth Base is used as a selective enrichment for the cultivation of *Salmonella* species that may be present in small numbers and compete with intestinal flora. *Salmonella* organisms may also be injured in food-processing procedures, which include exposure to low temperatures, sub-marginal heat, drying, radiation, preservatives and sanitizers.[1] Although injured cells may not form colonies on selective media, they can, if ingested, cause disease.[2] *Salmonella* species cause many types of infections, from mild self-limiting gastroenteritis to life-threatening typhoid fever.[3] The most common form of *Salmonella* disease is self-limiting gastroenteritis with fever lasting less than two days and diarrhea lasting less than 7 days.[3]

Mueller[4] demonstrated the effectiveness of Tetrathionate Broth for enriching typhoid and paratyphoid bacilli while inhibiting coliform organisms. Using modified Mueller's broth, Kauffmann[5,6] increased the number of positive isolates. Tetrathionate Broth was used in studies for the poultry industry[7,8] and in a collaborative study for rapid screening of *Salmonella* in food.[9]

Modifications of Tetrathionate Broth Base include TT Broth w/Brilliant Green, TT Broth Base, Hajna, Mueller Kauffmann Tetrathionate Broth Base and Tetrathionate with Novobiocin.[10]

Tetrathionate Broth Base is specified in standard methods[12,13,14,15] for *Salmonella* testing. Tetrathionate Broth is used in processing fecal cultures for bacteria.[16]

Principles of the Procedure

Proteose Peptone provides the nitrogen, carbon, vitamins and amino acids in Tetrathionate Broth Base. Selectivity is accomplished by the combination of Sodium Thiosulfate and tetrathionate, which suppresses commensal intestinal organisms.[17] (Tetrathionate is formed in the medium upon addition of the iodine and potassium iodide solution.) Organisms containing the enzyme tetrathionate reductase will proliferate in the medium. Bile Salts, a selective agent, suppresses coliform bacteria and inhibits gram-positive organisms. Calcium Carbonate neutralizes and absorbs toxic metabolites.

Formula

Tetrathionate Broth Base

Formula Per Liter

Bacto Proteose Peptone	5 g
Bacto Bile Salts	1 g
Sodium Thiosulfate	30 g
Calcium Carbonate	10 g

Final pH 8.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Tetrathionate Broth Base:**

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

Dehydrated Appearance:	White to off-white, may have a slight greenish tint, free-flowing, homogeneous.
Solution:	4.6% solution, insoluble in distilled or deionized water. Suspension is milky white, opaque. On standing, supernatant is nearly colorless to light yellow over a heavy white precipitate.
Prepared Medium:	Nearly colorless to light yellow supernatant over a heavy white precipitate.
Reaction of 4.6% Solution at 25°C:	pH 8.4 ± 0.2 (measured before iodine solution is added).

Cultural Response

Prepare Tetrathionate Broth Base per label directions and enrich with 2% iodine solution. Inoculate with 100-1,000 CFUs of test organism and incubate at 35 ± 2°C for 18-24 hours. Subculture onto MacConkey Agar and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	GROWTH	COLOR OF COLONY ON MACCONKEY AGAR
Escherichia coli	25922*	little or no increase in the number of colonies	pink w/bile precipitate
Salmonella typhimurium	14028*	good	colorless

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Storage

Store Tetrathionate Broth Base dehydrated below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Tetrathionate Broth Base

Materials Required But Not Provided

Glassware
Distilled or deionized water
Iodine solution (see Method of Preparation)
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile tubes

Method of Preparation

1. Suspend 4.6 grams in 100 ml distilled or deionized water.
2. Heat to boiling. DO NOT AUTOCLAVE.
3. Cool to below 60°C.
4. Add 2 ml iodine solution (6 grams iodine crystals and 5 grams potassium iodide in 20 ml water).
5. DO NOT REHEAT MEDIUM. DO NOT AUTOCLAVE.
6. Dispense into sterile tubes. Use immediately.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion of the isolation and identification of *Salmonella*, refer to appropriate procedures outlined in the references.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Hartman, P. A., and S. A. Minnich.** 1981. Automation for rapid identification of salmonellae in foods. J. Food Prot. **44**:385-386.
2. **Sorrells, K. M., M. L. Speck, and J. A. Warren.** 1970. Pathogenicity of *Salmonella gallinarum* after metabolic injury by freezing. Appl. Microbiol. **19**:39- 43.
3. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Muller, L.** 1923. Un nouveau milieu d'enrichissement pour la recherche du bacille typhique et des paratyphiques. C. R. Soc. Biol. **89**:434. Paris.
5. **Kauffmann, F.** 1930. Ein kombiniertes anreicherungsverfahren fur typhus-und-paratyphusbacillen. Zentralb. Bakteriol. Parasitenke. Infektionskr. Hyg. Abt.I orig. **113**:148.
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7. **Jones, F. T., R. C. Axtell, D. V. Rives, S. E. Scheideler, F. R. Tarver, Jr., R. L. Walker, and M. J. Wineland.** 1991. A survey of *Salmonella* contamination in modern broiler production. J. Food Prot. **54**:502-507.
8. **Barnhart, H. M., D. W. Dressen, R. Bastien, and O. C. Pancorbo.** 1991. Prevalence of *Salmonella enteritidis* and other serovars in ovaries of layer hens at time of slaughter. J. Food Prot. **54**:488-492.
9. **Eckner, K. F., W. A. Dustman, M. S. Curiale, R. S. Flowers, and B. J. Robison.** 1994. Elevated-temperature, colorimetric, monoclonal, enzyme-linked immunosorbent assay for rapid screening of *Salmonella* in foods: collaborative study. J. Assoc. Off. Anal. Chem. **77**:374-383.
10. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 751-754, Williams & Wilkins, Baltimore, MD.
11. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*. p 5.01-5.20. *In* Bacteriological analytical manual, 8th ed. AOAC International. Gaithersburg, MD.
12. **Russell, S. F., J.-Y. D'Aoust, W. H. Andrews, and J. S. Bailey.** 1992. *Salmonella*, p.371-422. *In* Vanderzant, C. and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
13. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall (ed.) Standard methods for the examination of dairy products. 16th ed., American Public Health Association, Washington, D.C.
14. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.
15. **Federal Register.** 1991. Animal and plant health inspection service: chicken affected by *Salmonella enteritidis*, final rule. Fed. Regist. **56**:3730-3743.
16. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 1, American Society for Microbiology, Washington, D. C.
17. **Knox, R., P. H. Gell, and M. R. Pollack.** 1942. Selective media for organisms of the *Salmonella* group. J. Pathol. Bacteriol. **54**:469-483.

Packaging

Tetrathionate Broth Base	500 g	0104-17
	2 kg	0104-07

Bacto® m Tetrathionate Broth Base

Intended Use

Bacto m Tetrathionate Broth Base is used for selectively enriching *Salmonella* by membrane filtration prior to isolation procedures.

Summary and Explanation

Salmonella spp. cause many types of infections, from mild self-limiting gastroenteritis to life-threatening typhoid fever.[2] The most common form of *Salmonella* disease is self- limiting gastroenteritis with fever lasting less than two days and diarrhea lasting less than 7 days.[2]

Tetrathionate Broth, in single strength and without calcium carbonate, was used by Kabler and Clark[1] for the preliminary enrichment of *Salmonella* other than *S. typhi*. Their investigation found that approximately 80% of *Salmonella* species recovered were from mixed cultures and that most coliforms were suppressed. The presence of calcium carbonate in the medium gave poor, erratic results. The authors[1] reported favorable results for enrichment of *S. typhimurium* in the membrane filtration technique. This study used a 3-hour preliminary incubation on pads saturated with Tetrathionate Broth followed by 15 hours incubation on m Brilliant Green Broth.

m Tetrathionate Broth Base has the same formulation as Tetrathionate Broth Base, except that calcium carbonate has been omitted.[1]

Principles of the Procedure

Proteose Peptone provides nitrogen, vitamins, amino acids and carbon in m Tetrathionate Broth Base. Selectivity is achieved by the combination of sodium thiosulfate and tetrathionate, which suppresses commensal intestinal organisms.[3] Tetrathionate is formed in the medium by the addition of iodine and potassium iodide solution. Organisms containing the enzyme tetrathionate reductase will proliferate in the medium. Bile Salts, a selective agent, is added to suppress coliform bacteria and inhibit gram-positive organisms.

Formula

m Tetrathionate Broth Base

Formula Per Liter

Bacto Proteose Peptone	5 g
Bacto Bile Salts	1 g
Sodium Thiosulfate	30 g

Final pH 8.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Lungs.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Use rehydrated medium within 24 hours.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

m Tetrathionate Broth Base

Materials Required But Not Provided

Glassware

Membrane filtration equipment
Iodine solution (see Method of Preparation, #4)
Incubator (35°C)
Sterile Petri dishes, 50 x 9 mm
m Brilliant Green Broth
Distilled or deionized water

Method of Preparation

1. Suspend 3.6 grams in 100 ml distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Cool medium to below 60°C.
4. Add 2 ml iodine solution (6 grams iodine crystals and 5 grams potassium iodide dissolved in 20 ml distilled or deionized water). Use the complete medium containing iodine within 24 hours.
5. Do not heat the medium after adding the iodine solution.
6. Dispense 2 ml amounts of medium onto sterile absorbent pads in 50-60 mm Petri dishes.

Specimen Collection and Preparation

Obtain and process samples according to the techniques and procedures established by laboratory policy.

Test Procedure

1. Perform membrane filtration with the inoculum to be tested.

Escherichia coli
ATCC® 25922

Salmonella typhimurium
ATCC® 14028

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige with greenish cast, free-flowing, homogeneous.
Solution:	3.6% solution, soluble in distilled or deionized water on boiling. Solution is light amber, clear, may have some precipitate.
Prepared Medium:	Light amber, clear, may have some precipitate.
Reaction of 3.6% Solution at 25°C:	pH 8.0 ± 0.2 (before adding iodine solution)

Cultural Response

Prepare m Tetrathionate Broth per label directions. Inoculate and incubate at 35 ± 2°C in a humid atmosphere for approximately 3 hours. Transfer filters to pads containing m Brilliant Green Broth and continue incubation to a total of 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR ON m BRILLIANT GREEN BROTH
Escherichia coli	25922*	30-300	good	yellow to green
Salmonella typhimurium	14028*	30-300	good	pink to red

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

2. Place the membrane filter on a pad soaked with m Tetrathionate Broth (with iodine) and incubate at 35 ± 2°C for 3 hours.
3. Aseptically transfer the filter to a pad soaked with 2 ml m Brilliant Green Broth in a 50-60 mm Petri dish.
4. Incubate at 35 ± 2°C for an additional 15-21 hours (total incubation to 18-24 hours) in a humid atmosphere.

Results

Examine for growth. *Salmonella* species produce pink to red colonies.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Kabler and Clark.** 1952. Am. J. Public Health **42**:390.
2. **Gray, L. D.** 1995. *Escherichia*, *Salmonella*, *Shigella*, and *Yersinia*, p. 450-456. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Knox, R., P. H. Gell, and M. R. Pollack.** 1942. Selective media for organisms of the *Salmonella* group. J. Pathol. Bacteriol. **54**:469-483.

Packaging

m Tetrathionate Broth Base	500 g	0580-17

Bacto® Thermoacidurans Agar

Intended Use

Bacto Thermoacidurans Agar is used for isolating and cultivating *Bacillus coagulans* (*Bacillus thermoacidurans*) from foods.

Summary and Explanation

Stern et al.[1] described a medium for isolating *B. coagulans* (*B. thermoacidurans*) which causes "flat sour" spoilage in tomato juice. "Flat sour" spoilage of canned foods can caused by *Bacillus coagulans* (*Bacillus thermoacidurans*). Bacterial growth results in a 0.3-0.5 drop in pH, while the ends of the can remain flat. *B. coagulans* is a soil microorganism that can be found in canned tomato products and dairy products. Conditions favorable to multiplication of the organism can result in spoilage of the food product.[2]

Thermoacidurans agar can also be used to isolate mesophilic spore forming anaerobes (*Clostridium* spp.) from foods.[2] These microorganisms tolerate high heat, grow in the absence of oxygen and grow over the range of temperatures used in canned and processed foods. They are of primary importance in spoilage of low-acid foods packed in hermetically sealed containers.[2]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	3.9% solution, soluble in distilled or deionized water on boiling; light amber, opalescent without significant precipitate.
Prepared Medium:	Light amber, opalescent without precipitate.
Reaction of 3.9% Solution at 25°C:	pH 5.0 ± 0.2

Cultural Response

Prepare Thermoacidurans Agar per label instructions. Inoculate and incubate the plates at 55 ± 1°C for 18-48 hours.

TEST ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacillus coagulans	7050	100-1,000	good

The culture listed is the minimum that should be used for performance testing.

Principles of the Procedure

Thermoacidurans Agar contains Proteose Peptone to provide the carbon and nitrogen for general growth requirements. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. Bacto Agar is a solidifying agent.

Formula

Formula Per Liter

Bacto Thermoacidurans Agar

Bacto Yeast Extract	5 g
Bacto Proteose Peptone	5 g
Bacto Dextrose	5 g
Dipotassium Phosphate	4 g
Bacto Agar	20 g

Final pH 5.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bacto Thermoacidurans Agar

Materials Required but not Provided

Glassware
Distilled or deionized water

Autoclave
Incubator (55°C)
Petri dishes

Method of Preparation

1. Suspend 39 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Avoid overheating which could cause a softer medium.
4. Cool to room temperature.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Consult appropriate references for recommended test procedures.[1,2]

Results

Growth is evident in the form of turbidity.

Limitations of the Procedure

Microorganisms other than *B. coagulans* may grow on this medium. Perform microscopic examination and biochemical tests to identify to genus and species if necessary.

References

1. **Stern, Hegarty, and Williams.** 1942. Food Research **7**:186.
2. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Thermoacidurans Agar	500 g	0303-17

Bacto® Thiamine Assay Medium
Bacto Thiamine Assay Medium LV

Intended Use

Bacto Thiamine Assay Medium is used for determining thiamine concentration by the microbiological assay technique.

Bacto Thiamine Assay Medium LV is used for determining thiamine concentration by the microbiological assay technique using *Lactobacillus viridescens* ATCC® 12706.

Also Known As

Thiamine is also know as Vitamin B1.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of medium are used for this purpose:

1. Maintenance Medium: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose.
2. Inoculum Medium: To condition the test culture for immediate use.
3. Assay Medium: To permit quantitation of the vitamin under test. Assay media contain all factors necessary for optimal growth of the test organism except the single essential vitamin to be determined.

Thiamine Assay Medium is prepared according to the formula by Sarett and Cheldelin.[1] *Lactobacillus fermentum* ATCC® 9338 is used as the test organism in the microbiological assay of thiamine.

Thiamine Assay Medium LV, patterned after APT medium, was described by Deibel, Evans and Niven[2] for the microbiological assay of thiamine using *Lactobacillus viridescens* ATCC® 12706.

Nutritional studies by Evans and Niven[3] on the heterofermentative lactobacilli that cause greening in cured meat products indicated that thiamine was an essential vitamin for growth of these organisms. Deibel, Evans and Niven[4] described APT medium for lactobacilli cultivation. They reported that lactobacilli required at least 10 ng thiamine per ml for growth in contrast to 0.2 to 3 ng per ml for thiamine-requiring streptococci, leuconostocs and staphylococci. Further, they suggested that lactobacilli requiring large amounts of thiamine might be employed in microbiological assay procedures. In 1957,[2] these authors described a medium for the microbiological assay of thiamine using *Lactobacillus viridescens* ATCC® 12706 as the test organism. This medium is known as Thiamine Assay Medium LV.

Principles of the Procedure

Thiamine Assay Medium and Thiamine Assay Medium LV are free from thiamine, but contain all other nutrients and vitamins essential for the growth of the test organisms. The addition of thiamine in specified increasing concentrations gives a growth response that can be measured turbidimetrically.

Formula

Thiamine Assay Medium

Formula Per Liter		
Thiamine-Free Tryptone	22	g
Bacto Vitamin Assay Casamino Acids	5	g
Bacto Dextrose	40	g
Sodium Acetate	15	g
L-Cystine	0.2	g
Adenine Sulfate	20	mg
Guanine Hydrochloride	20	mg
Uracil	20	mg
Riboflavin	200	µg
Calcium Pantothenate	200	µg
Niacin	200	µg
Pyridoxine Hydrochloride	200	µg
p-Aminobenzoic Acid	200	µg
Folic Acid	5	µg
Biotin	0.8	µg
Dipotassium Phosphate	1	g
Monopotassium Phosphate	1	g
Magnesium Sulfate	0.4	g
Sodium Chloride	20	mg
Ferrous Sulfate	20	mg
Manganese Sulfate	20	mg

Final pH 6.5 ± 0.2 at 25°C

Thiamine Assay Medium LV

Formula Per Liter

Thiamine-Free Yeast Extract	10 g
Thiamine-Free Tryptone	20 g
Bacto Dextrose	20 g
Sodium Citrate	10 g
Dipotassium Phosphate	10 g
Sodium Chloride	10 g
Magnesium Sulfate	1.6 g
Manganese Sulfate	0.28 g
Ferrous Sulfate	0.08 g
Tween 80	2 g

Final pH 6.0 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Thiamine Assay Medium

Dehydrated Medium:	Beige, homogeneous, tendency to clump.
Solution:	4.25% (single strength) and 8.5% (double strength) solution, soluble in distilled or deionized water on boiling 2-3 minutes. 4.25% solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	4.25% solution is light amber, clear, may have a slight precipitate.
Reaction of 4.25% Solution at 25°C:	pH 6.5 ± 0.2

Thiamine Assay Medium LV

Dehydrated Appearance:	Beige, homogeneous, tendency to clump.
Solution:	4.2% (single strength) and 8.4% (double strength) solution, soluble in distilled or deionized water on boiling 2-3 minutes. 4.2% solution is light amber, clear, may have a slight precipitate.
Prepared Medium:	4.2% solution is light amber, clear, may have a slight precipitate.
Reaction of 4.2% Solution at 25°C:	pH 6.0 ± 0.2

Cultural Response

Thiamine Assay Medium

Prepare single-strength Thiamine Assay Medium per label directions. Prepare a standard curve using a thiamine hydrochloride reference standard at 0.0 to 0.05 µg per 10 ml. Inoculate with *Lactobacillus fermentum* ATCC® 9338 and incubate with caps loosened at 35-37°C for 16-18 hours. Read percent transmittance using a spectrophotometer at 660 nm.

Thiamine Assay Medium LV

Prepare single-strength Thiamine Assay Medium LV per label directions. Prepare a standard curve using a thiamine hydrochloride reference standard at 0.0 to 25.0 µg per 10 ml. Inoculate with *Lactobacillus viridescens* ATCC® 12706 and incubate with caps loosened at 30 ± 2°C for 16-20 hours. Read percent transmittance using a spectrophotometer at 660 nm.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. Great care to avoid contamination of media or glassware must be taken in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
4. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Storage

Store the dehydrated media at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Thiamine Assay Medium
Thiamine Assay Medium LV

Materials Required But Not Provided

Glassware
Autoclave
Spectrophotometer or nephelometer
Centrifuge
Incubator, 30°C and 35°C
Sterile tubes and caps
Sterile 0.85% NaCl
Stock culture of *Lactobacillus viridescens* ATCC® 12706 or
Stock culture of *Lactobacillus fermentum* ATCC® 9338
Lactobacilli Agar AOAC
Micro Assay Culture Agar
APT Agar
Lactobacilli Broth AOAC
Micro Inoculum Broth
APT Broth
Thiamine hydrochloride

Method of Preparation

1. Suspend the medium in 100 ml distilled or deionized water:
   Thiamine Assay Medium - 8.5 grams;
   Thiamine Assay Medium LV - 8.4 grams.
2. Boil 2-3 minutes to dissolve completely.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 5 minutes.

Specimen Collection and Preparation

Prepare assay samples according to references given in the specific assay procedures. The samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Thiamine Assay Medium

Prepare stock cultures of the test organism, *Lactobacillus fermentum* ATCC® 9338, by stab inoculation on Lactobacilli Agar AOAC or Micro Assay Culture Agar. After 24-48 hours incubation at 35-37°C, keep the tubes in the refrigerator. Make transfers in triplicate at monthly intervals.

Prepare the inoculum by subculturing a stock culture of the test organism in 10 ml of Lactobacilli Broth AOAC or Micro Inoculum Broth. After 16-18 hours incubation at 35-37°C, centrifuge the cells under aseptic conditions and decant the supernatant liquid. Wash the cells three times with 10 ml sterile 0.85% NaCl. After the third wash, resuspend the cells in 10 ml sterile 0.85% NaCI. Add 0.5 ml of this suspension to 100 ml sterile 0.85% NaCl. Use one drop of the resulting suspension to inoculate the assay tubes.

A standard curve should be run with each assay because conditions of heating and incubation temperature that influence the standard curve readings cannot always be duplicated.

The tubes for the Thiamine Assay Medium standard curve contain 0.0, 0.005, 0.01, 0.015, 0.02, 0.03, 0.04 and 0.05 µg of thiamine hydrochloride per 10 ml tube. The most effective assay range for Thiamine Assay Medium is between 0.005 and 0.03 µg thiamine.

Prepare the stock solution of thiamine required for the preparation of the standard curve in Thiamine Assay Medium as follows:

1. Dissolve 0.1 gram of thiamine hydrochloride in 1,000 ml of distilled water (100 µg/ml).
2. Add 1 ml of the solution in Step 1 to 99 ml distilled water (1 µg/ml).
3. Add 1 ml of the solution in Step 2 to 99 ml distilled water to give a final concentration of 10 ng (0.010 µg/ml). Use 0.0, 0.5, 1, 1.5, 2, 3, 4 and 5 ml of this final solution per tube. Prepare fresh stock solution daily.

After 20-24 hours incubation at 35-37°C, *L. fermentum* ATCC® 9338 is capable of using the pyrimidine and thiazole moieties of the thiamine molecule. It is essential that the growth response be measured turbidimetrically prior to this time. Incubate the tubes at 35-37°C for 16-18 hours, then place in the refrigerator for 15-30 minutes to stop growth. The growth can then be measured by any suitable nephelometric method.

Thiamine Assay Medium LV

Prepare stock cultures of the test organism, *L. viridescens* ATCC® 12706, by stab inoculation on APT Agar or Lactobacilli Agar AOAC. After 24-48 hours incubation at 30 ± 2°C, keep the tubes in the refrigerator. Make transfers in triplicate at monthly intervals.

Prepare the inoculum by subculturing a stock culture of the test organism to 10 ml APT Broth or Lactobacilli Broth AOAC. After 16-20 hours incubation at 30 ± 2°C, centrifuge the cells under aseptic conditions and decant the supernatant liquid. Wash the cells three times with 10 ml sterile 0.85% NaCl. After the third wash, resuspend the cells in 10 ml sterile 0.85% NaCl. Add 1 ml of this cell suspension to 100 ml sterile 0.85% NaCl. Use one drop of this suspension to inoculate the assay tubes.

A standard curve should be run with each assay because conditions of heating and incubation temperature that influence the standard curve readings cannot always be duplicated.

The standard curve for Thiamine Assay Medium LV is obtained by using thiamine at levels of 0.0, 1, 2.5, 5, 7.5, 10, 15, 20 and 25 ng of thiamine hydrochloride per 10 ml tube. This is obtained by using 0.0, 0.2, 0.5, 1, 1.5, 2, 3, 4 and 5 ml of the standard solution, which contains 5 ng (0.005 µg) thiamine hydrochloride per ml. The most effective assay range is between 2.5 and 20 ng per tube.

The solution for preparing the standard curve for Thiamine Assay Medium LV may be prepared as follows:

1. Dissolve 50 mg of thiamine hydrochloride in 500 ml distilled water (100 µg/ml).
2. Add 1 ml of the solution in Step 1 to 99 ml distilled water (1 µg/ml).
3. Add 1 ml of the solution in Step 2 to 199 ml distilled water to give a final concentration of 5 ng (0.005 µg) per ml.

Following incubation of *L. viridescens* ATCC® 12706 at 30 ± 2°C for 16-20 hours, the growth response is measured turbidimetrically.

Results

Thiamine Assay Medium and Thiamine Assay Medium LV

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average and use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the microbiological assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements which will not give a satisfactory response.
4. For successful results, all conditions of the assay must be followed exactly.

References

1. **Sarett and Cheldelin.** 1944. J. Biol. Chem. **155**:153.
2. **Deibel, Evans, and Niven.** 1957. Paper presented 57th general meet. Soc. Am. Bacteriol. Detroit, MI.
3. **Evans and Niven.** 1951. J. Bacteriol. **62**:599.
4. **Diebel, Evans, and Niven.** 1955. Bacteriol. Proc.

Packaging

Thiamine Assay Medium	100 g	0326-15
Thiamine Assay Medium LV	100 g	0808-15

Thioglycollate Media

Bacto® Fluid Thioglycollate Medium · Bacto NIH Thioglycollate Broth · Bacto Brewer Thioglycollate Medium · Bacto Fluid Thioglycollate Medium w/Beef Extract · Bacto Fluid Thioglycollate Medium w/K Agar · Bacto Thioglycollate Medium w/o Dextrose · Bacto Thioglycollate Medium w/o Dextrose or Indicator · Bacto Thioglycollate Medium w/o Indicator

Intended Use

Bacto Fluid Thioglycollate Medium is used for detecting microorganisms in normally sterile materials. Fluid Thioglycollate Medium conforms to the formula specified by the US Pharmacopeia XXIII (USP)[1], the Code of Federal Regulations (21 CFR)[2] and European Pharmacopeia for sterility testing of pharmaceutical products, biologics and devices.

Bacto NIH Thioglycollate Broth is used in detecting microorganisms in normally sterile, turbid or viscous materials. This formula conforms with USP Alternate Thioglycollate Medium.[1]

Bacto Brewer Thioglycollate Medium is for detecting microorganisms in normally sterile materials.

Bacto Fluid Thioglycollate Medium w/Beef Extract is used in cultivating microorganisms from normally sterile biological products.

Bacto Fluid Thioglycollate Medium w/K Agar is for detecting microorganisms in normally sterile materials containing mercurial preservatives when clarity and early visualization of growth are desired.

Bacto Thioglycollate Medium w/o Dextrose and **Thioglycollate Medium w/o Dextrose or Indicator** are used for detecting microorganisms in normally sterile materials, especially those containing mercurial preservatives. These media formulations may be used with added carbohydrates for fermentation studies.

Bacto Thioglycollate Medium w/o Indicator is for detecting microorganisms in normally sterile materials, especially those containing mercurial preservatives, when no oxidation-reduction indicator is required.

Also Known As

Fluid Thioglycollate Medium is often referred to as Thioglycollate Medium and abbreviated as FTM.

NIH Thioglycollate Medium is also known as USP Thioglycollate Medium Alternative and Alternate Fluid Thioglycollate.

Thioglycollate Medium w/o Indicator and Thioglycollate Medium w/o Dextrose or Indicator have been called Thioglycollate Fermentation Media.

Summary and Explanation

Quastel and Stephenson[4] found that the presence of a small amount of a compound containing an -SH group (cysteine, thioglycollic acid, glutathione) permitted "aerobic" growth of *Clostridium sporogenes* in tryptic digest broth.

Falk, Bucca and Simmons[5] pointed out the advantages of using small quantities of agar (0.06-0.25%) in detecting contaminants during sterility testing of biologicals. The value of combining a small amount of agar and a reducing substance was demonstrated by Brewer.[6] Brewer's experiments revealed that in a liquid medium containing 0.05% agar, anaerobes grew equally well in the presence or absence of sodium thioglycollate. Marshall, Gunnish and Luxen[7] reported satisfactory cultivation of anaerobes in Brewer's Thioglycollate Medium in the presence of a mercurial preservative. Nungester, Hood and Warren[8] and Portwood[9] confirmed the neutralization of the bacteriostatic effect of mercurial compounds by sodium thioglycollate. Malin and Finn[10] reported the commonly used medium containing thioglycollate is inhibitory to some organisms in the presence of a carbohydrate. In 1941, the National Institutes of Health specified the use of two thioglycollate media in sterility testing, the Brewer Formula and the Linden Formula.[11] The Linden Formula was later referred to as Modified Brewer Thioglycollate Medium in which meat infusion was replaced by plant (soy) peptones.[12]

Fluid Thioglycollate Medium is prepared according to the formula in the FDA Bacteriological Analytical Manual (BAM)[13] and AOAC Official Methods of Analysis[14] for the examination of food, and for determining the phenol coefficient and sporicidal effects of disinfectants. Fluid Thioglycollate Medium is also specified for sterility checks on banked blood.[15]

Fluid Thioglycollate Medium w/ Beef Extract is recommended by Animal and Plant Health Inspection Service, USDA,[3] in the detection of viable bacteria in live vaccines. Thioglycollate Medium w/o Dextrose and Thioglycollate Medium w/o Dextrose or Indicator may be used with added carbohydrates for fermentation studies.

Thioglycollate Medium w/o Indicator is the medium of choice for diagnostic work because the lack of indicator avoids possible toxicity to organisms.[13] This medium supports a minimal inoculum with early visibility of growth.

When used as an enrichment broth to support plated media, thioglycollate media are often supplemented with hemin and vitamin K_1.[17] Several modifications of this medium, usually with the addition

User Quality Control

Identity Specifications

Fluid Thioglycollate Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.98% solution, soluble in distilled or deionized water on boiling. Appearance of solution immediately after sterilization - light amber, clear.
Prepared Medium:	Appearance of solution immediately after sterilization - light amber, clear. After cooling to room temperature, light amber, slightly opalescent with pink upper layer. If pink layer is greater than 10% of the tube, the medium may be restored once by heating on a steambath until the pink color disappears.
Reaction of 2.98% Solution at 25°C:	pH 7.1 ± 0.2

Brewer Thioglycollate Medium

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.05 % solution, soluble in distilled or deionized water on boiling; medium amber, clear to very slightly opalescent with upper 10% or less medium green on standing.
Prepared Medium:	Medium amber, clear to very slightly opalescent with upper 10% or less medium green.
Reaction of 4.05% Solution at 25°C:	pH 7.2 ± 0.2

NIH Thioglycollate Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	2.9% solution, soluble in distilled or deionized water on boiling; light amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Medium:	Light amber, clear to very slightly opalescent, may have a slight precipitate.
Reaction of 2.9% Solution at 25°C:	pH 7.1 ± 0.2

Fluid Thioglycollate Medium w/Beef Extract

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.47% solution, soluble upon boiling for 1-2 minutes. Immediately after sterilization, appearance is medium to dark amber, clear, becoming medium to dark amber with upper 10% or less of medium pink and slightly opalescent.
Prepared Medium:	Medium to dark amber with upper 10% or less medium pink, slightly opalescent.
Reaction of 3.47% Solution at 25°C:	pH 7.2 ± 0.2

Fluid Thioglycollate Medium w/K Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.9% solution, soluble in distilled or deionized water upon boiling. Appearance of solution immediately after sterilization-light amber, clear. After cooling to room temperature and shaking, solution becomes pink with slight opalescence.
Prepared Medium:	Appearance of solution immediately after sterilization - light amber, clear. After cooling to room temperature, light amber, with some opalescence with upper 10% or less of medium pink.
Reaction of 2.9% Solution at 25°C:	pH 7.2 ± 0.2

Thioglycollate Medium w/o Dextrose

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.4% solution, soluble in distilled or deionized water on boiling; light amber, clear to very slightly opalescent. Upper 10% or less medium green on standing.
Prepared Medium:	Light amber, very slightly opalescent with upper 10% or less medium turning green on cooling.
Reaction of 2.4% Solution at 25°C:	pH 7.2 ± 0.2

Thioglycollate Medium w/o Indicator

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.9% solution, soluble in distilled or deionized water upon boiling. Appearance of solution immediately after sterilization-light amber, clear without significant precipitate. After cooling to room temperature, solution may exhibit slight opalescence.
Prepared Medium:	Appearance of solution immediately after sterilization - light amber, clear without significant precipitate. After cooling to room temperature, solution may exhibit slight opalescence.
Reaction of 2.9% Solution at 25°C:	pH 7.2 ± 0.2

Thioglycollate Medium w/o Dextrose or Indicator

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.4% solution, soluble in distilled or deionized water upon boiling. Appearance of solution immediately after sterilization - light amber, clear with no significant precipitate. After cooling to room temperature - light, very slightly to slightly opalescent with no significant precipitate.
Prepared Medium:	Immediately after sterilization-light, clear with no significant precipitate. After cooling to room temperature - light, very slightly to slightly opalescent with no significant precipitate.
Reaction of 2.4% Solution at 25°C:	pH 7.2 ± 0.2

continued on following page

of sodium polyanetholesulfonate (SPS), are used in the inoculum of blood cultures specifically, for the isolation of anaerobes.[18]

The methodologies for the multiple applications using thioglycollate medium are outlined in the references.

Principles of the Procedure

Thioglycollate media support the growth of a large variety of fastidious microorganisms having a wide range of growth requirements. The nitrogen source, provided by Casitone, Infusion from Beef, Proteose Peptone, Beef Extract, Pancreatic Digest of Casein varies with the formula. Yeast Extract is added as a source of vitamins.

Sodium Thioglycollate, Thioglycollic Acid and L-Cystine lower the oxidation-reduction potential of the medium by removing oxygen to maintain a low Eh. By creating an environment with a low Eh, the reducing agents prevent the accumulation of peroxides which can be toxic to some organisms. The sulfhydryl groups (-SH) of these compounds also neutralize the antibacterial effect of mercurial preservatives, making thioglycollate media useful in testing material which contains heavy metals.

Resazurin or Methylene Blue are oxidation indicators. In the oxidized state, methylene blue appears green, resazurin turns pink. In the reduced state both compounds are colorless. Bacto Agar eliminates the need for seals because it retards dispersion of CO_2, diffusion of oxygen and reducing substances.[12] Substituting K Agar and Potassium Chloride for Bacto Agar and Sodium Chloride in Fluid Thioglycollate Medium w/K Agar produces a medium with greater clarity to facilitate earlier visual recognition of growth.

Dextrose is included in the formulations because many organisms show earlier and more vigorous growth. Sodium chloride is used to maintain the osmotic balance of the media. Potassium Chloride and Dipotassium Phosphate are used as buffering agents.

User Quality Control cont.

Cultural Response

Brewer Thioglycollate Medium (0236), **Thioglycollate Medium w/o Indicator** (0430), **Thioglycollate Medium w/o Dextrose** (0363), **Thioglycollate Medium w/o Dextrose or Indicator** (0432).

Medium was prepared per label directions. Tubes were inoculated with the test organisms and incubated at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Staphylococcus aureus	25923	10-100	good
Clostridium novyi	7659	10-100	good
Clostridium sporogenes	11437	10-100	good
Bacteroides fragilis	25285	10-100	good

Fluid Thioglycollate Medium w/K Agar (0607), **Fluid Thioglycollate Medium w/Beef Extract** (0697), **NIH Thioglycollate Broth** (0257).

Medium was prepared per label directions. Tubes were inoculated with the test organisms and incubated at 30-35°C for up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Bacillus subtilis	6633	10-100	good
Bacteroides vulgatus	8482	10-100	good
Candida albicans	10231	10-100	good
Clostridium sporogenes	19404	10-100	good

Uninoculated tube — *Bacteroides vulgatus* ATCC® 8482

USP and EP Growth Promotion Procedure[1]

Fluid Thioglycollate Medium. Prepare FTM per label directions. Inoculum of 10-100 CFU were used and incubated for up to 7 days at temperature specified.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	INC. TEMP
Bacillus subtilis	6633*	10-100	growth must be evident	30-35°C
Bacteroides vulgatus	8482*	10-100	growth must be evident	30-35°C
Candida albicans	10231*	10-100	growth must be evident	20-25°C
Candida albicans	2091*	10-100	growth must be evident	20-25°C
Clostridium sporogenes	19404*	10-100	growth must be evident	30-35°C
Staphylococcus aureus	6538P*	10-100	good	30-35°C
Staphylococcus aureus	25923	10-100	good	30-35°C
Clostridium novyi	7659	10-100	good	30-35°C
Clostridium perfringes	13124	10-100	good	30-35°C

*Pharmacopeia growth promotion

Mercurial Neutralization

This test is performed by recovering the test organisms in Fluid Thioglycollate Medium after exposure to 1% Merthiolate®

ORGANISM	ATCC®	INOCULUM CFU	RECOVERY
Staphylococcus aureus	6538P	1,000	good
Streptococcus pyogenes	19615**	1,000	good

The cultures listed are the minimum that should be used for performance testing.

**These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

Fluid Thioglycollate Medium

Formula Per Liter

Bacto Casitone	15 g
Bacto Yeast Extract	5 g
Bacto Dextrose	5.5 g
Sodium Chloride	2.5 g
L-Cystine	0.5 g
Sodium Thioglycollate	0.5 g
Bacto Agar	0.75 g
Resazurin	0.001 g

Final pH 7.1 ± 0.2 at 25ºC

Thioglycollate Medium w/o Dextrose

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Casitone	15 g
L-Cystine	0.25 g
Sodium Chloride	2.5 g
Sodium Thioglycollate	0.5 ml
Bacto Agar	0.75 g
Methylene Blue	0.002 g

Final pH 7.2 ± 0.2 at 25ºC

Brewer Thioglycollate Medium

Formula Per Liter

Beef, Infusion from	500 g
Sodium Chloride	5 g
Dipotassium Phosphate	2 g
Bacto Proteose Peptone	10 g
Bacto Dextrose	5 g
Sodium Thioglycollate	0.5 g
Bacto Agar	0.5 g
Methylene Blue	0.002 g

Final pH 7.2 ± 0.2 at 25ºC

NIH Thioglycollate Broth

Formula Per Liter

Bacto Casitone	15 g
Bacto Yeast Extract	5 g
Bacto Dextrose	5.5 g
Sodium Chloride	2.5 g
L-Cystine	0.5 g
Sodium Thioglycollate	0.5 g

Final pH 7.1 ± 0.2 at 25ºC

Fluid Thioglycollate Medium w/K Agar

Formula per liter

Bacto Casitone	15 g
Bacto Yeast Extract	5 g
Bacto Dextrose	5 g
Potassium Chloride	2.5 g
L-Cystine	0.5 g
Sodium Thioglycollate	0.5 ml
K Agar	0.45 g
Resazurin	0.001 g

Final pH 7.2 ± 0.2 at 25ºC

Thioglycollate Medium w/o Indicator

Formula per liter

Bacto Yeast Extract	5 g
Bacto Casitone	15 g
Bacto Dextrose	5 g
L-Cystine	0.25 g
Sodium Chloride	2.5 g
Sodium Thioglycollate	0.5 g
Bacto Agar	0.75 g

Final pH 7.2 ± 0.2 at 25ºC

Fluid Thioglycollate Medium w/Beef Extract

Formula Per Liter

Bacto Beef Extract	5 g
Bacto Yeast Extract	5 g
Pancreatic Digest of Casein	15 g
Bacto Dextrose	5.5 g
Sodium Chloride	2.5 g
L-Cystine	0.5 g
Sodium Thioglycollate	0.5 g
Bacto Agar	0.75 g
Resazurin	0.001 g

Final pH 7.2 ± 0.2 at 25ºC

Thioglycollate Medium w/o Dextrose or Indicator

Formula Per Liter

Bacto Yeast Extract	5 g
Bacto Casitone	15 g
L-Cystine	0.25 g
Sodium Chloride	2.5 g
Sodium Thioglycollate	0.5 ml
Bacto Agar	0.75 g

Final pH 7.2 ± 0.2 at 25ºC

Precautions

1. For Laboratory Use:
   Fluid Thioglycollate Medium
   NIH Thioglycollate Broth
   Fluid Thioglycollate Medium w/K Agar
   Brewer Thioglycollate Medium
   Fluid Thioglycollate Medium w/Beef Extract
   Thioglycollate Medium w/o Dextrose
   Thioglycollate Medium w/o Indicator
   Thioglycollate Medium w/o Dextrose or Indicator
2. Do not reheat the media more than once, because continued reheating gives rise to toxicity. Do not to reheat **NIH Thioglycollate Broth**.
3. When testing human serum, treat all specimens as infectious agents.
4. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

1. Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.
2. Store prepared media at 15-30°C.

3. For **Fluid Thioglycollate Medium, Fluid Thioglycollate Medium w/Beef Extract** and **Fluid Thioglycollate Medium w/K Agar,** if more than 30% of the medium is pink prior to use, reheat once (100°C) to drive off absorbed oxygen.

   For **Brewer Thioglycollate Medium**, if more than 20% of the medium is green prior to use, reheat once (100°C).

   After prolonged storage, reheat **Thioglycollate Medium w/o Indicator, Thioglycollate Medium w/o Dextrose or Indicator** and **Thioglycollate Medium w/o Dextrose** only once in flowing steam or a boiling water bath to drive off dissolved gases.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Fluid Thioglycollate Medium
NIH Thioglycollate Broth
Brewer Thioglycollate Medium
Fluid Thioglycollate Medium w/Beef Extract
Fluid Thioglycollate Medium w/K Agar
Thioglycollate Medium w/o Dextrose
Thioglycollate Medium w/o Dextrose or Indicator
Thioglycollate Medium w/o Indicator

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath
Sterile tubes

Method of Preparation

1. Suspend the appropriate amount of medium in 1 liter distilled or deionized water:

Fluid Thioglycollate Medium	29.8 g
Brewer Thioglycollate Medium	40.5 g
NIH Thioglycollate Broth	29 g
Fluid Thioglycollate Medium w/Beef Extract	34.7 g
Fluid Thioglycollate Medium w/K Agar	29 g
Thioglycollate Medium w/o Dextrose	24 g
Thioglycollate Medium w/o Indicator	29 g
Thioglycollate Medium w/o Dextrose or Indicator	24 g

2. Heat to boiling to dissolve completely. Avoid overheating.
3. Dispense as desired, using only clean, rust-free equipment.
4. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion on the isolation and identification of bacteria and yeasts, refer to appropriate procedures outlined in the references.

Results

Typically growth is visually observed in the media. Gram negative bacilli tend to grow diffusely, gram positive cocci exhibit puff-ball type growth and strict aerobes, such as pseudomonads and yeast, tend to grow in a thin layer on the surface of the broth.

References

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Packaging

Fluid Thioglycollate Medium	100 g	0256-15
	500 g	0256-17
	2 kg	0256-07
	10 kg	0256-08
NIH Thioglycollate Broth	500 g	0257-17
Brewer Thioglycollate Medium	500 g	0236-17
	10 kg	0236-08
Fluid Thioglycollate Medium w/ Beef Extract	500 g	0697-17
	10 kg	0697-08
Fluid Thioglycollate Medium w/K Agar	500 g	0607-17
	2 kg	0607-07
Thioglycollate Medium w/o Dextrose	500 g	0363-17
Thioglycollate Medium w/o Dextrose or Indicator	500 g	0432-17
Thioglycollate Medium w/o Indicator	500 g	0430-17

Bacto® Thiol Medium Bacto Thiol Broth

Intended Use

Bacto Thiol Medium is used for cultivating organisms from body fluids and other materials containing penicillin, streptomycin or sulfonamides.

Bacto Thiol Broth is used for cultivating organisms from body fluids and other materials containing penicillin, streptomycin or sulfonamides.

Summary and Explanation

While studying *Vibrio fetus* cultivation, Huddleson[1] found that vibrios remained viable in Thiol Medium at room temperature for at least 150 days without transfer. Christensen[2] tested Thiol Medium for the ability to neutralize penicillin and streptomycin. Ten milliliters (10 ml) of Thiol Medium can inactivate up to 100 units of penicillin and up to 1,000 micrograms of streptomycin, producing luxuriant growth of staphylococci and other organisms from dilute inocula in 24 hours.

Szawatkowski[3] and Shanson and Barnicoat[4] reported Thiol Broth to be superior in supporting the growth of *Bacteroides* species in blood cultures. Thiol Broth was used to study the optimum incubation period of blood culture broths.[5] Media containing thiol and thioglycollate are recommended for recovery of nutritionally variant streptococci (NVS).[6]

Thiol Broth has the same formulation as Thiol Medium, omitting the agar. Thiol is cited in Clinical Microbiology Procedures Handbook[7] as a medium specific for anaerobic bacteria in blood cultures.

Principles of the Procedure

Proteose Peptone No. 3 and Yeast Extract provide nitrogen, vitamins and amino acids in Thiol media. Dextrose is a carbon source. Sodium Chloride maintains osmotic balance. Para-aminobenzoic Acid is a preservative. Thiol Complex is rich in sulfhydryl (-SH) groups, which neutralize the bacteriostatic and bactericidal effects of penicillin, streptomycin and sulfonamides. Thiol Medium contains 0.1% Bacto Agar to maintain an Eh potential that facilities anaerobic growth and aids in dispersion of reducing substances and CO_2 formed in the environment.[8]

Formula

Thiol Medium

Formula Per Liter

Bacto Proteose Peptone No.3	10 g
Bacto Yeast Extract	5 g
Bacto Dextrose	1 g
Sodium Chloride	5 g
Thiol Complex	8 g
Bacto Agar	1 g
p-Aminobenzoic Acid	0.05 g

Final pH 7.1 ± 0.2 at 25°C

Thiol Broth

Formula Per Liter

Bacto Proteose Peptone No.3	10 g
Bacto Yeast Extract	5 g
Bacto Dextrose	1 g
Sodium Chloride	5 g
Thiol Complex	8 g
p-Aminobenzoic Acid	0.05 g

Final pH 7.1 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30%C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Use Thiol media within four days of preparation.

Procedure

Materials Provided

Thiol Medium
Thiol Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water:
   Thiol Medium - 30 grams;
   Thiol Broth - 29 grams.
2. Heat to boiling to dissolve completely.
3. Dispense as desired, using clean, rust-free equipment to prevent precipitate formation.
4. Autoclave at 121°C for 15 minutes. Cool to room temperature.

User Quality Control

Identity Specifications

Thiol Medium

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3% solution, soluble in distilled or deionized water on boiling. Very light to light amber, clear to very slightly opalescent when hot, opalescent after cooling.
Prepared Medium:	Very light amber; very slightly to slightly opalescent when hot, opalescent after cooling.
Reaction of 3% Solution at 25°C:	pH 7.1 ± 0.2

Thiol Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.9% solution, soluble in distilled or deionized water on boiling; very light to light amber, clear to slightly opalescent.
Prepared Medium:	Very light amber, clear to slightly opalescent.
Reaction of 2.9% Solution at 25°C:	pH 7.1 ± 0.2

Cultural Response

Prepare Thiol Medium and Thiol Broth per label directions. Test 5 unit, 100 unit and 1,000 unit concentrations of penicillin and 100 µg, 1,000 µg and 10,000 µg concentrations of streptomycin. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH w/o ANTIBIOTICS	GROWTH w/ANTIBIOTICS
Staphylococcus aureus	25923*	100-1,000	good	good†
Streptococcus pyogenes	19615*	100-1,000	good	good†

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

†Antibiotic concentrations up to 100 units of penicillin or 1000 µg of streptomycin.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion on processing and interpretation of blood cultures and body fluids from clinical specimens, refer to appropriate references.[7,9]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Strict reliance on blood culture bottles containing Thiol Broth is not recommended for aerobic microorganisms. Always use an aerobic medium, for example, a vented Tryptic Soy Broth, for optimum isolation of the broad spectrum of microorganisms that can cause bacteremia or septicemia.

References

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Packaging

Thiol Medium	500 g	0307-17
Thiol Broth	500 g	0434-17
	10 kg	0434-08

Tinsdale Agar

Bacto® Tinsdale Base · Bacto Tinsdale Enrichment Desiccated

Intended Use

Bacto Tinsdale Base is used with Bacto Tinsdale Enrichment Desiccated in isolating and differentiating *Corynebacterium diphtheriae*.

Also Known As

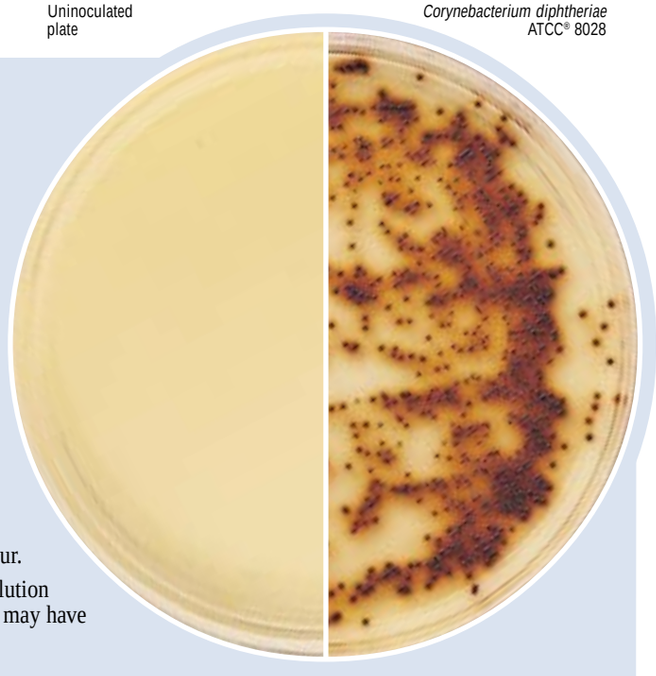
Tinsdale Base (TIN) is also called Tinsdale Selective Medium, Tinsdale Tellurite Medium and Tellurite Agar.

Summary and Explanation

Tinsdale Base, supplemented with Tinsdale Enrichment, is employed in the cultural diagnosis of diphtheria. Diphtheria, an acute infectious disease primarily of the upper respiratory tract but occasionally of the skin,[1] is caused by toxigenic strains of *Corynebacterium diphtheriae*. The three biotypes are mitis, intermedius and gravis.[1] The signs and symptoms of the disease are a pharyngeal membrane, sore throat, malaise, headache and nausea.[2] Death can result from respiratory obstruction by the membrane or myocarditis caused by the toxin.[2]

Tinsdale[3] developed a serum-cystine-thiosulfate-tellurite agar medium for the primary isolation and differentiation of *C. diphtheriae*. This formulation distinguished between *C. diphtheriae* and diphtheroids which exhibited similar characteristics. The differential principle is based on the capacity of *C. diphtheriae* to produce a brown or black halo around the colonies.

Billings[4] simplified Tinsdale Basal Medium by using Proteose Peptone No. 3 as a nutrient source. This modification improved the differential qualities and recovery of *Corynebacterium diphtheriae*. Tinsdale Base and Tinsdale Enrichment are prepared according to the Billings[4] modification. Moore and Parsons[5] confirmed the halo formation of *C. diphtheriae* with one exception; *C. ulcerans* occasionally produced colonies similar to *C. diphtheriae* and required biochemical identification.



User Quality Control

Identity Specifications

Tinsdale Base

Dehydrated Appearance:	Light beige, free flowing, homogeneous.
Solution:	4.5% solution, soluble in distilled or deionized water upon boiling. Solution is light to medium amber, slightly opalescent to opalescent, without significant precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent to opalescent without precipitate.
Reaction of 4.5% Solution at 25°C:	pH 7.4 ± 0.2

Tinsdale Enrichment Desiccated

Lyophilized Appearance:	Light to dark tan cake; variations may occur.
Solution:	Soluble in distilled or deionized water. Solution is light to dark amber, clear to opalescent, may have a slight precipitate.

Cultural Response

Prepare Tinsdale Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Corynebacterium diphtheriae type *gravis*	8028	100-1,000	good	brown with halos
Corynebacterium diphtheriae type *mitis*	8024	100-1,000	good	brown with halos
Klebsiella pneumoniae	13883*	100-1,000	marked to complete inhibition	–
Streptococcus pyogenes	19615*	100-1,000	fair	brown to black without halos

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Tinsdale Enrichment Desiccated contains Bovine Serum, Sodium Hydroxide, L-Cystine, Sodium Thiosulfate and Potassium Tellurite in the quantity and proportion described by Billings.[4]

Principles of the Procedure

Proteose Peptone No. 3 provides the nitrogen, vitamins, carbon and amino acids in Tinsdale Base. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is the solidifying agent.

Tinsdale Enrichment contains Bovine Serum, which provides essential growth factors. Sodium Hydroxide maintains the pH. L-Cystine and Sodium Thiosulfate are H_2S indicators. Potassium Tellurite is a selective agent. The formation of black to brown halos surrounding the colony result from the reduction of potassium tellurite to metallic tellurite.

Stabbing the medium with an inoculating needle accentuates darkening of the medium by *C. diphtheriae*.

Formula

Tinsdale Base

Formula Per Liter

Bacto Proteose Peptone No. 3	20 g
Sodium Chloride	5 g
Bacto Agar	20 g

pH 7.4 ± 0.2 at 25°C

Tinsdale Enrichment Desiccated

Contains Bovine Serum, L-Cystine, Sodium Hydroxide, Sodium Thiosulfate and Potassium Tellurite at pH 8.0-10.0 at 25°C.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store Tinsdale Enrichment Desiccated at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Tinsdale Base
Tinsdale Enrichment Desiccated

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C)
Sterile Petri dishes

Method of Preparation

1. Suspend 45 grams of Tinsdale Base in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense 100 ml amounts into flasks.
4. Autoclave at 121°C for 15 minutes.
5. Rehydrate Tinsdale Enrichment with 15 ml sterile distilled or deionized water and rotate in an end-over-end motion to dissolve completely.
6. Aseptically add 15 ml rehydrated Tinsdale Enrichment to each 100 ml of Tinsdale Base at 50-55°C. Mix well.
7. Dispense into sterile Petri dishes.

Specimen Collection and Preparation[1]

1. Both throat and nasopharyngeal specimens are necessary in cases of respiratory illness. If cutaneous diphtheria is suspected, collect skin, throat and nasopharyngeal specimens. Sterile silica gel is recommended for shipping when clinical specimens are not cultured locally.

Test Procedure

1. For a complete discussion on the collection, isolation and identification of *Corynebacterium diphtheriae* and other *Corynebacterium* species, refer to the appropriate procedures outlined in the references.
2. Inoculate plates with the test organisms in a manner to obtain discrete colonies and stab the medium several times with an inoculating needle.
3. Definitive identification of a strain of *C. diphtheriae* as a true pathogen requires demonstration of toxin production.[6] Characteristic colonies of *C. diphtheriae* may be inoculated directly onto KL Virulence Agar enriched with KL Virulence Enrichment and containing KL Antitoxin Strips for toxigenicity tests.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Tinsdale Agar is not suitable as a primary plating medium, since it may not support the growth of some strains of *C. diphtheriae*.[1]
3. *Corynebacterium ulcerans*, *Corynebacterium pseudotuberculosis* and (rarely) *Staphylococcus* species may produce a characteristic halo on Tinsdale Agar.[1]
4. Do not read Tinsdale Agar early because several organisms may exhibit slight browning on this medium in 18 hours.[1]
5. Incubation at 5-10% CO_2 retards the development of halos on Tinsdale Agar.[1]
6. On media containing tellurite, diphtheria bacilli are shorter and stain more uniformly; however, granules are less readily observed than when grown on Loeffler's medium.[7]
7. Further biochemical tests may be necessary to distinguish between *C. diphtheriae* and *C. ulcerans* due to similar reactions on this medium.

References

1. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.

2. **Clarridge, J. E., and C. A. Spiegel.** 1995. Corynebacterium and miscellaneous irregular gram-positive rod, *Erysipelothrix*, and *Gardnerella*, p. 357-377. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Tinsdale, G. F. W.** 1947. A new medium for the isolation and identification of *C. diphtheriae* based on the production of hydrogen sulphide. J. Pathol. Bacteriol. **59**:461-466.
4. **Billings, E.** 1956. An investigation of Tinsdale Tellurite medium: its usefulness and mechanisms of halo-formation. M.S. thesis. University of Michigan, Ann Arbor, MI.
5. **Moore, M. S., and E. I. Parsons.** 1958. A study of a modified Tinsdale's medium for the primary isolation of *Corynebacterium diphtheriae*. J. Infect. Dis. **102**:88.
6. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
7. **Bailey, R. W., and E. G. Scott.** 1966. Diagnostic microbiology, 2nd ed., p. 213. The C. V. Mosby Company, St. Louis, MO.

Packaging

Tinsdale Base	500 g	0786-17
Tinsdale Enrichment Desiccated	6 x 15 ml	0342-33

Bacto® Todd Hewitt Broth

Intended Use

Bacto Todd Hewitt Broth is used for cultivating streptococci, pneumococci and other fastidious organisms; for cultivating group A streptococci prior to serological typing.

Also Known As

Todd Hewitt Broth can be abbreviated as THB.

Summary and Explanation

Todd Hewitt Broth was originally developed for the production of antigenic streptococcal hemolysin.[1] Todd Hewitt Broth is prepared according to the formula described by Updyke and Nickle[2] who compared media for type specific extract production of group A streptococci. This study was performed using Todd Hewitt Broth prepared with infusion of fresh beef heart as a control. Results showed Todd Hewitt Broth was particularly satisfactory for growth of group A streptococci for serological typing.

Elliott[3] reported that Todd Hewitt Broth prepared with neopeptone was excellent for growing group A streptococci for the production of type specific M substance. This is possible because proteinase is not produced in this medium.

Moody, et al.[4] used Todd Hewitt Broth in the fluorescent-antibody identification of group A streptococci from throat cultures. Todd Hewitt Broth is recommended as an enrichment medium for the growth of streptococcal cells in the identification of groups A and B by IF staining.[5] Todd Hewitt Broth was used as an enrichment broth for group A streptococci in a comparison study of a rapid antigen test.[6]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in distilled or deionized water; light to medium amber, clear with no significant precipitate.
Prepared Medium:	Light to medium amber, clear with no significant precipitate.
Reaction of 3.0% Solution at 25°C:	pH 7.8 ± 0.2

Cultural Response

Prepare Todd Hewitt Broth per label directions. Incubate inoculated tubes at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090*	100-1,000	good
Staphylococcus aureus	25923*	100-1,000	good
Streptococcus pneumoniae	6303*	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedures

Infusion from Beef Heart and Neopeptone provides the nitrogen, vitamins and amino acids in Todd Hewitt Broth. Dextrose is the carbon source, and a stimulant for hemolysin production.[7] Disodium Phosphate and Sodium Carbonate act as buffers to aid in neutralizing acid production from dextrose fermentation, and protect hemolysin from inactivation.[7] Sodium Chloride maintains the osmotic balance of the medium.

Formula

Todd Hewitt Broth

Formula Per Liter

Beef Heart, Infusion from	500 g
Bacto Neopeptone	20 g
Bacto Dextrose	2 g
Sodium Chloride	2 g
Disodium Phosphate	0.4 g
Sodium Carbonate	2.5 g

Final pH 7.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Todd Hewitt Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile tubes

Method of Preparation

1. Suspend 30 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.
3. Cool to room temperature.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

For a complete discussion on the isolation, identification and serological procedures of fastidious microorganisms, refer to the procedures described in appropriate references.[4,5,8,9]

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Todd Hewitt Broth cannot be used unbuffered for bile solubility testing[10]

References

1. **Todd, E. W., and L. F. Hewitt**. 1932. A new culture medium for the production of antigenic streptococcal haemolysin. J. Pathol. Bacteriol. **35**:973.
2. **Updyke, E. L., and M. I. Nickle.** 1954. A dehydrated medium for the preparation of type specific extracts of group A streptococci. Appl. Microbiol. **2**:117.
3. **Elliott.** 1945. J. Exp. Med. **81**:573.
4. **Moody, M. D., A. C. Siegel, B. Pittman, and C. C. Winter.** 1963. Fluorescent- antibody identification of group A streptococci from throat swabs. Am. J. Public Health, **53**:1083.
5. **Facklam, R. R., and R. B. Carey**. 1985. Streptococci and Aerococci, p. 154-175. *In*, E. H.Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
6. **Bourbeau, P. P., B. J. Heiter, J. P. Anhalt, and D. W. Naumovitz.** 1993. Comparison of direct specimen testing utilizing testpack strep A with testing of specimens following a two-hour broth enrichment. Diagn. Microbiol. Infect. Dis. **17**:93-96.
7. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, p.772-775. vol. 1. Williams & Wilkins, Baltimore, MD.
8. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
9. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, American Society for Microbiology, Washington, D.C.

Packaging

Todd Hewitt Broth	100 g	0492-15
	500 g	0492-17
	2 kg	0492-07
	10 kg	0492-08

Tomato Juice Media

Bacto® Tomato Juice Agar · Bacto Tomato Juice Agar Special Bacto Tomato Juice Broth

Intended Use

Bacto Tomato Juice Agar is used for cultivating and enumerating *Lactobacillus* species.

Bacto Tomato Juice Agar Special is used for cultivating and enumerating lactobacilli and other acidophilic microorganisms from saliva and other specimens.

Bacto Tomato Juice Broth is used for cultivating yeasts and other aciduric microorganisms.

Summary and Explanation

In 1925, Mickle and Breed[1] reported the use of tomato juice in culture media used for cultivating lactobacilli. Kulp[2] investigated the use of tomato juice on bacterial development and found that the growth of *L. acidophilus* was enhanced. Tomato Juice Agar, prepared according to Kulp and White's[3] modification, is especially useful in cultivating *L. acidophilus* from clinical specimens and foodstuffs.[4]

Tomato Juice Agar Special is recommended for the direct plate count of lactobacilli from saliva and for cultivation of other acidophilic microorganisms. The number of lactobacilli in saliva is an index of a predisposition to dental caries as described by Jay.[5, 6] Many dentists use the direct count of lactobacilli for the diagnosis of caries.

The acidic pH of Tomato Juice Agar Special encourages growth of lactobacilli while inhibiting growth of accompanying bacteria. This medium is more selective for lactobacilli than Tomato Juice Agar.

Tomato Juice Broth is recommended for use in cultivating and isolating yeasts, lactobacilli and other aciduric microorganisms from clinical specimens and foods.

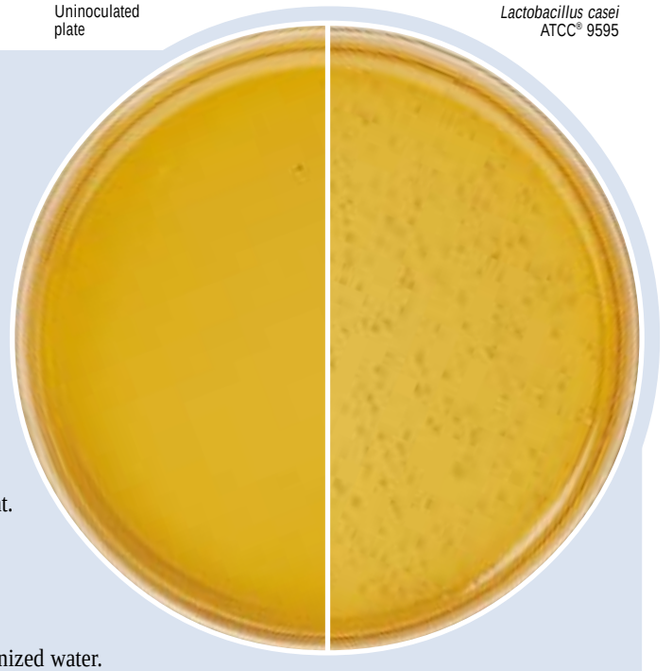
Principles of the Procedure

Tomato Juice Agar and Tomato Juice Agar Special

Tomato Juice is a source of carbon, protein and nutrients. Peptone provides a source of nitrogen, amino acids and carbon. Peptonized Milk contains lactose as an energy source. Bacto Agar is a solidifying agent.

Tomato Juice Broth

Tomato Juice is a source of carbon, protein and nutrients. Yeast Extract is a source of trace elements, vitamins and amino acids. Dipotassium Phosphate and Monopotassium Phosphate provide buffering capability. Magnesium Sulfate, Ferrous Sulfate and Manganese



User Quality Control

Identity Specifications

Tomato Juice Agar

Dehydrated Appearance: Tan, free-flowing, homogeneous.

Solution: 5.1% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, very slightly opalescent without precipitate.

Reaction of 5.1% Solution at 25°C: pH 6.1 ± 0.2

Tomato Juice Agar Special

Dehydrated Appearance: Tan, free-flowing, homogeneous.

Solution: 6.0% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, slightly opalescent.

Reaction of 6.0% Solution at 25°C: pH 5.0 ± 0.2

Tomato Juice Broth

Dehydrated Appearance: Tan, free-flowing, homogeneous.

Solution: 4.1% solution, soluble in distilled or deionized water. Solution is dark amber, clear without significant precipitate.

Reaction of 4.1% Solution at 25°C: pH 6.7 ± 0.2

Cultural Response

Tomato Juice Agar

Prepare Tomato Juice Agar per label directions. Inoculate using the pour plate technique and incubate at 35 ± 2°C for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus acidophilus	4356	100-1,000	good
Lactobacillus casei	9595	100-1,000	good
Lactobacillus delbrueckii	4797	100-1,000	good

Tomato Juice Agar Special

Prepare Tomato Juice Agar Special per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours (72 hours if necessary).

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus acidophilus	4356	100-1,000	good
Lactobacillus casei	9595	100-1,000	good
Lactobacillus delbrueckii	4797	100-1,000	good

Tomato Juice Broth

Prepare Tomato Juice Broth per label directions. Inoculate and incubate at 35 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Lactobacillus casei	9595	100-1,000	good
Lactobacillus delbrueckii	4797	100-1,000	good
Saccharomyces carlsbergensis	9080	100-1,000	good
Saccharomyces cerevisiae	9763	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Sulfate provide inorganic ions. Sodium Chloride is a source of essential ions that maintain the osmotic balance of the medium.

Formula

Tomato Juice Agar

Formula Per Liter

Tomato Juice (400 ml)	20 g
Bacto Peptone	10 g
Peptonized Milk	10 g
Bacto Agar	11 g

Final pH 6.1 ± 0.2 at 25°C

Tomato Juice Agar Special

Formula Per Liter

Tomato Juice (400 ml)	20 g
Bacto Peptone	10 g
Peptonized Milk	10 g
Bacto Agar	20 g

Final pH 5.0 ± 0.2 at 25°C

Tomato Juice Broth

Formula Per Liter

Tomato Juice (400 ml)	20 g
Bacto Yeast Extract	10 g
Bacto Dextrose	10 g
Dipotassium Phosphate	0.5 g
Monopotassium Phosphate	0.5 g
Magnesium Sulfate	0.2 g
Sodium Chloride	0.01 g
Ferrous Sulfate	0.01 g
Manganese Sulfate	0.01 g

Final pH 6.7 ± 0.2 at 25°C

Precautions

1. Tomato Juice Agar: For Laboratory Use.
   Tomato Juice Agar Special: For Laboratory Use.
   Tomato Juice Broth: For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Tomato Juice Agar
Tomato Juice Agar Special
Tomato Juice Broth

Materials Required but not Provided

Glassware
Autoclave
Distilled or deionized water

Method of Preparation

Tomato Juice Agar

1. Equilibrate the medium to room temperature before opening.
2. Suspend 51 grams in 1 liter distilled or deionized water.
3. Heat to boiling to dissolve completely.
4. Autoclave at 121°C for 15 minutes.

Tomato Juice Agar Special

1. Equilibrate the medium to room temperature before opening.
2. Suspend 60 grams in 1 liter distilled or deionized water.
3. Heat to boiling to dissolve completely.
4. Autoclave at 121°C for 15 minutes. Avoid overheating which could cause a softer medium.

Tomato Juice Broth

1. Dissolve 41 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Mickle and Breed.** 1925. Technical Bulletin 110. NY State Agriculture Exp. Station.
2. **Kulp, W. L.** 1927. Scientific apparatus and laboratory methods. An agar medium for plating L. acidophilus and L. bulgaricus. Science **66**:512-513.
3. **Kulp, J. W. L., and V. White.** 1932. Modified medium for plating *L. acidophilus*. Science **76**:17-18.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol 1, p. 776-778. Williams & Wilkins, Baltimore, MD.
5. **Jay, P., and S. Gordon (ed).** 1938. Bacteriology and immunology of dental caries and dental science and dental art. Lea and Febiger, Philadelphia, PA.
6. **Jay, P., W. J. Pelton, and J. M. Wisan.** 1949. Dentistry in public health. W. B. Saunders Company, Philadelphia, PA.

Packaging

Tomato Juice Agar	500 g	0031-17
Tomato Juice Agar Special	500 g	0389-17
Tomato Juice Broth	500 g	0517-17
	10 kg	0517-08

Transport Media

Bacto® Transport Medium Amies · Bacto Transport Medium Amies w/o Charcoal · Bacto Transport Medium Stuart · Bacto Cary-Blair Transport Medium

Intended Use

Bacto Transport Medium Amies, Transport Medium Amies w/o Charcoal and Transport Medium Stuart are used for collecting, transporting and preserving microbiological specimens.

Bacto Cary-Blair Transport Medium is used for collecting, transporting and preserving microbiological specimens, particularly those containing *Vibrio cholerae*.

Summary and Explanation

Transport media are chemically defined, semisolid, non-nutritive, phosphate buffered media that provide a reduced environment. Transport media are formulated to maintain the viability of microorganisms without significant increase in growth.

In 1948, Moffett, Young and Stuart described a medium for transporting gonococcal specimens to the laboratory.[1] Stuart, Toshach and Patsula improved this formulation, introducing what is now known as Stuart's Transport Medium.[2] The ability of Stuart's medium to maintain the viability of gonococci during transport[3,4] led other researchers to explore its use with a variety of specimens. This medium is currently recommended for throat, vaginal, and wound samples.

In 1964, Cary and Blair modified Stuart's medium by substituting inorganic phosphates for glycerophosphate and raising the pH to 8.4.[5] The modified medium was effective in maintaining the viability of *Salmonella* and *Shigella*[6,7] in fecal samples. Due to its high pH, Cary-Blair Transport Medium is also effective in maintaining the viability of *Vibrio* cultures for up to four weeks.[8] Cary-Blair Transport Medium is currently recommended for fecal and rectal samples.

Amies[9] confirmed Cary and Blair's observations that an inorganic salt buffer was superior to the glycerophosphate. He further modified the formulation by using a balanced salt solution containing inorganic phosphate buffer, omitting the methylene blue, and adding charcoal. This modified medium yielded a higher percentage of positive cultures than the transport medium of Stuart. Transport Medium Amies, available with and without charcoal, is recommended for throat, vaginal, and wound samples.

User Quality Control

Identity Specifications

Transport Medium Amies

Dehydrated Appearance:	Black, free-flowing, homogeneous.
Solution:	2.0% solution, soluble in distilled or deionized water on boiling. Solution is black, opaque.
Prepared Vials:	Black, opaque, semi-solid.
Reaction of 2.0% Solution at 25°C:	pH 7.3 ± 0.2

Transport Medium Amies w/o Charcoal

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	1.0% solution, soluble in distilled or deionized water on boiling. Solution is colorless to very light amber, opalescent with precipitate.
Prepared Vials:	Colorless to very light amber, opalescent with precipitate, semi-solid.
Reaction of 1.0% Solution at 25°C:	pH 7.3 ± 0.2

Transport Medium Stuart

Dehydrated Appearance:	Bluish white, free-flowing, homogeneous.
Solution:	1.41% solution, soluble in distilled or deionized water on boiling. Solution is light amber, opalescent with a bluish upper layer.
Prepared Vials:	Light amber, opalescent with blue upper layer, without precipitate, semi-solid.
Reaction of 1.41% Solution at 25°C:	pH 7.4 ± 0.1

continued on following page

Principles of the Procedure

In the formulations, potassium chloride, calcium chloride, magnesium chloride and sodium chloride provide essential ions that help maintain osmotic balance while controlling permeability of bacterial cells. Monopotassium phosphate and Disodium phosphate provide buffering capabilities. Sodium thioglycollate suppresses oxidative changes and provides a reduced environment. Sodium glycerophosphate is a buffer for use with calcium chloride. Methylene blue is a colorimetric pH indicator of the oxidation-reduction state. Charcoal neutralizes fatty acids that are toxic to microorganisms. Bacto Agar is a solidifying agent.

Formula

Transport Medium Amies

Formula Per Liter

Sodium Chloride	3 g
Potassium Chloride	0.2 g
Calcium Chloride	0.1 g
Magnesium Chloride	0.1 g
Monopotassium Phosphate	0.2 g
Disodium Phosphate	1.15 g
Sodium Thioglycollate	1 g
Charcoal	10 g
Bacto Agar	4 g

Final pH 7.3 ± 0.2 at 25°C

Transport Medium Amies w/o Charcoal

Formula Per Liter

Sodium Chloride	3 g
Potassium Chloride	0.2 g
Calcium Chloride	0.1 g
Magnesium Chloride	0.1 g
Monopotassium Phosphate	0.2 g
Disodium Phosphate	1.15 g
Sodium Thioglycollate	1 g
Bacto Agar	4 g

Final pH 7.3 ± 0.2 at 25°C

Transport Medium Stuart

Formula Per Liter

Sodium Thioglycollate	0.9 g
Sodium Glycerophosphate	10 g
Calcium Chloride	0.1 g
Bacto Methylene Blue	0.002 g
Bacto Agar	3 g

Final pH 7.4 ± 0.1 at 25°C

Cary-Blair Transport Medium

Formula Per Liter

Sodium Thioglycollate	1.5 g
Disodium Phosphate	1.1 g
Calcium Chloride	0.1 g
Sodium Chloride	5 g
Bacto Agar	5 g

Final pH 8.4 ± 0.2 at 25°C

User Quality Control cont.

Cary-Blair Transport Medium

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	1.27% solution, soluble in distilled or deionized water on boiling. Solution is colorless with a very light amber tint, very slightly to slightly opalescent, may have a slight, fine precipitate.
Prepared Vials:	Colorless to whitish gray, opalescent without significant precipitate, semi-solid.
Reaction of 1.27% Solution at 25°C:	pH 8.4 ± 0.2

Cultural Response

Transport Medium Amies, Transport Medium Amies w/o Charcoal and Transport Medium Stuart

Prepare media per label directions. Inoculate sterile swabs with suspensions of test organisms containing 1,000-10,000 CFU/0.1 ml. Place swabs in the medium and incubate at room temperature for 18-24 hours. Remove swabs, streak on prepared chocolate agar plates and incubate appropriately. All cultures should be viable.

ORGANISM	ATCC®
Bacteroides fragilis	25285*
Haemophilus influenzae Type b	10211
Neisseria meningitidis Group B	13090*
Neisseria gonorrhoeae	43069
Streptococcus pneumoniae	6305
Streptococcus pyogenes Group A	19615*

Cary-Blair Transport Medium

Prepare Cary-Blair Transport Medium per label directions. Inoculate sterile swabs with suspensions of test organisms containing 1,000-10,000 CFU/0.1 ml. Place in the medium, and incubate at room temperature for up to 48 hours. Remove swabs, streak on prepared TSA with 5% Sheep Blood plates and incubate appropriately. All cultures should be viable.

ORGANISM	ATCC®
Salmonella enteritidis	13076
Shigella dysenteriae	13313
Vibrio cholerae biotype *eltor*	15748
Vibrio parahaemolyticus EB 101	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Precautions

1. For Laboratory Use.
2. **Transport Medium Amies: IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   Transport Medium Amies w/o Charcoal: IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   Transport Medium Stuart: IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   Cary-Blair Transport Medium: IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact

with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated media below 30°C. The dehydrated media are very hygroscopic. Keep containers tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Transport Medium Amies, Transport Medium Amies w/o Charcoal, Transport Medium Stuart, or Cary-Blair Transport Medium.

Materials Required but not Provided

Glassware
Autoclave
Distilled or deionized water
Incubator

Method of Preparation

1. **Transport Medium Amies:** Suspend 20 grams in 1 liter distilled or deionized water. Invert vials just before solidification to uniformly distribute the charcoal.

   Transport Medium Amies w/o Charcoal: Suspend 10 grams in 1 liter distilled or deionized water.

   Transport Medium Stuart: Suspend 14.1 grams in 1 liter distilled or deionized water.

   Cary-Blair Transport Medium: Suspend 12.7 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into 6-8 ml capacity screw-cap vials to within 5mm of the top. Cap tightly.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and primary isolation technique recommendations.[10,11,12]

Test Procedure

1. Insert specimen swab(s) into the upper third of the medium in the transport container.
2. Cut or break off the protruding portion of the swab stick. Tightly screw the lid on the bottle or vial.
3. Label the bottle or vial and send to the laboratory with minimum delay. Specimens may be refrigerated until ready for shipment.
4. Submit to laboratory within 24 hours for culture and analysis.

Results

Survival of bacteria in a transport medium depends on many factors including the type and concentration of bacteria in the specimen, the formulation of the transport medium, the temperature and duration of transport, and inoculation to appropriate culture media within 24 hours.

Optimal growth and typical morphology can only be expected following direct inoculation and appropriate cultivation.

Limitations of the Procedure

1. Specimens taken from transport media will not exhibit the optimal or comparative growth as expected from direct inoculation and cultivation. These media do, however, provide an adequate degree of preservation for those specimens which cannot be forwarded immediately to the laboratory for prompt evaluation.
2. Viability of cells will diminish over time and some degree of multiplication or growth of contaminants can occur during prolonged periods of transit. This is particularly true of fecal specimens that contain substantial numbers of coliform organisms.
3. The condition of the specimen received by the laboratory for culture is a significant variable in recovery and final identification of the suspect pathogen. An unsatisfactory specimen (overgrown by contaminants, containing non-viable organisms, or having the number of pathogens greatly diminished) can lead to erroneous or inconclusive results.

References

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Packaging

Transport Medium Amies	500 g	0996-17
Transport Medium Amies w/o Charcoal	500 g	0832-17
Transport Medium Stuart	500 g	0621-17
Cary-Blair Transport Medium	500 g	0505-17

Trichophyton Agars

Bacto® Trichophyton Agar 1 · Trichophyton Agar 2 · Trichophyton Agar 3 · Trichophyton Agar 4 · Trichophyton Agar 6 Trichophyton Agar 7

Intended Use

Bacto Trichophyton Agars are used for differentiating *Trichophyton* species based on differing nutritional requirements.

Summary and Explanation

Trichophyton Agar media are prepared according to the formulations of Georg and Camp[1] and are based on the nutritional requirements of various species. The growth patterns of the different species are given in Table 1.[1] *(see Table 1 below)*

The authors divided the dermatophytes into four groups. Group 1 includes *T. verucosum*, *T. schoenleinii* and *T. concentricum*. Organisms within this group seldom produce spores or distinctive pigments and their colonies resemble each other so closely that they cannot be identified by morphological criteria. These species are differentiated as indicated in Table 1 by their growth in the absence or presence of inositol, thiamine, or a combination. *T. verrucosum* grows faster at 37°C than at room temperature while *T. schoenleinii* and *T. concentricum* are not stimulated by incubation at 37°C.

Group 2 includes *T. tonsurans*, *T. mentagrophytes* and *T. rubrum*. These species usually produce microconidia, but only occasionally produce macroconidia. Their colonial forms and pigments are so variable that differentiation by these means is inadequate. Ajello and Georg[2] based differentiation on their nutritional behavior on Trichophyton Agars 1 and 4 and by *in vitro* hair cultures. *T. mentagrophytes* forms perforating organs within 2-3 weeks in human hair samples suspended in water; *T. rubrum* does not.

Table 1. Growth patterns of *Trichophyton* species on Trichophyton Agars.[1]

DERMATOPHYTE TESTED	STRAINS STUDIED (1% REPORTING)	TRICHOPHYTON AGARS 1	2	3	4	6	7
T. verrucosum	100 (84%) 100 (16%)	0 0	± 0	4+ 4+	0 4+		
T. schoenleinii	50	4+	4+	4+	4+		
T. concentricum	19 (50%) 19 (50%)	4+ 2+	4+ 2+	4+ 4+	4+ 4+		
T. tonsurans	70	± to 1+			4+		
T. mentagraphytes	50	4+			4+		
T. rubrum	50	4+			4+		
T. ferrugineum	14	4+			4+		
T. violaceum	13	± to 1+			4+		
T. megninii	13					0	4+
T. gallinae	7					4+	4+
*T. equinum**	13	0					

* *T. equinum* will grow to a 4+ reaction on Trichophyton Agar 5, which is not commercially available from Difco Laboratories. Trichophyton Agar 5 is equivalent to Trichophyton Agar 1 (product code 0877) with added nicotinic acid (200 µg per liter).

Group 3 includes *T. violaceum*. It seldom produces microconidia but does develop characteristically pigmented colonies. *T. violaceum* has a similar nutritional pattern as *T. tonsurans*; however, it grows very slowly even in the presence of thiamine and produces a glabrous colony without spores. *T. tonsurans* grows rapidly in the presence of thiamine and shows numerous microconidia.

Group 4 includes *T. megninii* and *T. equinum*. Both can be identified solely from nutritional requirements. *T. megninii* requires histidine, as indicated on Trichophyton Agar 6 in Table 1. *T. equinum* requires nicotinic acid, as indicated in the table.

T. gallinae is differentiated morphologically as well as culturally from *T. megninii* and *T. equinum*. *T. gallinae* grows on Trichophyton Agar 1 or 6 without added vitamins.

Principles of the Procedure

Trichophyton Agars 1, 2, 3 and 4

Vitamin Assay Casamino Acids is the source of nutrients. Dextrose provides carbon. Monopotassium Phosphate provides buffering capability. Magnesium Sulfate is a source of divalent cations and sulfate. Bacto Agar is a solidifying agent. Where included in the formulations for Trichophyton Agars 2,3 and 4, Inositol and Dextrose provide carbon; Thiamin is present for organisms requiring the vitamin for growth.

Trichophyton Agars 6 and 7

Ammonium Nitrate is a source of nitrogen. Dextrose provides carbon. Monopotassium Phosphate provides buffering capability. Magnesium Sulfate is a source of divalent cations and sulfate. Bacto Agar is a solidifying agent. Histidine Hydrochloride is present in Trichophyton Agar 7 for organisms requiring the amino acid histidine.

Formula

Trichophyton 1

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	2.5 g
Bacto Dextrose	40 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Trichophyton Agars 1, 2, 3, 4, 6 and 7

Dehydrated Appearance:	White to off-white, free-flowing, homogeneous.
Solution:	5.9% solution, soluble in distilled or deionized water upon boiling. Solution is light to medium amber, slightly opalescent.
Reaction of 5.9% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Trimchophyton Agars 1, 2 and 3

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC®	GROWTH AGARS 1 & 2	GROWTH AGAR 3
Trichophyton concentricum	9358	good	good
Trichophyton schoenleinii	4822	good	good
Trichophyton verrucosum	34470	none to poor	good

Trichophyton Agar 4

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC®	GROWTH
Trichophyton rubrum	28188	good
Trichophyton verrucosum	34470	none to poor
Trichophyton violaceum	8376	good

Trichophyton Agar 6

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC®	GROWTH
Microsporum gallinae	22243	good
Trichophyton megninii	12106	none to poor

Uninoculated tube

Trichophyton schoenleinii ATCC® 4822

Trichophyton Agar 7

Prepare the medium per label directions. Inoculate and incubate at 30 ± 2°C for up to 2 weeks.

ORGANISM	ATCC®	GROWTH
Microsporum gallinae	12108	good
Trichophyton megninii	12106	good

The cultures listed are the minimum that should be used for performance testing.

Trichophyton 2

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	2.5 g
Bacto Inositol	50 mg
Bacto Dextrose	40 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Trichophyton 3

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	2.5 g
Bacto Inositol	50 mg
Thiamin	200 µg
Bacto Dextrose	40 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Trichophyton 4

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	2.5 g
Thiamin	200 µg
Bacto Dextrose	40 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Trichophyton 6

Formula Per Liter

Ammonium Nitrate	1.5 g
Bacto Dextrose	40 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Trichophyton 7

Formula Per Liter

Ammonium Nitrate	1.5 g
Histidine Hydrochloride	30 mg
Bacto Dextrose	40 g
Monopotassium Phosphate	1.8 g
Magnesium Sulfate	0.1 g
Bacto Agar	15 g

Final pH 6.8 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated Trichophyton Agars below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Trichophyton Agars 1, 2, 3, 4, 6 and 7

Materials Required but not Provided

Glassware
Autoclave

Method of Preparation

1. Suspend 59 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 12 minutes.

Specimen Collection and Preparation

1. Specimens should be collected in sterile containers or with sterile swabs and transported immediately to the laboratory according to recommended guidelines.[3]

Test Procedure[1,2,4]

1. Inoculate the media by placing a small particle (approximately 1 mm square) of a colony on each medium.
2. Incubate at 30°C for up to 2 weeks.

Results

Examine the media, comparing the amount of growth on each. A small amount of growth is documented as a + and heavy growth as 4+.

Limitations of the Procedure

1. It is important that pure cultures from a medium that is not vitamin enriched, such as Sabouraud Dextrose Agar of Mycobiotic Agar, be used for the inoculum.
2. If cultures are contaminated with bacteria, the cultures should be grown on a medium containing antibiotics, such as Mycobiotic Agar or Brain Heart CC Agar, for several generations to eliminate the bacteria. Many bacteria synthesize vitamins and may invalidate the test results.
3. When inoculating Trichophyton Agars, take care not to carry over growth substances from primary cultures to the tube media used in the differential tests. Inocula transferred to the nutrition tubes should be very small.

References

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Packaging

Trichophyton Agar 1	500 g	0877-17
Trichophyton Agar 2	500 g	0874-17
Trichophyton Agar 3	500 g	0965-17
Trichophyton Agar 4	500 g	0197-17
Trichophyton Agar 6	500 g	0524-17
Trichophyton Agar 7	500 g	0955-17

Bacto® Triple Sugar Iron Agar

Intended Use

Bacto Triple Sugar Iron Agar is used for differentiating gram-negative enteric bacilli based on fermentation of dextrose, lactose and sucrose and on hydrogen sulfide production.

Also Known As

Triple Sugar Iron Agar is also known as TSI.

Summary and Explanation

In 1911, Russell1 described the use of two sugars in an agar medium to differentiate gram-negative organisms of intestinal origin. Lead or iron salts were added to the Russell medium to detect the presence of hydrogen sulfide. Kligler[2,3] added lead acetate to Russell Double Sugar Agar, resulting in a medium that was capable of differentiating typhoid, paratyphoid and dysentery. A modification of this medium, Kligler Iron Agar, using phenol red as an indicator and iron salts to detect hydrogen sulfide production, was developed.

Krumweide and Kohn[4] modified Russell Double Sugar Agar by the addition of sucrose to the medium. This modification allowed for an earlier detection of coliform organisms that ferment lactose slowly, since many of these organisms attack sucrose more readily than lactose. The added sucrose also permitted the exclusion of certain coliform and *Proteus* organisms that can attack sucrose, but not lactose, in a 24-48 hour incubation period.

In 1940, Sulkin and Willet[5] described a triple sugar ferrous sulfate medium for use in the identification of enteric organisms. Difco Laboratories concurrently developed a similar medium by adding 1% sucrose to Kligler Iron Agar with phenol red as the indicator. Hajna[6] described a similar medium for the identification of bacteria of the intestinal group.

Triple Sugar Iron Agar is essentially the formula originally described by Sulkin and Willet.[5] Tryptone has been replaced by a combination of Bacto Peptone and Proteose Peptone. Yeast Extract has been added, and Phenol Red is used as an indicator instead of Brom Thymol Blue.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Pink, free flowing, homogeneous.
Solution:	6.5% solution, soluble in distilled or deionized water on boiling. Solution is red, very slightly opalescent, may have a slight dark precipitate.
Prepared Tubes:	Red, slightly opalescent, may have a slight precipitate.
Reaction 6.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Triple Sugar Iron Agar per label directions. Inoculate and incubate the tubes at 35°C for 18-24 hours.

ORGANISM	ATCC®	CFU	GROWTH	SLANT /BUTT	GAS	H_2S
Escherichia coli	25922*	undiluted	good	A/A	+	–
Pseudomonas aeruginosa	9027	undiluted	good	K/K	–	–
Salmonella enteritidis	13076	undiluted	good	K/A	+	+
Shigella flexneri	12022*	undiluted	good	K/A	–	–

A = acid reaction (yellow)
K = alkaline reaction (no color change)
+gas = cracks, splits or bubbles in medium
–gas = no cracks, splits or bubbles in medium
+H_2S = black precipitate in butt
–H_2S = no black precipitate in butt

Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Salmonella enteritidis* ATCC® 13076 | *Shigella flexneri* ATCC® 12022

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Triple Sugar Iron Agar is recommended for differentiation of enteric gram negative bacilli from clinical specimens,[7,8,9] dairy samples[10], and food samples.[11,12,13,14] Its use is also recommended for microbial limits testing of *Escherichia coli* and *Salmonella* spp.[15]

Principles of the Procedure

Beef Extract, Yeast Extract, Bacto Peptone, and Proteose Peptone provide nitrogen, vitamins, and minerals. Triple Sugar Iron Agar contains three carbohydrates (dextrose, lactose and sucrose). When these carbohydrates are fermented, the resulting production of acid is detected by the phenol red indicator. The color changes that result are yellow for acid production and red for alkalinization. Sodium thiosulfate is reduced to hydrogen sulfide. Hydrogen sulfide then reacts with an iron salt yielding the typical black iron sulfide. Sodium chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

Formula

Triple Sugar Iron Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Yeast Extract	3 g
Bacto Peptone	15 g
Bacto Proteose Peptone	5 g
Bacto Dextrose	1 g
Bacto Lactose	10 g
Sucrose	10 g
Ferrous Sulfate	0.2 g
Sodium Chloride	5 g
Sodium Thiosulfate	0.3 g
Bacto Agar	12 g
Bacto Phenol Red	0.024 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared tubes at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Triple Sugar Iron Agar

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Tubes with closures
Inoculating needle
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 65 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes with closures.
4. Autoclave at 121°C for 15 minutes. Cool in slanted position with deep butts.

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[7-14]
2. Process each specimen, using procedures appropriate for that specimen or sample.[7-14]

Test Procedure

1. Obtain a pure culture of the organism to be tested. Select well-isolated colonies.
2. With an inoculating needle, pick the center of well-isolated colonies obtained from solid culture media.
3. Stab the center of the medium into the deep of the tube to within 3 - 5 mm from the bottom.
4. Withdraw the inoculating needle, and streak the surface of the slant.
5. Loosen closure on the tube before incubating.
6. Incubate at 35°C for 18-24 hours.
7. Read tubes for acid production of slant/butt, gas, and hydrogen sulfide reactions.

Results

1. An alkaline slant-acid butt (red/yellow) indicates fermentation of dextrose only.
2. An acid slant-acid butt (yellow/yellow) indicates fermentation of dextrose, lactose and/or sucrose.
3. An alkaline slant-alkaline butt (red/red) indicates that neither dextrose nor lactose was fermented (non-fermenter).
4. Cracks, splits, or bubbles in the medium indicate gas production.
5. A black precipitate in the butt indicates hydrogen sulfide production.

Limitations of the Procedure

1. Hydrogen sulfide production may be evident on Kligler Iron Agar but negative on Triple Sugar Iron Agar. Studies by Bulmash and Fulton[15] showed that the utilization of sucrose could suppress the enzymatic mechanisms responsible for H_2S production. Padron and Dockstader[16] found that not all H_2S-positive *Salmonella* are positive on TSI.
2. Sucrose is added to TSI to eliminate some sucrose-fermenting non-lactose fermenters such as *Proteus* and *Citrobacter* spp.[17]
3. Further biochemical tests and serological typing must be performed for definite identification and confirmation of organisms.

4. Do not use an inoculating loop to inoculate a tube of Triple Sugar Iron Agar. While stabbing the butt, mechanical splitting of the medium occurs, causing a false positive result for gas production.[17]
5. A pure culture is essential when inoculating Triple Sugar Iron Agar. If inoculated with a mixed culture, irregular observations may occur.
6. Tubes should be incubated with caps loosened. This allows a free exchange of air, which is necessary to enhance the alkaline condition on the slant.[17]

References

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14. **Federal Register.** 1996. Pathogen reduction; hazard analysis and critical point (HACCP) systems; final rule. Fed. Regis. **61**:38917-38925.
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16. **Padron, A. P. and W. B. Dockstader.** 1972. Selective medium for hydrogen sulfide production. Appl. Microbiol. **23**:1107.
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Packaging

Triple Sugar Iron Agar	100 g	0265-15
	500 g	0265-17
	2 kg	0265-07

Bacto® Tryptic Nitrate Medium

Intended Use

Bacto Tryptic Nitrate Medium is used for differentiating microorganisms based on nitrate reduction.

Summary and Explanation

Tryptic Nitrate Medium is a differential, semi-solid, general purpose medium that supports growth of aerobes as well as facultative and obligate anaerobes.[1] The formulation includes potassium nitrate which can be reduced by certain organisms to either nitrite or nitrogen gas. Nitrate reduction can be detected by various test methods and is used in differentiating organisms from clinical samples, foods and dairy products.[1,2,3,4,5]

Principles of the Procedure

Tryptose is a source of nitrogen, amino acids, and vitamins. Dextrose provides carbohydrates. Potassium Nitrate provides the basis for nitrate reduction. Disodium Phosphate is a buffering agent. The low agar content, which allows varying degrees of anaerobiosis in the medium, supports growth of organisms with various oxygen requirements.

Formula

Tryptic Nitrate Medium

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	1 g
Disodium Phosphate	2 g
Potassium Nitrate	1 g
Bacto Agar	1 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Tryptic Nitrate Medium

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Test tubes with closures
Autoclave
Incubator (35°C)
SpotTest™ Nitrate Reagents A, B and C or equivalents

Method of Preparation

1. Suspend 25 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Dispense into tubes with closures.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect specimens or samples in sterile containers or with sterile swabs and transport immediately to the laboratory in accordance with recommended guidelines.[1-7]
2. Process each specimen, using procedures appropriate for that sample.[1-7]

Test Procedure

1. Obtain a pure culture of the organism to be tested from a solid culture medium. Select well-isolated colonies.
2. Inoculate a tube of Tryptic Nitrate Medium and incubate at 35 ± 2°C for 18-24 hours.
3. Read tubes for growth.
4. Test for nitrate reduction using SpotTest Nitrate Reagents A, B and C or equivalents per reagent instructions.

Results

1. After adding Nitrate Reagents A and B:
   Positive nitrate reduction reaction: Development of a red-violet color within 1-2 minutes indicates that nitrate has been reduced to nitrite.
   Presumptive negative nitrate reduction reaction: Lack of color development denotes an absence of nitrite in the medium; this should be confirmed by addition of Nitrate Reagent C (zinc dust).
2. After adding Nitrate Reagent C:
   Positive nitrate reduction reaction: Lack of color development indicates that nitrate has been reduced to nitrogen gas.
   Negative nitrate reduction reaction: Development of a red-vio let color within 5- 10 minutes indicates that unreduced nitrate is still present.

Limitations of the Procedure

1. This medium is not recommended for indole testing of coliforms and other enterics.[1]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free flowing, homogeneous.
Solution:	2.5% solution, soluble in distilled or deionized water on boiling. Solution is light amber, clear to slightly opalescent.
Prepared Medium:	Light amber, slightly opalescent; semisolid.
Reaction of 2.5% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Tryptic Nitrate Medium per label directions. Inoculate and incubate at 35 ± 2°C for 18-24 hours; incubate *Clostridium sporogenes* anaerobically.

ORGANISM	INOCULUM ATCC®	NITRATE CFU	GROWTH	REDUCTION
Clostridium sporogenes	11437	100-1,000	good	–
Escherichia coli	25922*	100-1,000	good	+
Staphylococcus aureus	25923*	100-1,000	good	+

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube | *Escherichia coli* ATCC® 25922 | *Clostridium sporogenes* ATCC® 11437

References

1. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
2. **FDA Bacteriological Analytical Manual, 8th ed.** AOAC International, Gaithersburg, MD.
3. **Pezzlo, M. (ed.).** 1992. Aerobic bacteriology, p. 1.0.0.-1.20.47. *In* H. D. Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
5. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall (ed.), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
6. **Murray, P. R., E. J. Baron, M. A., Pfaller, F. C. Tenover, and R. H. Yolken.** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
7. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey and Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Tryptic Nitrate Medium	500 g	0367-17
SpotTest Nitrate Reagent A	50 x 0.75 ml	3554-26
SpotTest Nitrate Reagent B	50 x 0.75 ml	3555-26
SpotTest Nitrate Reagent C	50 x 1 g	3556-26

Bacto® Tryptic Soy Agar

Intended Use

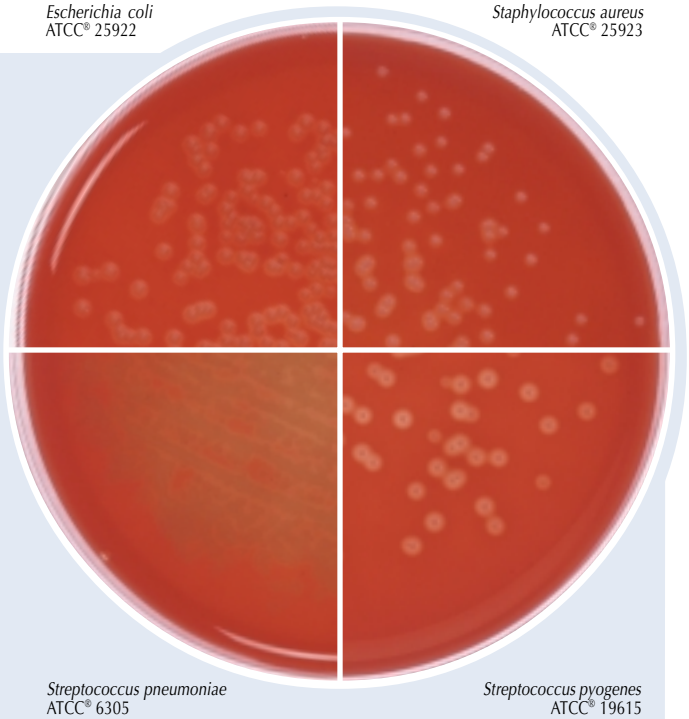
Bacto Tryptic Soy Agar is used for isolating and cultivating fastidious microorganisms and, with blood, in determining hemolytic reactions.

Also Known As

Tryptic Soy Agar (TSA) conforms with Soybean-Casein Digest Agar Medium, USP.

Summary and Explanation

In 1955, Leavitt et al.[1] demonstrated that Tryptic Soy Agar supports excellent growth of a both aerobic and anaerobic microorganisms. Tryptic Soy Agar is a general purpose medium used for multiple applications, e.g., as a blood culture medium, as maintenance medium for culture collections, in colony count methods[2], and for testing bacterial contaminants in cosmetics.[3]



User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in distilled or deionized water on boiling. Solution is light amber, slightly opalescent.
Plates Prepared Without Blood:	Light amber, slightly opalescent,with no significant precipitate.
Plates Prepared With Blood:	Cherry red, opaque with no hemolysis.
Reaction of 4.0% Solution at 25°C :	pH 7.3 ± 0.2

Cultural Response

Prepare Tryptic Soy Agar per label instructions. Inoculate and incubate the plates at 35 ± 2° under approximately 5-10% CO_2 for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	HEMOLYSIS
Escherichia coli	25922*	100-1,000	good	–
Staphylococcus aureus	25923*	100-1,000	good	beta
Streptococcus pneumoniae	6305	100-1,000	good	alpha
Streptococcus pyogenes	19615*	100-1,000	good	beta

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

CAMP test: Perform Camp test using *Staphylococcus aureus* ATCC® 33862 or ATCC® 25923. *Streptococcus agalactiae* ATCC® 12386 positive reaction (arrow head area of clearing) *Streptococcus pyogenes* ATCC® 19615 negative reaction (no arrow head formation)

TSA is recommended in multiple water and wastewater applications.[4] Tryptic Soy Agar conforms to the formula specified by US Pharmacopeia for use in Microbiological Tests[5], Antimicrobial Preservatives-Effectiveness and Microbial Limits Test.

Clinically, Tryptic Soy Agar is used in the differentiation of *Haemophilus* species, because it does not contain the X and V factors required for *Haemophilus* growth. With the addition of Differentiation Disks V, X and VX, *Haemophilus* growth can be observed around the appropriate disk.

Tryptic Soy Agar is a nutritious base to which a variety of supplements can be added. Addition of 5% sterile, defibrinated sheep, horse or rabbit blood provides an excellent general purpose medium that allows the growth of yeast species, staphylococci, enterococci and gram-negative bacilli.[6] TSA blood agars may be used to determine hemolytic reactions of bacteria. TSA supplemented with lecithin and polysorbate 80 is the formula for Microbial Content Test Agar (also known as TSALT) used in environmental monitoring.[7] For the examination of foods, Tryptic Soy Agar is supplemented with 3% NaCl for the isolation of *Vibrio* species and halophilic microorganisms.[8] Tryptic Soy Agar supplemented with 0.6% yeast extract is used for the isolation of *Listeria monocytogenes* and cultivation of a wide variety of heterotrophic microorganisms.[8] Addition of colistin and nalidixic acid to TSA is used for the selective isolation of gram-positive cocci.[9] Gunn et al.[10] used trimethoprim and sulfamethoxazole (SxT) supplementation to inhibit normal flora on throat specimens, allowing Groups A and B streptococci to grow well. Addition of iron salt and sodium thiosulfate to TSA aids in the identification of non-fermentating gram-negative bacilli, and with nitro blue tetrazolium (0.5% aqueous solution) allows for the selective isolation of *Corynebacterium diphtheriae*.[11]

Chocolate Agar for culturing *Haemophilus influenzae* and related organisms may be prepared by adding 1% Hemoglobin and Supplement B to Tryptic Soy Agar.

The methodologies for multiple applications using Tryptic Soy Agar are outlined in the references.

Principles of the Procedure

Tryptone and Soytone provide nitrogen, vitamins and minerals. The natural sugars from the soybean promote bacterial growth. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

Formula

Tryptic Soy Agar

Formula Per Liter

Bacto Tryptone Pancreatic Digest of Casein	15 g
Bacto Soytone Papaic Digest of Soybean Meal	5 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Tryptic Soy Agar

Materials Required But Not Provided

Glassware

Autoclave

Incubator (35°C)

Waterbath (45-50°C) (optional)

Defibrinated blood (optional)

Method of Preparation

1. Suspend 40 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. OPTIONAL: To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Leavitt, J. M., I. J. Naidorf and P. Shugaevsky.** 1955. The undetected anaerobe in endodontics; a sensitive medium for detection of both aerobes and anaerobes. The NY J. Dentist. **25**:377-382.
2. **Swanson, K. J., F. F. Busta, E. H. Peterson and M. G. Johnson.** 1992. Colony Count Methods, p.75-95. *In* C. Vanderzant, and D. F. Spittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Curry, A. S., G. G. Joyce, and G. N. McEwen, Jr.** 1993. CTFA Microbiology guidelines. The Cosmetic, Toiletry, and Fragrance Association, Inc., Washington, D.C.
4. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (ed).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.

5. **The United States Pharmacopeia.** 1995. Microbiological tests, p. 1681-1686. The United States pharmacopeia, 23rd ed. United States Pharmacopeial Convention, Rockville, MD.
6. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Etiological agents recovered from clinical material, p. 244. Bailey & Scott's Diagnostic Microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
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8. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
9. **Ellner, P.** 1966. J. of Clin. Pathol. **45**:502-504.
10. **Gunn, B. A., D. K. Ohashi, C. A. Gaydos, and E. S. Holt.** 1977. Selective and enhanced recovery of group A and B streptococci from throat cultures with sheep blood containing sulfamethoxazole and trimethoprim. J. Clin. Microbiol. **5**:650-655.
11. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification- maintenance of medical bacteria, vol. 1, p. 794-802. Williams & Wilkins, Baltimore, MD.

Packaging

Tryptic Soy Agar	100 g	0369-15
	500 g	0369-17
	2 kg	0369-07
	10 kg	0369-08

Bacto® Tryptic Soy Broth
Bacto Tryptic Soy Broth w/o Dextrose

Intended Use

Bacto Tryptic Soy Broth is used for cultivating a wide variety of microorganisms. Tryptic Soy Broth conforms with the formula specified in the US Pharmacopeia XXIII (USP)[1] and the Code of Federal Regulations (21 CFR)[2] for sterility testing of pharmaceutical products, biologics and devices.

Bacto Tryptic Soy Broth w/o Dextrose, a low carbohydrate formulation of Tryptic Soy Broth, is used for cultivating fastidious and non-fastidious microorganisms.

Also Known As

Tryptic Soy Broth is commonly referred to as Soybean-Casein Digest Medium, USP, and Fluid Soybean-Casein Digest Medium; it is abbreviated as TSB.

User Quality Control

Identity Specifications

Tryptic Soy Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.0% solution, soluble in distilled or deionized water. Solution is light amber, clear.
Prepared Medium:	Light amber, clear.
Reaction of 3.0% Solution at 25°C:	pH 7.3 ± 0.2

Tryptic Soy Broth w/o Dextrose

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.75% solution, soluble in distilled or deionized water; light amber, clear to very slightly opalescent.
Prepared Medium:	Light amber, clear to very slightly opalescent.
Reaction of 2.75% Solution at 25°C:	pH 7.3 ± 0.2

continued on following page

Summary and Explanation

Tryptic Soy Broth is a general purpose medium used for isolating fastidious and non-fastidious microorganisms. Tryptic Soy Broth was originally developed for use without blood in determining the effectiveness of sulfonamides against pneumococci and other organisms.[3] Tryptic Soy Broth is often used to support growth of non-typical isolates such as *Brucella*.[4] Clostridia and non-sporulating anaerobes grow luxuriantly in this broth when incubated under anaerobic conditions. Garrison[5] and Hedgecock[6] used TSB to support growth of *Histoplasma capsulatum*. Mashimo and Ellison[7] supplemented this medium with agar to enhance growth of anaerobic organisms. With the addition of 6.5% NaCl, TSB can be used for the selective growth of group D streptococci.

Tryptic Soy Broth was chosen by the USDA Animal and Plant Health Inspection Service for detecting viable bacteria in live vaccines.[9] It is used in the coliphage detection procedure, a proposed methodology in Standard Methods for the Examination of Water and Wastewater.[10] TSB is recommended for testing bacterial contaminants in cosmetics[11] and complies with established standards[12,13] in the food industry.

TSB is recommended by the National Committee for Clinical Laboratory Standards (NCCLS)[14] for inoculum preparation when performing the disk diffusion sensitivity test, also known as the Kirby-Bauer method.

The rich nutritional base of Tryptic Soy Broth is often modified to provide varying growth environments. With the addition of 1% Supplement B, TSB will support growth of *Neisseria, Haemophilus influenzae* and other related organisms. The medium is used as an enrichment broth in clinical applications and is an excellent blood culture medium when supplemented with SPS and CO_2.[15]

Tryptic Soy Broth w/o Dextrose, a modification of TSB, is a basal medium to which carbohydrates may be added for use in fermentation studies. Agar may be added (0.5-1.0 grams/liter) to enhance anaerobic growth.[16] Phenol red and other indicators may also be added.

Principles of the Procedure

Tryptone and Soytone are nitrogen sources in Tryptic Soy Broth and Tryptic Soy Broth w/o Dextrose. Dextrose is a carbon energy source that facilitates organism growth. Sodium chloride maintains osmotic balance, while dipotassium phosphate is a buffering agent.

Dextrose is omitted from the formula for Tryptic Soy Broth w/o Dextrose to permit use of the medium in fermentation studies. The carbohydrate concentration used most frequently in fermentation reactions is 0.5% or 1%.

Formula

Tryptic Soy Broth

Formula Per Liter

Bacto Tryptone	17 g
Bacto Soytone	3 g
Bacto Dextrose	2.5 g
Sodium Chloride	5 g
Dipotassium Phosphate	2.5 g

Final pH 7.3 ± 0.2 at 25°C

Tryptic Soy Broth w/o Dextrose

Formula Per Liter

Bacto Tryptone	17 g
Bacto Soyton	3 g
Sodium Chloride	5 g
Dipostassium Phosphate	2.5 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Tryptic Soy Broth
Tryptic Soy Broth w/o Dextrose

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Sterile tubes

Method of Preparation

1. Suspend the medium in 1 liter distilled or deionized water:
   Tryptic Soy Broth - 30 grams;
   Tryptic Soy Broth w/o Dextrose - 27.5 grams.
2. Dispense as desired.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

User Quality Control Cont.

Cultural Response

Prepare Tryptic Soy Broth or Tryptic Soy Broth w/o Dextrose per label directions. Inoculate medium and incubate at 35 ± 2°C for 18-48 hours or up to 76 hours if necessary.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090*	10-100	fair to good
Staphylococcus epidermidis	12228*	10-100	good
Streptococcus pneumoniae	6305	10-100	good
Streptococcus pyogenes	19615*	10-100	good

USP and EP Growth Promotion[1]

Prepare Tryptic Soy Broth per label directions. Inoculate medium and incubate at the temperature specified for up to 7 days.

ORGANISM	ATCC®	INOCULUM CFU	INCUBATION TEMPERATURE	RECOVERY
Bacillus subtilis	6633	10-100	20-25°C	growth must be evident
Candida albicans	2091	10-100	20-25°C	growth must be evident
Candida albicans	10231	10-100	20-25°C	growth must be evident
Clostridium sporogenes	19404	10-100	30-35°C	growth must be evident
Staphylococcus aureus	6538P	10-100	30-35°C	growth must be evident

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube

Staphylococcus epidermidis ATCC® 12228

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

Methodologies for the multiple applications using Tryptic Soy Broth and Tryptic Soy Broth w/o Dextrose are outlined in the references.

Results

Refer to appropriate references and procedures for results.

References

1. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia**,** 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
2. **Federal Register**. 1992. General biological products standards. Fed. Regist. **21**:610.12.
3. **McCullough, N. B.** 1949. Laboratory tests in the diagnosis of brucellosis. Amer. J. of Public Health **39**:866-869.
4. **Baron, E. J., L. R. Peterson, and S. M. Finegold**. 1994. Microorganisms encountered in the blood, p. 205. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
5. **Garrison, R. G.** 1961. Studies of the respiratory activity of *Histoplasma capsulatum.* J. of Infect. Dis. **108**:120-124.
6. **Hedgecock, L. W.** 1971. Effect of vaccines prepared from *Histoplasma capsulatum* and other yeast on experimental tuberculosis. J. Bacteria. **82**:115- 123.
7. **Mashimo, P. A., and S. A. Ellison.** 1959. Simple method for the isolation of anaerobic oral vibrios. J. Bacteria. **78**:636-639.
8. **Sherman, J. M., and P. Stark.** 1961. Streptococci which grow at high temperatures. J. Bacteria. **22**:275-285.
9. **Federal Register**. 1992. Detection of viable bacteria and fungi except in live vaccine. Fed. Regist. **21**:113.26.
10. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (eds).** 1992. Coliphage detection, 9,22-23. Standard methods for the examination of water and wastewater, 18th ed. American Public Health Association, Washington, D.C.
11. **Curry, A. S., G. G. Joyce, and G. N. McEwen, Jr.** 1993. CTFA Microbiology guidelines. The Cosmetic, Toiletry, and Fragrance Association, Inc., Washington, D.C.
12. **Association of Official Analytical Chemists.** 1995**.** Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
13. **Cunnif, P.** 1995. Official methods of analysis AOAC International, 16th ed. AOAC International, Arlington, VA.
14. **National Committee for Clinical Laboratory Standards.** 1994. Performance standards for antimicrobial disk susceptibility tests, M2-A5, vol. 13, no. 24. National Committee for Clinical Laboratory Standards, Villanova, PA.
15. **Isenberg, H. D. (ed.).** 1992. Processing and interpretation of blood cultures, p. 1.7.1-1.7.2. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
16. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, p. 797, vol. 1. Williams & Wilkins, Baltimore, MD.

Packaging

Tryptic Soy Broth	100 g	0370-15
	500 g	0370-17
	2 kg	0370-07
	10 kg	0370-08
Tryptic Soy Broth w/o Dextrose	500 g	0862-17
	10 kg	0862-08

Bacto® Tryptone Peptone

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous powder.
Solution:	1%, 2% and 10% solutions are soluble in distilled or deionized water: 1%-Very light to light amber, clear without precipitate; 2%-Light to medium amber, clear without precipitate; 10%-Medium to dark amber, clear to slightly opalescent, may have a slight precipitate.
Nitrogen (Kjeldahl Method):	11.4-13.9%
Amino Nitrogen (Modified Sorensen Method):	4.0-6.6%
Reaction of 2% Solution at 25°C:	pH 6.9-7.4

continued on following page

Intended Use

Bacto Tryptone Peptone is used in preparing microbiological culture media.

Also Known As

Tryptone Peptone is also referred to as Peptone C, Peptone 50 and Tryptone T.

Summary and Explanation

Tryptone Peptone is a pancreatic digest of casein used as a nitrogen source in culture media formulated for isolating and cultivating fastidious and nonfastidious bacteria and fungi.

Tryptone Peptone was developed by Difco Laboratories while investigating a peptone particularly suitable for the elaboration of indole by bacteria. The high tryptophane content of Tryptone Peptone makes it valuable for use in detecting indole production.[1,2,3] The absence of detectable levels of carbohydrates in Tryptone Peptone makes it a suitable peptone in differentiating bacteria on the basis of their ability to ferment various carbohydrates.

Several media containing Tryptone Peptone are specified in standard methods[4,5,6,7] for multiple applications.

Principles of the Procedure

Tryptone Peptone is a pancreatic digest of casein especially rich in tryptophane. Casein, a milk protein, is a rich source of amino acid nitrogen.

Typical Analysis

Physical Characteristics

Ash (%)	6.8	Loss on Drying (%)	3.7
Clarity, 1% Solution (NTU)	0.5	pH, 1% Solution	7.2
Filterability (g/cm^2)	1.3		

Carbohydrate (%)

Total	7.7

Nitrogen Content (%)

Total Nitrogen	13.0	AN/TN 20.7	40.0
Amino Nitrogen	5.2		

Amino Acids (%)

Alanine	2.86	Lysine	6.70
Arginine	3.03	Methionine	2.57
Aspartic Acid	6.11	Phenylalanine	3.71
Cystine	0.42	Proline	7.45
Glutamic Acid	17.05	Serine	4.29
Glycine	1.75	Threonine	3.58
Histidine	2.02	Tryptophan	0.71
Isoleucine	4.40	Tyrosine	1.42
Leucine	7.11	Valine	5.00

Inorganics (%)

Calcium	0.013	Phosphate	2.669
Chloride	0.186	Potassium	0.229
Cobalt	<0.001	Sodium	2.631
Copper	<0.001	Sulfate	0.241
Iron	<0.001	Sulfur	0.740
Lead	<0.001	Tin	<0.001
Magnesium	0.017	Zinc	0.003
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.1	PABA	3.7
Choline (as Choline Chloride)	350.0	Pantothenic Acid	5.3
Cyanocobalamin	<0.1	Pyridoxine	0.6
Folic Acid	0.3	Riboflavin	<0.1
Inositol	1400.0	Thiamine	0.4
Nicotinic Acid	97.8	Thymidine	93.4

Biological Testing (CFU/g)

Coliform	negative
Salmonella	negative
Spore Count	73
Standard Plate Count	870
Thermophile Count	8

The values presented are "typical". This information is for broad comparison use only and is not indicative of the makeup of any particular lot of material. No guarantee is made, either expressed or implied, that any specific lot of product will match the values presented.

Procedure

Materials Provided

Tryptone Peptone

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Tryptone Peptone in the formula of the medium being prepared. Add Tryptone Peptone as required.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures using Tryptone Peptone.

Results

See appropriate references and procedures for results.

References

1. J. Bacteriol. 1933. **25**:623.
2. Pure Culture Study of Bacteria. 1947. No. 3
3. Centr. Bakt. , I Abt. 1915. **76**:1.

User Quality Control cont.

Cultural Response

For each Test specified, prepare a Test Solution of Tryptone Peptone and, if necessary, adjust to pH 7.2-7.4; sterilize, inoculate and incubate according to standard test procedure.

TEST	TEST SOLUTION	ORGANISM	ATCC®	INOCULUM	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922*	1 drop, undiluted	negative; red color
Indole Production	0.1%	*Escherichia coli*	25922*	1 drop, undiluted	positive; pink color on Indole Test Strip
Acetylmethylcarbinol Production	0.1% w/ 0.5% dextrose	*Enterobacter aerogenes*	13048*	1 drop, undiluted	positive; pink color upon adding reagents
Hydrogen Sulfide Production	1%	*Salmonella typhi*	6539	1 drop, undiluted	positive; brownish blackening of H_2S Test Strip
Growth Production	2% w/1.5% agar and 0.5% NaCl	*Escherichia coli*	25922*	100-1,000 CFU	good growth
Growth Production	2% w/1.5% agar and 0.5% NaCl	*Staphylococcus aureus*	25923*	100-1,000 CFU	good growth

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

4. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
5. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual , 8th ed. AOAC International, Gaithersburg, MD.
6. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
7. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.

Packaging

Tryptone Peptone	100 g	0123-15
	500 g	0123-17
	2 kg	0123-07
	10 kg	0123-08

Bacto® Tryptone Glucose Extract Agar
Bacto m TGE Broth

Intended Use

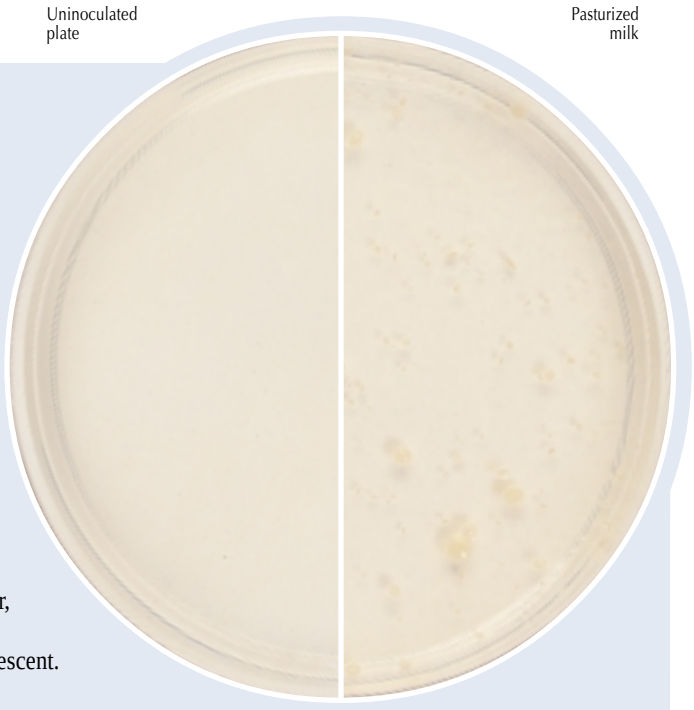
Bacto Tryptone Glucose Extract Agar is used for cultivating and enumerating microorganisms in water and dairy products.

Bacto m TGE Broth is used for enumerating microorganisms by membrane filtration.

Also Known As

Tryptone Glucose Extract Agar is also known as Yeast Dextrose Agar and may be abbreviated as TGEA.

m TGE is an abbreviation for membrane Tryptone Glucose Extract.



User Quality Control

Identity Specifications

Tryptone Glucose Extract Agar

Dehydrated Appearance:	Light to medium tan, free-flowing, homogeneous.
Solution:	2.4% solution, soluble in distilled or deionized water on boiling. Solution is light amber, clear to slightly opalescent, no significant precipitate.
Prepared Medium:	Light amber, clear to slightly opalescent, no precipitate.
Reaction of 2.4% Solution at 25°C:	pH 7.0 ± 0.2

m TGE Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	1.8% solution, soluble in distilled or deionized water. Solution is medium amber, clear to very slightly opalescent.
Prepared Medium:	Medium amber, clear to very slightly opalescent.
Reaction of 1.8% Solution at 25°C:	pH 7.0 ± 0.2

Cultural Response

Tryptone Glucose Extract Agar
Prepare Tryptone Glucose Extract Agar per label directions in parallel with a reference control. Inoculate with pasteurized and raw milk samples using the pour plate technique and incubate at 32 ± 1°C for 47-49 hours. Recovery of bacteria from the milk samples should be comparable for both the test and reference lots.

m TGE Broth
Prepare mTGE Broth per label directions. Inoculate using the membrane filter technique and incubate in a humid atmosphere at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	100-1,000	good
Staphylococcus aureus	25923*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Summary and Explanation

In the 1930's, Bower and Hucker[1] developed a medium for detecting bacteria in milk and other dairy products. Many investigators compared the performance of Tryptone Glucose Skim Milk Agar to Nutrient Agar for estimating bacteria in milk and other dairy products.[2,3,4] Prickett[5] used a glucose agar containing Tryptone to study thermophilic bacteria in milk. This medium, described in *Standard Methods of Milk Analysis*[6], was prepared in the dehydrated form as Yeast Dextrose Agar. The American Public Health Association (APHA) adopted Tryptone Glucose Extract Agar for use in testing milk and dairy products in 1948.[7] For many years, Tryptone Glucose Extract Agar with added milk remained the Standard Methods medium for dairy products[8] and was also adopted for testing water.[9] Currently, the APHA specifies using Tryptone Glucose Extract Agar for the heterotrophic plate count procedure in testing bottled water.[10]

m TGE Broth is a nonselective nutrient medium for the determination of bacterial counts by the membrane filter method. The broth has the same formulation as Tryptone Glucose Extract Agar, except that the broth contains no agar and the ingredients are at twice the concentration.

Principles of the Procedure

Tryptone Glucose Extract Agar and m TGE Broth contain Beef Extract and Tryptone as sources of carbon, nitrogen, vitamins and minerals. Dextrose (Glucose) is a carbohydrate. Tryptone Glucose Extract Agar contains Bacto Agar as a solidifying agent.

Formula

Tryptone Glucose Extract Agar

Formula Per Liter

Bacto Beef Extract	3 g
Bacto Tryptone	5 g
Bacto Dextrose (Glucose)	1 g
Bacto Agar	15 g

Final pH 7.0 ± 0.2 at 25°C

m TGE Broth

Formula Per Liter

Bacto Beef Extract	6 g
Bacto Tryptone	10 g
Bacto Dextrose	2 g

Final pH 7.0 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Tryptone Glucose Extract Agar
m TGE Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Petri dishes (Tryptone Glucose Extract Agar)
Sterile membranes (m TGE Broth)
Filter apparatus (m TGE Broth)
Sterile absorbent pads (m TGE Broth)
Incubator (TGEA - 32 ± 1°C; mTGE Broth - 35°C)

Method of Preparation

Tryptone Glucose Extract Agar (TGEA)

1. Suspend 24 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

m TGE Broth

1. Dissolve 18 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

Collect samples in accordance with laboratory procedures.[10]

Test Procedure

1. Use the appropriate culture method, as follows:
   TGEA - pour plate method;
   m TGE - membrane filtration.
2. Incubate the inoculated medium in a humid atmosphere, as follows:
   TGEA - 32 ± 1°C for 47-49 hours;
   m TGE - 35 ± 2°C for 18-24 hours.

Results

Count total colonies and record results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Bowers and Hucker**. 1935. Tech. Bull. 228. NY State Agr. Exp. Sta.
2. **Yale**. 1938. Am. J. Pub. Health **28**:148.
3. **Proc. 36th Cong. Intern. Assoc. Ice Cream Manufacturers**. 1936. **2**:132.
4. **Dennis and Weiser**. 1937. J. Dairy Science **20**:445.
5. **Prickett**. 1928. Tech. Bull. 147. NY State Agr. Exp. Sta.
6. **Standard Methods of Milk Analysis, 6th ed.** 1934.
7. **American Public Health Association**. 1948. Standard methods for the examination of dairy products, 9th ed. American Public Health Association, Washington, D.C.
8. **American Public Health Association**. 1972. Standard methods for the examination of dairy products, 13th ed. American Public Health Association, Washington, D.C.

9. **American Public Health Association**. 1980. Standard methods for the examination of water and wastewater, 15th ed. American Public Health Association, Washington, D.C.
10. **Cowman, S., and R. Kelsey.** 1992. Bottled water, p. 1031-1036. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

Tryptone Glucose Extract Agar	100 g	0002-15
	500 g	0002-17
	2 kg	0002-07
m TGE Broth		
	100 g	0750-15
	500 g	0750-17

Bacto® Tryptone Water

Intended Use

Bacto Tryptone Water is recommended for use in the detection of *Escherichia coli* in food and water samples based on indole production.

Summary and Explanation

Tryptone Water is based on the Tryptone Water formula described in AFNOR and ISO Standards.[1] In these procedures, Tryptone Water is used with Brilliant Green Bile 2% to determine the most probable number (MPN) of *E. coli* present in meat and meat products. Growth and gas production in Brilliant Green Bile 2% and indole production in Tryptone Water following incubation of both media at 44 ± 1°C is used as the basis for this presumptive *E. coli* test.

Tryptone Water may also be used for differentiation of bacteria based on indole production. Production of indole using pure cultures in tryptophan containing media is recommended as an aid in the differentiation of bacteria and the identification of *E. coli* isolated from food and water samples.[2,3]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free flowing, homogeneous.
Solution:	1.5 % solution, soluble in distilled or deionized water. Solution is light to medium amber, clear to slightly opalescent with no significant precipitate.
Prepared Medium:	Light to medium amber, clear to slightly opalescent with no significant precipitate.
Reaction of 1.5% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Tryptone Water per label directions. Inoculate and incubate the tubes at 35 ± 2°C for 18-24 hours. Add 0.5 ml SpotTest™ Indole Reagent Kovacs to the tubes to test for indole production. Formation of a red color denotes a positive indole test.

ORGANISM	ATCC®	INOCULUM CFU	INDOLE PRODUCTION	GROWTH
Escherichia coli	25922*	100-300	positive	good
Enterobacter cloacae	13047	100-300	negative	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Tryptone Water is similar to Tryptone Broth, containing both Tryptone (1%) and Sodium Chloride. Due to its high tryptophan content, Tryptone is suitable for use in detecting indole production by bacteria. Tryptophan is hydrolyzed and deaminated to produce indole, pyruvic acid and ammonia.[4] Indole can then be detected by the addition of either Kovac's or Ehrlich's Reagent, which contain an aldehyde group. The aldehyde group combines with indole to produce a red color in the alcohol layer. Sodium Chloride is added to the medium to provide a suitable osmotic environment.

Formula

Tryptone Water

Formula Per Liter

Bacto Tryptone	10 g
Sodium Chloride	5 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The powder is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Tryptone Water

Materials Required But Not Provided

Brilliant Green Bile 2%
SpotTest™ Indole Reagent Kovacs
Flasks with closures
Distilled or deionized water
Autoclave
Incubator (30 ± 1°C)
Incubator (44 ± 1°C)
Diluent

Method of Preparation

1. Suspend 15 grams in 1 liter of distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

1. Collect food and water samples in sterile containers and transport immediately to the laboratory following recommended guidelines.[1-4]
2. Process each food and water sample using procedures appropriate for that sample.[1-4]

Test Procedure

Presumptive Test For *E. coli* in Meats and Meat Products[1]

1. Suspend one part sample in 9 parts diluent. Homogenize sample.
2. Dilute homogenate in duplicate using serial 10-fold dilutions to 10^{-6} using 1 ml of material to be diluted to 9 ml of diluent. Mix each dilution thoroughly.
3. Transfer 1 ml of homogenate (step 1) to 6 tubes containing 10 ml Brilliant Green Bile 2% and group in 2 sets of 3 tubes each. If the number of coliforms is expected to be high, transfer 10 ml of homogenate into 6 tubes containing double strength Brilliant Green Bile 2%.
4. Transfer 1 ml from each of the dilutions prepared in step 2 into each of 3 tubes containing 10 ml Brilliant Green Bile 2%.
5. Incubate Brilliant Green Bile 2% tubes (prepared in steps 3 and 4) at 30 ± 1°C for 48 ± 2 hours. Subculture all tubes containing gas, using 1 drop of inoculum, into Tryptone Water and Brilliant Green Bile 2%.
6. Incubate tubes prepared in step 5 at 44 ± 1°C for 48 ± 2 hours.

Indole Determination Using Pure Cultures

1. Inoculate Tryptone Water using a light inoculum of an 18-24 hour pure culture.
2. Incubate the tubes at 35 ± 2°C with loosened caps for 18-24 hours.
3. Add 0.5 ml of SpotTest™ Indole Reagent Kovacs directly to the tube and agitate. Allow tubes to stand for 5-10 minutes.

Results

Presumptive Test For *E. coli* in Meats and Meat Products

Examine and record the tubes of Brilliant Green Bile 2% tubes containing gas. Add 0.5 ml SpotTest™ Indole Reagent Kovacs to the Tryptone Water tubes. Observe tubes for the formation of a red ring at the top of medium indicating indole production. Record the tubes for positive indole production. Determine the MPN (Most Probable Number) of *E. coli* present in the sample based on the number of tubes that are positive for both gas and indole. Consult the appropriate 3-tube MPN table.[2]

Indole Determination Using Pure Cultures

Examine tubes for the formation of a red ring at the top of the tube indicating indole production.

Limitations of the Procedure

1. Detection of *E. coli* in meats using Tryptone Water is a presumptive test. If confirmatory testing is required, please consult appropriate references.
2. Indole testing is recommended as an aid in the differentiation of microorganisms based on indole production. For complete identification of the organism, further biochemical evaluation is necessary.

References

1. **International Organization for Standardization:** Meat and meat products. Detection and enumeration of presumptive coliform bacterial and presumptive *E. coli* (Reference method ISO/DIS 3811-1979).
2. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Greenberg, A. E., L. S. Clesceri, and A. D. Eaton (ed.).** 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. American Public Health Association, Washington, D.C.
4. **MacFaddin, J. F.** Biochemical Test for Identification of Medical Bacteria, 3rd ed. 1985. Williams and Wilkens, Baltimore, MD.

Packaging

Tryptone Water	500 g	0644-17

Bacto® Tryptose

Intended Use

Bacto Tryptose is an enzymatic digest of protein for use in preparing microbiological culture media.

Also Known As

Tryptose is also referred to as Polypeptone™ Peptone.

Summary and Explanation

Tryptose is a mixed enzymatic hydrolysate with distinctive nutritional properties. Tryptose was originally developed as a peptone particularly adapted to the growth requirements of *Brucella*. An agar medium containing Tryptose, sodium chloride and dextrose, without liver or other infusions, was shown to be an excellent medium for propagation of these organisms.[1] Culture media prepared with Tryptose were superior to the meat infusion peptone media previously used for the cultivation of *Brucella*, streptococci, pneumococci, meningococci and other fastidious bacteria.[2,3]

An agar medium prepared with 2% Tryptose and 0.5-0.8% sodium chloride, without tissue infusion, is an excellent blood agar base. The growth of organisms on Tryptose Blood Agar Base is luxuriant and the zones of hemolysis produced are distinct and clear. Huddleson[1] used a broth containing 2% Tryptose as an enrichment medium in the isolation of *Brucella* from clinical specimens.

Principles of the Procedure

Tryptose is a mixed enzymatic hydrolysate with distinctive nutritional properties. The digestive process in Tryptose results in assorted peptides, including those of higher molecular weight.

Typical Analysis

Physical Characteristics

Ash (%)	9.7	Loss on Drying (%)	3.2
Clarity, 1% Soln (NTU)	0.8	pH, 1% Soln	7.4
Filterability (g/cm^2)	2.3		

Carbohydrate (%)

Total	7.1

Nitrogen Content (%)

Total Nitrogen	13.4	AN/TN	32.5
Amino Nitrogen	4.4		

Amino Acids (%)

Alanine	4.45	Lysine	4.64
Arginine	4.65	Methionine	1.92
Aspartic Acid	6.34	Phenylalanine	7.52
Cystine	0.44	Proline	6.33
Glutamic Acid	13.92	Serine	4.09
Glycine	2.84	Threonine	3.55
Histidine	<0.01	Tryptophan	0.62
Isoleucine	0.34	Tyrosine	2.21
Leucine	3.67	Valine	1.93

Inorganics (%)

Calcium	0.001	Phosphate	2.144
Chloride	0.886	Potassium	0.679
Cobalt	<0.001	Sodium	3.410
Copper	<0.001	Sulfate	0.308
Iron	0.002	Sulfur	0.737
Lead	<0.001	Tin	<0.001
Magnesium	0.022	Zinc	0.005
Manganese	<0.001		

Vitamins (µg/g)

Biotin	0.2	PABA	11.4
Choline (as Choline Chloride)	2700.0	Pantothenic Acid	16.0
Cyanocobalamin	<0.1	Pyridoxine	1.4
Folic Acid	0.4	Riboflavin	4.3
Inositol	5400.0	Thiamine	0.1
Nicotinic Acid	47.4	Thymidine	769.0

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	825
Salmonella	negative	Thermophile Count	100
Spore Count	875		

User Quality Control

Identity Specifications

Dehydrated Appearance: Tan, free-flowing granules.

Solution: 1, 2 and 10% solutions are soluble in distilled or deionized water:
1% - Light amber, clear.
2% - Medium amber, clear to slightly opalescent.
10% - Medium to dark amber, very slightly opalescent to opalescent, may have precipitation.

Reaction of 1% Solution at 25°C: pH 7.1 to 7.5

Cultural Response

TEST	SOLUTION	ORGANISM	ATCC®	RESULT
Fermentable Carbohydrates	2%	*Escherichia coli*	25922*	negative
Indole Production	0.1%	*Escherichia coli*	25922*	positive
Acetylmethyl-carbinol	0.1% w /0.5% dextrose	*Enterobacter aerogenes*	13048*	positive
Hydrogen Sulfide Production	1%	*Salmonella typhi*	6539	positive
Growth Response	2% w/ 0.5% NaCl, 0.1% agar, and 0.1% dextrose	*Brucella suis*	4314	good growth
Growth Response	2% w/ 0.5% NaCl, 0.1% agar, and 0.1% dextrose	*Staphylococcus aureus*	25923*	good growth
Growth Response	2% w/ 0.5% NaCl, 0.1% agar, and 0.1% dextrose	*Streptococcus pneumoniae*	6303*	good growth
Growth Response	2% w/ 0.5% NaCl, 0.1% agar, and 0.1% dextrose	*Streptococcus pyogenes*	19615*	good growth

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated ingredient below 30°C. The dehydrated ingredient is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Tryptose

Materials Required But Not Provided

Materials vary depending on the medium being prepared.

Method of Preparation

Refer to the final concentration of Tryptose in the formula of the medium being prepared. Add Tryptose as required.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Huddleson, I. F.** 1939. Brucellosis in man and animals. **14**. Oxford University Press, Oxford.

2. **Casman, E. P.** 1942. A dehydrated medium to supplement meat infusion as a base for blood agar. J. Bacteriol. **43**:33.
3. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. **17**:281-289.

Packaging

Tryptose	100 g	0124-15
	500 g	0124-17
	2 kg	0124-07
	10 kg	0124-08

Bacto® Tryptose Agar
Bacto Tryptose Broth

Intended Use

Bacto Tryptose Agar is used for cultivating a wide variety of fastidious microorganisms, particularly for isolating *Brucella* according to Huddleson and Castañeda.

Bacto Tryptose Broth is used for cultivating *Brucella* and other fastidious microorganisms.

User Quality Control

Identity Specifications

Tryptose Agar

Dehydrated Appearance:	Light beige, homogeneous, free-flowing.
Solution:	4.1% solution, soluble on boiling in distilled or deionized water; light amber, very slightly to slightly opalescent, without significant precipitate.
Prepared Medium:	Light amber, slightly opalescent, without precipitate.
Reaction of 4.1% Solution at 25°C:	pH 7.2 ± 0.2

Tryptose Broth

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	2.6% solution, soluble in distilled or deionized water; light amber, clear, without significant precipitate.
Prepared Medium:	Light amber, clear without significant precipitate.
Reaction of 2.6% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare Tryptose Agar and Tryptose Broth per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 40-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Brucella abortus	4315	100-1,000	good
Brucella melitensis	4309	100-1,000	good
Brucella suis	6597	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Summary and Explanation

Tryptose media, prepared without extract or infusion of meat, are recommended for the cultivation and isolation of pathogenic and saprophytic bacteria. Historically, it was considered necessary to include meat extract or infusion as a nutritional supplement in culture media. Tryptose was developed while studying the growth requirements of *Brucella*. Huddleson[1] found tryptose media to be equal or superior to meat infusion media, providing uniformity for the cultivation and differentiation of fastidious organisms.

Tryptose media are particularly well suited for the isolation of *Brucella* from blood. Castañeda[2] studied the isolation of *Brucella* species using a broth containing 2% Tryptose and 2% sodium citrate. Sodium citrate serves as an anticoagulant and assists in inactivating complement in the blood specimen.

Tryptose Broth can be used as a complete basal medium or supplemented with enrichments. Huddleson[3] used a broth containing 2% Tryptose as an enrichment medium in the isolation of *Brucella* from clinical specimens. McCullough *et al*. reported that addition of thiamine, dextrose and iron salts increased growth of *Brucella suis*.[4] Addition of 0.1% agar to Tryptose Broth can increase growth of aerobes and anaerobes in liquid media. Blood agar may be prepared by adding 5% sterile, defibrinated sheep, horse or rabbit blood to the sterile medium.

The high productivity of tryptose media in the isolation and cultivation of *Brucella* supports use of these formulas as general purpose media, especially when avoidance of animal tissue products is desired. Tryptose Agar with 5% bovine serum, with or without antibiotics, remains a standard plating medium for the isolation of brucellae.[5] For isolation of *Brucella* stains from contaminated milk, crystal violet (gentian violet) can be added to Tryptose Agar to suppress gram-positive organisms.[6] Tryptose media can be supplemented with thiamine or citrate for the cultivation and maintenance of fastidious aerobic and facultative microorganisms.[7]

Tryptose Agar is specified in the Compendium of Methods for the Microbiological Examination of Food.[8] Tryptose media are recommended in FDA Bacteriological Analytical Manual for serological testing.[9]

Principles of the Procedure

In Tryptose media, Tryptose is a source of nitrogen and carbon. Dextrose is a source of carbohydrate. Sodium Chloride maintains osmotic balance. Bacto Agar is the solidifying agent in Tryptose Agar.

Formula

Tryptose Agar

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	1 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.2 ± 0.2 at 25°C

Tryptose Broth

Formula Per Liter

Bacto Tryptose	20 g
Sodium Chloride	5 g
Bacto Dextrose	1 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Tryptose Agar
Tryptose Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes and/or tubes
Bacto Agar (optional)
Sterile defibrinated blood

Method of Preparation

Tryptose Agar

1. Suspend 41 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Distribute into tubes, bottles or flasks.
4. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
5. To prepare blood agar, aseptically add 5% sterile defibrinated sheep, horse or rabbit blood. Dispense into sterile Petri dishes.

Tryptose Broth

1. Dissolve 26 grams in 1 liter distilled or deionized water.
2. Distribute into tubes, bottles or flasks.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.

Specimen Collection and Preparation

Specimens should be collected in sterile containers or with sterile swabs and transported immediately to the laboratory in accordance with recommended guidelines outlined in the references.

Test Procedure

Methodologies for the multiple applications using tryptose media are outlined in the references.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Tryptose media are general purpose, non-selective media. Although certain diagnostic tests may be performed directly on the medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification.
2. When preparing blood agar, hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood.
3. Atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[10] For optimal performance, incubate tryptose media supplemented with blood under increased CO_2 or anaerobic conditions.
4. Dextrose has been shown to inhibit hemolysin production by some organisms.

References

1. **Huddleson, I. F.** 1943. Brucellosis in man and animals. Rev. Ed. The Commonwealth Fund, New York.
2. **Castañeda, M. R.** 1947. A practical method for routine blood cultures in brucellosis. Proc. Soc. Exp. Biol. Med. **64**:114-115.
3. **Huddleson, I. F.** 1939. Brucellosis in man and animals. 14. Oxford University Press, Oxford.
4. **McCullough, W. G., R. C. Mills, E. J. Herbst, W. G. Roessler, and C. R. Brewer.** 1947. Studies on the nutritional requirements of *Brucella suis*. J. Bacteriol. **53**:5-15.
5. **Moyer, N. P., and L. A. Holcomb.** 1995. Brucella. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
6. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 802-806. Williams & Wilkins, Baltimore, MD.
7. **Atlas, R. M.** 1995. Handbook of microbiology media for the examination of food, p. 266-268. CRC Press, Boca Raton, FL.
8. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
9. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel.** 1995. FDA Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
10. **Ruoff, K. L.** 1995. Streptococcus, p. 299-305**.** ***In*** P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Tryptose Agar	500 g	0064-17
	2 kg	0064-07
	10 kg	0064-08
Tryptose Broth	500 g	0062-17
	10 kg	0062-08

Bacto® Tryptose Blood Agar Base
Bacto Tryptose Blood Agar Base w/Yeast Extract

Intended Use

Bacto Tryptose Blood Agar Base is used with blood in isolating, cultivating and determining the hemolytic reactions of fastidious microorganisms.

Bacto Tryptose Blood Agar Base w/Yeast Extract is used with or without blood in cultivating a wide variety of microorganisms.

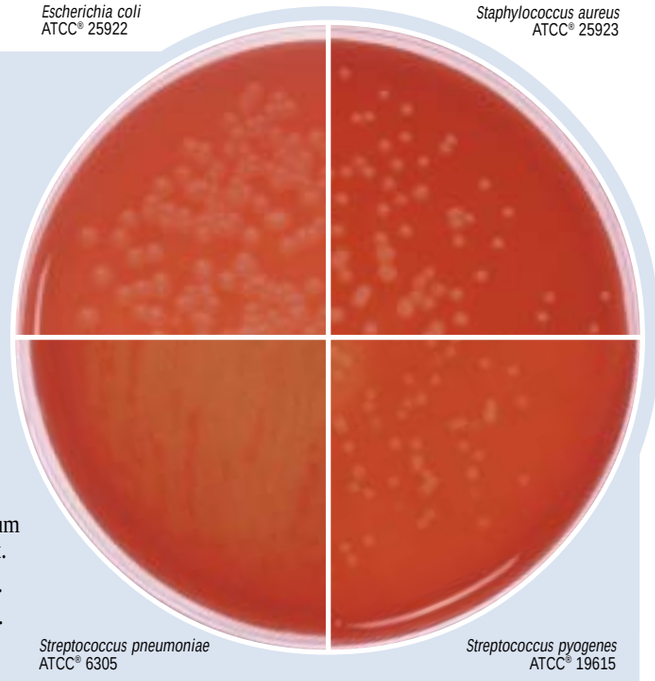
Also Known As

"Blood Agar Base" may be abbreviated as BAB.

Summary and Explanation

Investigations of the nutritive properties of Tryptose demonstrated that culture media prepared with this peptone were superior to the meat infusion peptone media previously used for the cultivation of *Brucella*, streptococci, pneumococci, meningococci and other fastidious bacteria. Casman[1,2] reported that a medium consisting of 2% Tryptose, 0.3% Beef Extract, 0.5% NaCl, 1.5% Bacto Agar and 0.03% dextrose equaled fresh beef infusion base with respect to growth of organisms. The small amount of carbohydrate was noted to interfere with hemolytic reactions, unless the medium was incubated in an atmosphere of carbon dioxide.

Tryptose Blood Agar Base and Tryptose Blood Agar Base w/ Yeast Extract are nutritious infusion-free basal media typically supplemented with 5-10% sheep, rabbit or horse blood for use in isolating, cultivating and determining hemolytic reactions of fastidious pathogenic microorganisms. Without enrichment, these bases can be used as general purpose media. Tryptose Blood Agar Base is specified in FDA Bacteriological Analytical Manual.[4] Tryptose Blood Agar Base w/ Yeast Extract was formulated to provide a base with additional nutrients to improve the growth of fastidious organisms.



User Quality Control

Identity Specifications

Tryptose Blood Agar Base

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 3.3% solution, soluble in distilled or deionized water on boiling; light amber, slightly opalescent.

Prepared Medium: Light amber, slightly opalescent. With 5% sheep blood-cherry red, opaque.

Reaction of 3.3% Solution at 25°C: pH 7.2 ± 0.2

Tryptose Blood Agar Base w/Yeast Extract

Dehydrated Appearance: Beige, free-flowing, homogeneous.

Solution: 3.4% solution, soluble in distilled or deionized water on boiling; light to medium amber, very slightly to slightly opalescent.

Prepared Medium: Light to medium amber, slightly opalescent. With 5% sheep blood-cherry red, opaque.

Reaction of 3.4% Solution at 25°C: pH 7.3 ± 0.2

Cultural Response

Prepare Tryptose Blood Agar Base and Tryptose Blood Agar Base w/ Yeast Extract with and without 5% sterile defibrinated sheep blood per label directions. Inoculate and incubate at 35 ± 2°C under 5-10% CO_2 for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH w/o BLOOD	GROWTH W/BLOOD	HEMOLYSIS 18-48 H
Escherichia coli	25922*	100-1,000	good to excellent	good to excellent	beta
Neisseria meningitidis	13090*	100-1,000	fair to good	good	N/A
Staphylococcus aureus	25923*	100-1,000	good	good	beta
Streptococcus pneumoniae	6305	100-1,000	fair to good	good	alpha
Streptococcus pyogenes	19615*	100-1,000	fair to good	good	beta

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Tryptose is the source of nitrogen, carbon and amino acids in Tryptose Blood Agar Base and Tryptose BAB w/ Yeast Extract. Beef Extract provides additional nitrogen. Bacto Agar is the solidifying agent. Sodium Chloride maintains osmotic balance. Yeast Extract supplies additional vitamins and cofactors for growth.

Supplementation with 5-10% blood provides additional growth factors for fastidious microorganisms, and is used to determine hemolytic patterns of bacteria.

Formula

Tryptose Blood Agar Base

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	3 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.2 ± 0.2 at 25°C

Tryptose Blood Agar Base w/Yeast Extract

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	3 g
Bacto Yeast Extract	1 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.3 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Tryptose Blood Agar Base

Tryptose Blood Agar Base w/ Yeast Extract

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes
5% sterile defibrinated blood (optional)

Method of Preparation

1. Suspend the appropriate amount of medium in 1 liter distilled or deionized water.

Tryptose Blood Agar Base	33 g
Tryptose Blood Agar Base w/ Yeast Extract	34 g

2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Anaerobic bacteria are overlooked or missed unless the specimen is properly collected and transported to the laboratory.[7] Obtain and process specimens according to the procedures established by institutional policy. Specimens should be collected in sterile containers or with sterile swabs, and transported immediately to the laboratory in accordance with recommended guidelines outlined in the references.

Test Procedure

1. Process each specimen as appropriate, and inoculate directly onto the surface of the medium. Streak for isolation with an inoculating loop, then stab the agar several times to deposit beta-hemolytic streptococci beneath the agar surface. Subsurface growth will display the most reliable hemolytic reactions of both oxygen-stable and oxygen-labile streptolysins.[3]
2. Incubate plates aerobically, anaerobically or under conditions of increased CO_2 (5-10%) in accordance with established laboratory procedures.
3. Examine plates for growth and hemolytic reactions after 18-24 and 48-hour incubation. Four different types of hemolysis on blood agar media can be described:[5]
   a. Alpha (α)-hemolysis is the reduction of hemoglobin to methemoglobin in the medium surrounding the colony. This causes a greenish discolorization of the medium.
   b. Beta (β)-hemolysis is the lysis of red blood cells, resulting in a clear zone surrounding the colony.
   c. Gamma (γ)-hemolysis indicates no hemolysis. No destruction of red blood cells occurs, and there is no change in the medium.
   d. Alpha-prime (ὰ)-hemolysis is a small zone of complete hemolysis that is surrounded by area of partial lysis.

Limitations of the Procedure

1. Blood Agar Base Media are intended for use with blood supplementation. Although certain diagnostic tests may be performed directly on this medium, biochemical and, if indicated, immunological testing using pure cultures are recommended for complete identification. Consult appropriate references for further information.
2. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
3. Hemolytic reactions of some strains of group D streptococci have been shown to be affected by differences in animal blood. Such strains are beta-hemolytic on horse, human and rabbit blood agar and alpha-hemolytic on sheep blood agar.[3]

4. Colonies of *Haemophilus haemolyticus* are beta-hemolytic on horse and rabbit blood agar, and must be distinguished from colonies on beta-hemolytic streptococci using other criteria. The use of sheep blood has been suggested to obviate this problem since sheep blood is deficient in pyridine nucleotides and does not support growth of *H. haemolyticus*.[6]
5. The atmosphere of incubation has been shown to influence hemolytic reactions of beta-hemolytic streptococci.[3] For optimal performance, incubate blood agar base media under increased CO_2 or anaerobic conditions.
6. Hemolytic patterns may vary with the source of animal blood or type of base medium used.[3]

References

1. **Casman, E. P.** 1942. A dehydrated medium to supplement meat infusion as a base for blood agar. J. Bacteriol. **43**:33.
2. **Casman, E. P.** 1947. A noninfusion blood agar base for neisseriae, pneumococci and streptococci. Am. J. Clin. Pathol. **17**:281-289.
3. **Ruoff, K. L.** 1995. *Streptococcus*, p. 299-305. *In P. R.* Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel**. 1995. FDA Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
5. **Isenberg, H. D.** (ed.). 1992. Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
6. **Baron, E. J., L. R. Peterson, and S. M. Finegold**. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.

Packaging

Tryptose Blood Agar Base	500 g	0232-17
	2 kg	0232-07
Tryptose Blood Agar Base w/ Yeast Extract	500 g	0662-17

Bacto® Tryptose Phosphate Broth

Intended Use

Bacto Tryptose Phosphate Broth is used for cultivating fastidious microorganisms.

Also Known As

Tryptose Phosphate Broth is abbreviated as TPB.

Summary and Explanation

Tryptose Phosphate Broth is an infusion-free buffered medium recommended for the cultivation of fastidious, pathogenic microorganisms. In a study by Waisbren, Carr, and Dunnett,[1] Tryptose Phosphate Broth was used in the tube method for antibiotic sensitivity testing.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, homogeneous, free-flowing.
Solution:	2.95% solution, soluble in distilled or deionized water; light amber, may be very slightly opalescent with a very slight precipitate.
Prepared Medium:	Light amber, clear to very slightly opalescent, may have a very slight precipitate.
Reaction of 2.95% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Prepare Tryptose Phosphate Broth per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090*	100-1,000	good
Staphylococcus epidermidis	12228*	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube

Streptococcus pneumoniae ATCC® 6305

Tryptose Phosphate Broth is valuable in tissue culture procedures, as shown by Ginsberg, Gold and Jordan.[2] The proteose content of Tryptose Phosphate Broth is considered to be a stimulating factor for cells. Tryptose Phosphate Broth is specified in the FDA Bacteriological Analytical Manual for cell culture procedures.[3]

Principles of the Procedure

Tryptose provides carbon and nitrogen. Dextrose is a carbon source. Sodium Chloride maintains osmotic balance. Buffering capacity is provided by Disodium Phosphate.

The addition of 0.1-0.2% agar to Tryptose Phosphate Broth facilitates anaerobic growth and aids in dispersion of reducing substances and CO_2 formed in the environment.[4] The low agar concentration provides suitable conditions for both aerobic growth in the upper zone and for microaerophilic and anaerobic growth in the lower zone.

Formula

Tryptose Phosphate Broth

Formula Per Liter

Bacto Tryptose	20 g
Bacto Dextrose	2 g
Sodium Chloride	5 g
Disodium Phosphate	2.5 g

Final pH 7.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Tryptose Phosphate Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Tubes with closures
Bacto Agar (optional)

Method of Preparation

1. Dissolve 29.5 grams in 1 liter distilled or deionized water.
2. If a medium containing 0.1% agar is desired, add 1 gram of Bacto Agar. Heat to boiling to dissolve completely.
3. Dispense as desired.
4. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Waisbren, B. A., M. S. Carr, and J. Dunnett.** 1951. The tube dilution method of determining bacterial sensitivity to antibiotics. Am. J. Clin. Pathol. **21**:884.
2. **Ginsberg, Gold, and Jordan.** 1955. Proc. Soc. Exp. Biol. Med. **89**:66.
3. **Harmon, S. M., D. A. Kautter, D. A. Golden, and E. J. Rhodehamel.** 1995. FDA Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
4. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 802-804. Williams & Wilkins, Baltimore, MD.

Packaging

Tryptose Phosphate Broth	500 g	0060-17
	2 kg	0060-07
	10 kg	0060-08

Bacto® UBA Medium

Intended Use

Bacto UBA Medium is used for cultivating microorganisms of significance in the brewing industry.

Also Known As

UBA is also referred to as Universal Beer Medium or Universal Beer Agar.

Summary and Explanation

UBA Medium is a basal medium to which beer is added. It is based on the formula developed by Kozulis and Page[1] who compared it with other media commonly used in breweries for detecting microbial contamination.[2] The characteristics of UBA Medium are closer to the natural environmental conditions found in the typical brewery than other media studied. UBA Medium supports growth of more varieties of lactic acid bacteria and yields larger colonies in a shorter time than traditional brewer's media. Due to the presence of beer

in the medium, it is selective for growth of microorganisms that have adapted themselves to existent conditions in the brewery. The presence of hop constituents and alcohol inhibits growth of many airborne microorganisms not adapted to this environment.[3]

UBA Medium supports growth of *Lactobacillus*, *Pediococcus*, *Acetobacter* and yeast strains which may be found contaminating the wort and beer.

Principles of the Procedure

Yeast Extract is a source of trace elements, vitamins and amino acids. Peptonized Milk contains lactose as an energy source. Tomato Juice is a source of carbon, protein and nutrients. Dextrose provides additional carbon. Dipotassium and Monopotassium Phosphate provide buffering capability. Magnesium Sulfate, Ferrous Sulfate and Manganese Sulfate are sources of ions that stimulate metabolism. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

Formula

UBA Medium

Formula Per Liter

Bacto Yeast Extract	6.1 g
Bacto Peptonized Milk	15 g
Tomato Juice (244 ml)	12.2 g
Bacto Dextrose	16.1 g
Dipotassium Phosphate	0.31 g
Monopotassium Phosphate	0.31 g
Magnesium Sulfate	0.12 g
Sodium Chloride	0.006 g
Ferrous Sulfate	0.006 g
Manganese Sulfate	0.006 g
Bacto Agar	12 g

Final pH 6.3 ± 0.2 at 25°C

User Quality Control

Identity Specifications

Dehydrated Appearance:	Medium beige, homogeneous, free-flowing.
Solution:	6.2% solution, soluble in distilled or deionized water on boiling. Solution is medium to dark amber, very slightly opalescent.
Reaction of 6.2% Solution at 25°C:	pH 6.3 ± 0.2

Cultural Response

Prepare UBA Medium per label directions. Inoculate the medium and incubate at 30 ± 2°C for up to 3 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Acetobacter pasteurianus	12879	100-1,000	good
Lactobacillus fermentum	9338	100-1,000	good
Pediococcus acidilactici	8081	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

UBA Medium

Materials Required but not Provided

Glassware

Autoclave

Method of Preparation

1. Suspend 62 grams in 750 ml distilled or deionized water or halogen-free tap water.
2. Heat to boiling to dissolve completely.
3. Add 250 ml of commercial beer (not degassed) and mix well.
4. Autoclave at 121°C for 10 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Kozulis, J. A., and H. E. Page.** 1968. A new universal beer agar medium for the enumeration of wort and beer microorganisms. Proc. Am. Soc. Brew. Chem. 1968:52-58.
2. **Murphy, D. T., and L. T. Saletan.** 1970. Use of microbiological media in the brewery. Tech. Q. Master Brew. Assoc. Am. **7**:182-187.
3. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance medical bacteria, vol. 1, p. 819-820. Williams & Wilkins, Baltimore, MD.

Packaging

UBA Medium	500 g	0856-17

Bacto® UVM Modified Listeria Enrichment Broth

Intended Use

Bacto UVM Modified Listeria Enrichment Broth is used for rapidly isolating *Listeria monocytogenes*.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[1] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[2] The first reported food-borne outbreak of listeriosis was in 1985,[3] and since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[4]

Implicated vehicles of transmission include turkey frankfurters,[5] coleslaw, pasteurized milk, Mexican-style cheese, paté and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[6]

Listeria species grow over a pH range of 5.0-9.6 and survive in food products with pH levels outside these parameters.[7] *Listeria* spp. are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.2% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber with a faint bluish-green ring at the surface, very slightly opalescent with a fine precipitate.
Reaction of 5.2% Solution at 25°C:	pH 7.2 ± 0.2

Cultural Response

Prepare UVM Modified Listeria Enrichment Broth per label directions. Inoculate tubes and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia faecalis	29212*	1,000-2,000	suppressed at 18-24 hours
Escherichia coli	25922*	1,000-2,000	marked to complete inhibition
Listeria monocytogenes	19114	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are: streptococci, especially the enterococci, micrococci and *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[8]

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

UVM Modified Listeria Enrichment Broth is a modification of the formula described by Donnelly and Baigent.[9] It is used for selective enrichment of *Listeria* spp. from food[7,11] and clinical specimens.[10]

Principles of the Procedure

Tryptose, Beef Extract and Yeast Extract in UVM Modified Listeria Enrichment Broth provide nitrogen, vitamins and minerals. Sodium chloride maintains the osmotic balance of the medium. Phosphate acts as a buffering agent. Nalidixic acid inhibits growth of gram-negative organisms. Acriflavine hydrochloride inhibits many gram-positive bacteria. Esculin is hydrolyzed by *Listeria* species.

Formula

UVM Modified Listeria Enrichment Broth

Formula Per Liter

Bacto Tryptose	10 g
Bacto Beef Extract	5 g
Bacto Yeast Extract	5 g
Sodium Chloride	20 g
Sodium Phosphate, Dibasic	9.6 g
Potassium Phosphate, Monobasic	1.35 g
Esculin	1 g
Nalidixic Acid	0.02 g
Acriflavine HCl	0.012 g

Final pH 7.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store the prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

UVM Modified Listeria Enrichment Broth

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Test tubes with closures
Autoclave
Incubator (30°C)
Incubator (35°C)

Method of Preparation

1. Suspend 52 grams in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.

Specimen Collection and Preparation

1. Collect specimens or food samples in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[7,10,11,12]
2. Clinical specimens obtained from nonsterile sites, foods and specimens obtained from the environment should be selectively enriched for *Listeria* species before being plated.[10]
3. Process each specimen using procedures appropriate for that specimen or sample.[7,10,11,12]

Test Procedure

The USDA method[11] involves enrichment of the specimen in UVM Modified Listeria Enrichment Broth (one part sample in nine parts broth) at 30°C. After incubation, a portion of the enrichment mixture is added to an enrichment broth or plated onto the final isolation agar.[7] For further information when testing food samples or clinical specimens, refer to appropriate references.[7,10,11,12]

Results

Refer to appropriate references and procedures for results.

References

1. **Murray, E. G. D., R. A. Webb, and M. B. R. Swann.** 1926. A disease of rabbits characterized by large mononuclear leucocytosis caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n. sp.). J. Path. Bact. **29:**407-439.
2. **Monk, J. D., R. S. Clavero, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1994. Irradiation inactivation of *Listeria monocytogenes* and *Staphylococcus aureus* in low-and high-fat, frozen and refrigerated ground beef. J. Food Prot. **57**:969-974.
3. **Wehr, H. M.** 1987. *Listeria monocytogenes* - a current dilemma special report. J. Assoc. Off. Anal. Chem. **70**:769-772.
4. **Bremer, P. J., and C. M. Osborne.** 1995. Thermal-death times of *Listeria monocytogenes* in green shell mussels (*Perna canaliculus*) prepared for hot smoking. J. Food Prot. **58**:604-608.
5. **Grau, F. H., and P. B. Vanderlinde.** 1992. Occurrence, numbers, and growth of *Listeria monocytogenes* on some vacuum-packaged processed meats. J. Food Prot. **55**:4-7.
6. **Patel, J. R., C. A. Hwang, L. R. Beuchat, M. P. Doyle, and R. E. Brackett.** 1995. Comparison of oxygen scavengers for their ability to enhance resuscitation of heat-injured *Listeria monocytogenes*. J. Food Prot. **58**:244-250.
7. **Donnelly, C. W., R. E. Bracket, D. Doores, W. H. Lee, and J. Lovett.** 1992. *Listeria*, p. 637-663. *In* C. Vanderzant, and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
8. **Kramer, P. A., and D. Jones**. 1969. Media selective for *Listeria monocytogenes*. J. Appl. Bacteriol. **32:**381-394.
9. **Donnelly, C. W., and G. J. Baigent.** 1986. Method for flow cytometric detection of *Listeria monocytogenes* in milk. Appl. Environ. Microbiol. **52:**689-695.
10. **Swaminathan, B., J. Rocourt, and J. Bille.** 1995. *Listeria*. *In* P. R. Murray, *et al*. (ed), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
11. **Lee, W. H., and D. McClain.** 1989. *Laboratory Communication No. 57* (revised May 24, 1989). U.S.D.A., F.S.I.S. Microbiology Division, Bethesda, MD.
12. **Hayes, P. S., L. M. Graves, B. Swaminathan, G. W. Ajello, G. B. Marcolm, R. E. Weaver, R. Ransom, K. Deaver, B. D. Plikaytis, A. Schuchat, J. D. Wenger, R. W. Pinner, C. V. Broome, and The *Listeria* Study Group.** 1992. Comparison of three selective enrichment methods for the isolation of *Listeria monocytogenes* from naturally contaminated foods. J. Food. Prot. **55:**952-959.

Packaging

UVM Modified Listeria Enrichment Broth	500 g	0223-17
	2 kg	0223-07
	10 kg	0223-08

Bacto® Universal Preenrichment Broth

Intended Use

Bacto Universal Preenrichment Broth is used for recovering sub-lethally injured *Salmonella* and *Listeria* from food products.

Summary and Explanation

Traditional methods for recovering *Salmonella* and *Listeria* from food products require separate preenrichment media for each microorganism.[1, 2]

Some broth media recommended for preenrichment contain antibiotic inhibitors[3] or have insufficient buffering capacity which hinder recovery of sublethally injured cells.[3, 4, 5]

Bailey and Cox[3] formulated Universal Preenrichment Broth to permit simultaneous resuscitation of sublethally injured *Salmonella* and *Listeria*. The broth medium provides sufficient buffering capacity to prevent rapid decreases in pH and allows for repair of injured cells that might be sensitive to low pH values or inhibitory substances.

Principles of the Procedure

Universal Preenrichment Broth contains Tryptone and Proteose Peptone as sources of carbon, nitrogen, vitamins and minerals. Sodium and Potassium Phosphates buffer the medium. Sodium Chloride, Magnesium Sulfate and Ferric Ammonium Citrate provide essential ions. Dextrose is an energy source. Sodium Pyruvate helps stimulate the metabolism of stressed organisms.

Formula

Universal Preenrichment Broth

Formula Per Liter	
Bacto Tryptone	5 g
Bacto Proteose Peptone	5 g
Potassium Phosphate Monobasic	15 g
Sodium Phosphate Dibasic	7 g
Sodium Chloride	5 g
Bacto Dextrose	0.5 g
Magnesium Sulfate	0.25 g
Ferric Ammonium Citrate	0.1 g
Sodium Pyruvate	0.2 g

Final pH 6.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Universal Preenrichment Broth

User Quality Control

Identity Specifications

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.8% solution, soluble in distilled or deionized water on warming. Solution is light to medium amber, slightly opalescent to opalescent, may have precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent to opalescent, may have precipitate.
Reaction of 3.8% Solution at 25°C:	pH 6.3 ± 0.2

Cultural Response

Prepare Universal Preenrichment Broth per label directions. Inoculate and incubate the tubes at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Listeria monocytogenes	19115	10-100	good to excellent
Salmonella enteritidis	13076	10-100	good to excellent
Salmonella typhimurium	14028*	10-100	good to excellent

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Uninoculated tube

Salmonella typhimurium ATCC® 14028

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (35°C)
Sterile tubes with closures

Method of Preparation

1. Suspend 38 grams in 1 liter distilled or deionized water.
2. Heat gently to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to room temperature.
4. Store prepared medium at 2-8°C.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Substitute Universal Preenrichment Broth for preenrichment media as specified for *Salmonella* and *Listeria* [1,2] and follow recommended procedures.

Results

Salmonella and *Listeria* demonstrate good growth and recovery following preenrichment in this broth.

References

1. **Vanderzant, C., and D.F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
2. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
3. **Bailey, J. S., and N. A. Cox.** 1992. Universal preenrichment broth for the simultaneous detection of *Salmonella* and *Listeria* in foods. J. Food Protect. **55**:256-259.
4. **Bailey, J. S., D. L. Fletcher, and N. A. Cox.** 1990. Efficacy of enrichment media for recovery of heat-injured *Listeria monocytogenes*. J. Food Prot. **53**:473-477.
5. **Juven, B. J., N. A. Cox, J. S. Bailey, J. E. Thomson, O. W. Charles, and J. V. Shutze.** 1984. Recovery of *Salmonella* from artificially contaminated poultry feed in non-selective and selective broth media. J. Food Prot. **47**:299-302.

Packaging

Universal Preenrichment Broth	500 g	0235-17

Bacto® Urea Agar Base · Bacto Urea Agar Base Concentrate Bacto Urea Broth · Bacto Urea Broth Concentrate

User Quality Control

Identity Specifications

Urea Agar Base

Dehydrated Appearance:	Light orange-red to orange-red, homogeneous, inherently lumpy.
Solution:	2.9% solution, soluble in distilled or deionized water. Solution is orange, clear.
Prepared Agar Medium:	Reddish orange, very slightly opalescent.
Reaction of 2.9% Solution at 25°C:	pH 6.8 ± 0.1

Urea Agar Base Concentrate

Solution:	29% solution is reddish orange, clear liquid.
Reaction of 29% Solution at 25°C:	pH 6.75 ± 0.15

Urea Broth

Dehydrated Appearance:	Light orange to light pink, homogeneous, inherently lumpy.
Solution:	3.87% solution, soluble in distilled or deionized water. Solution is orange-yellow, clear.
Reaction of 3.87% Solution at 25°C:	pH 6.8 ± 0.1

continued on following page

Uninoculated tube | *Proteus vulgaris* ATCC® 13315 | *Escherichia coli* ATCC® 25922

Intended Use

Bacto Urea Agar Base, when combined with Bacto Agar, is used for differentiating microorganisms based on urease activity.

Bacto Urea Agar Base Concentrate is a sterile 10X solution of Urea Agar Base which, when combined with Bacto Agar, is used for preparing Urea Agar.

Bacto Urea Broth is used for differentiating microorganisms, particularly *Proteus* species, based on urease production.

Bacto Urea Broth Concentrate is a sterile 10X solution of Urea Broth ready to use as recommended. It is suggested for laboratories that require only small amounts of medium.

Also Known As

Urea Agar Base is also known as Urea Agar Base, Christensen or Christensen's Urea Agar.

Urea Broth is also referred to as Stuart's Urea Broth.

Summary and Explanation

Christensen[1] devised a urea agar medium containing peptone and dextrose that had a reduced buffer content. The medium supported a more vigorous growth of many of the gram-negative enteric bacilli and readily permitted observation of urease production.

Ewing[2] used Urea Agar as a differential medium in the examination of many cultures from stool specimens. Urea Agar may be used as a screening medium (along with Triple Sugar Iron Agar) for the selection of *Salmonella* and *Shigella* cultures for serologic classification.[3] Qadri et al.[4] developed a spot test for the rapid detection of urease activity by applying diluted Urea Agar Base Concentrate to filter paper and inoculating the paper with a loopful of 24-48 hour culture. Urease-positive results were obtained within 2 minutes. When combined with results of other rapid screening methods, Urea Agar is the most common way to detect the production of urease by yeasts.[5] Urea Agar Base Concentrate has also been used in differentiating mycobacteria species.[6]

Urea Broth, prepared according to the formula of Stuart, Van Stratum and Rustigian[7] is a highly buffered urea medium that provides all the essential growth requirements for *Proteus*. Stuart et al.[7] noted that by decreasing the amount of buffer in their standard medium to one-tenth or one-hundredth of the original concentration, the incubation time for *Proteus* could be decreased from 12-48 hours to 2-4 hours. When the amount of buffer is decreased, however, other organisms capable of urease production give a positive test. Rustigian and Stuart[8] used urea decomposition as a limiting characteristic for the identification of *Proteus* strains from other members of the family *Enterobacteriaceae*. Ferguson and Hook[9] reported that urease production could be used to differentiate between members of the *Proteus* and *Salmonella* groups. The medium is positive for *Proteus*, *Morganella morganii*, *Providencia rettgeri* and a few *Providencia stuartii* strains.

The detection of urease production is an important differential test in microbiology and is outlined in standard references.[10-16]

Principles of the Procedure

Bacto Peptone provides carbon and nitrogen required for good growth of a wide variety of organisms. Yeast Extract provides vitamins and cofactors required for growth and as an additional source of nitrogen and carbon. Dextrose is included as an energy source. Sodium Chloride maintains the osmotic balance of the medium. Potassium Phosphate, Monobasic and Potassium Phosphate, Dibasic provide buffering capability. Urea provides a source of nitrogen for those organisms producing urease. This is indicated by a color change of the pH indicator, Phenol Red, from yellow (pH 6.8) to red to pink-red (pH 8.1).

User Quality Control cont.

Urea Broth Concentrate

Appearance:	38.7% solution is reddish-orange, clear liquid.
Reaction of 38.7% Solution at 25°C:	pH 6.8 ± 0.2

Cultural Response

Urea Agar Base and Urea Agar Base Concentrate

Prepare Urea Agar per label directions. Inoculate and incubate at 35 ± 2°C for 6-48 hours.

Urea Broth and Urea Broth Concentrate

Prepare Urea Broth per label directions. Inoculate and incubate at 35 ± 2°C for 8-48 hours.

ORGANISM	ATCC®	UREASE PRODUCTION
Escherichia coli	25922*	negative, no color change in the medium
Proteus vulgaris	13315*	positive, red or cerise medium

The cultures listed are the minimum that should be used for performance testing.

Uninoculated tube | *Proteus vulgaris* ATCC® 13315 | *Escherichia coli* ATCC® 25922

Urea Broth

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Formula

Urea Agar Base

Formula Per Liter

Bacto Peptone	1 g
Bacto Dextrose	1 g
Sodium Chloride	5 g
Potassium Phosphate, Monobasic	2 g
Urea	20 g
Bacto Phenol Red	0.012 g

Final pH 6.8 ± 0.1 at 25°C

Urea Agar Base Concentrate

A liquid, 10X concentrate of Urea Agar Base.

Urea Broth

Formula Per Liter

Bacto Yeast Extract	0.1 g
Potassium Phosphate, Monobasic	9.1 g
Potassium Phosphate, Dibasic	9.5 g
Bacto Urea	20 g
Bacto Phenol Red	0.01 g

Final pH 6.8 ± 0.1 at 25°C

Urea Broth Concentrate

A liquid, 10X concentrate of Urea Broth.

Precautions

1. For Laboratory Use.
2. **Urea Broth**: **IRRITANT.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated media at 2-8°C. The dehydrated media are very hygroscopic. Keep containers tightly closed. Store the prepared media also at 2-8°C.

Store Urea Agar Base Concentrate and Urea Broth Concentrate at 2-8°C.

Expiration Date

The expiration date applies to the products in their intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Urea Agar Base
Urea Agar Base Concentrate
Urea Broth
Urea Broth Concentrate

Materials Required But Not Provided

Glassware
Autoclave
Refrigerator (2-8°C)
Waterbath (50-55°C) (optional)
Incubator (35°C)
Bacto Agar
Filter sterilization apparatus

Method of Preparation

Urea Agar Base

Equilibrate this medium to room temperature before opening.

The presence of urea in this medium renders it inherently lumpy. This condition will not adversely affect a properly stored medium.

1. Dissolve 29 grams in 100 ml distilled or deionized water.
2. Filter sterilize. DO NOT BOIL OR AUTOCLAVE.
3. Suspend 15 grams of Bacto Agar in 900 ml distilled or deionized water.
4. Boil to dissolve completely.
5. Autoclave at 121°C for 15 minutes.
6. Cool to 50-55°C.
7. Aseptically add 100 ml of the filter sterilized Urea Agar Base to the cooled Bacto Agar. Mix thoroughly. DO NOT HEAT THE COMPLETE MEDIUM.
8. Distribute in sterile test tubes. Slant the tubes to have a butt about 2 cm in depth and a slant about 3 cm in length.

Urea Agar Base Concentrate

If crystals have formed in the concentrate prior to preparing the final medium, place the tube(s) in a water bath at 40-50°C for a few moments. Agitate to dissolve the crystals.

1. Suspend 1.5 grams of Bacto Agar in 90 ml distilled or deionized water.
2. Boil to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 50-55°C. Aseptically add 10 ml of Urea Agar Base Concentrate.
5. Mix thoroughly; dispense into tubes and slant.

Urea Broth

Equilibrate this medium to room temperature before opening.

The presence of urea in this medium renders it inherently lumpy. This condition will not adversely affect a properly stored medium.

1. Dissolve 38.7 grams in 1 liter distilled or deionized water. Mix thoroughly to dissolve completely.
2. Filter sterilize. DO NOT BOIL OR AUTOCLAVE THE MEDIUM.
3. Aseptically distribute 3 ml amounts into sterile test tubes (14 x 125 mm or equivalent).

Urea Broth Concentrate

If crystals have formed in the concentrate prior to preparing the final medium, place the tube(s) in a water bath at 40-50°C for a few moments. Agitate to dissolve the crystals.

Do no heat Urea Broth above 50°C during preparation or sterilization.

1. To prepare 100 ml of final medium, sterilize 90 ml of distilled or deionized water at 121-124°C for 15 minutes.
2. Cool to 50-55°C. Aseptically add 10 ml of Urea Broth Concentrate. Mix thoroughly.
3. Distribute 3 ml amounts into sterile test tubes (14 x 125 mm).

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Urea Agar

1. Use a heavy inoculum of growth from a pure 18-24 hour culture. Inoculate by streaking back and forth over the entire slant surface. Do not stab the butt because it serves as a color control.
2. Incubate tubes with loosened caps at 35 ± 2°C.
3. Observe reactions after 6 and 24 hours and every day thereafter for a total of 6 days.[1] Longer periods of incubation may be necessary.

Urea Broth

1. Inoculate with a heavy inoculum, using a straight needle or a drop from an 18-24 hour culture. Shake tube gently to resuspend the bacteria.
2. Incubate aerobically at 35 ± 2°C.
3. Record reactions after 8, 12, 24 and 48 hours of incubation.

Results

Urea Agar

Positive: The production of urease is indicated by an intense red or cerise color on the slant which may penetrate into the butt.

Negative: No color change of the medium.

Urea Broth

Positive: The production of urease is indicated by an intense red or cerise color throughout the broth.

Negative: No color change of the broth.

Limitations of the Procedure

Urea Agar Base

1. The alkaline reaction produced in this medium after prolonged incubation may not be caused by urease activity. False positive reactions may occur due to the utilization of peptones (especially in slant agar by *Pseudomonas aeruginosa*, for example) or other proteins which raise the pH due to protein hydrolysis and the release of excessive amino acid residues. To eliminate possible protein hydrolysis, perform a control test with the same test medium without urea.[17]
2. Do not heat or reheat the medium because urea decomposes very easily.
3. Urea Agar detects rapid urease activity of only the urease-positive *Proteus* species. For results to be valid for the detection of *Proteus*, the results must be read within the first 2 to 6 hours after incubation. Urease-positive *Enterobacter, Citrobacter* or *Klebsiella*, in contrast, hydrolyze urea much more slowly, showing only slight penetration of the alkaline reaction into the butt of the medium in 6 hours and requiring 3 to 5 days to change the reaction of the entire butt.

Urea Broth

1. To rule out false positives due to protein hydrolysis (as opposed to urea hydrolysis) that may occur in the medium after prolonged incubation, perform a control test with the same test medium without urea.[17]
2. Do not heat or reheat the medium because urea decomposes very easily.
3. The high buffering system in this medium masks urease activity in organisms that are delayed positive. This medium is therefore recommended for the detection of urease activity in all *Proteus* spp., *Providencia rettgeri* and urease- positive *Providencia stuartii*.[1] *M. morganii* slowly hydrolyzes urea and may require approximately a 36 hour incubation for a strong urease-positive reaction to occur.[1] If in doubt as to a result, compare with an uninoculated tube or incubate for an additional 24 hours.
4. Variations in the size of the inoculum can affect the time required to reach positive (alkaline, pH 8.1) results. The accepted standard inoculum is 0.1 ml.[1]

References

1. **Christensen, W. B.** 1946. Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. J. Bacteriol. **52**:461.
2. **Ewing, W. H.** 1946. An additional *Shigella paradysenteriae* serotype. J. Bacteriol. **51**:433-445.
3. **Ewing, W. H., and D. W. Bruner.** 1947. Selection of *Salmonella* and *Shigella* cultures for serologic classification. Am. J. Clin. Path. **17**:1-12.
4. **Qadri, S. M. Hussain, S. Zubairi, H. P. Hawley, and E. G. Ramirez.** 1984. Simple spot test for rapid detection of urease activity. J. Clin. Microbiol. **20(6)**:1198-1199.
5. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's Diagnostic Microbiology, 9th edition. Mosby-Year Book, Inc., St. Louis, MO.
6. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology - A guide for the level III laboratory. U.S. Public Health Service, Atlanta, GA.
7. **Stuart, C. A., E. Van Stratum, and R. Rustigian.** 1945. Further studies on urease production by *Proteus* and related organisms. J. Bacteriol. **49**:437.
8. **Rustigian, R., and C. A. Stuart.** 1941. Decomposition of urea by *Proteus*. Proc. Soc. Exptl. Biol. Med. **47**:108-112.
9. **Ferguson, W. W., and A. E. Hook.** 1943. Urease activity of *Proteus* and *Salmonella* organisms. J. Lab. Clin. Med. **28**:1715-1719.
10. **Vanderzant, C., and D. F. Splittstoesser.** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Assoc., Washington, D.C.
11. **Marshall, R. T. (ed.)** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Assoc., Washington, D.C.
12. **Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley, and S. T. Williams.** 1994. Bergey's manual of determinative bacteriology, 9th edition. Williams & Wilkins, Baltimore, MD.

13. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken.** 1995. Manual of clinical microbiology, 6th ed. ASM Press, Washington, D.C.
14. **Bacteriological Analytical Manual,** 8th ed. 1995. AOAC International, Gaithersburg, MD.
15. **Oberhofer, T. R.** 1985. Manual of nonfermenting gram-negative bacteria. Churchill Livingstone, New York, NY.
16. **Ewing, W. H.** 1986. Edwards and Ewing's Identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., Inc., New York, NY.
17. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria. Williams & Wilkins, Baltimore, MD.

Packaging

Urea Agar Base	100 g	0283-15
	500 g	0283-17
Urea Agar Base Concentrate 10X	12 x 10 ml	0284-61
Urea Broth	500 g	0272-17
Urea Broth Concentrate	12 x 10 ml	0280-61

Bacto® VJ Agar

Intended Use

Bacto VJ Agar is used with Bacto Chapman Tellurite Solution 1% for isolating coagulase-positive, mannitol-fermenting staphylococci.

Also Known As

VJ Agar is also known as Vogel and Johnson Agar, Modification of Tellurite-Glycine Agar[1], and Tellurite-Glycine-Phenol Red Agar Base[2]

Summary and Explanation

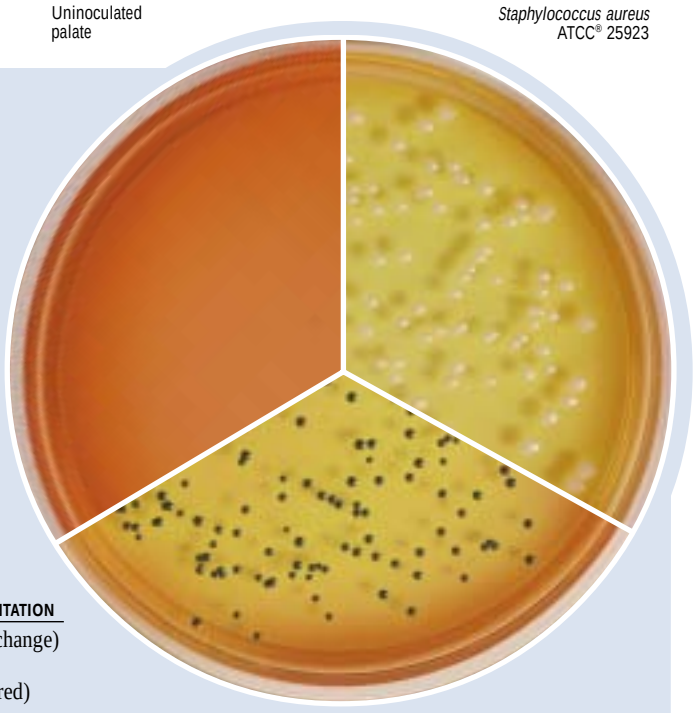
Coagulase-positive staphylococci, primarily *Staphylococcus aureus*, are among the microorganisms that can cause spoilage or chemical changes in cosmetic products.[4]

To isolate coagulase-positive, mannitol fermenting staphylococci, Vogel and Johnson[3] modified Tellurite-Glycine Agar by Zebovitz et al.[1] by increasing the mannitol content and adding a pH indicator. Vogel-Johnson (VJ) Agar selects and differentiates the coagulase-positive staphylococci which ferment mannitol and reduce tellurite.[2]

VJ Agar is specified as a standard methods medium for cosmetics,[4,5] pharmaceutical articles[6] and nutritional supplements.[6]

Principles of the Procedure

VJ Agar contains Tryptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Mannitol is the carbohydrate. Chapman



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pink, homogenous, free-flowing.
Solution:	6.0% solution, soluble in distilled or deionized water on boiling. Solution is red, slightly opalescent with a white precipitate.
Prepared Medium:	Red, slightly opalescent, may have slight white precipitate.
Reaction of 6.0% Solution at 25°C:	pH 7.2 ± 0.1

Cultural Response

Prepare VJ Agar per label directions with the addition of Chapman Tellurite Solution, 1%. Inoculate and incubate the plates at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	TELLURITE GROWTH	MANNITOL REDUCTION	MANNITOL FERMENTATION
Escherichia coli	25922*	marked to complete inhibition	– (no change)	– (no change)
Proteus mirabilis	25933	partial to complete inhibition	+ (black)	– (red)
Staphylococcus aureus	25923*	good	+ (black)	+ (yellow)
Staphylococcus epidermidis	12228*	none to fair	± (translucent to black)	– (red)

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Tellurite Solution 1% contains Potassium Tellurite which, along with Lithium Chloride and Glycine, inhibits most microorganisms except the staphylococci. Phenol Red is the pH indicator. Bacto Agar is the solidifying agent.

Formula

Bacto VJ Agar

Formula Per Liter

Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
Bacto Mannitol	10 g
Dipotassium Phosphate	5 g
Lithium Chloride	5 g
Glycine	10 g
Bacto Agar	15 g
Bacto Phenol Red	0.025 g

Final pH 7.2 ± 0.1 at 25°C

Precautions

1. For Laboratory Use.
2. **HARMFUL.** IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Kidneys, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated VJ Agar below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

VJ Agar

Materials Required but not Provided

Chapman Tellurite Solution 1%
Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

VJ Agar

1. Suspend 60 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Add 20 ml Chapman Tellurite Solution 1%. Mix well.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Coagulase-positive strains of *S. aureus* reduce tellurite and form black colonies on the medium. These strains typically ferment mannitol and exhibit yellow halos around the black colonies.

References

1. **Zebovitz, E., J. B. Evans, and C. F. Niven, Jr**. 1955. Tellurite-Glycine Agar: a selective plating medium for the quantitative detection of coagulase-positive staphylococci. J. Bacteriol. **70**:686.
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6. **United States Pharmacopeial Convention**. 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention. Rockville, MD.

Packaging

VJ Agar	100 g	0562-15
	500 g	0562-17

Bacto® Veal Infusion Agar
Bacto Veal Infusion Broth

Intended Use

Bacto Veal Infusion Agar is used for cultivating fastidious microorganisms with or without added enrichment.

Bacto Veal Infusion Broth is used for cultivating fastidious microorganisms.

Summary and Explanation

The nutritive factors of Veal Infusion media permit luxuriant growth of fastidious microorganisms. Veal Infusion Agar may be used as a base with blood, ascitic fluid, serum or other enrichments. Veal Infusion media are specified for use in the examination of food.[1,2] Veal Infusion Agar is specified in AOAC Official Methods of Analysis for culturing eggs and egg products, and as a maintenance medium for *E. coli*.[3] Veal Infusion Broth is recommended for culturing *E. coli* in the AOAC procedure for invasiveness of mammalian cells.[3]

User Quality Control

Identity Specifications

Veal Infusion Agar

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	4.0% solution, soluble in distilled or deionized water on boiling. Light to medium amber, very slightly to slightly opalescent without significant precipitate.
Prepared Medium:	Light to medium amber, slightly opalescent without precipitate.
Reaction of 4.0% Solution at 25°C:	pH 7.4 ± 0.2

Veal Infusion Broth

Dehydrated Appearance:	Very light beige, free-flowing, homogeneous.
Solution:	2.5% solution, soluble in distilled or deionized water, very light amber, clear to very slightly opalescent.
Prepared Medium:	Very light amber, clear to very slightly opalescent with no more than very slight precipitation.
Reaction of 2.5% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Veal Infusion Agar per label directions with and without 5% sterile defibrinated sheep blood. Inoculate medium with the test organisms. Incubate inoculated plates at 35 ± 2°C for 18-48 hours under approximately 10% CO_2.

Prepare Veal Infusion Broth per label directions. Inoculate tubes with the test organisms. Incubate inoculated tubes at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090*	100-1,000	good
Staphylococcus epidermidis	12228*	100-1,000	good
Streptococcus mitis	9895	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Infusion from Lean Veal and Proteose Peptone No. 3 provides the nitrogen, vitamins, carbon and amino acids in Veal Infusion media. Sodium Chloride maintains the osmotic balance of the formulations. Bacto Agar is the solidifying agent in Veal Infusion Agar.

Formula

Veal Infusion Agar

Formula Per Liter

Lean Veal, Infusion from	500 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	5 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C

Veal Infusion Broth

Formula Per Liter

Lean Veal, Infusion from	500 g
Bacto Proteose Peptone No. 3	10 g
Sodium Chloride	5 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Veal Infusion Agar
Veal Infusion Broth

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile defibrinated blood (optional)
Sterile Petri dishes
Sterile tubes with closures

Method of Preparation

1. Suspend the appropriate amount of medium in 1 liter distilled or deionized water:
   Veal Infusion Agar 40 g/l
   Veal Infusion Broth 25 g/l
2. Heat to boiling to dissolve completely (Veal Infusion Agar).
3. Autoclave at 121°C for 15 minutes.

4. OPTIONAL: To prepare blood agar, aseptically add 5% sterile defibrinated blood to the medium at 45-50°C. Mix well.
5. Dispense as desired.

Specimen Collection and Preparation

Obtain and process specimens according to the procedures established by laboratory policy.

Test Procedure

For a complete discussion on the examination of fastidious microorganisms in food refer to the procedures outlined in the references.[1,2,3]

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
2. **Vanderzant, C., and D. F Splittstoesser. (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
3. **Association of Official Analytical Chemists.** 1995. Official methods of analysis of AOAC International, 16th ed. AOAC International, Arlington, VA.

Packaging

Veal Infusion Agar	500 g	0343-17
Veal Infusion Broth	500 g	0344-17
	10 kg	0344-08

Bacto® Veillonella Agar

Intended Use

Bacto Veillonella Agar is used with added vancomycin in isolating *Veillonella*.

Summary and Explanation

Veillonella Agar is prepared according to the formula described by Rogosa[1,2] as modified by Rogosa, Fitzgerald, MacKintosh and Beaman.[3] Rogosa's[2] experiments with oral specimens from humans and rats demonstrated the medium to be highly selective for *Veillonella* species. Streptomycin was originally employed as the selective agent. Later, Rogosa et al.[3] demonstrated vancomycin to be superior to streptomycin in reducing growth of extraneous organisms without restricting growth of *Veillonella*.

Veillonella parvula is part of the normal human fecal flora.[4] *V. parvula*, *V. atypica* and *V. dispar* are flora colonizing the oral cavity.[4] *Veillonella* species have been encountered in patients with bite wound, head, neck, oral and miscellaneous soft tissue infections.[5] *Veillonella* species are anaerobic gram negative diplococci and appear as clumps of diplococci when stained.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous with small dark particles.
Solution:	3.6% solution, soluble in distilled or deionized water on boiling.
Prepared Medium:	Pink, slightly opalescent without precipitate.
Reaction of 3.6% Solution at 25°C	pH 7.5 ± 0.2

Cultural Response

Prepare Veillonella Agar per label directions. Using the pour plate technique, inoculate plates with 1 ml of the diluted test organisms and 1 ml of the specimen. Pour 20 ml medium per plate, mix well. Incubate plates at 35 ± 2°C anaerobically for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Veillonella criceti	17747	100-1,000	good
Veillonella dispar	17748	100-1,000	good
Veillonella ratti	17746	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	inhibited

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Tryptone and Yeast Extract provide the nitrogen, vitamins, amino acids and carbon in Veillonella Agar. Sodium Thioglycollate is a reducing agent, and lowers the oxidation-reduction potential of the medium by removing oxygen to maintain a low pH. Basic Fuchsin and Vancomycin are the selective agents. Sodium Lactate, 60% provides nutrients and selective properties. Bacto Agar is the solidifying agent.

Formula

Veillonella Agar

Formula Per Liter

Bacto Tryptone	5 g
Bacto Yeast Extract	3 g
Sodium Thioglycollate	0.75 g
Bacto Basic Fuchsin	0.002 g
Sodium Lactate, 60%	21 ml
Bacto Agar	15 g

Final pH 7.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Veillonella Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator (35°C)
Waterbath (45-50°C) (optional)
Sterile Petri dishes
Tween® 80 (optional)
Vancomycin

Method of Preparation

1. Suspend 36 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. OPTIONAL: Add 1 gram Tween® 80, if desired.
4. Autoclave at 121°C for 15 minutes.
5. Add 7.5 mcg vancomycin per ml of sterile medium at 50-55°C. Mix thoroughly.

Specimen Collection and Preparation

Anaerobic bacteria are overlooked or missed unless the specimen is properly collected and transported to the laboratory.[6] Obtain and process specimens according to the techniques and procedures established by institutional policy.

Test Procedure

1. Rogosa[1,2,3] recommends that one ml of the diluted specimen be added to a sterile Petri dish.
2. Pour approximately 20 ml of medium to the Petri dish, and rotate to mix well with the inoculum.
3. Incubate plates anaerobically at 35 ± 2°C for 40-48 hours; 72 hours if necessary.

For a complete discussion on *Veillonella* species from clinical specimens, refer to the appropriate procedures outlined in the references.[4,6,7] For the examination of anaerobic bacteria in food refer to standard methods.[8, 9, 10]

Limitations of the Procedure

1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.
2. Clinical specimens must be obtained properly and transported to the laboratory in a suitable anaerobic transport container.[6]
3. The microbiologist must be able to verify quality control of the medium and determine whether the environment is anaerobic.[6]
4. The microbiologist must perform aerotolerance testing on each isolate recovered to ensure the organism is an anaerobe.[6]

References

1. **Rogosa, M.** 1955. Nutrition of the *Vellonella*. J. Dent. Res. **34**:721-722.
2. **Rogosa, M.** 1956. A selective medium for the isolation and enumeration of the *Veillonella* from the oral cavity. J. Bacteriol. **72**:533-536.
3. **Rogosa, M., R. J. Fitzgerald, M. E. MacKintosh, and A. J. Beaman.** 1958. Improved medium for selective isolation of *Veillonella*. J. Bacteriol. **76**:455-456.
4. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
5. **Summanen, P., E. J. Baron, D. M. Citron, C. Strong, H. M. Wexler, and S. M. Finegold.** 1993. Wadsworth anaerobic bacteriology manual, 5th ed. Star Publishing Co., Belmont, CA.
6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
7. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Etiological agents recovered from clinical material, p. 474-503. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
8. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
9. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
10. **Marshall, R. T. (ed.).** 1992. Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington. D.C.

Packaging

Veillonella Agar	500 g	0917-17

Bacto® Violet Red Bile Agar

Intended Use

Bacto Violet Red Bile Agar is used for enumerating coliform organisms in dairy products.

Also Known As

Violet Red Bile Lactose Agar

Summary and Explanation

The coliform group of bacteria includes aerobic and facultatively anaerobic gram-negative non-sporeforming bacilli that ferment lactose and form acid and gas at 35°C within 48 hours. Members of the *Enterobacteriaceae* comprise the majority of the group but other lactose fermenting organisms may also be included.

Procedures to detect, enumerate and presumptively identify coliforms are used in testing foods and dairy products.[1,2,3] One method for performing the presumptive test for coliforms uses Violet Red Bile Agar (VRBA). If typical coliform colonies appear, they are tested further to confirm their identification as coliforms.

Principles of the Procedure

Violet Red Bile Agar (VRBA) contains Bacto Peptone to provide carbon and nitrogen sources for general growth requirements. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Bile Salts No. 3 and Crystal Violet inhibit most gram-positive microorganisms. Lactose is the carbohydrate source and Neutral Red is the pH indicator. Bacto Agar is the solidifying agent.

Formula

Violet Red Bile Agar

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Peptone	7 g
Bacto Bile Salts No. 3	1.5 g
Bacto Lactose	10 g
Sodium Chloride	5 g
Bacto Agar	15 g
Neutral Red	0.03 g
Bacto Crystal Violet	0.002 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Violet Red Bile Agar

Materials Required but not Provided

Flask with closure
Distilled or deionized water
Autoclave
Incubator (35°C or 32°C for dairy products)

Method of Preparation

1. Suspend 41.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. Do not boil for more than 2 minutes. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

Presumptive test for coliforms using solid medium:

1. Transfer a 1 ml aliquot of test sample to a Petri dish.
2. Add 10 ml of Violet Red Bile Agar (at 48°C) and swirl to mix.
3. Allow medium to solidify before incubating at 35°C for 18 to 24 hours; use 32°C for dairy products.
4. Examine for purple-red colonies, 0.5 mm in diameter (or larger), surrounded by a zone of precipitated bile acids.
5. Continue with confirmatory testing of typical coliform colonies.[1,2,3]

User Quality Control

Identity Specifications

Dehydrated Appearance:	Reddish-beige, homogeneous, free-flowing.
Solution:	4.15% solution, reddish-purple, very slightly to slightly opalescent.
Prepared plates:	Reddish-purple, slightly opalescent.
Reaction of 4.15% solution at 25°C:	7.4 ± 0.2

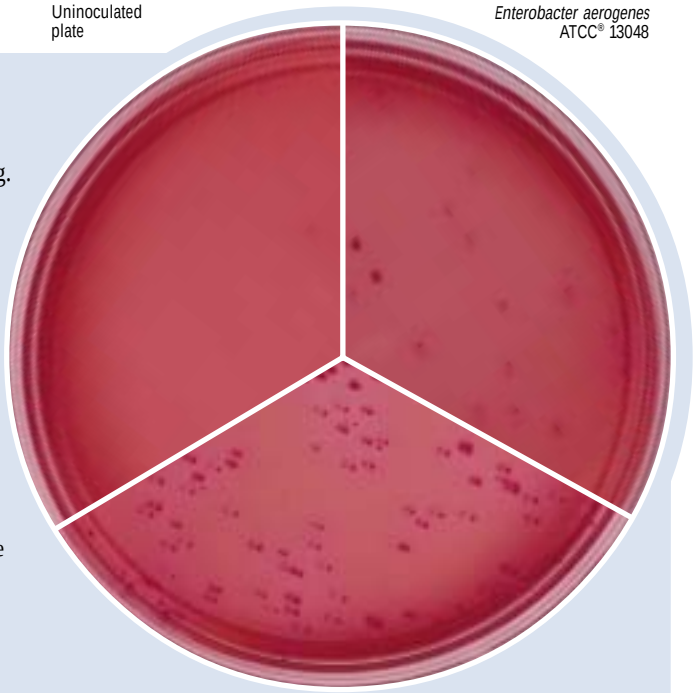
Cultural Response

Prepare Violet Red Bile Agar per label directions. Inoculate and incubate the plates at 32 ± 1°C for 24 ± 2 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR
Enterobacter aerogenes	13048*	30-300	good	red, may have slight red precipitate around colonies
Escherichia coli	25922*	30-300	good	deep red with red precipitate around colonies
Staphylococcus aureus	25923*	1,000-2,000	markedly to completely inhibited	

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Results

Lactose fermenters: Purple-red colonies, with or without a zone of precipitate around the colonies

Lactose non-fermenters: Colorless to transparent colonies

Gram-positive cocci: Colorless, pinpoint colonies

Limitations of the Procedure

1. Violet Red Bile Agar may not be completely inhibitory to gram-positive organisms. Perform Gram stain and biochemical tests as necessary to identify isolates.
2. The medium will grow gram-negative bacilli other than members of the *Enterobacteriaceae*. Perform biochemical tests to identify isolates to genus and species.
3. Boiling the medium for longer than 2 minutes can decrease the ability to support growth.
4. Plates of Violet Red Bile Agar should not be incubated longer than 24 hours because microorganisms that are only partially inhibited may grow after extended incubation.
5. For optimum performance, prepare and use the medium within 24 hours.

References

1. **Christen, G. L., P. M. Davidson, J. S. McAllister, and L. A. Roth.** 1993. Coliform and other indicator bacteria, p. 247-252. *In* Marshall, R. T. (ed.). Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
2. **Hitchins, A. D., P. A. Hartman, and E. C. D. Todd.** 1992. Coliforms - *Escherichia coli* and its toxins, p. 325-369. *In* Vanderzant, C., and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Hitchins, A. D., P. Feng, W. D. Watkins, S. R. Rippey, and L. A. Chandler.** 1995. *Escherichia coli* and the coliform bacteria, p. 4.01-4.29. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.

Packaging

Violet Red Bile Agar	100 g	0012-15
	500 g	0012-17
	2 kg	0012-07

Bacto® Violet Red Bile Agar with MUG

Intended Use

Bacto Violet Red Bile Agar with MUG is used for enumerating *Escherichia coli* and total coliform bacteria in food and dairy products.

Also Known As

VRBA with MUG

Summary and Explanation

Violet Red Bile Agar is specified in Standard Methods procedures to enumerate coliforms in food and dairy products.[1,2,3] In 1982, Feng and Hartman developed a rapid fluorogenic assay for *Escherichia coli* by incorporating 4-methylumbelliferyl-ß-D-glucuronide (MUG) into Lauryl Tryptose Broth.[4] Incorporating MUG into Violet Red Bile Agar permits the detection of *E. coli* among the coliform colonies.[2, 3]

Standard Methods procedures specify Violet Red Bile Agar with MUG for detecting *E. coli* in food and dairy products by fluorescence.[1,2,3]

Principles of the Procedure

Violet Red Bile Agar contains Bacto Peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Bile Salts No. 3 and Crystal Violet inhibit gram-positive bacteria. Lactose is a carbohydrate source. Neutral Red is a pH indicator. MUG (4-methylumbelliferyl-ß-D-glucuronide) is a substrate used for detecting glucuronidase activity. Bacto Agar is a solidifying agent.

E. coli produces the enzyme glucuronidase which hydrolyzes MUG to yield a fluorogenic compound detectable with long-wave UV light (366 nm). Typical strains of *E. coli* (red colonies surrounded by a bile precipitate) exhibit blue fluorescence. Non-*E. coli* coliforms may produce red colonies with zones of precipitated bile but they are MUG negative.

Formula

Violet Red Bile Agar with MUG

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Peptone	7 g
Bacto Bile Salts No. 3	1.5 g
Bacto Lactose	10 g
Sodium Chloride	5 g
Bacto Agar	15 g
Neutral Red	0.03 g
Bacto Crystal Violet	0.002 g
MUG (4-methylumbelliferyl-ß-D-glucuronide)	0.1 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Violet Red Bile Agar with MUG

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (32°C)
Waterbath (45°C)

Method of Preparation

1. Suspend 41.6 grams in 1 liter distilled or deionized water.
2. Heat to boiling and boil no more than 2 minutes to dissolve completely. DO NOT AUTOCLAVE.
3. Cool to 45°C.
4. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory in accordance with recommended guidelines.[1,2,3]

Test Procedure

1. Process each specimen as appropriate for that specimen.[1,2,3]
2. Incubate plates at 35°C for 22-26 hours.
3. Examine plates for growth and fluorescence.

Results

Coliform organisms form purplish-red colonies that are generally surrounded by a reddish zone of precipitated bile. When examined under long-wave fluorescent light, MUG-positive colonies are surrounded by a bluish fluorescent halo. MUG-negative colonies lack the fluorescent halo.

E. coli colonies are red surrounded by a zone of precipitated bile and fluoresce blue under long-wave UV light.

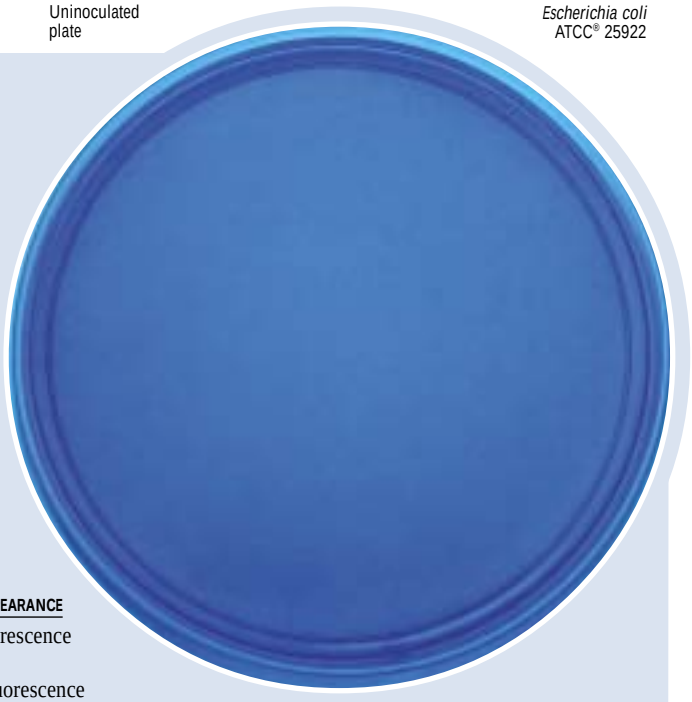
Salmonella and *Shigella* strains that produce glucuronidase may be encountered infrequently but these are generally lactose negative and appear as colorless colonies which may fluoresce.

Limitations of the Procedure

1. Glucuronidase-negative strains of *E. coli* have been encountered.[5,6,7] Similarly, glucuronidase-positive strains of *E. coli* that do not fluoresce have been reported.[8]
2. Strains of *Salmonella* and *Shigella* that produce glucuronidase may infrequently be encountered.[9] These strains must be distinguished from *E. coli* on the basis of other parameters, e. g., gas production, lactose fermentation or growth at 44.5°C.
3. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.

References

1. **Christen, G. L., P. M. Davidson, J. S. McAllister, and L. A. Roth.** 1993. Coliform and other indicator bacteria, p. 247-269. *In* R. T. Marshall (ed.). Standard methods for the microbiological examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.



User Quality Control

Identity Specifications

Dehydrated Appearance:	Reddish beige, free-flowing, homogeneous.
Solution:	4.16% solution, soluble in distilled or deionized water on boiling. Solution is reddish purple, slightly opalescent, without significant precipitate. Very slight surface material may be present.
Prepared Medium:	Reddish purple, very slightly to slightly opalescent, no significant precipitate.
Reaction of 4.16% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Violet Red Bile Agar with MUG per label directions. Inoculate and incubate at 32 ± 2°C for 22-26 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	COLONY COLOR	APPEARANCE
Escherichia coli	25922*	30-300	good	deep red, with a bile ppt.	fluorescence
Enterobacter aerogenes	13048*	30-300	good	pink, may have a bile ppt.	no fluorescence
Staphylococcus aureus	25923*	1,000	marked to complete inhibition	–	–

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

2. **Hitchins, A. D., P. A. Hartman, and E. C. D. Todd.** 1992. Coliforms-*Escherichia coli* and its toxins, p. 325-369. *In* C. Vanderzant and D. F. Splittstoesser (ed.). Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
3. **Hitchins, A. D., P. Feng, W. D. Watkins, S. R. Rippey, and L. A. Chandler.** 1995. *Escherichia coli* and the coliform bacteria, p. 4.01-4.29. *In* Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
4 **Feng, P. C. S., and P. A. Hartman.** 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. **43**:1320-1329.
5. **Chang, G. W., J. Brill, and R. Lum.** 1989. Proportion of ß-D-glucuronidase- negative *Escherichia coli* in human fecal samples. Appl. Environ. Microbiol. **55**:335-339.
6. **Hansen, W., and E. Yourassowsky.** 1984. Detection of ß-glucuronidase in lactose fermenting members of the family Enterobacteriaceae and its presence in bacterial urine cultures. J. Clinical Microbiol. **20**:1177-1179.
7. **Kilian, M., and P. Bulow.** 1976. Rapid diagnosis of Enterobacteriaceae. Acta Pathol. Microbiol. Scand. Sect. B **84**:245-251.
8. **Mates, A., and M. Shaffer.** 1989. Membrane filtration differentiation of *E. coli* from coliforms in the examination of water. J. Appl. Bacteriology **67**:343-346.
9. **Damare, J. M., D. F. Campbell, and R. W. Johnston.** 1985. Simplified direct plating method for enhanced recovery of *Escherichia coli* in food. Journal of Food Science **50**:1736-1746.

Packaging

Violet Red Bile Agar with MUG	500 g	0029-17

Bacto® Violet Red Bile Glucose Agar

Intended Use

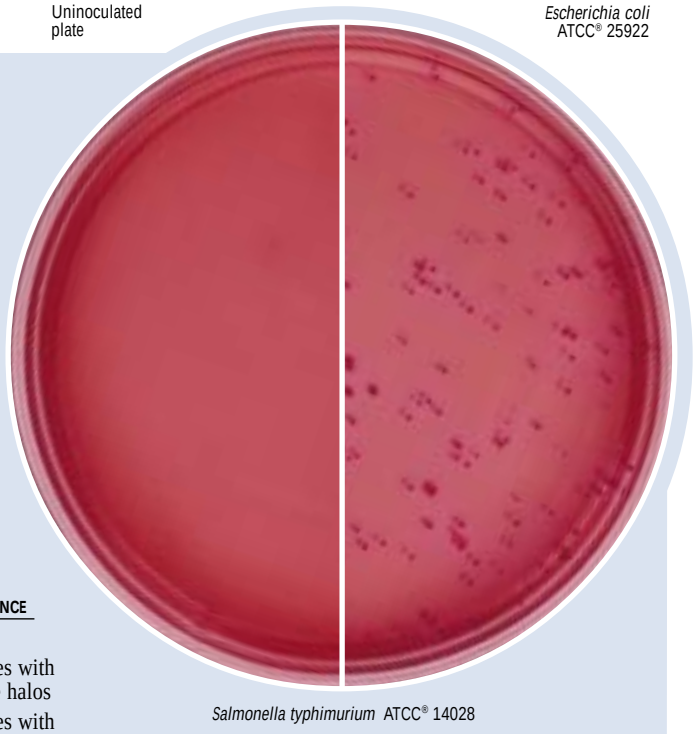
Bacto Violet Red Bile Glucose Agar is used for detecting and enumerating *Enterobacteriaceae* in foods and dairy products.

Also Known As

Violet Red Bile Glucose Agar is also known as VRBGA.

Summary and Explanation

The *Enterobacteriaceae* group includes lactose-fermenting coliform bacteria, nonlactose-fermenting strains of *E. coli*, and nonlactose-fermenting species such as *Salmonella* and *Shigella*. When examining



User Quality Control

Identity Specifications

Dehydrated Appearance:	Pink-beige, free-flowing, homogeneous.
Solution:	4.15% solution, soluble in distilled or deionized water on boiling. Solution is reddish-purple, very slightly to slightly opalescent, without significant precipitate.
Prepared Medium:	Reddish-purple, very slightly to slightly opalescent.
Reaction of 4.15% Solution at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare Violet Red Bile Glucose Agar per label directions. Using the pour plate method, inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Acinetobacter baumanii	19606	1,000-2,000	none to poor	–
Escherichia coli	25922*	30-300	good	red colonies with red-purple halos
Salmonella typhimurium	14028*	30-300	good	red colonies with red-purple halos
Staphylococcus aureus	25923*	1,000-2,000	none to poor	–

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

some foods, it is desirable to detect *Enterobacteriaceae* rather than the coliform bacteria.[1,2]

Enterobacteriaceae are glucose-fermenting bacteria. Mossel et al[3] modified Violet Red Bile Agar, suggested by MacConkey[4], that contains lactose by adding glucose to improve the recovery of *Enterobacteriaceae*. Later work by Mossel et al.[5,6] demonstrated that lactose could be omitted, resulting in the formulation known as Violet Red Bile Glucose Agar.

Principles of the Procedure

Violet Red Bile Glucose Agar contains Bacto Peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Glucose is a carbohydrate. Bile Salts No. 3 and Crystal Violet inhibit gram positive bacteria. Glucose fermenters produce red colonies with red-purple halos in the presence of Neutral Red, a pH indicator. Bacto Agar is a solidifying agent.

Formula

Violet Red Bile Glucose Agar

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Peptone	7 g
Bacto Bile Salts No. 3	1.5 g
Glucose	10 g
Sodium Chloride	5 g
Neutral Red	0.03 g
Bacto Crystal Violet	0.002 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper, established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Violet Red Bile Glucose Agar

Materials Required but not Provided

Glassware
Petri dishes
Distilled or deionized water
Autoclave
Incubator (35°C)

Method of Preparation

1. Suspend 41.5 grams in 1 liter distilled or deionized water.
2. Heat to boiling and boil for no more than 2 minutes to dissolve completely.
3. DO NOT AUTOCLAVE.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

This medium can be used in spread or pour plate procedures, with or without an overlay. In addition, this medium can be used as an overlayer for spread plates to both prevent swarming colonies and to provide semi-anaerobic conditions that suppress the growth of nonfermentative gram negative organisms. Stab inoculation procedures can also be used with this medium.

Results

Enterobacteriaceae ferment glucose, produce acid products and form red to dark purple colonies surrounded by red-purple halos.

Limitations of the Procedure

1. When used in the pour plate procedure, the medium should be freshly prepared, tempered to 47°C, and used within 3 hours.

References

1. Draft Standard Methods for Microbiological Examination of Meat Products. Part 3: Detection and enumeration of Enterobacteriaceae. BS5393: Part 3 1977 ISO/DIS 5552.
2. **Mossel, D. A. A.** 1985. Media for *Enterobacteriaceae.* Int. J. Food Microbiol. **2**:27.
3. **Mossel, D. A. A., W. H. J. Mengerink, and H. H. Scholts.** 1962. Use of a modified MacConkey agar medium for the selective growth and enumeration of *Enterobacteriaceae.* J. Bacteriol. **84**:381.
4. **MacConkey, A.** 1905. Lactose-fermenting bacteria in faeces. J. Hyg. **5**:333-378.
5. **Mossel, D. A. A., I. Eelderink, M. Koopmans, and F. van Rossem.** 1978. Lab Practice **27**:1049-1050.
6. **Mossel, D. A. A., I. Eelderink, M. Koopmans, and F. van Rossem.** 1979. Influence of carbon source, bile salts and incubation temperature on recovery of *Enterobacteriaceae* from foods using macconkey-type agars. J. Food Protect. **42**:470-475.

Packaging

Violet Red Bile Glucose Agar	500 g	1866-17

Bacto® Vitamin B_{12} Assay Medium

Intended Use

Bacto Vitamin B_{12} Assay Medium is used for determining vitamin B_{12} concentration by the microbiological assay technique.

Summary and Explanation

Vitamin Assay Media are prepared for use in the microbiological assay of vitamins. Three types of media are used for this purpose:

1. Maintenance Media: For carrying the stock culture to preserve the viability and sensitivity of the test organism for its intended purpose.
2. Inoculum Media: To condition the test culture for immediate use.
3. Assay Media: To permit quantitation of the vitamin under test.

Vitamin B_{12} Assay Medium is prepared according to the formula described by Capp, Hobbs and Fox.[1] This medium is used in the microbiological assay of vitamin B_{12} using *Lactobacillus delbrueckii* subsp. *lactis* ATCC® 4797 or 7830 (*Lactobacillus leichmannii*).

Principles of the Procedure

Vitamin B_{12} Assay Medium is a vitamin B_{12}-free medium containing all other nutrients and vitamins essential for the cultivation of *L. delbrueckii* subsp. *lactis* ATCC® 4797 or 7830. To obtain a standard curve, USP Cyanocobalamin Reference is added in specified increasing concentrations providing a growth response that can be measured titrimetrically or turbidimetrically.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Very light to light beige, homogeneous, with a tendency to clump.
Solution:	3.8% solution (single-strength), 7.6% (double strength), soluble in distilled or deionized water on boiling 2-3 minutes. Solution (single strength) is light amber, clear, may have a slight precipitate.
Prepared Medium:	Very light amber, clear, may have a very slight precipitate.
Reaction of 3.8% Solution at 25°C:	pH 6.3 ± 0.2

Cultural Response

Prepare Vitamin B_{12} Assay Medium per label directions. Dispense into tubes with a titration from 0 to 0.25 ng of USP Cyanocobalamin Reference Standard. Inoculate with *L. delbrueckii* subsp. *lactis* ATCC® 4797 and incubate at 35-37°C for 18-24 hours. Turbidimetric measurements are taken using a spectrophotometer. The curve is then constructed from the values obtained.

Formula

Vitamin B_{12} Assay Medium

Formula Per Liter

Bacto Vitamin Assay Casamino Acids	12 g
Bacto Dextrose	40 g
Sodium Acetate	20 g
L-Cystine	0.2 g
DL-Tryptophane	0.2 g
Adenine	20 mg
Guanine	20 mg
Uracil	20 mg
Xanthine	1 mg
Thiamine Hydrochloride	2 mg
Riboflavin	2 mg
Niacin	2 mg
Calcium Pantothenate	200 µg
Pyridoxine Hydrochloride	4 mg
p-Aminobenzoic Acid	200 µg
Biotin	10 µg
Folic Acid	100 µg
Sorbitan Monooleate Complex	2 g
Dipotassium Phosphate	1 g
Monopotassium Phosphate	1 g
Magnesium Sulfate	0.4 g
Sodium Chloride	20 mg
Ferrous Sulfate	20 mg
Manganese Sulfate	20 mg

Final pH 6.3 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.
3. Great care must be taken to avoid contamination of media or glassware in microbiological assay procedures. Extremely small amounts of foreign material may be sufficient to give erroneous results. Scrupulously clean glassware free from detergents and other chemicals must be used. Glassware must be heated to 250°C for at least 1 hour to burn off any organic residues that might be present.
4. Take precautions to keep sterilization and cooling conditions uniform throughout the assay.

Storage

Store the dehydrated medium at 2-8°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Vitamin B_{12} Assay Medium

Materials Required But Not Provided

Glassware
Autoclave
Stock culture of *Lactobacillus delbrueckii* subsp. *lactis* ATCC® 4797 or 7830
Sterile 0.85% saline
Distilled or deionized water
Centrifuge
0.1 N NaOH
Cyanocobalamin USP
Spectrophotometer or nephalometer
Lactobacilli Agar AOAC or B_{12} Culture Agar USP
Lactobacilli Broth AOAC or B_{12} Inoculum Broth USP

Method of Preparation

1. Suspend 7.6 grams in 100 ml distilled or deionized water.
2. Boil 2-3 minutes.
3. Dispense 5 ml amounts into tubes, evenly dispersing the precipitate.
4. Add standard or test samples.
5. Adjust tube volume to 10 ml with distilled or deionized water.
6. Autoclave at 121°C for 5 minutes.

Specimen Collection and Preparation

Assay samples are prepared according to references given in the specific assay procedures. For assays, the samples should be diluted to approximately the same concentration as the standard solution.

Test Procedure

Stock cultures of the test organism, *L. delbrueckii* subsp. *lactis* ATCC® 4797 or 7830, are prepared by stab inoculation of Lactobacilli Agar AOAC or B_{12} Culture Agar USP. Following incubation at 37°C for 24-48 hours, the tubes are stored in the refrigerator. Transfers are made at 2 week intervals.

Inoculum for the assay is prepared by subculturing a stock of *L. delbrueckii* subsp. *lactis* ATCC 4797 or 7830 into a tube containing 10 ml of Lactobacilli Broth AOAC or B_{12} Inoculum Broth USP. After incubation at 35-37°C for 18-24 hours, the cells are centrifuged under aseptic conditions and the supernatant liquid decanted. The cells are washed by resuspending in 10 ml of sterile 0.85% saline solution and centrifuging. The washing is repeated for a total of 3 times. Finally the cells are resuspended in 10 ml of sterile 0.85% saline. The cell suspension is then diluted 1:100 with sterile 0.85% saline. One drop is used to inoculate each assay tube.

It is essential that a standard curve be constructed each time an assay is run. Conditions of autoclaving and temperature of incubation that influence the standard curve readings cannot always be duplicated.

The concentrations required for the preparation of the standard curve are obtained by adding sufficient 25% ethanol to an accurately weighed amount of USP Cyanocobalamin Reference Standard (resulting in a solution containing 1.0 µg of cyanocobalamin per ml). This stock solution is stored in the refrigerator and should be used within 60 days.

In the preparation of the standard curve, further dilutions of this stock solution (1 µ/ml) are made as follows:

A. Add 1 ml stock solution to 99 ml distilled water (1 ml = 10 ng).
B. Add 1 ml of the solution from step A to 199 ml distilled water (1 ml = 0.05 ng).

An acceptable standard curve can be obtained by using the USP Cyanocobalamin Reference Standard at levels of 0.0, 0.025, 0.05, 0.1, 0.15, 0.2 and 0.25 ng per assay tube. This is accomplished by adding 0, 0.5, 1, 2, 3, 4 and 5 ml of the 0.05 ng/ml solution per assay tube and sufficient distilled or deionized water to make 10 ml volume per tube.

A standard concentration is used which, after incubation, gives a transmittance value at the 5 ml level of not less than that which corresponds to a dry cell weight of 1.25 mg (see USP[2] for method of calibration of a spectrophotometer and determination of dry cell weight). For the titrimetric method, a standard concentration should be used which, after incubation, will give a titration at the 5 ml level of 8-12 ml 0.1N sodium hydroxide.

Inoculate and incubate at 35-37°C for 18-24 hours. For turbidimetric determinations, place tubes in a refrigerator at 2-8°C for 15-20 minutes to stop growth. The growth can be measured by a nephelometric method. Titrimetric determinations of growth are made after incubation at 37°C for 72 hours. The curve is then constructed from the values obtained.

Results

1. Prepare a standard concentration response curve by plotting the response readings against the amount of standard in each tube, disk or cup.
2. Determine the amount of vitamin at each level of assay solution by interpolation from the standard curve.
3. Calculate the concentration of vitamin in the sample from the average of these volumes. Use only those values that do not vary more than ±10% from the average. Use the results only if two thirds of the values do not vary more than ±10%.

Limitations of the Procedure

1. The test organism used for inoculating an assay medium must be cultured and maintained on media recommended for this purpose.
2. Aseptic technique should be used throughout the assay procedure.
3. The use of altered or deficient media may cause mutants having different nutritional requirements that will not give a satisfactory response.
4. For successful results of these procedures, all conditions of the assay must be followed precisely.

References

1. **Capps, Hobbs, and Fox.** 1949. J. Biol. Chem. **178**:517.
2. **The United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention Inc. Rockville, MD.

Packaging

Vitamin B_{12} Assay Medium	100 g	0360-15*

*Store at 2-8°C

Bacto® WL Nutrient Medium · Bacto WL Nutrient Broth Bacto WL Differential Medium

Intended Use

Bacto WL Nutrient Medium and Bacto WL Nutrient Broth are used for cultivating yeasts, molds and bacteria encountered in brewing and industrial fermentation processes.

Bacto WL Differential Medium is used for isolating bacteria encountered in brewing and industrial fermentation processes.

Also Known As

WL Nutrient Medium is also referred to as "Wallerstein Laboratory Medium".

WL Nutrient Broth is also referred to as "Wallerstein Laboratory Nutrient Broth".

WL Differential Medium is also referred to as "Wallerstein Laboratory Differential Medium".

Summary and Explanation

WL Nutrient Media were developed by Green and Gray[1,2] in their study of various fermentation processes. An exhaustive study examining the methods of fermentation control procedures in worts, beers, liquid yeasts and similar fermentation products led to the development of WL Nutrient Media.

User Quality Control

Identity Specifications

WL Nutrient Medium and WL Differential Medium

Dehydrated

Media Appearance:	Light beige with a greenish tint, free-flowing, homogeneous.
Solution:	8.0% solution, soluble in distilled or deionized water on boiling. Solution is blue to greenish blue, slightly opalescent without significant precipitate.
Prepared Media:	Blue to greenish blue, slightly opalescent without significant precipitate.
Reaction of 8.0% Solution at 25° C:	pH 5.5 ± 0.2

WL Nutrient Broth

Dehydrated

Media Appearance:	Light beige with a greenish tint, free-flowing, homogeneous.
Solution:	6.0% solution, soluble in distilled or deionized water. Solution is blue, clear without precipitate.
Prepared Medium:	Blue, clear without precipitation.
Reaction of 6.0% Solution at 25°C:	pH 5.5 ± 0.2

continued on following page

At a pH of 5.5, counts of viable bakers' yeast may be made on the WL Nutrient Medium. By adjusting the pH to 6.5, the medium is suitable for obtaining counts of bakers' and distiller's yeast. The medium can support the growth of bacteria, but unless the number of yeast cells is small the bacteria may not be detected. Due to this limitation, Green and Gray developed WL Differential Medium that inhibits the growth of yeasts without inhibiting the growth of bacteria present in beers.

WL Nutrient Agar and WL Differential Medium are used simultaneously as a set or three plates. One plate is prepared from WL Nutrient Agar and two plates from WL Differential Medium.[3] The WL Nutrient Agar plate is incubated aerobically to obtain a total count of mainly yeast colonies. A differential agar plate is incubated aerobically for growth of acetic acid bacteria, *Flavobacterium*, *Proteus* and thermophilic bacteria. Another differential agar plate is incubated anaerobically for growth of lactic acid bacteria and *Pediococcus*.

Principles of the Procedure

Yeast Extract is a source of trace elements, vitamins and amino acids. Casitone provides nitrogen, amino acids, and carbon. Dextrose is the source of carbohydrate. Monopotassium Phosphate buffers the media. Potassium Chloride, Calcium Chloride and Ferric Chloride are essential ions and help to maintain osmotic balance. Magnesium Sulfate and Manganese Sulfate are sources of divalent cations. Brom Cresol Green is a pH indicator.

Agar is the solidifying agent in WL Nutrient Medium and WL Differential Medium.

Actidione (cycloheximide) inhibits yeasts and molds in WL Differential Medium.

Formula

WL Nutrient Medium

Formula Per Liter

Bacto Yeast Extract	4 g
Bacto Casitone	5 g
Bacto Dextrose	50 g
Monopotassium Phosphate	0.55 g
Potassium Chloride	0.425 g
Calcium Chloride	0.125 g
Magnesium Sulfate	0.125 g
Ferric Chloride	0.0025 g
Manganese Sulfate	0.0025 g
Bacto Agar	20 g
Brom Cresol Green	0.022 g

Final pH 5.5 ± 0.2 at 25°C

WL Nutrient Broth

Formula Per Liter

Bacto Yeast Extract	4 g
Bacto Casitone	5 g
Bacto Dextrose	50 g
Monopotassium Phosphate	0.55 g
Potassium Chloride	0.425 g
Calcium Chloride	0.125 g
Magnesium Sulfate	0.125 g

Ferric Chloride ..... 0.0025 g
Manganese Sulfate ..... 0.0025 g
Brom Cresol Green ..... 0.022 g

Final pH 5.5 ± 0.2 at 25°C

WL Differential Medium

Formula Per Liter

Bacto Yeast Extract ..... 4 g
Bacto Casitone ..... 5 g
Bacto Dextrose ..... 50 g
Monopotassium Phosphate ..... 0.55 g
Potassium Chloride ..... 0.425 g
Calcium Chloride ..... 0.125 g
Magnesium Sulfate ..... 0.125 g
Ferric Chloride ..... 0.0025 g
Manganese Sulfate ..... 0.0025 g
Bacto Agar ..... 20 g
Brom Cresol Green ..... 0.022 g
Actidione® ..... 0.004 g

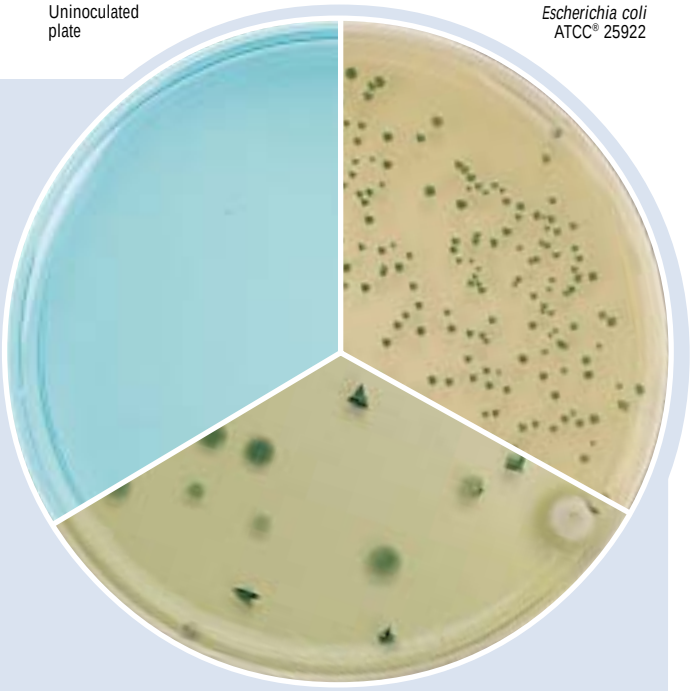
Final pH 5.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.



User Quality Control cont.

Cultural Response

WL Nutrient Medium

Prepare WL Nutrient Medium per label directions. Inoculate and incubate for 40-48 hours at 35 ± 2°C for bacteria and at 30 ± 2°C for yeasts.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	100-1,000	fair to good
Lactobacillus fermentum	9338	100-1,000	fair to good
Saccharomyces cerevisiae	9763	100-1,000	good

Escherichia coli ATCC® 25922 | *Lactobacillus fermentum* ATCC® 9338 | *Saccharomyces cerevisiae* ATCC® 9763

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

WL Nutrient Broth

Prepare WL Nutrient Broth per label directions. Inoculate and incubate for 40-48 hours at 35 ± 2°C for bacteria and at 30 ± 2°C for yeasts.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	ACID	GAS
Escherichia coli	25922*	100-1,000	fair to good	+	+
Lactobacillus fermentum	9338	100-1,000	fair to good	+	sl. +
Saccharomyces cerevisiae	9763	100-1,000	good	+	+

Acid + = positive, yellow Acid – = negative, no color change

WL Differential Medium

Prepare WL Differential Medium per label directions. Inoculate and incubate for 40-48 hours at 35 ± 2°C for bacteria and at 30 ± 2°C for yeasts.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	100-1,000	good
Lactobacillus fermentum	9338	500-1,000	good
Saccharomyces cerevisiae	9763	1,000-2,000	inhibited

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

WL Nutrient Medium
WL Nutrient Broth
WL Differential Medium

Materials Required but not Provided

Glassware
Autoclave
Petri dishes
Tubes with closures
Fermentation tubes

Method of Preparation

WL Nutrient Medium and WL Differential Medium

1. Suspend 80 grams in 1 liter distilled or deionized water. OPTIONAL: To adjust the pH to 6.5, add the amount of 1% sodium carbonate solution specified on the product label to the rehydration water before dissolving the medium.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

WL Nutrient Broth

1. Dissolve 60 grams in 1 liter distilled or deionized water. OPTIONAL: Add fermentation tubes before sterilizing to assess gas production.
2. Dispense into tubes.
3. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Refer to appropriate references for specimen collection and preparation.

Test Procedure

See appropriate references for specific procedures.

Results

Refer to appropriate references and procedures for results.

References

1. **Green, S. R., and P. P. Gray.** 1950. Paper read at American Society of Brewing Chemists Meeting. Wallerstein Lab. Commun. **12**:43.
2. **Green, S. R., and P. P. Gray.** 1950. A differential procedure applicable to bacteriological investigation in brewing. Wallerstein Lab. Commun. **13**:357.
3. **MacFaddin, J. D.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1, p. 854-856. Williams & Wilkins, Baltimore, MD.

Packaging

WL Nutrient Medium	500 g	0424-17
	10 kg	0424-08
WL Nutrient Broth	500 g	0471-17
WL Differential Medium	500 g	0425-17

Bacto® XL Agar Base · Bacto XLD Agar

User Quality Control

Identity Specifications

XL Agar Base

Dehydrated Appearance:	Pink, homogeneous, free-flowing.
Solution:	4.7% solution, soluble in distilled or deionized water upon boiling. Solution is red, very slightly opalescent.
Prepared Plates:	Red, slightly opalescent.
Reaction of 4.7% Solution at 25°C:	pH 7.4 ± 0.2

XLD Agar

Dehydrated Appearance:	Pink, homogeneous, free-flowing.
Solution:	5.7% solution, soluble in distilled or deionized water upon boiling. Solution is red, very slightly opalescent.
Prepared Plates:	Red, slightly opalescent.
Reaction of 5.7% Solution at 25°C:	pH 7.4 ± 0.2

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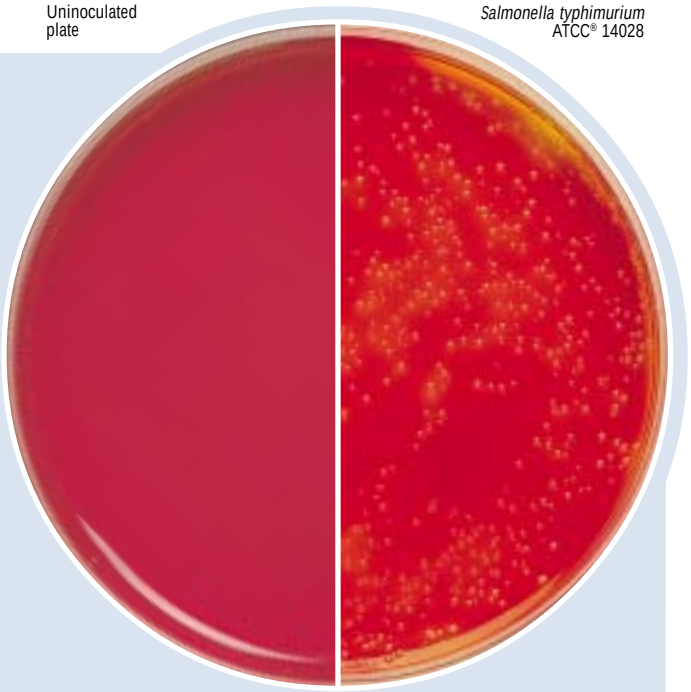


Intended Use

Bacto XL Agar Base is used with or without selective agents for isolating, differentiating and enumerating enteric bacteria.

Bacto XLD Agar is used for isolating and differentiating gram-negative enteric bacilli, especially *Shigella* and *Providencia*.

Also Known As

XL Agar with added brilliant green is referred to as XLBG Agar.

Summary And Explanation

XL (Xylose Lysine) Agar Base was developed by Taylor[1] for isolating and differentiating gram-negative enteric bacilli. The medium is nonselective, allowing growth of most enteric bacteria. XL Agar is recommended for performing counts of enteric organisms.

XL Agar Base can be supplemented with sodium thiosulfate and ferric ammonium citrate and, further, with sodium desoxycholate (2.5 grams per liter) to make XLD Agar. XL Agar Base can be made selective for *Salmonella* by adding brilliant green (1.25 ml of a 1% aqueous solution per liter) prior to autoclaving. The resulting XLBG Agar inhibits coliforms and *Shigella* and is recommended for isolating *Salmonella* following selenite or tetrathionate enrichment in food analysis.[1]

XLD Agar was developed principally for isolating *Shigella* and *Providencia*. It has been shown to be more effective than other enteric differential media.[2,3,4]

Principles of the Procedure

Yeast Extract provides sources of nitrogen and carbon, as well as vitamins and cofactors required for growth. Xylose, Lactose, and Sucrose (Saccharose) provide sources of fermentable carbohydrate. Xylose is fermented by most enteric organisms except *Shigella* and *Providencia*. Lysine is added to differentiate *Salmonella*. As xylose is exhausted, *Salmonella* organisms decarboxylate lysine causing a reversion to alkaline conditions. Alkaline reversion by other lysine-positive organisms is prevented by excess acid production from fermentation of lactose and sucrose.

Sodium Thiosulfate and Ferric Ammonium Citrate allow visualization of hydrogen sulfide production under alkaline conditions. Acidic conditions inhibit the reaction. Phenol Red is an indicator. Sodium Chloride maintains osmotic balance in the medium. Bacto Agar is a solidifying agent.

Sodium Desoxycholate in XLD Agar inhibits growth of gram-positive organisms.

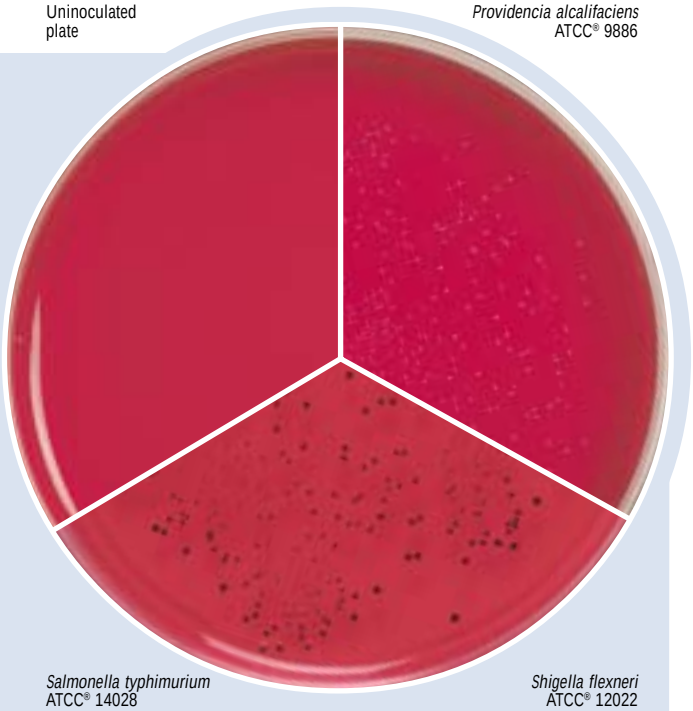
Formula

XL Agar Base

Formula Per Liter

Bacto Yeast Extract	3 g
L-Lysine	5 g
Bacto Xylose	3.75 g
Bacto Lactose	7.5 g
Bacto Saccharose	7.5 g
Sodium Chloride	5 g
Bacto Phenol Red	0.08 g
Bacto Agar	15 g

Final pH 7.4 ± 0.2 at 25°C



User Quality Control cont.

Cultural Response

XL Agar Base

Prepare XL Agar Base per label directions. Inoculate the medium and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	100-1,000	poor to fair	yellow
Escherichia coli	25922*	100-1,000	good	yellow
Salmonella typhimurium	14028*	100-1,000	good	red w/black centers
Shigella flexneri	12022*	100-1,000	good	red

XLD Agar

Prepare XLD Agar per label directions. Inoculate the medium and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	1,000-2,000	partial inhibition	–
Escherichia coli	25922*	1,000-2,000	partial inhibition	yellow, may have a bile precipitate
Salmonella typhimurium	14028*	100-1,000	good	red w/black centers
Shigella flexneri	12022*	100-1,000	good	red

The organisms listed are the minimum used for performance testing.

*These cultures are available as Bactrol™ Disks and are to be used as directed in Bactrol Disks Technical Information.

XLD Agar

Formula Per Liter

Bacto Yeast Extract	3 g
L-Lysine	5 g
Bacto Xylose	3.75 g
Bacto Lactose	7.5 g
Bacto Saccharose	7.5 g
Sodium Desoxycholate	2.5 g
Ferric Ammonium Citrate	0.8 g
Sodium Thiosulfate	6.8 g
Sodium Chloride	5 g
Bacto Agar	15 g
Bacto Phenol Red	0.08 g

Final pH 7.4 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. XLD Agar:

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated media below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

XL Agar Base
XLD Agar

Materials Required But Not Provided

Glassware
Autoclave
Incubator
Petri dishes
34% sodium thiosulfate, sterile (XL Agar)
4% ferric ammonium citrate, sterile (XL Agar)
1% brilliant green (XLBG)

Method of Preparation

XL Agar Base

1. Suspend 47 grams in 1 liter distilled or deionized water. (OPTIONAL: To prepare XLBG Agar, add 1.25 ml aqueous 1% solution of brilliant green.)
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
3. Cool to 55-60°C.
4. Aseptically add 20 ml sterile aqueous 34% solution of sodium thiosulfate and 4% solution of ferric ammonium citrate. Mix thoroughly.
5. Dispense as desired.

XLD Agar

1. Suspend 57 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely. Avoid overheating. **DO NOT AUTOCLAVE.**
3. Cool to 55-60°C. Dispense as desired.

Specimen Collection and Preparation

XLD Agar is listed in several procedures as a plating medium for the testing of food samples.[8,9,10,11] Refer to the appropriate references regarding specimen preparation.

Test Procedure

Feces or rectal swabs may be plated directly; selective enrichment broths, such as Selenite Broth or Tetrathionate Broth, may be used prior to streaking.[5,6,7]

Results

Degradation of xylose, lactose and sucrose generates acid products, causing a color change in the medium from red to yellow.

Hydrogen sulfide production under alkaline conditions causes colonies to develop black centers. This reaction is inhibited by the acid conditions that accompany carbohydrate fermentation.

Lysine decarboxylation in the absence of lactose and sucrose fermentation causes reversion to an alkaline condition and the color of the medium changes back to red.

Limitations of the Procedure

1. Red, false-positive colonies may occur with some *Proteus* and *Pseudomonas* species.
2. Incubation in excess of 48 hours may lead to false-positive results.
3. *S. paratyphi A, S. choleraesuis, S. pullorum* and *S. gallinarum* may form red colonies without black centers, thus resembling *Shigella* species.
4. Some *Proteus* strains will give black-centered colonies on XLD Agar.

References

1. **Taylor, W. I.** 1965. Isolation of shigellae. I. Xylose lysine agars; new media for isolation of enteric pathogens. Am. J. Clin. Pathol. **44**(4):471-475.
2. **Rollender, W., O. Beckford, R. D. Belsky, and B. Kostroff.** 1969. Comparison of xylose lysine deoxycholate agar and MacConkey agar for the isolation of *Salmonella* and *Shigella* from clinical specimens. Tech. Bull. Reg. Med. Tech. **39**(1):8-10.
3. **Pollock, H. M., and B. J. Dahlgren.** 1974. Clinical evaluation of enteric media in the primary isolation of *Salmonella* and *Shigella*. Appl. Microbiol. **27**(1):197-201.

4. **Bhat, P., and D. Rajan.** 1975. Comparative evaluation of desoxycholate citrate medium and xylose lysine desoxycholate medium in the isolation of shigellae. Am. J. Clin. Pathol. **64**:399-404.
5. **MacFaddin, J. F.** 1985. Media for isolation-cultivation-identification-maintenance of medical bacteria, vol. 1. Williams & Wilkins, Baltimore, MD.
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7. **United States Pharmacopeial Convention.** 1995. The United States pharmacopeia, 23rd ed. The United States Pharmacopeial Convention, Rockville, MD.
8. **Smith, J. L., and R. L. Buchanan.** 1992. *Shigella*, p. 423-431. *In* C. Vanderzant and D. F. Splittstoesser (ed.), Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
9. **Flowers, R. S., W. Andrews, C. W. Donnelly, and E. Koenig.** 1993. Pathogens in milk and milk products, p. 103-212. *In* R. T. Marshall, (ed.), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
10. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*, p. 5.01-5.20. *In* Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, MD.
11. **Association of Official Analytical Chemists.** 1996. Official methods of analysis of AOAC International, Supplement March 1996. AOAC International, Arlington, VA.

Packaging

XL Agar Base	500 g	0555-17
XLD Agar	100 g	0788-15
	500 g	0788-17
	2 kg	0788-07
	10 kg	0788-08

Bacto® XLT4 Agar Base
Bacto XLT4 Agar Supplement

Intended Use

Bacto XLT4 Agar Base is used with Bacto XLT4 Agar Supplement in isolating non-*typhi Salmonella*.

User Quality Control

Identity Specifications

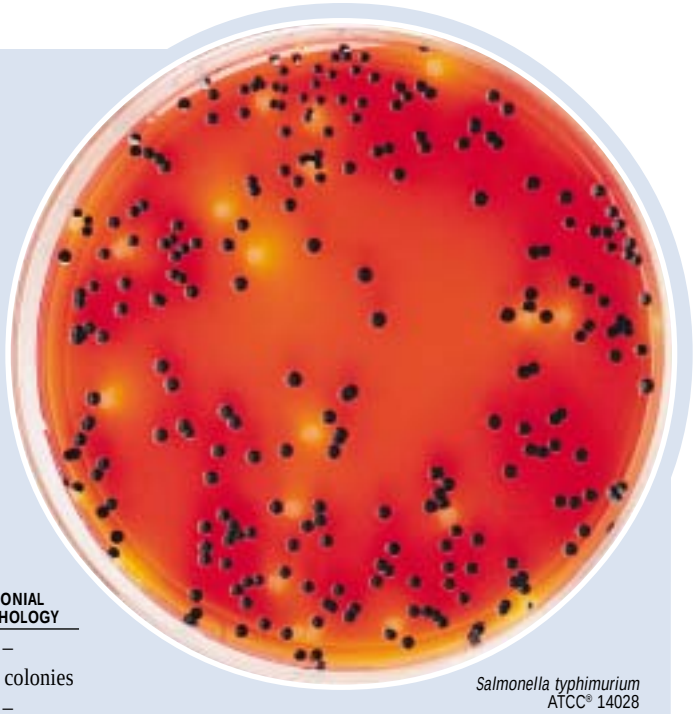
Dehydrated Appearance:	Pink, free flowing, homogeneous.
Base Solution:	5.9% solution; soluble in distilled or deionized water on boiling. Solution is red, very slightly to slightly opalescent.
XLT4 Agar Supplement:	Colorless to slightly yellow, clear, slightly viscous solution.
Prepared Medium:	Reddish-orange, very slightly to slightly opalescent.
Reaction of Final Medium at 25°C:	pH 7.4 ± 0.2

Cultural Response

Prepare XLT4 Agar per label directions. Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	CFU	INOCULUM GROWTH	COLONIAL MORPHOLOGY
Enterococcus faecalis	29212*	1,000	markedly inhibited	–
Escherichia coli	25922*	1,000	partially inhibited	yellow colonies
Proteus mirabilis	25933	1,000	inhibited	–
Salmonella typhimurium	14028*	100-1,000	good	yellow to red colonies with black centers
Staphylococcus aureus	25923*	1,000	inhibited	–

The cultures listed are the minimum that should be used for performance testing.
*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.



Salmonella typhimurium ATCC® 14028 on XLT4 Agar Base with XLT4 Supplement

Summary and Explanation

Numerous media have been developed for isolating and differentiating enteric pathogens. The majority were designed to recover a broad spectrum of enteric pathogens.[1] Consequently, overgrowth of nuisance or contaminating organisms can be a major problem when recovery of a specific organism or species is desired. This is particularly true for *Salmonella* isolation media where overgrowth of *Proteus*, *Providencia* and *Pseudomonas* can dramatically interfere with the detection and isolation of *Salmonella*.

In 1990, Miller and Tate described a new medium, XLT4 Agar, for isolating *Salmonella*.[1] The authors established the selectivity of XLT4 Agar using pure cultures of a variety of enteric organisms. They also evaluated its sensitivity in detecting and isolating *Salmonella* using fecal-contaminated farm samples containing high numbers of competing bacteria. In follow-up studies, Miller[2,3] and Tate[4] reported that XLT4 Agar significantly improved the recovery of non-*typhi Salmonella* from chicken and farm environmental drag-swab samples.

XLT4 Agar can be used clinically to screen stool samples for non-typhoid *Salmonella*.[5,6]

Principles of the Procedure

XLT4 Agar Base contains Proteose Peptone No. 3 as a source of complex nitrogen compounds. Yeast Extract is added as a source of vitamins and other cofactors. Differentiation of *Salmonella* from other organisms that also grow on this medium is based on fermentation of Xylose, Lactose and Sucrose, decarboxylation of Lysine, and the production of hydrogen sulfide. Hydrogen sulfide production is detected by the addition of ferric ions. Sodium Thiosulfate is added as a source of inorganic sulfur. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is the solidifying agent. Phenol Red is added as an indicator of pH changes resulting from fermentation and decarboxylation reactions. XLT4 Agar Supplement is added to inhibit growth of non-*Salmonella* organisms.

Formula

XLT4 Agar Base

Formula Per Liter

Bacto Proteose Peptone No. 3	1.6 g
Bacto Yeast Extract	3 g
L-Lysine	5 g
Bacto Xylose	3.75 g
Bacto Lactose	7.5 g
Bacto Saccharose	7.5 g
Ferric Ammonium Citrate	0.8 g
Sodium Thiosulfate	6.8 g
Sodium Chloride	5 g
Bacto Agar	18 g
Bacto Phenol Red	0.08 g

Final pH 7.4 ± 0.2 at 25°C

XLT4 Agar Supplement

A 27% solution (approximate) of the surfactant 7-ethyl-2-methyl-4-undecanol hydrogen sulfate, sodium salt, formerly produced by Union Carbide under the tradename of Tergitol 4.

Precautions

1. For Laboratory Use.
2. **XLT4 Agar Base**

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   XLT4 Agar Supplement

   CORROSIVE. CAUSES BURNS. HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing, gloves and eye/face protection. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store XLT4 Agar Base below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed. Store prepared medium at 2-8°C.

Store XLT4 Agar Supplement at 15-30°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

XLT4 Agar Base
XLT4 Agar Supplement

Materials Required But Not Provided

Flasks with closures
Distilled or deionized water
Bunsen burner or magnetic hot plate
Waterbath (45-50°C)
Petri dishes
Incubator (35°C)

Method of Preparation

1. Suspend 59 grams of XLT4 Agar Base in 1 liter distilled or deionized water.
2. Add 4.6 ml XLT4 Agar Supplement.
3. Heat to boiling to dissolve completely. Avoid overheating. **DO NOT AUTOCLAVE**. Cool to 45-50°C in a waterbath.
4. Dispense into sterile Petri dishes.

Specimen Collection and Preparation

1. Collect specimens in sterile containers or with sterile swabs and transport immediately to the laboratory following recommended guidelines.[7-10]

2. Process each specimen, using procedures appropriate for that sample.[7,8,10,11]

Test Procedure

1. Inoculate a suitable *Salmonella* enrichment broth (such as Tetrathionate Broth) and incubate at 35°C for 18-24 hours.
2. Following enrichment, subculture onto XLT4 Agar. Streak for isolation.
3. Incubate plates aerobically at 35 ± 2°C. Examine for growth after 18-24 and 48 hours incubation.

Results

Typical *Salmonella* colonies (H_2S-positive) appear black or black-centered with a yellow periphery after 18-24 hours of incubation. Upon continued incubation, the colonies become entirely black or pink to red with black centers.

Colonies of H_2S-negative *Salmonella* strains appear pinkish-yellow.

Most *Citrobacter* colonies that grow on this medium are yellow without evidence of blackening. Growth of *Enterobacter aerogenes* and *Escherichia coli* is markedly inhibited; colonies that do grow appear yellow without evidence of blackening. Growth of *Proteus*, *Pseudomonas*, *Providencia*, *Alteromonas putrefaciens*, *Yersinia enterocolitica* and *Acinetobacter calcoaceticus* is markedly to completely inhibited on XLT4 Agar. *Shigella* species are partially inhibited and colonies appear red.

Limitations of the Procedure

1. XLT4 Agar is intended for detecting and isolating *Salmonella* based on selectivity and colonial characteristics. Presumed *Salmonella* colonies must be confirmed by biochemical and/or immunological methods. Consult appropriate references for further information.[5,7,8,12]
2. Since the nutritional requirements of organisms vary, some strains of *Salmonella* may be encountered that fail to grow or grow poorly on this medium.
3. Non-*Salmonella* strains that are not completely inhibited on this medium may be encountered and must be differentiated from *Salmonella*. Consult appropriate references.[7,8,10,12]
4. Freshly inoculated plates and plates held over several days may develop multicolored, metallic looking crystals/flecks on the surface. These crystals/flecks do not interfere with the performance of the medium.

References

1. **Miller, R. G., and C. R. Tate**. 1990. XLT4: A highly selective plating medium for the isolation of *Salmonella*. The Maryland Poultryman April:2-7.
2. **Miller, R. G., C. R. Tate, E. T. Mallinson, and J. A. Schemer**. 1991. Xylose-Lysine-Tergitol 4: An improved selective agar medium for the isolation of *Salmonella*. Poultry Science **70**:2429-2432.
3. **Miller, R. G., C. R. Tate, E. T. Mallinson, and J. A. Schemer**. 1992. *Erratum*. Xylose-Lysine-Tergitol 4: An improved selective agar medium for the isolation of *Salmonella*. Poultry Science **71**:398.
4. **Tate, C. R., R. G. Miller, and E. T. Mallinson**. 1992. Evaluation of two isolation and two non-isolation methods for detecting naturally occurring salmonellae from broiler flock environmental drag-swab samples. J. Food Prot. **55**:964-967.
5. **Dusch, H., and M. Altwegg**. 1994. Evaluation of Xylose-Lysine-Tergitol 4 (XLT4) Agar and Modified Semisolid Rappaport Vassiliadis (MSRV) Medium for the isolation of non-typhoid salmonellae from stool samples. Abstr. Annu. Meet. Am. Soc. Microbiol. **C5**:557.
6. **Dusch, H., and M. Altwegg**. 1995. Evaluation of five new plating media for the isolation of *Salmonella* species. J. Clin. Microbiol. **33**:802-804.
7. **Andrews, W. H., G. A. June, P. S. Sherrod, T. S. Hammack, and R. M. Amaguana.** 1995. *Salmonella*. *In* FDA Bacteriological analytical manual, 8th ed. AOAC International, Arlington, VA.
8. **Vanderzant, C., and D. F. Splittstoesser (ed.)** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
9. **Miller, J. M., and H. T. Holmes.** 1995. Specimen collection, transport, and storage, p. 19-31. *In* P. R. Murray, *et al.* (ed). Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
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11. **Forbes, B. A., and P. A. Granato.** 1995. Processing specimens for bacteria, p. 265-281. *In* P. R. Murray, *et al.* (ed), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
12. **Gray, L. D.** 1995. *Escherichia, Salmonella, Shigella and Yersinia*. *In* P. R. Murray, *et al.* (ed), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

XLT4 Agar Base	500 g	0234-17
XLT4 Agar Supplement	l00 ml	0353-72

Bacto® YM Agar
Bacto YM Broth

Intended Use

Bacto YM Agar and YM Broth are used for cultivating yeasts, molds and other aciduric microorganisms.

Also Known As

YM is an abbreviation for Yeast Extract and Malt Extract.

Summary and Explanation

YM Agar and YM Broth are prepared according to the formulation published by Wickerham.[1,2,3] Wickerham suggested that YM Broth acidified to pH 3.0-4.0 be used as an enrichment medium for yeasts from populations also containing bacteria and molds. To favor isolation of fermentative species, add a layer of sterile paraffin oil

1 cm deep on the surface of the inoculated broth. Incubate the culture until growth appears and then streak onto YM Agar to obtain isolated yeast colonies. To isolate fermentative and oxidative strains, place acidified inoculated YM Broth on a rotary shaker for 1 or 2 days. This favors yeast recovery while preventing the sporulation of molds.

Media selectivity may be enhanced through acidification or through addition of selective agents. YM Broth may be acidified prior to sterilization. YM Agar should be sterilized without pH adjustment and sterile acid added to the sterile molten medium cooled to 45-50°C. Acidified YM Agar should not be heated. Antibiotics may be aseptically added to the sterile media. Other fungistatic materials, such as sodium propionate and diphenyl may be added to YM Agar to eliminate molds and permit the enumeration of yeasts to mixed populations.

User Quality Control

Identity Specifications

YM Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	4.1% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly opalescent, without significant precipitate.
Prepared Plates:	Light to medium amber, slightly opalescent without precipitate.
Reaction of 4.1% Solution at 25°C:	pH 6.2 ± 0.2

YM Broth

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	2.1% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear to very slightly opalescent without significant precipitate.
NOTE:	At pH adjusted to 3.0-4.0, medium becomes slightly opalescent.
Prepared Tubes:	Light - medium amber, clear to very slightly opalescent without significant precipitate.
Reaction of 2.1% Solution at 25°C:	pH 6.2 ± 0.2

Cultural Response

Prepare 2 sets of YM Agar plates or YM Broth tubes (one set pH 6.2, one set adjusted to pH 3.0-4.0) per label directions. Inoculate and incubate at 30 ± 2°C for 18-72 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH PH 3.0-4.0	GROWTH PH 6.2
Aspergillus niger	16404	100-1,000	good	good
Candida albicans	10231	100-1,000	good	good
Escherichia coli	25922*	100-1,000	markedly to completely inhibited	good
Lactobacillus casei	7469	100-1,000	poor to fair	good
Saccharomyces cerevisiae	9763	100-1,000	good	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Yeast Extract is a source of trace elements, vitamins and amino acids. Malt Extract is a source of carbon, protein and nutrients. Bacto Peptone is an additional source of carbon and provides nitrogen and amino acids. Dextrose provides carbon. Bacto Agar is a solidifying agent.

Formula

YM Agar

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Malt Extract	3 g
Bacto Peptone	5 g
Bacto Dextrose	10 g
Bacto Agar	20 g

Final pH 6.2 ± 0.2 at 25°C

YM Broth

Formula Per Liter

Bacto Yeast Extract	3 g
Bacto Malt Extract	3 g
Bacto Peptone	5 g
Bacto Dextrose	10 g

Final pH 6.2 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

YM Agar
YM Broth

Materials Required but not Provided

Glassware
Autoclave
Antibiotics
Sterile 10% HCl, Tartaric Acid or 10% Citric Acid

Method of Preparation

YM Agar

1. Suspend 41 grams in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes. Cool to 45-50°C.
4. Dispense into sterile Petri dishes.

YM Broth

1. Dissolve 21 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

   ***Optional* (for Agar or Broth):** If desired, acidify the medium to pH 3.0-4.0 by adding sterile 10% HCl, Tartaric Acid or 10% Citric Acid. Selective agents, e.g., penicillin (20 units per ml final concentration) or streptomycin (40 micrograms per ml final concentration) may be added to the medium after sterilization using aseptic technique.

Test Procedure

1. Inoculate YM Agar plates or YM Broth tubes with sample to evaluated for the presence of yeasts, molds, or aciduric microorganisms.
2. Incubate at 30 ± 2°C for 18-72 hours.

Results

Examine the plates or tubes for growth. Record YM Agar results as colony forming units (CFU) per volume of sample. Record YM Broth results as growth or no growth.

Limitations of the Procedure

1. Acidified YM Agar should not be overheated.

References

1. 1951. U. S. Dept. Agricult. Tech. Bull. No. 1029.
2. 1939. J. Tropical Med. Hyg. **42:**176.
3. **Jong, S. C., and M. J. Edwards**. 1991. American Type Culture Collection Catalog of filamentous fungi, 18th ed. American Type Collection, Rockville, MD.

Packaging

YM Agar	500 g	0712-17
YM Broth	500 g	0711-17
	10 kg	0711-08

Bacto® YPD Agar
Bacto YPD Broth

User Quality Control

Identity Specifications

YPD Agar

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	6.5% solution, soluble in distilled or deionized water on boiling. Solution is light to medium amber, very slightly to slightly opalescent.
Prepared Medium:	Light to medium amber, slightly opalescent.
Reaction of 6.5% Solution at 25°C:	pH 6.5 ± 0.2

YPD Broth

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	5.0% solution, soluble in distilled or deionized water. Solution is light to medium amber, clear to very slightly opalescent.
Prepared Medium:	Light to medium amber, clear to very slightly opalescent.
Reaction of 5.0% Solution at 25°C:	pH 6.5 ± 0.2

Cultural Response

Prepare YPD Agar or YPD Broth per label directions. Inoculate and incubate the plates or tubes at 25 ± 2°C for 42-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Kluyveromyces lactis	8563	100-1,000	good
Saccharomyces cerevisiae	18790	100-1,000	good
Saccharomyces pastorianus	9080	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

Intended Use

Bacto YPD Agar and Bacto YPD Broth are used for maintaining and propagating yeasts in molecular microbiology procedures.

Also Known As

YPD media are also known as Yeast Extract-Peptone-Glucose media and may be abbreviated as YEPD.

Summary and Explanation

General methods in yeast genetics specify using yeast extract-peptone-glucose (YPD) medium for cultivating *Saccharomyces cerevisiae* and other yeasts.[1] Yeasts grow well on a minimal medium containing only glucose and salts. The addition of protein and yeast cell extract hydrolysates allow faster growth so that during exponential or log-phase growth, the cells divide every 90 minutes.[1]

Principles of the Procedure

YPD Agar and YPD Broth contain Bacto Peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast Extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate source. YPD Agar contains Bacto Agar as the solidifying agent.

Formula

YPD Agar

Formula Per Liter

Bacto Yeast Extract	10 g
Bacto Peptone	20 g
Bacto Dextrose	20 g
Bacto Agar	15 g

Final pH 6.5 ± 0.2 at 25°C

YPD Broth

Formula Per Liter

Bacto Yeast Extract	10 g
Bacto Peptone	20 g
Bacto Dextrose	20 g

Final pH 6.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

YPD Agar
YPD Broth

Materials Required but not Provided

Glassware
Distilled or deionized water
Autoclave
Incubator (25°C)

Method of Preparation

YPD Agar

1. Suspend 65 grams of YPD Agar in 1 liter of distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.

YPD Broth

1. Suspend 50 grams in 1 liter distilled or deionized water.
2. Autoclave at 121°C for 15 minutes.

Specimen Collection and Preparation

Collect specimens according to recommended guidelines.

Test Procedure

See appropriate references for specific procedures.

Results

Growth of colonies on the agar or in the broth (turbidity).

References

1. **Ausubel, F. M. , R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl**. 1994. Current Protocols in Molecular Biology., Current Protocols, Brooklyn, NY.

Packaging

YPD Agar	500 g	0427-17
	2 kg	0427-07
YPD Broth	500 g	0428-17
	2 kg	0428-07

Yeast Extract

Bacto® Yeast Extract · Bacto Yeast Extract, Technical Bacto Autolyzed Yeast

Intended Use

Bacto Yeast Extract, Bacto Yeast Extract, Technical and Bacto Autolyzed Yeast are used in preparing microbiological culture media.

Summary and Explanation

Yeast Extract is the water soluble portion of autolyzed yeast. The autolysis is carefully controlled to preserve the naturally occurring B-complex vitamins. Yeast Extract is prepared and standardized for bacteriological use. It is an excellent stimulator of bacterial growth and used in culture media in place of, or in addition to, beef extract. Yeast Extract is generally employed in the concentration of 0.3-0.5%.

Yeast Extract has been used successfully in culture media for studies of bacteria in milk and other dairy products. The advantage of Yeast Extract for this purpose is documented by Prickett[1] on the thermophilic and thermoduric bacteria of milk. Since publication of Prickett's[1] study, Yeast Extract has been used more frequently in the study of bacterial flora in milk. Hutner[2] used this product in a stock broth for streptococci. Partansky and McPherson[3] used Yeast Extract in combination with Bacto Malt Extract and Bacto Agar for testing mold resistant properties of oil paints.

Yeast Extract is an excellent source of B-complex vitamins and is often used to supply these factors in bacteriological culture media. Snell and Strong[4] used Yeast Extract for the preparation of yeast supplement in their medium for riboflavin assay. It has been a valuable ingredient for carrying stock cultures, and for preparation of inocula of lactobacilli for microbiological assay of vitamins. This product is also of value in the assay of antibiotics. A growth substance, B factor for *Norcardia*, can be isolated from Yeast Extract.[5] Yeast Extract supplies this factor necessary for a rifampin mutant to product rifampin.[5]

Several media containing Yeast Extract are specified in standard methods for multiple applications.[6,7,8,9]

Yeast Extract, Technical is a water soluble portion of autolyzed yeast containing vitamin B complex. Yeast Extract, Technical is used in bacterial culture media when a standardized yeast extract is not essential. It demonstrates acceptable clarity and growth promoting characteristics.

Autolyzed Yeast is a desiccated product containing both the soluble and insoluble portions of autolyzed bakers' yeast. It is recommended for preparation of yeast supplements used in microbiological assay for riboflavin and pantothenic acid.[10,11]

Principles of the Procedure

Yeast Extract is typically prepared by growing baker's yeast, *Saccharomyces* sp., in a carbohydrate-rich plant medium. The yeast is harvested, washed and resuspended in water, where is undergoes autolysis, i.e., self digestion using yeast's enzymes. Yeast Extract is the total soluble portion of this autolytic action. The autolytic activity is stopped by a heating step. The resulting Yeast Extract is then filtered clear and subsequently made a powder by the spray drying process.

Yeast Extract, Yeast Extract, Technical and Autolyzed Yeast provide vitamins, nitrogen, amino acids and carbon in microbiological culture media.

User Quality Control

Identity Specifications

Yeast Extract

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	1% solution - soluble in distilled or deionized water. Solution is light to medium amber, clear, may have a very slight precipitate. 2% solution-medium amber, clear, may have a very slight precipitate.
Reaction of 1% Solution at 25°C:	pH 6.6 ± 0.2

Yeast Extract, Technical

Dehydrated Appearance:	Light to medium beige, free-flowing, homogeneous.
Solution:	1% solution - soluble in distilled or deionized water. Solution is light to medium amber in color, clear to very slightly opalescent, may have a precipitate.

Autolyzed Yeast

Dehydrated Appearance:	Medium to dark brown, homogenous, free-flowing.
Solution:	1% solution - not completely soluble in distilled or deionized water upon boiling. Solution is amber, opaque, may have a precipitate.
Reaction of 1% Solution at 25°C:	pH 4.9-6.3

Cultural Response

Yeast Extract

Prepare a solution containing 1% Yeast Extract and 0.5% sodium chloride. Adjust the pH to 7.2 ± 0.2 using dilute NaOH. Inoculate tubes with the test organisms and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Neisseria meningitidis	13090*	100-1,000	fair to good
Staphylococcus aureus	25923*	100-1,000	good
Streptococcus pneumoniae	6305	100-1,000	good

Yeast Extract, Technical

Prepare a solution containing 2% Yeast Extract with 0.5% sodium chloride. Adjust the pH to 7.2 ± 0.2 using dilute NaOH. Inoculate tubes with the test organisms and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	100-1,000	good
Streptococcus pyogenes	19615*	100-1,000	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Typical Analysis

Yeast Extract

Physical Characteristics

Ash (%)	11.2	Loss on Drying (%)	3.1
Clarity, 1% Soln (NTU)	1.5	pH, 1% Soln	6.7
Filterability (g/cm2)	2.7		

Carbohydrate (%)

Total	17.5

Nitrogen Content (%)

Total Nitrogen	10.9	AN/TN	55.0
Amino Nitrogen	6.0		

Amino Acids (%)

Alanine	5.36	Lysine	5.15
Arginine	3.02	Methionine	1.05
Aspartic Acid	6.69	Phenylalanine	2.53
Cystine	0.74	Proline	2.60
Glutamic Acid	14.20	Serine	2.84
Glycine	3.25	Threonine	2.95
Histidine	1.20	Tryptophan	1.36
Isoleucine	3.23	Tyrosine	1.20
Leucine	4.69	Valine	3.79

Inorganics (%)

Calcium	0.013	Phosphate	3.270
Chloride	0.380	Potassium	3.195
Cobalt	<0.001	Sodium	1.490
Copper	<0.001	Sulfate	0.091
Iron	<0.001	Sulfur	0.634
Lead	<0.001	Tin	<0.001
Magnesium	0.075	Zinc	0.011
Manganese	<0.001		

Vitamins (µg/g)

Biotin	3.3	PABA	763.0
Choline (as Choline Chloride)	300.0	Pantothenic Acid	273.7
Cyanocobalamin	<0.1	Pyridoxine	43.2
Folic Acid	1.5	Riboflavin	116.5
Inositol	1400.0	Thiamine	529.9
Nicotinic Acid	597.9	Thymidine	17.5

Biological Testing (CFU/g)

Coliform	negative	Standard Plate Count	60
Salmonella	negative	Thermophile Count	<5
Spore Count	9		

Precautions

1. For Laboratory Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage
Store the dehydrated ingredient below 30°C. The dehydrated ingredient is very hygroscopic. Keep container tightly closed.

Expiration Date
The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure
Materials Provided
Yeast Extract
Yeast Extract, Technical
Autolyzed Yeast

Materials Required But Not Provided
Materials vary depending on the medium being prepared.

Method of Preparation
Refer to the final concentration of Yeast Extract, Yeast Extract, Technical or Autolyzed Yeast in the formula of the medium being prepared. Add Yeast Extract, Yeast Extract, Technical or Autolyzed Yeast as required.

Specimen Collection and Preparation
Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Test Procedure
See appropriate references for specific procedures using Yeast Extract, Yeast Extract, Technical or Autolyzed Yeast.

Results
Refer to appropriate references and procedures for results.

Limitations of the Procedure
1. Since the nutritional requirements of organisms vary, some strains may encountered that fail to grow or grow poorly on prepared medium.

References

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3. **Partansky and McPherson.** 1940. Ind. Eng. Chem., Anal. Ed. **12**:443.
4. **Snell and Strong.** 1939. Ind. Eng. Chem., Anal. Ed. **11**:346.
5. **Kawaguchi, T., T. Asahi, T. Satoh, T. Uozumi, and T. Beppu.** 1984. B-factor, an essential regulatory substance inducing the production of rifamycin in a *Nocardia* sp. J. Antibiot. **37**:1587-1594.
6. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of food, 3rd ed. American Public Health Association, Washington, D.C.
7. **Association of Official Analytical Chemists.** 1995. Bacteriological analytical manual, 8th ed. AOAC International, Gaithersburg, MD.
8. **Eaton, A. D., L. S. Clesceri, and A. E. Greenberg (ed.).** 1995. Standard methods for the examination of water and wastewater, 19th ed. American Public Health Association, Washington, D.C.
9. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
10. **J. Ind. Eng. Chem., Anal. Ed.** 1941. **13**:567.
11. **J. Ind. Eng. Chem., Anal. Ed.** 1942. **14**:909.

Packaging
Yeast Extract	100 g	0127-15
	500 g	0127-17
	2 kg	0127-07
	10 kg	0127-08
Yeast Extract, Technical	500 g	0886-17
	10 kg	0886-08
Autolyzed Yeast	500 g	0229-17
	10 kg	0229-08

Bacto® Yeast Extract Glucose Chloramphenicol Agar

Intended Use
Bacto Yeast Extract Glucose Chloramphenicol Agar is a selective agar recommended by the International Dairy Federation[1,2] for enumerating yeasts and molds in milk and milk products.

Also Known As
Yeast Extract Glucose Chloramphenicol Agar is also known as YGC Agar.

Summary and Explanation
The antibiotic method for enumerating yeasts and molds in dairy products has become the method of choice, replacing the traditional acidified method.[2] The use of antibiotics for suppressing bacteria results in better recovery of injured fungal cells, which are sensitive to an acid environment, and in less interference from precipitated food particles during the counting.[3-7]

Yeast Extract Glucose Chloramphenicol Agar is a nutrient medium that inhibits the growth of organisms other than yeasts and molds due to the presence of Chloramphenicol. When a sample contains predominantly yeasts and/or injured yeasts, the use of Yeast Extract Glucose Chloramphenicol Agar may offer some advantage.[2] After incubation at 25°C, colonies are counted and yeast colonies are distinguished from molds by colony morphology.

Principles of the Procedure
Yeast Extract provides basic nutrients. Glucose is a carbon energy source. Chloramphenicol inhibits bacterial growth.

Formula
Yeast Extract Glucose Chloramphenicol Agar

Formula per liter

Bacto Yeast Extract	5 g
Glucose	20 g
Chloramphenicol	0.1 g
Bacto Agar	13 g

Final pH 6.6 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **TOXIC.** MAY CAUSE CANCER. POSSIBLE RISK OF HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Blood, Nerves, Lymph Glands, Eyes.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Yeast Extract Glucose Chloramphenicol Agar
Materials Required but not Provided
Glassware
Distilled or deionized water
Autoclave
Petri dishes
Ringer's solution
2% Dipotassium phosphate
2% Sodium citrate

Method of Preparation

1. Suspend 38.1 grams in 1 liter distilled or deionized water.
2. Boil gently to dissolve completely.
3. Dispense 10-12 ml aliquots into tubes or other final containers and cap loosely.
4. Autoclave at 121°C for 15 minutes.

Test Procedure

1. Prepare initial sample dilutions using 10 grams or 10 ml of sample in 90 ml of diluent, as listed below:

SAMPLE 10 grams or 10 ml	DILUENT 90 ml	PREPARATION
Milk Liquid milk product	1/4-strength Ringer's solution	Mix.
Dried Milk Whey powder Buttermilk powder Lactose	1/4-strength Ringer's solution	Shake at 47°C.
Casein	2% dipotassium phosphate solution	Shake at 47°C.
Cheese	2% sodium citrate solution	Shake at 47°C.
Butter Edible ice	1/4-strength Ringer's solution	Shake at 47°C.
Custard dessert Fermented milk Yogurt	1/4-strength Ringer's solution	Shake.

2. Add 10 ml from the initial dilution prepared above (#1) to 90 ml of 1/4-strength Ringer's solution. One milliliter (1 ml) of this dilution corresponds to 0.01 gram/ml of sample.
3. Prepare further dilutions by adding 10 ml of the 0.01 gram/ml dilution above (#2) to 90 ml of diluent.
4. Pipette 1 ml of each dilution into two Petri dishes.
5. Pour 10 ml of sterile molten agar (cooled to 45°C) into each dish. Mix thoroughly.
6. Incubate at 25°C for 4 days.

Results

1. Select plates containing 10-300 colonies and count the colonies. Distinguish yeasts from molds by colony morphology.
2. Express results as yeasts and molds "per gram" or "per milliliter."

User Quality Control

Identity Specifications

Dehydrated Appearance:	Beige, free-flowing, homogeneous.
Solution:	3.81% solution, soluble in distilled or deionized water on boiling. Solution is light amber, clear to slightly opalescent.
Reaction of 3.81% Solution at 25°C:	pH 6.6 ± 0.2

Cultural Response

Prepare the medium per label directions. Inoculate by the pour plate technique and incubate at 25 ± 2°C for up to 4 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Aspergillus niger	16404	30-300	good
Candida albicans	10231	30-300	good
Escherichia coli	25922*	1,000-2,000	inhibited
Saccharomyces cerevisiae	9763	30-300	good

*This culture is available as a Bactrol™ Disk and should be used as directed in Bactrol Disks Technical Information.

References

1. **International Dairy Federation.** Standard Method ISO/DIS 6611.
2. **Frank, J. F., G. L. Christen, and L. B. Bullerman.** 1993. Tests for groups of microorganisms, p. 281-283. *In* R. T. Marshall, (ed.), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
3. **Beuchat, L. R.** 1979. Comparison of acidified and antibiotic-supplemented potato dextrose agar from three manufacturers for its capacity to recover fungi from foods. J. Food Prot. **42**:427-428.
4. **Cooke, W. B., and A. R. Brazis.** 1968. Occurrence of molds and yeasts in dairy products. Mycopathol. Mycol. Appl. **35**:281-289.

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6. **Koburger, J. A.** 1973. Fungi in foods: 5. Response of natural populations to incubation temperatures between 12 and 32°C. J. Milk Food Technol. **36**:434- 435.
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Packaging

Yeast Extract Glucose Chloramphenicol Agar	500 g	1900-17
	5 kg	1900-03

Yeast Media

Bacto® Yeast Morphology Agar · Bacto Yeast Carbon Base Bacto Yeast Nitrogen Base · Bacto Yeast Nitrogen Base w/o Amino Acids · Bacto Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

User Quality Control

Identity Specifications

Yeast Morphology Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	3.5% solution, soluble in distilled or deionized water on boiling. Solution is very light amber, very slightly to slightly opalescent.
Prepared Medium:	Very light amber, slightly opalescent without significant precipitate.
Reaction of 3.5% Solution at 25°C:	pH 5.6 ± 0.2

Yeast Carbon Base

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	1.17% (single-strength) and 11.7% (10X) solution, soluble in distilled or deionized water with slight warming. Single-strength solution is colorless to very light amber, clear after filter-sterilization.
Prepared Medium:	Colorless to very light amber, clear, no precipitate.
Reaction of 1.17% Solution at 25°C:	pH 5.5 ± 0.2

Yeast Nitrogen Base

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	0.67% (single strength) and 6.7% (10X) solution, soluble in distilled or deionized water with agitation. Single-strength solution is almost colorless and clear; 10X solution is yellow and clear.
Prepared Medium:	Colorless, clear without precipitate.
Reaction of 0.67% Solution at 25°C:	pH 5.4 ± 0.2

continued on following page

Intended Use

Bacto Yeast Morphology Agar is used for classifying yeasts based on colonial characteristics and cell morphology.

Bacto Yeast Carbon Base is used for classifying yeasts based on nitrogen assimilation.

Bacto Yeast Nitrogen Base is used for classifying yeasts based on carbon assimilation.

Bacto Yeast Nitrogen Base w/o Amino Acids is used for classifying yeasts based on amino acid and carbohydrate requirements.

Bacto Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate is used for classifying yeasts based on carbon and nitrogen requirements.

Summary and Explanation

Yeasts are unicellular, eukaryotic, budding cells that are generally round-to-oval or elongate in shape.[1] They multiply principally by the production of blastoconidia (buds).[1] Yeast colonies are moist and creamy or glabrous to membranous in texture.[1] Yeasts are considered opportunistic pathogens.[1]

The yeast media cited are prepared according to the formulas of Wickerham.[2,3,4,5,6]

Yeast Carbon Base tests the ability of yeasts to assimilate nitrogen by the addition of various nitrogen sources. The inclusion of vitamins aids in the utilization of nitrogen-containing compounds by certain yeasts which cannot assimilate these compounds in the absence of vitamins.

Yeast Nitrogen Base is a suitable medium for studying strains of yeast that require certain vitamins.

Yeast Nitrogen Base w/o Amino Acids, which lacks the amino acids histidine, methionine and tryptophane, and Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate, which lacks amino acids and ammonium sulfate, are prepared according to Guenter's[7] modification of Wickerham's Yeast Nitrogen Base formulation.

These media are included in many applications for the study of yeasts in molecular genetics.[8,9]

Principles of the Procedure

Yeast Morphology Agar contains all essential nutrients and vitamins necessary for the cultivation of yeasts, including a source of carbohydrate.

Yeast Carbon Base contains all essential nutrients and vitamins necessary for the cultivation of yeasts except a source of nitrogen.

Yeast Nitrogen Base contains all essential nutrients and vitamins necessary for the cultivation of yeasts except a source of carbohydrate.

Yeast Nitrogen Base w/o Amino Acids contains all essential vitamins and inorganic salts necessary for the cultivation of yeasts except histidine, methionine, tryptophane and a source of carbohydrate.

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate contains all essential nutrients and vitamins necessary for the cultivation of yeasts except amino acids and a source of nitrogen and carbohydrate.

Formula

Yeast Morphology Agar

Formula per Liter

Nitrogen Sources

Ammonium Sulfate	3.5 g
Asparagine	1.5 g

Carbon Source

Dextrose	10 g

Amino Acids

L-Histidine Monohydrochloride	10 mg
LD-Methionine	20 mg
LD-Tryptophan	20 mg

Vitamins

Biotin	2 µg
Calcium Pantothenate	400 µg
Folic Acid	2 µg
Inositol	2,000 µg
Niacin	400 µg
p-Aminobenzoic Acid	200 µg
Pyridoxine Hydrochloride	400 µg
Riboflavin	200 µg
Thiamine Hydrochloride	400 µg

User Quality Control cont.

Yeast Nitrogen Base w/o Amino Acids

Dehydrated Appearance:	Off-white, free-flowing, homogeneous.
Solution:	0.67% (single strength) or 6.7% (10X) solution, soluble in distilled or deionized water with agitation. Single-strength solution is colorless to very pale yellow and clear; 10X solution is yellow and clear.
Prepared Medium:	Colorless, clear without precipitate.
Reaction of 0.67% Solution at 25°C:	pH 5.4 ± 0.2

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Dehydrated Appearance:	Light yellowish-beige, free-flowing, homogeneous.
Solution:	0.17% (single-strength) and 1.7% (10X) solution, soluble in distilled or deionized water. Single-strength solution is colorless to very pale yellow and clear; 10X solution is yellow and clear.
Prepared Medium:	Colorless, clear without precipitate.
Reaction of 0.17% Solution at 25°C:	pH 4.5 ± 0.2

Cultural Response

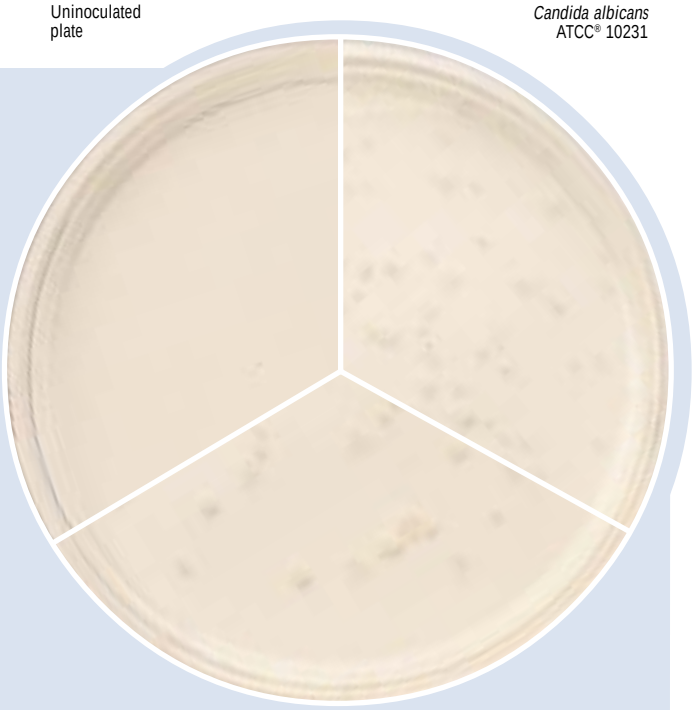
Yeast Morphology Agar

Prepare Yeast Morphology Agar per label directions. Inoculate using the pour plate technique and incubate at 25-30°C for 18-48 hours. Also, inoculate by the Dolman technique (streak and point) and incubate at 25-30°C for 6-7 days.

ORGANISM	ATCC®	GROWTH	DOLMAN PLATE TEST
Kloeckera apiculata	9774	good	–
Saccharomyces pastorianus	9080	good	–
Candida albicans	10231	good	hyphae

Uninoculated plate

Candida albicans ATCC® 10231



Saccharomyces pastorianus ATCC® 9080

Yeast Carbon Base (with and without 5% ammonium sulfate)
Yeast Nitrogen Base (with and without 5% dextrose)
Yeast Nitrogen Base w/o Amino Acids (with and without 5% dextrose, 0.02% DL-methionine, 0.02% DL-tryptophane and 0.015% L-histidine)
Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate (with and without 5% dextrose, 5% ammonium sulfate, 0.02% DL-methionine, 0.02% DL-tryptophane and 0.01% L-histidine)

Prepare the medium per label directions with and without the supplements indicated above. Inoculate and incubate at 25-30°C for 2-5 days.

ORGANISM	ATCC®	GROWTH WITHOUT SUPPLEMENT(S)	GROWTH WITH SUPPLEMENT(S)
Kloeckera apiculata	9774	none to poor	good
Saccharomyces pastorianus	9080	none to poor	good

The cultures listed are the minimum that should be used for performance testing.

Compounds Supplying Trace Elements
Boric Acid ..... 500 µg
Copper Sulfate ..... 40 µg
Potassium Iodide ..... 100 µg
Ferric Chloride ..... 200 µg
Manganese Sulfate ..... 400 µg
Sodium Molybdate ..... 200 µg
Zinc Sulfate ..... 400 µg

Salts
Potassium Phosphate Monobasic ..... 1 g
Magnesium Sulfate ..... 0.5 g
Sodium Chloride ..... 0.1 g
Calcium Chloride ..... 0.1 g
Bacto Agar ..... 18 g

Final pH 5.6 ± 0.2 at 25°C

Yeast Carbon Base

Formula per Liter

Carbon Source
Dextrose ..... 10 g

Amino Acids
L-Histidine Monohydrochloride ..... 1 mg
LD-Methionine ..... 2 mg
LD-Tryptophan ..... 2 mg

Vitamins
Biotin ..... 2 µg
Calcium Pantothenate ..... 400 µg
Folic Acid ..... 2 µg
Inositol ..... 2,000 µg
Niacin ..... 400 µg
p-Aminobenzoic Acid ..... 200 µg
Pyridoxine Hydrochloride ..... 400 µg
Riboflavin ..... 200 µg
Thiamine Hydrochloride ..... 400 µg

Compounds Supplying Trace Elements
Boric Acid ..... 500 µg
Copper Sulfate ..... 40 µg
Potassium Iodide ..... 100 µg
Ferric Chloride ..... 200 µg
Manganese Sulfate ..... 400 µg
Sodium Molybdate ..... 200 µg
Zinc Sulfate ..... 400 µg

Salts
Potassium Phosphate Monobasic ..... 1 g
Magnesium Sulfate ..... 0.5 g
Sodium Chloride ..... 0.1 g
Calcium Chloride ..... 0.1 g

Final pH 5.5 ± 0.2 at 25°C

Yeast Nitrogen Base

Formula per Liter

Nitrogen Sources
Ammonium Sulfate ..... 5 g

Amino Acids
L-Histidine Monohydrochloride ..... 10 mg
LD-Methionine ..... 20 mg
LD-Tryptophan ..... 20 mg

Vitamins
Biotin ..... 2 µg
Calcium Pantothenate ..... 400 µg
Folic Acid ..... 2 µg
Inositol ..... 2,000 µg
Niacin ..... 400 µg
p-Aminobenzoic Acid ..... 200 µg
Pyridoxine Hydrochloride ..... 400 µg
Riboflavin ..... 200 µg
Thiamine Hydrochloride ..... 400 µg

Compounds Supplying Trace Elements
Boric Acid ..... 500 µg
Copper Sulfate ..... 40 µg
Potassium Iodide ..... 100 µg
Ferric Chloride ..... 200 µg
Manganese Sulfate ..... 400 µg
Sodium Molybdate ..... 200 µg
Zinc Sulfate ..... 400 µg

Salts
Potassium Phosphate Monobasic ..... 1 g
Magnesium Sulfate ..... 0.5 g
Sodium Chloride ..... 0.1 g
Calcium Chloride ..... 0.1 g

Final pH 5.4 ± 0.2 at 25°C

Yeast Nitrogen Base w/o Amino Acids

Formula per Liter

Nitrogen Sources
Ammonium Sulfate ..... 5 g

Vitamins
Biotin ..... 2 µg
Calcium Pantothenate ..... 400 µg
Folic Acid ..... 2 µg
Inositol ..... 2,000 µg
Niacin ..... 400 µg
p-Aminobenzoic Acid ..... 200 µg
Pyridoxine Hydrochloride ..... 400 µg
Riboflavin ..... 200 µg
Thiamine Hydrochloride ..... 400 µg

Compounds Supplying Trace Elements
Boric Acid ..... 500 µg
Copper Sulfate ..... 40 µg
Potassium Iodide ..... 100 µg
Ferric Chloride ..... 200 µg
Manganese Sulfate ..... 400 µg
Sodium Molybdate ..... 200 µg
Zinc Sulfate ..... 400 µg

Salts
Potassium Phosphate Monobasic ..... 1 g
Magnesium Sulfate ..... 0.5 g
Sodium Chloride ..... 0.1 g
Calcium Chloride ..... 0.1 g

Final pH 5.4 ± 0.2 at 25°C

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Formula per Liter

Vitamins
Biotin ..... 2 µg
Calcium Pantothenate ..... 400 µg
Folic Acid ..... 2 µg
Inositol ..... 2,000 µg
Niacin ..... 400 µg
p-Aminobenzoic Acid ..... 200 µg

Pyridoxine Hydrochloride	400 µg
Riboflavin	200 µg
Thiamine Hydrochloride	400 µg

Compounds Supplying Trace Elements

Boric Acid	500 µg
Copper Sulfate	40 µg
Potassium Iodide	100 µg
Ferric Chloride	200 µg
Manganese Sulfate	400 µg
Sodium Molybdate	200 µg
Zinc Sulfate	400 µg

Salts

Potassium Phosphate Monobasic	1 g
Magnesium Sulfate	0.5 g
Sodium Chloride	0.1 g
Calcium Chloride	0.1 g

Final pH 4.5 ± 0.2 at 25°C

Precautions

1. For Laboratory Use.
2. **Yeast Morphology Agar**

   IRRITANT. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Lungs, Intestines.

   Yeast Nitrogen Base

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. HARMFUL IF SWALLOWED. (EC) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   Yeast Nitrogen Base w/o Amino Acids

   HARMFUL. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. HARMFUL IF SWALLOWED. (EC) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store the dehydrated medium below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Yeast Morphology Agar
Yeast Carbon Base
Yeast Nitrogen Base
Yeast Nitrogen Base w/o Amino Acids
Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Materials Required But Not Provided

Glassware
Filter sterilization equipment
Sterile distilled or deionized water
Sterile test tubes
Spectrophotometer
Petri dishes
Pipettes
Dextrose or an equivalent carbohydrate (Yeast Nitrogen Base)
Nitrogen source (Yeast Carbon Base)

Method of Preparation

Yeast Morphology Agar

1. Suspend 35 grams of Yeast Morphology Agar in 1 liter distilled or deionized water.
2. Heat to boiling to dissolve completely.
3. Autoclave at 121°C for 15 minutes.
4. Pour the sterile medium into plates to a depth of approximately 1.5 mm.
5. Allow the plates to stand at room temperature.

Yeast Carbon Base

1. Prepare a 10X solution by dissolving 11.7 grams of Yeast Carbon Base and a nitrogen source in 100 ml distilled or deionized water.

   NOTE: When using potassium nitrate, an important nitrogen-containing compound in nitrogen assimilation testing, add 0.78 grams.
2. Warm to dissolve, if necessary. Mix well.
3. Filter-sterilize the solution.
4. Store at 2-8°C.
5. Prepare the final medium by aseptically pipetting 0.5 ml of the 10X solution into 4.5 ml of sterile distilled water.
6. Mix the solution thoroughly by shaking before inoculation.

Yeast Nitrogen Base

1. Prepare a 10X solution by dissolving 6.7 grams of Yeast Nitrogen Base and 5 grams of Dextrose or an equivalent amount of other carbohydrate in 100 ml distilled or deionized water.
2. Warm slightly to dissolve. Mix well.
3. Filter-sterilize the solution.
4. Store at 2-8°C.
5. Prepare the final medium by aseptically pipetting 0.5 ml of the 10X solution into 4.5 ml of sterile distilled water.
6. Mix the solution thoroughly by shaking before inoculation.

Yeast Nitrogen Base w/o Amino Acids

1. Prepare a 10X solution by dissolving 6.7 grams of Yeast Nitrogen Base w/o Amino Acids and 5 grams of Dextrose or an equivalent amount of other carbohydrate and 5-10 mg% of the desired amino acid in 100 ml of distilled or deionized water.
2. Filter sterilize the solution.
3. Store at 2-8°C.
4. Prepare the final medium by aseptically pipetting 0.5 ml of the 10X solution into 4.5 ml of sterile distilled water.
5. Mix the solution thoroughly by shaking before inoculation.

Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

1. Prepare a 10X solution by suspending 1.7 grams of Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate and nitrogen and carbon sources, as required, in 100 ml distilled or deionized water.
2. Filter sterilize the solution.
3. Store at 2-8°C.
4. Prepare the final medium by aseptically pipetting 0.5 ml of the 10X solution into 4.5 ml sterile distilled water.
5. Mix the solution thoroughly by shaking before inoculation.

Specimen Collection and Preparation

Obtain and process specimens according to the techniques and procedures established by laboratory policy.

Procedure

Yeast Morphology Agar

Inoculate plates using the Dolman technique (as follows) described by Wickerham and Rettger.[1] This is an excellent method for studying the hyphae of filamentous yeasts.

1. Near one side of the plate (from the relative positions of 10 o'clock to 2 o'clock), lightly inoculate a single streak taken from a slant culture.
2. In addition to the single streak, inoculate two points near the other side of the plate (at the 4 o'clock and 8 o'clock positions).
3. Cover a central section of the streak inoculation and one point inoculation with cover glasses, as follows:
   a. With forceps, remove a cover glass from absolute alcohol, drain momentarily, and burn off excess alcohol by passing over a low flame.
   b When the cover glass has cooled, place one edge on the agar and allow it to fall across the central portion of the inoculated streak. Place a second cover glass over one point inoculation.
4. Incubate at 25-30°C for 6-7 days.
5. After incubation, observe with a high dry objective.

Yeast Carbon Base, Yeast Nitrogen Base, Yeast Nitrogen Base w/o Amino Acids, Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

1. Inoculate the prepared tubed medium very lightly with the test organism.
2. Incubate at 25°C for 6-7 days.
3. After incubation (6-7 days and, if necessary, 20-24 days), shake the tubes to suspend growth.
4. Read for growth.

Carbon Assimilation Test

Refer to the procedure described in the Manual of Clinical Microbiology.[10]

Nitrogen Assimilation Test

Refer to the procedure described in the Manual of Clinical Microbiology.[10]

Results

Yeast Morphology Agar

Using the high-dry objective, observe for hyphae of filamentous yeasts.

Yeast Carbon Base, Yeast Nitrogen Base, Yeast Nitrogen Base w/o Amino Acids, Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

Measure growth turbidimetrically at 660 nm wavelength using a spectrophotometer. Turbidimetric readings on assay tubes should be comparable to the control.

Limitations of the Procedure

1. Because the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on a medium.
2. Yeasts grown on a rich medium may carry a reserve of nitrogen in the form of protein. Possible errors due to this reserve are eliminated by making two serial transfers in the complete medium. When the first transfer is seven days old, the culture is shaken and one loopful is transferred to a second tube of the complete medium containing the same source of nitrogen. If a positive test is obtained when the second culture is seven days old, the organism being tested assimilates this particular nitrogen source.

References

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3. **Wickerham, L. J.** 1939. J. Tropical Med. Hyg. **42**:176.
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5. **Wickerham, L. J.** 1948. J. Bacteriol. **56**:363.
6. **Wickerham, L. J.** 1943. J. Bacteriol. **46**:501.
7. **Guenter.** Personal Communication.
8. **Sherman F., G. R. Fink, and J. B. Hicks.** 1986. Methods in yeast genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
9. **Brownstein, B. H., G. A. Silverman, R. D. Little, D. T. Burke, S. J. Korsmeyer, D. Schlessinger, and M. V. Olson.** 1989. Isolation of single-copy human genes from a library of yeast artificial chromosomes clones. Science. **244**:1348-1351.
10. **Warren, N. G., and H. J. Shadomy.** 1991. Yeasts of medical importance, p. 617- 629. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.). Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.

Packaging

Yeast Morphology Agar	100 g	0393-15
Yeast Carbon Base	100 g	0391-15
Yeast Nitrogen Base	100 g	0392-15
Yeast Nitrogen Base w/o Amino Acids	100 g	0919-15
	2 kg	0919-07
	10 kg	0919-08
Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate	100 g	0335-15
	10 kg	0335-08

Yersinia Selective Agar

Bacto® Yersinia Selective Agar Base · Bacto Yersinia Antimicrobic Supplement CN

Intended Use

Bacto Yersinia Selective Agar Base is used with Bacto Yersinia Antimicrobic Supplement CN in isolating and cultivating *Yersinia enterocolitica*.

Also Known As

Yersinia Selective Agar is also known as CIN Agar, Modified or Cefsulodin-Irgasan-Novobiocin Agar, Modified.

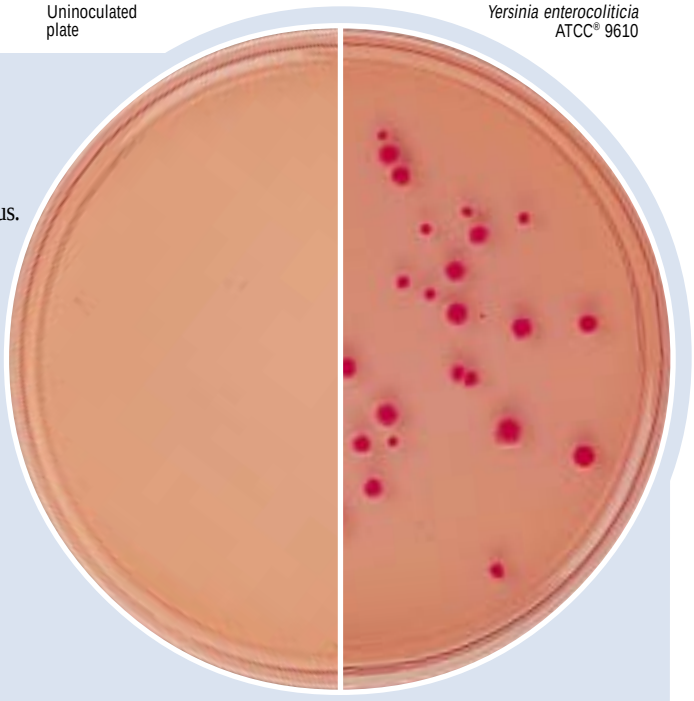
Summary and Explanation

Yersinia enterocolitica is a significant enteric pathogen[6] and can be food- or water- borne.[7]

Yersinia Selective Agar is a selective and differential medium that supports good growth of *Y. enterocolitica* and some other *Yersinia* species. The formulation is based on the Cefsulodin-Irgasan-Novobiocin (CIN) Agar formulation of Schiemann.[2-5] In comparison with MacConkey Agar and Salmonella-Shigella Agar, Schiemann found that CIN Agar provided better inhibition of normal enteric organisms and provided improved direct recovery of *Y. enterocolitica* from feces.[3] Schiemann later modified his original formula by substituting 0.5 grams of deoxycholate for the bile salts mixture and by reducing the content of novobiocin to 2.5 mg/liter for improved growth of strains of *Y. enterocolitica* serogroup 0:8.[5] The concentration of cefsulodin in the antimicrobic supplement was reduced from that described by Schiemann to further improve growth and recovery of *Y. enterocolitica*.

Principles of the Procedure

Selectivity of Yersinia Selective Agar Base is due to the presence of bile salts, crystal violet and Irgasan®, which markedly inhibit growth of gram-positive and many gram-negative organisms. Supplementation with Yersinia Antimicrobic Supplement CN (Cefsulodin and Novobiocin) improves inhibition of normal enteric organisms. Differentiation is based on mannitol fermentation. Organisms capable of fermenting



User Quality Control

Identity Specifications

Yersinia Selective Agar Base

Dehydrated Appearance: Light pinkish beige, free-flowing, homogeneous.

Solution: 5.95% solution, soluble in distilled or deionized water upon boiling. Reddish purple, very slightly to slightly opalescent.

Prepared Medium: Reddish orange, very slightly to slightly opalescent.

Reaction of a 5.95% Solution at 25°C: pH 7.4 ± 0.2

Yersinia Antimicrobic Supplement CN

Dehydrated Appearance: Lyophilized, white, homogeneous cake.

Solution: Soluble on rehydration with distilled or deionized water, colorless, clear.

Reaction of Solution at 25°C: pH 5.2-6.3

Cultural Response

Prepare Yersinia Selective Agar according to label directions. Inoculate and incubate at 30 ± 2°C for 18-24 hours or at 22-25°C for 48 hours.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH	APPEARANCE
Enterococcus faecalis	29212*	2,000-10,000	marked to complete inhibition	
Escherichia coli	25922*	2,000-10,000	marked to complete inhibition	
Pseudomonas aeruginosa	27853*	2,000-10,000	marked to complete inhibition	
Yersinia enterocolitica	9610	100-1,000	good	colorless with dark pink centers; bile precipitate may be present

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

mannitol produce a pH decrease around the colony which allows absorption of neutral red, giving the colony a red color. Due to the localized pH decrease, a zone of precipitated bile may also be present. Organisms that do not metabolize mannitol to acid end products will form colorless, translucent colonies.

Formula

Yersinia Selective Agar Base

Formula Per Liter	
Bacto Yeast Extract	2 g
Bacto Peptone	17 g
Bacto Proteose Peptone	3 g
Mannitol	20 g
Sodium Deoxycholate	0.5 g
Sodium Cholate	0.5 g
Sodium Chloride	1 g
Sodium Pyruvate	2 g
Magnesium Sulfate Heptahydrate	10 mg
Bacto Agar	13.5 g
Neutral Red	30 mg
Crystal Violet	1 mg
Irgasan	4 mg

Yersinia Antimicrobic Supplement CN

Formula Per 10 ml Vial	
Cefsulodin	4 mg
Novobiocin	2.5 mg

Precautions

1. For Laboratory Use.
2. MAY CAUSE ALLERGIC EYE, RESPIRATORY SYSTEM AND SKIN REACTION. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. Target Organs: Liver, Blood.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store Yersinia Selective Agar Base dehydrated below 30°C. The dehydrated medium is very hygroscopic. Keep container tightly closed

Store Yersinia Antimicrobic Supplement CN lyophilized and rehydrated at 2-8°C. Do not open or rehydrate vials until ready to use. Use the rehydrated product within 24 hours.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Yersinia Selective Agar Base
Yersinia Antimicrobic Supplement CN

Materials Required But Not Provided

Glassware
Distilled or deionized water
Incubator (22-25°C or 30 ± 2°C)
Autoclave
Sterile Petri dishes

Method of Preparation

Yersinia Antimicrobic Supplement CN

1. To rehydrate the supplement, aseptically add 10 ml sterile distilled or deionized water to the vial.
2. Invert the vial gently several times to dissolve the powder.

Yersinia Selective Agar Base

1. Suspend 59.5 grams in 1 liter distilled or deionized water and boil to dissolve completely.
2. Autoclave at 121°C for 15 minutes. Avoid overheating.
3. Cool the medium to 45-50°C. Aseptically add 10 ml rehydrated Yersinia Antimicrobic Supplement CN to the medium. Mix well.
4. Dispense into Petri dishes.

Specimen Collection and Preparation

All specimens should be collected in sterile containers in accordance with recommended guidelines and should be transported immediately to the laboratory. For specific information on collection and storage of specimens consult appropriate references.

Test Procedure

For a complete discussion on the isolation and identification of *Yersinia*, consult appropriate references.

Results

Y. enterocolitica colonies appear translucent or translucent with dark pink centers. Colony edges are entire or irregular. After 48 hours incubation, colonies appear dark pink with a translucent border and may be surrounded by a zone of precipitated bile.

Growth of non-*Yersinia* organisms is markedly to completely inhibited.

Limitations of the Procedure

1. Yersinia Selective Agar Base and Yersinia Antimicrobic Supplement CN are intended for use in the preparation of Yersinia Selective Agar. Although this medium is selective for *Yersinia*, biochemical testing using pure cultures is necessary for complete identification.
2. Due to the selective properties of the medium, some *Yersinia* strains may be encountered that fail to grow or grow poorly on the complete medium. Some strains of normal enteric organisms may be encountered that are not inhibited or are only partially inhibited on the complete medium, such as *Citrobacter freundii*, *Serratia liquefaciens* and *Enterobacter agglomerans*.
3. Growth of *Yersinia frederiksenii*, *Y. kristensenii*, *Y. pseudotuberculosis* and *Y. intermedia* is not inhibited on the complete medium. Colonies of these organisms must be differentiated from *Y. enterocolitica* on the basis of additional characteristics.

References

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6. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook. American Society for Microbiology, Washington, D.C.
7. **Vanderzant, C., and D. F. Splittstoesser (ed.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
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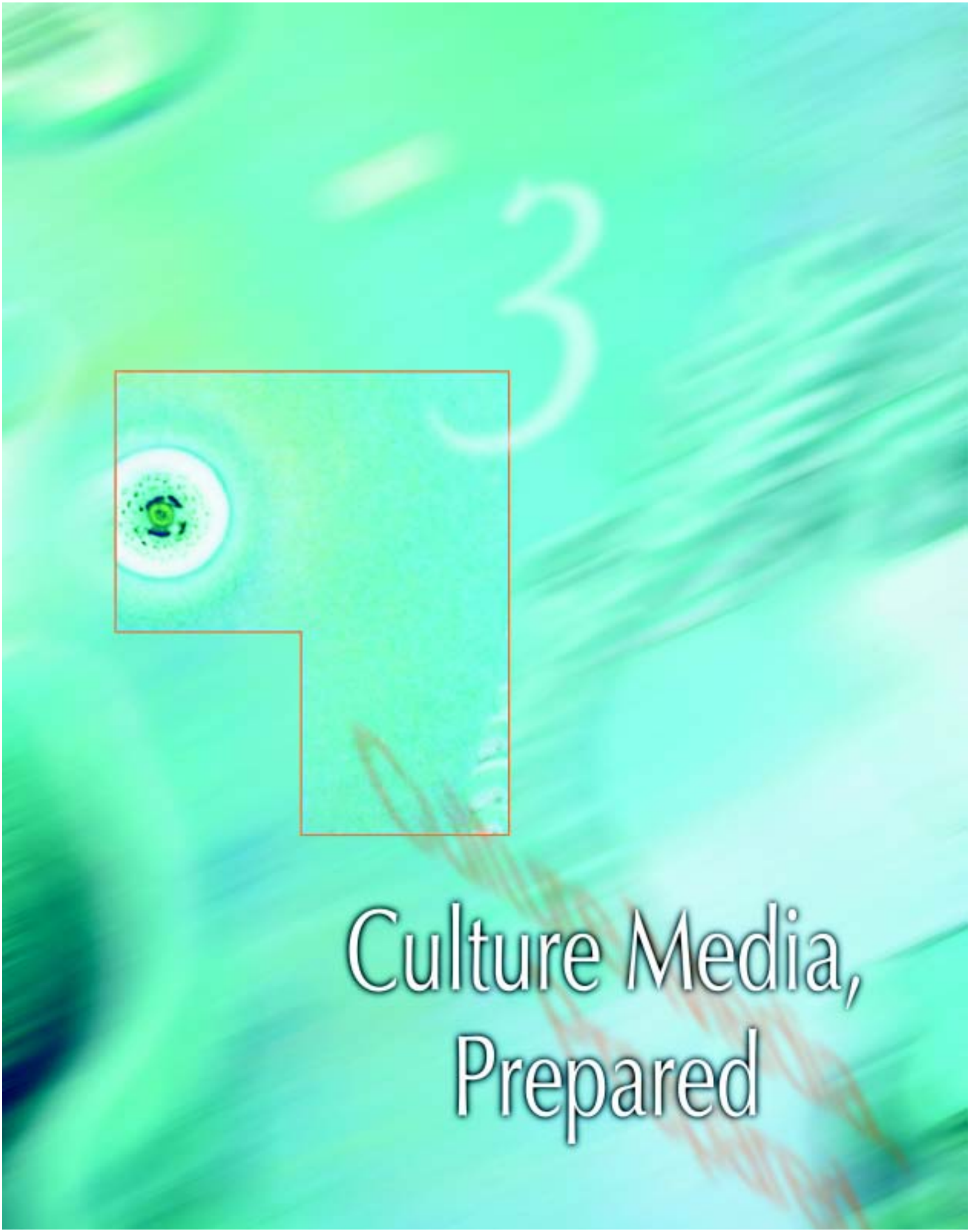
Packaging

Yersinia Selective Agar Base	500	g	1817-17
	10	kg	1817-08
Yersinia Antimicrobic Supplement CN	6 x 10	ml	3196-60


Culture Media,
Prepared

Bacto® ATS Medium

Intended Use

Bacto ATS Medium is used for the isolation and cultivation of mycobacteria.

Also Known As

ATS Medium is also known as American Trudeau Society Medium.

Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.[1] During the mid 1980's the number of tuberculosis (TB) cases in the U.S. began increasing. Before this time, the number of cases in the U.S. had been decreasing, reaching a low in 1984. Non-tuberculous mycobacteria infections have also increased since the mid 1980's.[2]

Two types of semi-solid culture media are available for the isolation of mycobacteria: egg-based media and agar-based media. Most formulations for the isolation of mycobacteria include malachite green, which is used to inhibit contaminating organisms.

Principles of the Procedure

ATS Medium is prepared according to the formula described by the committee on evaluation of Laboratory Procedures of the American Trudeau Society.[3] ATS Medium is an egg-based medium containing a small amount of Malachite Green. Due to the low concentration of Malachite Green, this formulation permits the relatively early detection of mycobacteria colonies. This medium is well suited to specimens that are not heavily contaminated such as cerebral spinal fluid (CSF) and pleural fluid.[4,5] When present, contaminating microorganisms may liquefy the medium.

User Quality Control

Identity Specifications

Dehydrated Appearance:	Not applicable
Prepared Medium:	Light green, opaque
Reaction of Medium at 25°C:	pH 6.4 -7.5

Cultural Response

Inoculate and incubate at 35 ± 2°C under CO_2 for up to three weeks.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Mycobacterium fortuitum	6841	100-1,000	good
Mycobacterium intracellulare	13950	100-1,000	good
Mycobacterium kansasii	12478	100-1,000	good
Mycobacterium tuberculosis H37Ra	25177	100-1,000	good
Mycobacterium scrofulaceum	19981	100-1,000	good

These cultures are the minimum that should be used for performance testing.

Formula

ATS Medium

Formula Per Liter

Egg Yolk Suspension	500 ml
Glycerol Extract of Potatoes	500 ml
Malachite Green	0.2 ml

Final pH 6.4-7.5 at 25°C

Precautions

1. For In Vitro Diagnostic Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store prepared medium at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

ATS Medium

Materials Required But Not Provided

Incubator

Specimen Collection and Preparation[5]

1. Collect specimens in sterile containers and transport immediately to the laboratory following recommended guidelines.
2. Process each specimen as appropriate for that specimen.
3. Inoculate the specimen into medium.

Test Procedure

1. Incubate tubes for up to eight weeks.
2. Examine tubes for growth.

Results

Observe for colonies that may or may not be pigmented. Colony morphology is dependent on the species isolated.

Limitations of the Procedure

Negative culture results do not rule out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures include the following:

1. The specimen was not representative of the infectious material, i.e. saliva instead of sputum.
2. The mycobacteria were destroyed during digestion and decontamination of the specimen.
3. Gross contamination interfered with the growth of the mycobacteria.
4. Proper aerobic CO_2 tension was not provided during incubation.

References

1. **Musser, J. M.** 1995. Antimicrobial resistance in Mycobacteria: Molecular genetic insights. Clin. Microbiol. Rev. **8**:496-514.

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3. **Woodruff, C. E., D. Crombie, J. S. Woolley, E. Medlar, and W. Steeken**. 1946. Report of the committee on evaluation of laboratory procedures. Am. Rev. Tuberc. **54**:428-432.
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Packaging

ATS Medium	100 tubes	1019-79*

Bacto® Dubos Albumin Broth

See page 163 of Section II for complete listing of this product.

HYcheck™

HYcheck D/E Neutralizing Agar · HYcheck for Disinfection Control · HYcheck for Enterobacteriaceae · HYcheck Plate Count Agar With TTC · HYcheck for Total Count · HYcheck for Yeasts and Molds · HYcheck for Yeasts and Molds with TTC

Intended Use

HYcheck is a hygiene contact slide which is used for assessing the microbiological contamination of surfaces or fluids.

Summary and Explanation

Monitoring the microbial flora of environmental surfaces, walls, ceilings, and equipment is an important stage in achieving good manufacturing practices in factories handling foods, cosmetics or pharmaceuticals.[1,2,3] To maintain good hygiene standards in hotels and restaurant kitchens, microbiological contamination must also be monitored.[4] Methods to monitor the environmental flora have been described using either swabbing techniques[5] or contact plates.[6] Contact slides were created to monitor the microbial flora of liquids (e.g. urine, milk) and equipment surfaces in the clinical and food industries.[1] Contact slides are statistically comparable to swab and contact plates for surface sampling.[1] The HYcheck contact slides were developed for the testing of fluids and surfaces for microbial cleanliness.

HYcheck is a double sided, hinged plastic paddle containing two agar surfaces. The agar surface extends above the paddle allowing for contact with test surfaces. The hinged paddle allows the agar surface to be easily held against each test area during sampling. The surface area of the paddle is clearly divided into seven units of one centimeter each to allow direct counting of microbial density per unit area.

The HYcheck range of hygiene control slides consists of seven media combinations designed to meet the various needs for monitoring different types of microbial contamination.

HYcheck D/E Neutralizing Agar has both sides coated with D/E Neutralizing Agar, a medium developed by Dey and Engley[7] to neutralize a broad spectrum of disinfectants and preservative antimicrobial chemicals. D/E Neutralizing Agar neutralizes higher concentrations of residual antimicrobials, when compared with other standard neutralizing formulas, such as Letheen media, Thioglycollate media, and Neutralizing Buffer.[8,9] Complete neutralization of disinfectants is important because disinfectant carryover can result in a false no growth result. D/E Neutralizing media effectively neutralize the inhibitory effects of disinfectant carryover,[10,11] allowing differentiation between bacteriostasis and true bactericidal actions of disinfectant chemicals.

User Quality Control

Identity Specifications

HYcheck D/E Neutralizing Agar

Medium:	**D/E Neutralizing Agar**
Appearance:	Lavender
Microbial Limits Test:	Satisfactory
pH at 25°C:	7.6 ± 0.2

HYcheck for Disinfection Control

Media:	**D/E Neutralizing Agar**	**Tryptic Soy Agar**
Appearance:	Lavender	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.6 ± 0.2	7.3 ± 0.2

continued on following page

HYcheck for Enterobacteriaceae

Media:	**Violet Red Bile Glucose Agar**	**Tryptic Soy Agar**
Appearance:	Reddish purple	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.4 ± 0.2	7.3 ± 0.2

HYcheck Plate Count Agar with TTC

Medium:	**Plate Count Agar with 0.01% TTC**
Appearance:	Light amber
Microbial Limits Test:	Satisfactory
pH at 25°C:	7.0 ± 0.2

HYcheck for Total Count

Media:	**Plate Count Agar**	**Plate Count Agar with 0.01% TTC**
Appearance:	Light amber	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.0 ± 0.2	7.0 ± 0.2

HYcheck for Yeasts and Molds

Media:	**Rose Bengal Chloramphenicol Agar**	**Tryptic Soy Agar**
Appearance:	Rose pink	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.2 ± 0.2	7.3 ± 0.2

HYcheck for Yeast and Molds with TTC

Media:	**Rose Bengal Chloramphenicol Agar**	**Tryptic Soy Agar with 0.01% TTC**
Appearance:	Rose pink	Light amber
Microbial Limits Test:	Satisfactory	Satisfactory
pH at 25°C:	7.2 ± 0.2	7.3 ± 0.2

Cultural Response (approx inoculum 30-300 CFU)

HYcheck D/E Neutralizing Agar

Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH ON D/E AGAR
Aspergillus niger NCPF 2275	–	good
Bacillus subtilis	6633	good
Candida albicans	2091	good
Escherichia coli	25922*	good
Pseudomonas aeruginosa	27853*	good
Staphylococcus aureus	25923*	good
Staphylococcus epidermidis	12228*	good

HYcheck for Disinfection Control

Inoculate and incubate at 35 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH ON D/E	GROWTH ON TSA
Aspergillus niger NCPF 2275	–	good	good
Bacillus subtilis	6633	good	good
Candida albicans	2091	good	good
Escherichia coli	25922*	good	good
Pseudomonas /nosa	27853*	good	good
Staphylococcus aureus	25923*	good	good
Staphylococcus epidermidis	12228*	good	good

HYcheck for Enterobacteriaceae

Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	GROWTH ON VRBGA	GROWTH ON TSA
Enterobacter aerogenes	13048*	good	good
Enterococcus faecalis	19433*	none to poor	good
Escherichia coli	25922*	good	good
Proteus mirabilis NCTC 11938	–	good	good
Salmonella typhimurium	14028*	good	good
Shigella sonnei	25931*	good	good
Staphylococcus aureus	25923*	none to poor	good

HYcheck Plate Count Agar with TTC

Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	GROWTH ON PCA W/TTC
Enterococcus faecalis	19433*	good
Escherichia coli	25922*	good
Proteus vulgaris	13315	good
Salmonella typhimurium	14028*	good
Staphylococcus aureus	25923*	poor

HYcheck for Total Count

Inoculate and incubate at 35 ± 2°C for 18-24 hours.

ORGANISM	ATCC®	GROWTH ON PCA	GROWTH ON PCA W/TTC
Enterococcus faecalis	19433*	good	good
Escherichia coli	25922*	good	good
Proteus vulgaris	13315	good	good
Salmonella typhimurium	14028*	good	good
Staphylococcus aureus	25923*	good	poor

HYcheck for Yeasts and Molds

Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH ON RBCA	GROWTH ON TSA
Aspergillus niger NCPF 2275	–	good	good
Candida albicans	2091	good	good
Escherichia coli	25922*	none to poor	good
Saccharomyces cerevisiae NCYC 1211	–	good	good
Serratia marcescens	8100	none to poor	good
Staphylococcus aureus	25923*	none to poor	good
Streptococcus pyogenes	19615*	none to poor	good

HYcheck for Yeasts and Molds with TTC

Inoculate and incubate at 30 ± 2°C for 18-48 hours.

ORGANISM	ATCC®	GROWTH ON RBCA	GROWTH ON TSA W/TTC
Aspergillus niger NCPF 2275	—	good	good
Candida albicans	2091	good	good
Escherichia coli	25922*	none to poor	good
Saccharomyces cerevisiae NCYC 1211	–	good	poor
Serratia marcescens	8100	none to poor	good
Staphylococcus aureus	25923*	none to poor	poor
Streptococcus pyogenes	19615*	none to poor	good

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

HYcheck for Disinfection Control has side one coated with D/E Neutralizing Agar (D/E) (see above), and side two coated with Tryptic Soy Agar (TSA). In 1955, Leavitt et al.[12] demonstrated that Tryptic Soy Agar supports excellent growth of a both aerobic and anaerobic microorganisms. Tryptic Soy Agar is a general purpose medium that is recommended in multiple water and wastewater applications.[13]

HYcheck for Enterobacteriaceae has side one coated with Violet Red Bile Glucose Agar and side two coated with Tryptic Soy Agar, a general purpose growth medium. Violet Red Bile Glucose Agar is a selective medium used for the enumeration of *Enterobacteriaceae* in foods. Coliform bacteria have long been used as an index of fecal contamination in waters, and their presence in milk is used as an index of sanitation in milk processing.[14] The presence of *Enterobacteriaceae*, coliforms, *Salmonellae*, *Klebsiella* or *Citrobacter*, in raw foodstuffs is an indicator of fecal contamination. Their presence after processing may indicate a failure in the manufacturing process.

HYcheck Plate Count Agar with TTC has both sides coated with Plate Count Agar with TTC (0.01% 2,3,5-Triphenyl Tetrazolium Chloride).

HYcheck for Total Count has side one coated with Plate Count Agar and side two coated with Plate Count Agar with 0.01% TTC. Plate Count Agar is used for enumerating bacteria in water, wastewater, food and dairy products.[13,15-18] TTC is a redox indicator that is colorless in the oxidized form. TTC is reduced to insoluble triphenylformazan by certain actively metabolizing bacteria, resulting in a red color in the presence of bacterial growth.

There are two HYcheck products for yeasts and molds: 1) **HYcheck for Yeasts and Molds** has side one coated with Rose Bengal Chloramphenical Agar and side two coated with Tryptic Soy Agar; 2) **HYcheck for Yeasts and Molds with TTC** has side one coated with Rose Bengal Chloramphenical Agar and side two coated with Tryptic Soy Agar with 0.01% TTC. Rose Bengal Chloramphenical Agar is recommended in the selective isolation and enumeration of yeasts and molds from environmental materials and foodstuffs. The pH of the medium is near neutrality for improved growth and recovery of acid sensitive strains. [19-21]

Principles of the Procedure

HYcheck D/E Neutralizing Agar

Tryptone provides carbon and nitrogen. Yeast Extract provides vitamins, cofactors and additional nitrogen and carbon. Dextrose provides fermentable carbohydrate. Sodium Thioglycollate neutralizes mercurials. Sodium Thiosulfate neutralizes iodine and chlorine. Sodium Bisulfite neutralizes formaldehyde and glutaraldehyde. Lecithin neutralizes quaternary ammonium compounds and Polysorbate 80 neutralizes phenols, hexachlorophene, formalin and, with lecithin, ethanol. Brom Cresol Purple is a colorimetric indicator. Bacto Agar is a solidifying agent.

HYcheck for Disinfection Control

D/E Neutralizing Agar (D/E) - side one

Tryptone provides carbon and nitrogen. Yeast Extract provides vitamins, cofactors and additional nitrogen and carbon. Dextrose provides fermentable carbohydrate. Sodium Thioglycollate neutralizes mercurials. Sodium Thiosulfate neutralizes iodine and chlorine. Sodium Bisulfite neutralizes formaldehyde and glutaraldehyde. Lecithin neutralizes quaternary ammonium compounds and Polysorbate 80 neutralizes phenols, hexachlorophene, formalin and, with lecithin, ethanol. Brom Cresol Purple is a colorimetric indicator. Bacto Agar is a solidifying agent.

Tryptic Soy Agar (TSA) - side two

Tryptone and Soytone provide nitrogen, vitamins and minerals. The natural sugars from the soybean promote bacterial growth. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

HYcheck for Enterobacteriaceae

Violet Red Bile Glucose Agar (VRBGA) - side one

Yeast Extract provides vitamins, cofactors, nitrogen and carbon. Glucose provides a source of fermentable carbohydrate. Bacto Agar is a solidifying agent.

Tryptic Soy Agar - side two

Tryptone and Soytone provide nitrogen, vitamins and minerals. The natural sugars from the soybean promote bacterial growth. Sodium Chloride maintains the osmotic balance of the medium. Bacto Agar is a solidifying agent.

HYcheck Plate Count Agar (PCA) with TTC

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. TTC is a redox indicator. Bacto Agar is a solidifying agent.

HYcheck for Total Count

Plate Count Agar - side one

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. Bacto Agar is a solidifying agent.

Plate Count Agar with TTC - side two

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. TTC is a redox indicator. Bacto Agar is a solidifying agent.

HYcheck for Yeasts and Molds

Rose Bengal Chloramphenicol Agar (RBCA) - side one

Soytone provides carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. Rose Bengal and Chloramphenicol inhibit bacterial growth and restrict size and height of rapidly growing mold colonies. Bacto Agar is a solidifying agent.

Tryptic Soy Agar - side two

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. Bacto Agar is a solidifying agent.

HYcheck for Yeasts and Molds with TTC

Rose Bengal Chloramphenicol Agar - side one

Soytone provides carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. Rose Bengal suppresses bacterial growth and restricts size and height of rapidly growing mold colonies. Chloramphenicol inhibits bacteria. Bacto Agar is a solidifying agent.

Tryptic Soy Agar - side two

Tryptone and Yeast Extract provide carbon and nitrogen. Dextrose provides a source of fermentable carbohydrate. TTC is a redox indicator. Bacto Agar is a solidifying agent.

Precautions

1. Do not touch agar surface.
2. Do not use if there are signs of dehydration or contamination.

Storage

Store HYcheck slides at 2-15°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

(One type is provided per package.)

HYcheck D/E Neutralizing Agar
HYcheck for Disinfection Control
HYcheck for Enterobacteriaceae
HYcheck Plate Count Agar with TTC
HYcheck for Total Count
HYcheck for Yeasts and Molds
HYcheck for Yeasts and Molds with TTC.

Test Procedure

Surfaces

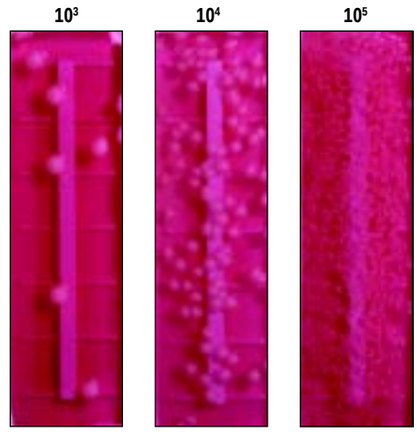
1. Loosen cap and remove HYcheck slide from the container.
2. Examine for dehydration or contamination.
3. Hold terminal spike against surface to be tested.
4. Press down on the spike to bend the paddle around the hinge line.
5. Gently lower the slide and press agar into contact with the test surface.
6. Apply firm and even pressure on the test surface for a few seconds.
7. Repeat procedure using the second agar surface on an area adjacent to the initial test site.
8. Replace slide in the container and close tightly.
9. Incubate in an upright position at indicated temperature.

Liquids

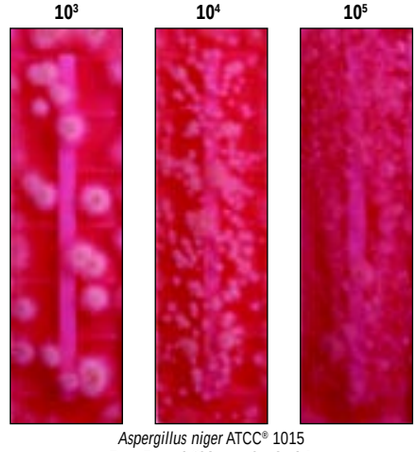
1. Loosen cap and remove HYcheck Slide from the container.
2. Examine for dehydration or contamination.
3. Immerse slide into test fluid so that agar surface becomes totally covered (if insufficient liquid is available, pour over surface of the slide).
4. Allow to drain.
5. Replace slide in the container and close tightly.
6. Incubate in an upright position at indicated temperature.

Results

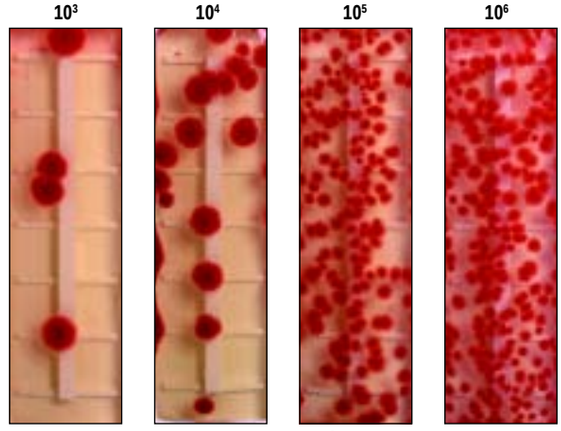
The following photos are reactions to *Candida albicans, Aspergillus niger and Escherichia coli.*



Candida albicans ATCC® 60193
on Rose Bengal Chloramphenicol Agar



Aspergillus niger ATCC® 1015
on Rose Bengal Chloramphenicol Agar



Escherichia coli ATCC® 11229
on Tryptic Soy Agar with 0.01% TTC

Limitations of the Procedure

1. Do not use the HYcheck Slide if it is contaminated or the agar medium is significantly dehydrated.

References

1. **Restaino, L.** 1994. HYcheck Slides versus contact plates compared to the swab technique. Dairy, Food and Environ. Sanit. **14**:528-530.
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16. **Marshall, R. T. (ed.).** 1993. Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
17. **Association of Official Agricultural Chemists.** 1995. Official methods of analysis, 16th ed. Association of Official Agricultural Chemists, Washington, D.C.
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Packaging

HYcheck D/E Neutralizing Agar	20 units	9041-36
HYcheck for Disinfection Control	20 units	9039-36
HYcheck for Enterobacteriaceae	20 units	9037-36
HYcheck Plate Count Agar with TTC	20 units	9045-36
HYcheck for Total Count	20 units	9053-36
HYcheck for Yeasts and Molds	20 units	9038-36
HYcheck for Yeasts and Molds with TTC	20 units	9046-36

Bacto® Petragnani Medium

Intended Use

Bacto Petragnani Medium is used for isolating and cultivating mycobacteria.

Summary and Explanation

Mycobacterial infections, particularly tuberculosis, are a worldwide health problem. Almost three million people worldwide die of tuberculosis each year.[1] During the mid 1980s. the number of tuberculosis (TB) cases in the U.S. began increasing. Before this time, the number of cases in the U.S. had been decreasing, reaching a low in 1984.[2] Non-tuberculous mycobacterial infections have also increased since the mid 1980s.[3]

Two types of semi-solid culture media are available for the isolation of mycobacteria, egg-based media and agar-based media. Most formulations for the isolation of mycobacteria include malachite green, which is used to inhibit contaminating organisms.

Petragnani Medium is an egg-based medium that is a modification of Petragnani[4] medium described by Norton, Thomor and Broom.[5] The formulation contains a large amount of malachite green which

inhibits the growth of contaminating organisms. This medium is well suited to specimens that are from nonsterile areas that may be heavily contaminated.[3, 6]

Principles of the Procedure

Whole Milk, Whole Eggs and Egg Yolks are protein sources. Potatoes and Potato Flour are starches that provide a carbohydrate source. Glycerol is a carbon source. Malachite Green inhibits contaminating organisms.

Formula

Petragnani Medium

Formula Per Liter

Whole Milk	900	ml
Potato Flour	36	g
Potato	500	g
Whole Eggs	1200	ml
Egg Yolks	115	ml
Bacto Glycerol	70	ml
Bacto Malachite Green	1.2	g

pH 7.2 ± 0.2 at 25°C

Precautions

1. For In Vitro Diagnostic Use.
2. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store prepared medium at 2-8°C

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use product if it fails to meet specifications for identity and performance.

User Quality Control

Identity Specifications

Prepared Appearance: Light to medium green, opaque, smooth slants with no visible contamination.

Reaction of Medium at 25°C: pH 7.2 ± 0.2

Cultural Response

Inoculate and incubate at 35 ± 2°C under CO_2 for up to 21 days.

ORGANISM	ATCC®	INOCULUM CFU	GROWTH
Escherichia coli	25922*	1,000-2,000	partial to complete inhibition
Mycobacterium fortuitum	6841	100-1,000	good
Mycobacterium intracellulare	13950	100-1,000	good
Mycobacterium kansasii	12478	100-1,000	good
Mycobacterium tuberculosis H37Ra	25177	100-1,000	good

These cultures are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Procedure

Materials Provided

Petragnani Medium

Materials Required But Not Provided

Specimen decontaminant and digestant
Buffer
Bovine albumin
Centrifuge
Inoculating Needles
CO_2 Incubator (35°C)

Method of Preparation

Supplied ready to use

Specimen Collection and Preparation[4]

1. Collect specimens in sterile containers and transport immediately to the laboratory following recommended guidelines.
2. Process each specimen as appropriate for that specimen.
3. Inoculate the specimen onto the medium.

Test Procedure

1. Incubate tubes for up to eight weeks.
2. Examine tubes for growth.

Results

Observe for colonies that may or may not be pigmented. Colony morphology is dependent on the species isolated.

Limitations of the Procedure

Negative culture results do not rule out active infection by mycobacteria. Some factors that are responsible for unsuccessful cultures are:

- The specimen was not representative of the infectious material, i.e., saliva instead of sputum.
- The mycobacteria were destroyed during digestion and decontamination of the specimen.
- Gross contamination interfered with the growth of the mycobacteria.
- Proper aerobic CO_2 tension was not provided during incubation.

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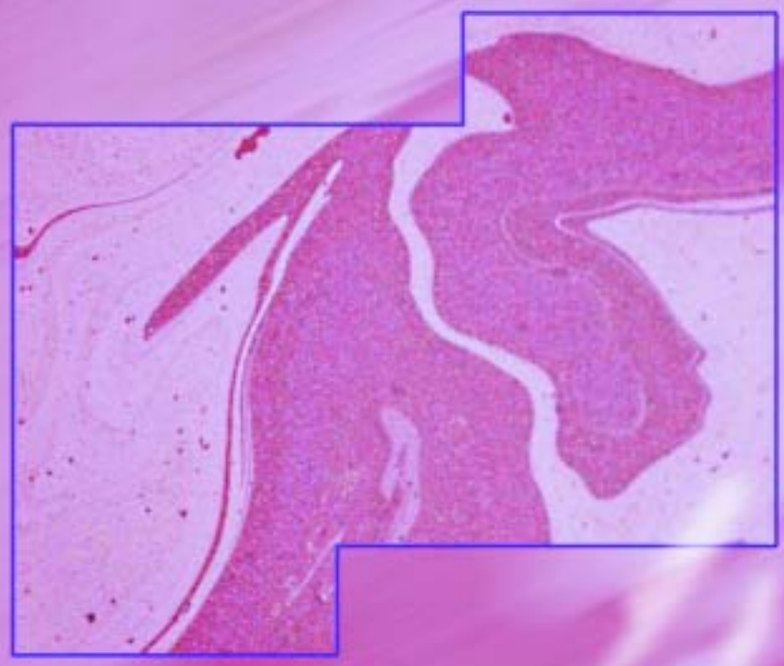
Packaging

Petragnani Medium	100 tubes	1010-79

Stains and Indicators



4

Bacto® Acridine Orange Stain
SpotTest™ Acridine Orange Stain

Intended Use

Bacto Acridine Orange Stain and SpotTest™ Acridine Orange Stain are used for detecting microorganisms in direct smears by the fluorescent staining technique.

Summary and Explanation

Fluorochromatic staining of microorganisms using acridine orange was first described by Strugger and Hilbrich in 1942[1] and has been used in the microscopic examination of soil and water.[2,3] Acridine orange possesses differential staining properties with regard to clinical materials when prepared at a low pH.[4] Bacteria stain bright orange and are differentiated from human cells and tissue debris which stain pale green to yellow.

Acridine orange staining is a simple, rapid, inexpensive alternative to blind subcultures.[5] The stain is more sensitive than the Gram stain for detecting microorganisms in clinical materials at concentrations of approximately 1 x 10^4 colony-forming units per ml.[6]

Acridine orange at a low pH has been used for the detection of *Trichomonas vaginalis*[7] and *Neisseria gonorrhoeae*[8] in clinical materials and for the enumeration of mycoplasmas.[9] The stain may be useful in the rapid screening of normally sterile specimens, such as cerebrospinal fluid where few organisms may be present, and in the rapid examination of blood smears or smears containing proteinaceous material, where differentiation of organisms from background material may be difficult.[10]

User Quality Control

Identity Specifications

Acridine Orange Stain
SpotTest™ Acridine Orange Stain

Solution:	The solution should be clear, orange, and without evidence of a precipitate.
Reaction at 25°C:	pH 3.5-4.0

Cultural Response

Prepare slides of the test organisms and sheep blood stained using Acridine Orange Stain or SpotTest™ Acridine Orange Stain. Examine slides using a fluorescent microscope at 1000X magnification.

ORGANISM	ATCC®	STAINED BACTERIA
Escherichia coli	25922*	orange to red-orange rods
Enterococcus faecalis	33186	orange to red-orange cocci

Background for both organisms: staining is hazy black or green; red blood cell "ghosts" stain pale green or have a green periphery.

The cultures listed are the minimum that should be used for performance testing.

*This culture is available as a Bactrol™ Disk and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Acridine orange is a fluorochromatic dye that binds to the nucleic acids of bacteria and other cells.[11] Under UV light, Acridine Orange stains RNA and single-stranded DNA orange; double-stranded DNA appears green.

Formula

Acridine Orange Stain
SpotTest™ Acridine Orange Stain

Formula Per Liter

Acridine Orange	0.1 g
Acetate Buffer, 0.5M	1 liter

Precautions

1. For In Vitro Diagnostic Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store at 15-30°C. Acridine Orange Stain is light sensitive. Protect from light.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Acridine Orange Stain
SpotTest™ Acridine Orange Stain
Ampule Crusher

Materials Required But Not Provided

Glass microscope slides
Methanol
Fluorescent microscope suitable for use with Acridine Orange

Method of Preparation

Not applicable

Specimen Collection and Preparation

Not applicable

Preparation, Staining, and Examination of Smears

1. Prepare a smear of the specimen to be stained on a clean glass slide.
2. Allow to air dry.
3. Fix smear with 50% or 100% methanol for 1 to 2 minutes.
4. Drain excess methanol and allow smear to dry.
5. If using SpotTest™ Acridine Orange Stain, hold the dispenser upright with the tip pointing in an outward direction. Using the provided ampule crusher, squeeze gently to crush the glass ampule inside the dispenser. Invert and squeeze slightly to dispense the stain on a per drop basis.

6. Flood the slide with Acridine Orange Stain for 2 minutes.
7. Rinse thoroughly with tap water and allow to dry.
8. Smears may be initially examined at 100X to 400X magnification using a fluorescent microscope. Findings should be confirmed by examination at 1000X with an oil immersion objective.

Results

Bacteria and fungi stain bright orange. The background appears black to yellow green. Human epithelial and inflammatory cells and tissue debris stain pale green to yellow. Activated leukocytes will stain yellow, orange or red depending on the level of activation and the amount of RNA produced. Erythrocytes either do not stain or stain pale green.

Limitations of the Procedure

1. Acridine Orange staining provides presumptive information on the presence and identification of microorganisms in the specimen. Because microorganisms seen in smears, including nonviable organisms, may arise from external sources (i.e., specimen collection devices, slides or water used for rinsing), all positive smears should be confirmed by culture.
2. Approximately 10^4 colony-forming units per ml are required for detection by the Acridine Orange staining method.
3. Acridine orange staining does not distinguish between gram-positive and gram-negative organisms. The gram reaction may be determined by performing the Gram stain procedure directly over the acridine orange stain after removing the immersion oil with xylene.[12]
4. Nuclei or granules from disintegrated, activated leukocytes may resemble cocci at lower magnifications (e.g., 100X-400X). They may be distinguished on the basis of morphology at higher magnifications (e.g., 1000X).
5. Certain types of debris may fluoresce in Acridine Orange stained smears. This debris may be distinguished from microorganisms on the basis of morphology when viewed at higher magnification.

References

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Packaging

Acridine Orange Stain	1 x 250 ml	3336-75
	6 x 250 ml	3336-76
SpotTest™ Acridine Orange Stain	50 x 0.75 ml	3561-26

Bacto® Gram Stain Sets and Reagents

Gram Stain Set · Gram Stain Set (with Stabilized Iodine) 3-Step Gram Stain Set-S · 3-Step Gram Stain Set-T

Intended Use

Bacto Gram Stain Sets and reagents are used to stain microorganisms from cultures or specimens by the differential Gram method.

Summary and Explanation

The Gram stain was devised in 1884 by Christian Gram[1] in an attempt to differentiate bacterial cells from infected tissue. Although Gram observed what is now called the "Gram reaction," he did not recognize the taxonomic value of his technique.[2]

The Hucker[3] modification of the Gram stain is now used to differentiate intact, morphologically similar bacteria into two groups based on cell color after staining. In addition, cell form, size and structural details are evident. Such preliminary information provides important clues to the type of organism(s) present, the further techniques required

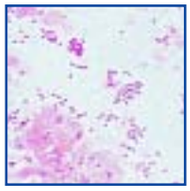
to characterize them, and the therapy to initiate while awaiting test results.

Principles of the Procedure

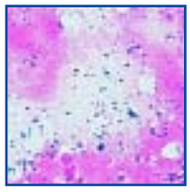
The Gram stain procedure consists of [4,5,6]:

1. Staining a fixed smear with crystal violet;
2. Applying iodine as a mordant;
3. Decolorizing the primary stain with alcohol/acetone; and,
4. Counterstaining with safranin or basic fuchsin.

A crystal violet-iodine complex forms in the protoplast (not the cell wall) of all organisms stained by this procedure. Organisms able to retain this dye complex after decolorization are classified as gram-positive while those that can be decolorized and counterstained are classified as gram-negative.[2,4,5,6]



Positive Blood Culture Bottle
Specimen containing numerous gram-negative rods with shape and size of enteric rods. The culture grew *Klebsiella pneumoniae.*



Ground Beef
Sample containing *E. coli*: H7 and *Staphylococcus aureus.*

Upon disruption or removal of the cell wall, the protoplast of gram-positive (as well as gram-negative) cells can be decolorized and the gram-positive attribute lost. Thus, the mechanism of the Gram stain appears to be related to the presence of an intact cell wall able to act as a barrier to decolorization of the primary stain.

User Quality Control

Run controls daily using 18-24 hour cultures of known gram-positive and gram-negative microorganisms. It is very important that controls be included in each staining run, preferably on the same slide. When performing the Gram stain on a clinical specimen, particularly when the results will be used as a guide to the selection of a therapeutic agent, such a control system furnishes assurance that the iodine solution is providing proper mordant activity and that decolorization was performed properly.

ORGANISM*	ATCC®	EXPECTED RESULTS
Staphylococcus aureus	25923	gram-positive cocci
Escherichia coli	25922	gram-negative rods

* Available as Bactrol™ Disks.

Generally, the cell wall is nonselectively permeable. It is theorized that during the Gram stain procedure, the cell wall of gram-positive cells is dehydrated by the alcohol in the decolorizer and loses permeability, thus retaining the primary stain. However, the cell wall of gram-negative cells has a higher lipid content and becomes more permeable when treated with alcohol, resulting in loss of the primary stain.

The principles of the 3-Step Gram Stain procedure are identical to the 4-step procedure described above. However, the decolorizing and counterstaining steps have been combined into one reagent.

The molecular basis for the Gram stain has not yet been determined.

Formula

Reagents are provided in two sizes, a 250 ml plastic dispensing bottle with a dropper cap and a one-gallon container with a dispensing tap.

Standardization may include adjustment to meet performance specifications.

3329-Gram Crystal Violet
PRIMARY STAIN
Aqueous solution of Crystal Violet.

3331-Gram Iodine
MORDANT
(Working solution prepared from Gram Diluent and Gram Iodine 100X)

Iodine Crystals	3.3 g
Potassium Iodide	6.6 g
Distilled Water	1 liter

3342-Stabilized Gram Iodine
MORDANT

Polyvinylpyrrolidone-Iodine Complex	100 g
Potassium Iodide	19 g
Distilled Water	1 liter

3330-Gram Decolorizer
DECOLORIZER

Acetone	250 ml
Isopropanol	750 ml

3332-Gram Safranin
COUNTERSTAIN

Safranin O Powder (pure dye)	4 g
Denatured Alcohol	200 ml
Distilled Water	800 ml

3343-Gram Basic Fuchsin
COUNTERSTAIN

Basic Fuchsin	0.08 g
Phenol	2.6 g
Isopropyl Alcohol	4.5 ml
Distilled Water	993 ml

3335-3-Step Gram Safranin-S
DECOLORIZER/COUNTERSTAIN
Alcohol-based solution of safranin.*

3341-3-Step Gram Safranin-T
DECOLORIZER/COUNTERSTAIN
Alcohol-based solution of safranin.*

* Patent Pending

Precautions

1. For In Vitro Diagnostic Use.
2. **3329-Gram Crystal Violet**
   IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] Avoid contact with skin and eyes. Do not breathe spray. Wear suitable protective clothing. Keep container tightly closed.

   3331-Gram Iodine 100X
   HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. MAY CAUSE HARM TO THE UNBORN CHILD. Avoid contact with skin and eyes. Do not breathe fumes. Wear suitable protective clothing. Keep container tightly closed.

   3342-Stabilized Gram Iodine
   HARMFUL IN CONTACT WITH SKIN. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF HARM TO THE UNBORN CHILD.[US] Avoid contact with skin and eyes. Do not breathe fumes. Wear suitable protective clothing. Keep container tightly closed.

   3330 - Bacto Gram Decolorizer
   HIGHLY FLAMMABLE. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe mist or vapor. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

   3332 - Bacto Gram Safranin
   FLAMMABLE.[EC] HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] Avoid contact with skin and eyes. Do not breathe vapor. Wear suitable protective clothing. Keep container tightly closed.

   3335 - Bacto 3-Step Gram Safranin-S
   HIGHLY FLAMMABLE. HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] POSSIBLE RISK OF HARM TO THE UNBORN CHILD.[US] Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

   3341 - Bacto 3-Step Gram Safranin-T
   HIGHLY FLAMMABLE. HARMFUL BY INHALATION, IN CONTACT WITH SKIN AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] POSSIBLE RISK OF HARM TO THE UNBORN CHILD.[US] Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

   FIRST AID:

   In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

   Gram Iodine 100X: Take off immediately all contaminated clothing.

   After contact with skin, wash immediately with plenty of water.

   If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice.

   If swallowed seek medical advice immediately and show this container or label.
3. Studies demonstrate that the traditional Gram Iodine working solution (Gram Iodine 100X dissolved in Gram Diluent) is relatively unstable and may cause variability in the Gram stain when sufficient iodine is no longer available to the solution. Protect the iodine solution from undue exposure to air and heat. Include controls in all staining runs or at least once daily (see USER QUALITY CONTROL) to ensure that the solution is providing proper mordant activity.

Storage

Store Gram Stain reagents at 15-30°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed.

Use the traditional Gram Iodine working solution within three months of preparation, not exceeding the Expiry of either component.

Specimen Collection and Preparation

1. Apply the test specimen to a clean glass slide in a manner that will yield a thin, uniform smear. Emulsify colonies from an 18-24 hour culture in saline to obtain the proper density.
2. Allow the smear to air dry.
3. Fix the smear to the slide using one of the following techniques:
   A. Heat fix by passing the slide through a low flame 2-3 times. Cool the slide to room temperature before staining. NOTE: Do not overheat the slide; excessive heating will cause atypical staining.
   B. Methanol fix[6,7] the slide by flooding with absolute methanol for 1-2 minutes and rinse with tap water before staining. NOTE: For proper fixation, store absolute methanol in a brown screw-capped bottle and replenish the working supply every two weeks.

Reagent Preparation

Prepare the traditional Gram Iodine working solution by adding an entire 2.5 ml ampule of Gram Iodine 100X to 250 ml Gram Diluent or an entire 40 ml vial of Gram Iodine 100X to 1 gallon of Gram Diluent; mix thoroughly.

4-Step Staining Procedure[4]

Materials Provided

4-Step Technique

Gram Crystal Violet
Gram Iodine or Bacto Stabilized Gram Iodine
Gram Decolorizer
Gram Safranin or Bacto Gram Basic Fuchsin

Materials Required but not Provided

Microscope slides
Bunsen burner or methanol
Bacteriological loop

Swabs
Blotting paper
Microscope with oil immersion lens
Bactrol™ Gram Slide
Bactrol™ Disks

1. Flood the fixed smear with primary stain (Gram Crystal Violet) and stain for 1 minute.
2. Remove the primary stain by gently washing with cold tap water.
3. Flood the slide with mordant (either Gram Iodine or Stabilized Gram Iodine) and retain on the slide for 1 minute.
4. Remove the mordant by gently washing with tap water.
5. Decolorize (Gram Decolorizer) until solvent running from the slide is colorless (30-60 seconds).
6. Wash the slide gently in cold tap water.
7. Flood the slide with counterstain (either Gram Safranin or Gram Basic Fuchsin) and stain for 30-60 seconds.
8. Wash the slide with cold tap water.
9. Blot with blotting paper or paper towel or allow to air dry.
10. Examine the smear under an oil immersion lens.

3-Step Staining Procedure

Materials Provided

3-Step Stabilized Iodine Technique

Gram Crystal Violet
Stabilized Gram Iodine
3-Step Gram Safranin-S

3-Step Traditional Iodine Technique

Gram Crystal Violet
Gram Iodine
3-Step Gram Safranin-T

Materials Required but not Provided

Microscope slides
Bunsen burner or methanol
Bacteriological loop
Swabs
Blotting paper
Microscope with oil immersion lens
Bactrol™ Gram Slide
Bactrol™ Disks

1. Flood the fixed smear with primary stain (Gram Crystal Violet) and stain for 1 minute.
2. Remove the primary stain by gently washing with cold tap water.
3. Flood the slide with mordant (Stabilized Gram Iodine or Gram Iodine [traditional formulation]) and retain on the slide for 1 minute. (Refer to LIMITATIONS OF THE PROCEDURE, #5.)
4. Wash off the mordant with decolorizer/counterstain (3-Step Gram Safranin-S or 3-Step Gram Safranin-T). (NOTE: Do not wash off iodine with water.) Add more decolorizer/counterstain solution to the slide and stain 20-50 seconds.
5. Remove the decolorizer/counterstain solution by gently washing the slide with cold tap water.
6. Blot with blotting paper or paper towel or allow to air dry.
7. Examine the smear under an oil immersion lens.

Results

REACTION	4-STEP TECHNIQUE USING GRAM SAFRANIN	4-STEP TECHNIQUE USING BASIC FUCHSIN	3-STEP TECHNIQUE USING EITHER GRAM SAFRANIN-S OR GRAM SAFRANIN-T
Gram-positive	Purple-black cells	Bright purple to purple-black cells	Purple-black to purple cells
Gram-negative	Pink to red cells	Bright pink to fuchsia cells	Red-pink to fuchsia cells

Limitations of the Procedure

1. The Gram stain provides preliminary identification information only and is not a substitute for cultural studies of the specimen.
2. Prior treatment with antibacterial drugs may cause gram-positive organisms from a specimen to appear gram-negative.
3. Use of an 18-24 hour culture is advisable for best results since fresh cells have a greater affinity than old cells for most dyes. This is particularly true of many spore formers, which are strongly gram-positive when examined in fresh cultures but which later become gram-variable or gram-negative.
4. The Gram stain reaction, like the acid-fast reaction, is altered by physical disruption of the bacterial cell wall or protoplast. The cell walls of gram-positive bacteria interpose a barrier which prevents leaching of the dye complex from the cytoplasm. Cell walls of gram-negative bacteria contain lipids soluble in organic solvents, which are then free to decolorize the cytoplasm. Therefore, a microorganism that is physically disrupted by excess heating will not react to Gram staining as expected.
5. 3-Step Gram Safranin-S is intended for use with stabilized iodine. 3-Step Gram Safranin-T is intended for use with traditional iodine. Unsatisfactory results may occur if other combinations of iodine and 3-Step Gram Safranin are used.
6. Over time, a fine precipitate may develop in Gram Basic Fuchsin, 3-Step Gram Safranin-S and 3-Step Gram Safranin-T. Product performance will not be affected.

References

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4. **Bartholomew, J. W.** 1962. Variables influencing results, and the precise definition of steps in gram staining as a means of standardizing the results obtained. Stain Technol. **37**:139-155.
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6. **Murray, P. R. (ed.).** 1995. Manual of Clinical Microbiology, 6th ed. American Society of Microbiology, Washington, D.C.
7. **Mangels, J. I., M. E. Cox, and L. H. Lindley.** 1984. Methanol fixation. An alternative to heat-fixation of smear. Diag. Microbiol. Infect. Dis. **2**:129-137.

Packaging

Gram Crystal Violet	6 x 250 ml	3329-76
PRIMARY STAIN	1 gallon	3329-83
Gram Iodine	6 x 250 ml	3331-76
MORDANT	1 gallon	3331-83
Stabilized Gram Iodine	6 x 250 ml	3342-76
MORDANT	1 gallon	3342-83
Gram Decolorizer	6 x 250 ml	3330-76
DECOLORIZER	1 gallon	3330-83
Gram Safranin	6 x 250 ml	3332-76
COUNTERSTAIN	1 gallon	3332-83
Gram Basic Fuchsin	6 x 250 ml	3343-76
COUNTERSTAIN	1 gallon	3343-83
3-Step Gram Safranin-S	6 x 250 ml	3335-76
DECOLORIZER/COUNTERSTAIN	1 gallon	3335-83
3-Step Gram Safranin-T	6 x 250 ml	3341-76
DECOLORIZER/COUNTERSTAIN	1 gallon	3341-83
Gram Stain Set	4 x 250 ml	3328-32
Contents: Gram Crystal Violet	250 ml	
Gram Iodine	250 ml	
Gram Decolorizer	250 ml	
Gram Safranin	250 ml	
Gram Stain Set (with Stabilized Iodine)	4 x 250 ml	3338-32
Contents: Gram Crystal Violet	250 ml	
Stabilized Gram Iodine	250 ml	
Gram Decolorizer	250 ml	
Gram Safranin	250 ml	
3-Step Gram Stain Set-S	3 x 250 ml	3334-3
Contents: Gram Crystal Violet	250 ml	
Stabilized Gram Iodine	250 ml	
3-Step Gram Safranin-S	250 ml	
3-Step Gram Stain Set-T	3 x 250 ml	3337-32
Contents: Gram Crystal Violet	250 ml	
Gram Iodine	250 ml	
3-Step Gram Safranin-T	250 ml	
Bactrol™ Gram Slide	50 slides	3140-26

Bacto® TB Stain Sets and Reagents

TB Stain Set K · TB Stain Set ZN · TB Fluorescent Stain Set M TB Fluorescent Stain Set T

Intended Use

Bacto TB Stain Sets are used to stain smears prepared from specimens suspected of containing mycobacteria for early presumptive diagnosis of mycobacterial infection.

Also Known As

TB Stain Set K is also known as the Kinyoun Stain.

TB Stain Set ZN is also known as the Ziehl-Neelsen Stain.

TB Fluorescent Stain Set M is also known as the Morse Stain.

TB Fluorescent Stain Set T is also known as the Truant Stain.

Summary and Explanation

The microscopic staining technique is one of the earliest methods devised for detecting the tubercle bacillus and it remains a standard procedure.[1-7] The unique acid-fast characteristic of mycobacteria makes the staining technique valuable in early presumptive diagnosis, and provides information about the number of acid-fast bacilli present. Fluorescent microscopy offers many advantages over classic methods for detecting mycobacteria because of its speed and simplicity, the ease of examining the slide, and the reliability and superiority of the method.[8]

TB Stain Set K uses the Kinyoun (cold) acid-fast procedure described by Kinyoun.[4,9]

TB Stain Set ZN uses the Ziehl-Neelsen (hot) acid-fast procedure described by Kubica and Dye.[4,10]

TB Fluorescent Stain Set M uses the auramine O acid-fast fluorescent procedure described by Morse, Blair, Weiser and Sproat.[4,11]

TB Fluorescent Stain Set T uses the acid-fast fluorescent procedure described by Truant, Brett and Thomas.[4,12]

Principles of the Procedure

The lipid content of the cell wall of acid fast bacilli makes staining of these organisms difficult. In acid fast stains, the phenol allows penetration of the primary stain, even after exposure to acid-alcohol decolorizers. For an organism to be termed acid fast, it must resist decolorizing by acid-alcohol. A counterstain is then used to emphasize the stained organisms, so they may be easily seen microscopically.

When using Stain Set K, acid fast bacilli (AFB) appear red against a green background if Brilliant Green K is used as the counterstain or red against a blue background if Methylene Blue is the counterstain.

When using Stain Set ZN, AFB appear red against a blue background because Methylene Blue is used as the counterstain.

When using Stain Set M, AFB have a bright yellow-green fluorescence.

When using Stain Set T, AFB have a reddish-orange fluorescence.

Formula

3326-TB Stain Set K

Formulas per Liter

3321-TB Carbolfuchsin KF

Basic Fuchsin	15 g
Phenol USP	45 g
Isopropanol	200 ml
Ethanol	50 ml
Distilled Water	750 ml

3318-TB Decolorizer

Hydrochloric Acid	30	ml
Denatured Ethanol	970	ml

3327-TB Brilliant Green K

Brilliant Green	2	g
Sodium Hydroxide	0.02	g
Distilled Water	1000	ml

User Quality Control

It is recommended that a positive and negative control slide, such as Bactrol™ TB Slide, be included with each batch of slides stained with acid fast stains.

Identity Specifications

3313-TB Carbolfuchsin ZN
Appearance: Reddish-purple suspension with no visible precipitate.

3314-TB Decolorizer TM
Appearance: Colorless, clear suspension.

3315-TB Potassium Permanganate
Appearance: Purple solution.

3316-TB Auramine M
Appearance: Yellow suspension.

3317-TB Auramine-Rhodamine T
Appearance: Red, viscous solution.

3318-TB Decolorizer
Appearance: Colorless, clear solution.

3319-TB Methylene Blue
Appearance: Blue solution with no visible precipitation.

3321-TB Carbolfuchsin KF
Appearance: Reddish purple suspension.

3327-TB Brilliant Green
Appearance: Green solution.

Stain Value

Stain Bactrol™ TB Slides (3139) using the appropriate TB stain procedure. Examine slides using a light or fluorescent microscope at a total magnification of 1000X (oil immersion).

ORGANISM	ATCC®	TB STAIN SET K USING TB BRILLIANT GREEN	TB STAIN SET K USING TB METHYLENE BLUE	TB STAIN SET ZN
Positive Control				
M. tuberculosis H37 Ra	25177	Dark pink to red	Dark pink to red	Dark pink to red
Negative Control				
S. aureus	25923	Green	Blue	Blue
K. pneumoniae	13883	Green	Blue	Blue

ORGANISM	ATCC®	TB FLUORESCENT STAIN SET M	TB FLUORESCENT STAIN SET T
Positive Control			
M. tuberculosis H37 Ra	25177	Bright yellow-green fluorescence	Reddish, orange fluorescence
Negative Control			
S. aureus	25923	No fluorescence	No florescence
K. pneumoniae	13883	No fluorescence	No florescence

3324-TB Stain Set ZN

Formulas per Liter

3313-TB Carbolfuchsin ZN

Basic Fuchsin	1.7	g
Phenol USP	50	g
Isopropanol	95	ml
Distilled Water	905	ml

3318-TB Decolorizer

Hydrochloric Acid	30	ml
Denatured Ethanol	970	ml

3319-TB Methylene Blue

Methylene Blue USP	2.4	g
Ethanol	300	ml
Distilled Water	700	ml

3323-TB Fluorescent Stain Set M

Formulas per Liter

3316-TB Auramine M

Auramine O	2	g
Phenol USP	4	g
Glycerine USP	100	ml
Isopropanol	250	ml
Distilled Water	650	ml

3314-TB Decolorizer TM

Hydrochloric Acid	5	ml
Isopropanol	700	ml
Distilled Water	300	ml

3315-TB Potassium Permanganate

Potassium Permanganate	5	g
Distilled Water	1000	ml

3325-TB Fluorescent Stain Set T

Formulas per Liter

3317-TB Auramine-Rhodamine T

Auramine O	12	g
Rhodamine B	6	g
Phenol USP	80	g
Glycerine USP	600	ml
Isopropanol	140	ml
Distilled Water	260	ml

3314-TB Decolorizer TM

Hydrochloric Acid	5	ml
Isopropanol	700	ml
Distilled Water	300	ml

3315-TB Potassium Permanganate

Potassium Permanganate	5	g
Distilled Water	1000	ml

Precautions

1. For In Vitro Diagnostic Use.
2. **3313-TB Carbolfuchsin ZN**

   IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. TOXIC IN CONTACT WITH SKIN AND IF SWALLOWED.[EC] CAUSES BURNS.[EC] POSSIBLE RISK OF IRREVERSIBLE

EFFECTS.[EC] Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed.

3314-TB Decolorizer TM

HIGHLY FLAMMABLE. CAUSES BURNS. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

3315-TB Potassium Permanganate

IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed.

3316-TB Auramine M

FLAMMABLE. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

3317-TB Auramine-Rhodamine T

FLAMMABLE. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. TOXIC IN CONTACT WITH SKIN AND IF SWALLOWED.[EC] CAUSES BURNS.[EC] POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

3318-TB Decolorizer

HIGHLY FLAMMABLE. HARMFUL BY INHALATION AND IF SWALLOWED. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.[US] POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] POSSIBLE RISK OF HARM TO THE UNBORN CHILD.[US] Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

3319-TB Methylene Blue

FLAMMABLE. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. HARMFUL BY INHALATION AND IF SWALLOWED. POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] Avoid contact with skin and eyes. Do not breathe vapors. Wear suitable protective clothing. Keep container tightly closed.

3321-TB Carbolfuchsin KF

FLAMMABLE. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. HARMFUL BY INHALATION AND IF SWALLOWED.[EC] POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[US] POSSIBLE RISK OF HARM TO THE UNBORN CHILD.[US] Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking.

FIRST AID:

In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

After contact with skin, wash immediately with plenty of water.

If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice.

If swallowed seek medical advice immediately and show this container or label.

Storage

Store TB Stain Sets and reagents at 15-30°C. Reagents that have been removed from the packing carton should be stored in the dark.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

TB Stain Set K*
TB Stain Set ZN*
TB Fluorescent Stain Set M*
TB Fluorescent Stain Set T*
Bactrol™ TB Slides

*Individual reagents available separately. See Packaging.

Materials Required but not Provided

Microscope slides–new or cleaned in acid dichromate solution
Staining rack
Microscope with oil immersion lens, OR
Fluorescent microscope
(See #8 of each fluorescent staining procedure for a complete description of the appropriate assembly required.)

Specimen Collection and Preparation

1. Acid fast stains may be performed on any type of clinical specimen suspected of containing mycobacteria.[4-5] Smears from sputum and other respiratory tract secretions are usually made from concentrated specimens. For procedures used in concentrating specimens for acid fast bacilli, please consult appropriate references.[4-7]
2. Apply a thin smear of the specimen directly on a clear microscope slide.
3. Allow smear to air dry.
4. Fix the smear to the slide by passing the slide through a low flame 2-3 times, avoiding excessive heat.

Test Procedure

See appropriate references for specific procedures.

Kinyoun Stain

TB Stain Set K

1. Place slides on a staining rack and flood with TB Carbolfuchsin KF for 4 minutes. Do not heat.
2 Wash gently in running water.
3. Decolorize with TB Decolorizer for 3-5 seconds, or until no more red color appears in washing.
4. Wash gently in running water.
5. Counterstain with either TB Brilliant Green K or TB Methylene Blue (available separately) for 30 seconds.

6. Wash gently in running water.
7. Air dry. If using TB Methylene Blue, dry over gentle heat.

Ziehl-Neelsen Stain

TB Stain Set ZN

1. Place slides on a staining rack and flood with TB Carbolfuchsin ZN. Heat gently to steaming and allow to steam for 5 minutes.
2. Wash gently in running water.
3. Decolorize with TB Decolorizer for 3-5 seconds or until no more red color appears in washing.
4. Wash gently in running water.
5. Counterstain with either TB Methylene Blue or TB Brilliant Green K for 30 seconds.
6. Wash gently in running water.
7. Dry over gentle heat.

Morse Stain

Fluorescent Stain Set M

1. Place slides on a staining rack and flood with TB Auramine M for 15 minutes.
2. Wash gently in running water.
3. Decolorize with TB Decolorizer TM for 30-60 seconds.
4. Wash slides gently in running water.
5. Counterstain with TB Potassium Permanganate for 2 minutes.
6. Wash gently in running water.
7. Air dry.
8. Examine under a microscope fitted, as described by Morse et al.,[11] with an incandescent bulb, a KG 1 heat filter, a 3-4 mm thick BG excitation filter, an ordinary substage condenser and a No. 51 bright field or GG barrier filter.

Truant Stain

TB Fluorescent Stain Set T

1. Place slides on a staining rack and flood with TB Auramine-Rhodamine T that has been thoroughly shaken prior to use. Leave undisturbed for 20-25 minutes at room temperature.
2. Wash gently in running water.
3. Decolorize with TB Decolorizer TM for 2-3 minutes.
4. Wash gently in running tap water.
5. Counterstain with TB Potassium Permanganate for 4-5 minutes.
6. Wash gently in running water.
7. Blot lightly. Dry in air or very gently over a flame.
8. Examine under a microscope fitted, as described by Truant et al.,[13] with 25X objective, an HBO L2 bulb heat filter, a BG 12 primary filter and OG 1 barrier filter.

Results

Refer to appropriate references and procedures for results.

Limitations of the Procedure

1. A positive staining reaction provides presumptive evidence of the presence of *M. tuberculosis* in the specimen. A negative staining reaction does not necessarily indicate that the specimen will be culturally negative for *M. tuberculosis*. For positive identification of *M. tuberculosis*, cultural methods must be employed.
2. Rapidly growing mycobacteria may retain acid-fast stains to a varying degree. Most rapidly growing mycobacteria will not fluoresce in fluorochrome-stained smears.[4]
3. Organisms other than mycobacteria, such as *Rhodococcus* spp., *Nocardia* spp., *Legionella micdadei*, and the cysts of *Cryptosporidium* spp. and *Isospora* spp., may display various degrees of acid-fastness.[4]
4. When decolorizing with acid-alcohol, avoid under-decolorization. It is difficult to over-decolorize acid-fast organisms.
5. During the counterstaining step with potassium permanganate, timing is critical. Quenching the fluorescing bacilli occurs when counterstaining for a longer period of time.[4]
6. If fluorochrome stained slides cannot be observed immediately, they may be stored at 2-8°C in the dark for up to 24 hours. This is required to prevent fading of the fluorescence.[4]
7. Prolonged counterstaining in non-fluorochrome stains may mask the presence of acid-fast bacilli. Use of brilliant green may help to minimize this problem.[4]

References

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Packaging

TB Stain Set K	3 x 250 ml	3326-32
Contains:		
TB Carbolfuchsin KF	250 ml	
TB Decolorizer	250 ml	
TB Brilliant Green K	250 ml	
TB Stain Set ZN	3 x 250 ml	3324-32
Contains:		
TB Carbolfuchsin ZN	250 ml	
TB Decolorizer	250 ml	
TB Methylene Blue	250 ml	
TB Fluorescent Stain Set M	3 x 250 ml	3323-32
Contains:		
TB Auramine M	250 ml	
TB Decolorizer TM	250 ml	
TB Potassium Permanganate	250 ml	
TB Fluorescent Stain Set T	3 x 250 ml	3325-32
Contains:		
TB Auramine-Rhodamine T	250 ml	
TB Decolorizer TM	250 ml	
TB Potassium Permanganate	250 ml	
TB Auramine M	6 x 250 ml	3316-76
TB Auramine-Rhodamine T	6 x 250 ml	3317-76
TB Brilliant Green K	6 x 250 ml	3327-76
TB Carbolfuchsin KF	6 x 250 ml	3321-76
TB Carbolfuchsin ZN	6 x 250 ml	3313-76
TB Decolorizer	6 x 250 ml	3318-76
TB Decolorizer TM	6 x 250 ml	3314-76
TB Methylene Blue	6 x 250 ml	3319-76
TB Potassium Permanganate	6 x 250 ml	3315-76
Bactrol™ TB Slides	50 slides	3139-26

Serology and Immunology

Bacto® Bordetella Antigens and Antiserum

Bordetella Pertussis Antiserum · Bordetella Parapertussis Antiserum · Bordetella Pertussis Antigen

Intended Use

Bacto Bordetella Pertussis Antiserum and Bacto Bordetella Parapertussis Antiserum are used in the slide agglutination test for identifying *Bordetella pertussis* and *Bordetella parapertussis*.

Bacto Bordetella Pertussis Antigen is used to demonstrate a positive quality control test in the slide agglutination test.

Summary and Explanation

All members of the genus *Bordetella* are respiratory pathogens of warm-blooded animals. Two species, *B. pertussis* and *B. parapertussis*, are uniquely human pathogens. These organisms adhere to, multiply among and remain localized in the ciliated epithelial cells of the respiratory tract. *B. pertussis* is the major cause of whooping cough or pertussis. *B. parapertussis* is associated with a milder, less frequently occurring form of the disease.[1] Person-to-person transmission occurs by the aerosol route. Pertussis is a highly contagious disease that, more than 90% of the time, attacks unimmunized populations.[2] Toxin production remains the major distinction between *B. pertussis* and *B. parapertussis*.

Classic pertussis caused by *B. pertussis* occurs in three stages. The first (catarrhal) stage is characterized by nonspecific symptoms similar to a cold or viral infection. The disease is highly communicable during this stage, which lasts 1-2 weeks. During the second (paroxysmal) stage, the cough increases in intensity and frequency. This stage is marked by sudden attacks of severe, repetitive coughing, often culminating with the characteristic whoop. The whooping sound is caused by the rapid inspiration of air after the clearance of mucus-blocked airways.[3] This stage may last 1-4 weeks. The beginning of the convalescent stage is marked by a reduction in frequency and severity of coughing spells. Complete recovery may require weeks or months.

User Quality Control

Identity Specifications

Bordetella Pertussis Antiserum

Bordetella Parapertussis Antiserum

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated Appearance: Light gold to amber, clear liquid.

Bordetella Pertussis Antigen

Appearance: Light gray to white suspension, may settle upon standing.

Performance Response

Rehydrate Bordetella Pertussis and Parapertussis Antiserum per label directions. Test as described (see Test Procedure). Bordetella Pertussis Antigen or known positive and negative control cultures must give appropriate reactions.

Despite the availability of an effective whole-cell vaccine, pertussis remains a disease of worldwide distribution because many developing nations do not have the resources for vaccinating their populations.[4] Major outbreaks have occurred even in developed nations such as Great Britain and Sweden. Pertussis is endemic in the United States, with most disease occurring as isolated cases. There has been a shift in the age group affected by the disease. In the past, children in the 1-5 year age group were more prone to pertussis. Since adults do not receive booster vaccinations, children less than one year of age[2] have become more susceptible because of a decrease in passively transferred maternal antibodies.

Bordetella are tiny, gram-negative, strictly aerobic coccobacilli that occur singly or in pairs and may exhibit a bipolar appearance. While some species are motile, *B. pertussis* and *B. parapertussis* are nonmotile. They do not produce acid from carbohydrates. *B. pertussis* will not grow on common blood agar bases or chocolate agar, while *B. parapertussis* will grow on blood agar and sometimes on chocolate agar. Media for primary isolation must include starch, charcoal, ion-exchange resins or a high percentage of blood to inactivate inhibitory substances.[3] *B. pertussis* may be recovered from secretions collected from the posterior nasopharynx, bronchoalveolar lavage and transbronchial specimens.

Principles of the Procedure

Identification of *Bordetella* species includes isolation of the microorganisms, biochemical identification and serological confirmation.

Serological confirmation involves the reaction in which the microorganism (antigen) reacts with its corresponding antibody. The *in vitro* reaction produces macroscopic clumping called agglutination. The desired homologous reaction is rapid, does not dissociate (has high avidity), and binds strongly (has high affinity).

Because a microorganism (antigen) may agglutinate with an antibody produced in response to some other species, heterologous reactions are possible. These are weak in strength or slow in formation. Such unexpected and, perhaps, unpredictable reactions may lead to some confusion in serological identification. Therefore, a positive homologous agglutination reaction should support the morphological and biochemical identification of the microorganism.

Homologous reactions are rapid and strong. Heterologous reactions are slow and weak.

Reagents

Bordetella Pertussis and Parapertussis Antisera are lyophilized, polyclonal rabbit antiglobulins containing approximately 0.04% Thimerosal as a preservative. When rehydrated and used as described, each 1 ml vial of Bordetella Pertussis or Parapertussis Antiserum diluted 1:10 contains sufficient reagent for 200 slide tests.

Bordetella Pertussis Antigen is a ready-to-use suspension of killed, whole organisms adjusted to a density approximating two times a McFarland Barium Sulfate Standard No. 3 (9 x 10^8 organisms per ml). Bordetella Pertussis Antigen contains 0.04% Thimerosal. When used as described, each 5 ml vial contains sufficient reagent to perform approximately 140 slide tests.

Because antigen density may vary, it is adjusted to ensure optimum performance when the antigen is standardized with hyperimmune sera obtained from laboratory animals.

Precautions

1. For In Vitro Diagnostic Use.
2. **Bordetella Pertussis Antiserum**
   Bordetella Parapertussis Antisera
   The Packaging of This Product Contains Dry Natural Rubber.
3. Bordetella Pertussis Antigen is not intended for use in the immunization of humans or animals.
4. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated Bordetella Pertussis Antiserum and Bordetella Parapertussis Antiserum at 2-8°C.

Store Bordetella Pertussis Antigen at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Bordetella Pertussis Antiserum
Bordetella Parapertussis Antiserum
Bordetella Pertussis Antigen

Materials Required But Not Provided

Agglutination slides
Applicator sticks
Sterile 0.85% NaCl solution
Distilled or deionized water
Inoculating loop

Reagent Preparation

Equilibrate all materials to room temperature before performing the test. Ensure that all glassware and pipettes are clean and free of detergent residues.

Bordetella Pertussis and Parapertussis Antiserum: To rehydrate, add 1 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents. Dilute the rehydrated antiserum 1:10 with sterile 0.85% NaCl solution. Dilute only enough for 1-2 days testing requirements.

Bordetella Pertussis Antigen is ready to use.

Specimen Collection and Preparation

Isolation of *Bordetella* from clinical specimens requires the use of certain media such as Bordet-Gengou Agar. Colonies of *B. pertussis* are very small, white, glistening, convex, entire and usually exhibit tiny zones of hazy hemolysis. Colonies of *B. parapertussis* are usually larger than those of *B. pertussis*, may have a slightly brown color, and do not have a glistening surface. For specific recommendations for culture and identification, consult appropriate references.[3,5] Determine that a pure culture of the microorganism has been obtained and that biochemical test reactions are consistent with the identification of the organism as *Bordetella*. After these criteria are met, serological identification can proceed.

Test Procedure

1. **Bordetella Antiserum**: On an agglutination slide, dispense 2 separate drops (approximately 35 µl, each) of the antiserum to be tested, the first to be used for the test isolate and the second for the positive control.
2. **Negative control**: Dispense 1 drop of sterile 0.85% NaCl solution.
3. **Test isolate**: Transfer a loopful of isolated colony from a solid agar medium to the first drop of antiserum and a second loopful to the negative control (0.85% NaCl solution).
4. **Positive control**: As appropriate, add 1 drop of Bordetella Pertussis Antigen or a small amount of a known *B. pertussis* or *B. parapertussis* culture to the second drop of antiserum.
5. Mix each test and control serum reaction area using separate applicator sticks.
6. Rotate the slide for 1 minute and read for agglutination.

Results

1. Read and record results as follows.
   4+ 100% agglutination; background is clear to slightly hazy.
   3+ 75% agglutination; background is slightly cloudy.
   2+ 50% agglutination; background is moderately cloudy.
   1+ 25% agglutination; background is cloudy.
   – No agglutination.
2. **Positive control**: Should produce 3+ or greater agglutination.
   Negative control: Should produce no agglutination.
   Positive test result: Agglutination of 3+ or greater within 1 minute.

Limitations of the Procedure

1. Correct interpretation of serological reactions depends on culture purity as well as morphological characteristics and biochemical reactions that are consistent with identification of the microorganism as a *Bordetella* species.
2. Serological methods alone cannot identify the isolate as *Bordetella*.
3. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent making a smooth suspension of the microorganism or may cause evaporation or precipitation of the test mixture. False-positive reactions may occur.
4. Rough culture isolates do occur and will agglutinate spontaneously causing agglutination of the negative control (autoagglutination). Smooth colonies must be selected and tested in serological procedures.
5. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
6. A rehydrated Bordetella Antiserum that is cloudy or develops a precipitate during use should be discarded.

7. Some *Hemophilus* species will grow on *Bordetella* isolation media and may cross-react with *B. pertussis* antisera. Rule out X- and V-factor dependence using Differentiation Disks V, X and VX.[5]
8. Shake the antigen vial well before use to obtain a smooth, uniform suspension. Occasionally, a *Bordetella* suspension may settle out during storage.
9. *Bordetella* antigen will display irreversible autoagglutination if, at anytime during shipment or storage, it is subjected to freezing temperatures. Do not allow to freeze.

References

1. **Linneman, C. C., and E. B. Pery.** 1977. *Bordetella parapertussis:* recent experience and a review of the literature. Am. J. Dis. Child **131:**560-563.
2. **Bass, J. W., and S. R. Stephenson**. 1987. The return of pertussis. Pediatr. Infect. Dis. J. **6:**141-144.
3. **Marcon, M. J.** 1995. *Bordetella*, p. 566-573. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
4. **Wright, P. F.** 1991. Pertussis in developing countries: definition of the problem and prospects for control. Rev. Infect. Dis. **13:**S228-S234.
5. **Pezzlo, M.** 1992. Aerobic bacteriology, p. 1.0.1-1.20.47. *In* H. D. Isenberg (ed.) Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
6. **Rose, N. R., H. Friedman, and J. L. Fahey (ed.).** 1986. Manual of clinical laboratory immunology, 3rd ed. American Society for Microbiology, Washington, D.C.

Packaging

Bordetella Pertussis Antiserum	1 ml	2309-50
Bordetella Parapertussis Antiserum	1 ml	2310-50
Bordetella Pertussis Antigen	5 ml	2585-56

Bacto® Brucella Antigens and Antisera

Brucella Abortus Antigen (Slide) · Brucella Abortus Antigen (Tube) Brucella Melitensis Antigen (Slide) · Brucella Suis Antigen (Slide) Brucella Abortus Antiserum · Febrile Negative Control

Intended Use

Bacto Brucella Abortus Antigen (Slide) and (Tube), Brucella Melitensis Antigen (Slide) and Brucella Suis Antigen (Slide) are used in the detection of antibodies by the slide and tube agglutination tests (as indicated).

Bacto Brucella Abortus Antiserum is used to demonstrate a positive quality control test reaction in the slide and tube agglutination tests.

Bacto Febrile Negative Control is used to demonstrate a negative quality control test reaction in the slide agglutination test.

Summary and Explanation

Brucellae are intracellular parasites that, upon invasion, produce fever in their host. Consequently, they are often called “Febrile Antigens.” Brucellae also cause localized infection of bone, tissue and organ systems in humans.[1,2] These organisms are intracellular pathogens of the reticuloendothelial system. They form granulomatous masses in various organs.

Brucellosis is the disease state caused by these organisms. The disease has an abrupt onset, usually three to four weeks after exposure. Symptoms of the disease include fever, arthralgia, malaise, chills and sweating. Approximately 70% of patients with acute brucellosis have a tube agglutination titer of 1:160 or greater.[3] Osteomyelitis is the most frequent complication in humans.[4,5]

Most cases of brucellosis are due to exposure to animals, including cattle, sheep, goats and swine, or to laboratory cultures of *Brucella* species.[3] Patient history usually includes exposure to livestock or to meat processing. Routes of infection are nasopharyngeal, gastrointestinal, conjunctival, respiratory and through abraded skin.[5] Sporadic episodes of food-associated brucellosis have been caused by *B. melitensis*.[6,7]

The human immune response to a particular microorganism results in measurable antibody production which, in some cases, can assist in completing the patient’s clinical diagnosis. In blood samples, the antibody titer during the initial phase of the infection (acute) is compared to the antibody titer 7-14 days later (convalescent). A high acute phase antibody titer (1:320) and paired acute and convalescent samples that show an increase in antibody titer are helpful in assisting the diagnosis of brucellosis.

A preliminary test using either the rapid slide test and/or the macroscopic tube test may be performed on the initial serum specimen and reported to the physician at that time. An aliquot of the serum should be transferred to a sterile test tube, sealed tightly, and kept in the freezer. When the second serum is obtained, it should be run in parallel with the original specimen. In this manner, the original serum will serve as a control. Any difference in titer will be more credible because the bias associated with the performance of the test and determining the endpoint will be reduced.

Brucellae are small, nonmotile, nonencapsulated gram-negative coccobacilli. The organisms grow aerobically and their growth is enhanced by incubation in CO_2.

Six species of *Brucella* have been recognized. Among these, *B. abortus* (cattle), *B. suis* (swine), *B. melitensis* (goats and sheep) and *B. canis* (dogs) are infective for humans, although *B. canis* infections in humans are rare.[8]

The rapid slide procedure is a screening test designed to detect agglutinins. The tube test is a confirmatory procedure designed to quantitate febrile agglutinins. It is, therefore, necessary that any positive results obtained in the screening (slide test) of specimens be confirmed by a tube test. The tube agglutination test is used clinically in the United States.[9,10,11]

Certain organisms may share cross-reacting antigens leading to the production of heterologous antibodies. These heterologous antibodies may react with one or more antigens in a febrile antibody test procedure, causing low-level antibody titers that may not singly be indicative of disease. Cross reactions between *Brucella* and *Francisella tularensis*, *Yersinia enterocolitica* and *Vibrio cholerae* can occur.

Principles of the Procedure

Agglutination tests involving the use of *Brucella* antigens detect the presence of antibodies that react with the test antigen. The serological procedure involves serially diluting the patient serum and then adding a standard volume of antigen. The endpoint of the test is the last dilution of the serum that shows a specific amount of agglutination. The end point, reported as a dilution of the serum, is called the patient's antibody "titer."

Reagents

Brucella Abortus Antigen (Slide), Brucella Melitensis Antigen (Slide) and Brucella Suis Antigen (Slide) are ready-to-use, chemically inactivated and stabilized suspensions of *Brucella abortus* 1119-3,[12] *Brucella melitensis* and *Brucella suis*, respectively. The slide antigens contain approximately 2% packed cells and 20% glycerin, as well as 0.5% phenol and approximately 0.002% crystal violet and 0.005% brilliant green as preservatives. When used as described, each 5 ml vial contains sufficient reagent for 20 slide tests.

Brucella Abortus Antigen (Tube) is a ready-to-use suspension of *Brucella abortus* 1119-3[12] adjusted to a density approximating a McFarland Barium Sulfate Standard No. 3 (9 x 10^8 organisms per ml). Brucella Abortus Antigen (Tube) contains 0.5% phenol as a preservative but does not contain dye. When used as directed, each 25 ml vial contains sufficient reagent for 6 tests.

Because antigen density may vary, density is adjusted to ensure optimum performance when the antigen is standardized with hyperimmune sera obtained from laboratory animals. Variation in antigen color intensity is normal and will not affect the outcome of the test.

Brucella Abortus Antiserum is a lyophilized, polyclonal rabbit antiserum containing approximately 0.04% Thimerosal as a preservative.

Brucella Abortus Antiserum is unabsorbed. Serological cross-reactions occur in unabsorbed sera from *Brucella* species because *B. abortus*, *B. suis* and *B. melitensis* are antigenically related, containing common A (*abortus*) and M (*melitensis*) substances. (Monospecific sera prepared by absorption produce weak, unstable reagents that make interpretation of agglutination results difficult.)

When rehydrated and used as described, each 3 ml vial of Brucella Abortus Antiserum contains sufficient reagent for 19 slide tests or 30 tube tests.

Febrile Negative Control is a standard protein solution containing approximately 0.04% Thimerosal as a preservative. When used as described, each 3 ml vial contains sufficient reagent for 32 slide tests.

User Quality Control

Identity Specifications

Brucella Abortus Antigen (Slide), Brucella Suis Antigen (Slide), Brucella Melitensis Antigen (Slide)
Appearance: Turquoise, blue-violet suspension.

Brucella Abortus Antigen (Tube)
Appearance: Light gray to white suspension.

Brucella Abortus Antiserum
Lyophilized Appearance: Light gold to amber, button to powdered cake.
Rehydrated Appearance: Light gold to amber, clear liquid.

Febrile Negative Control
Lyophilized Appearance: Colorless to light gold, button to powdered cake.
Rehydrated Appearance: Colorless to light gold, clear liquid.

Performance Response

Rehydrate Brucella Abortus Antiserum and Febrile Negative Control per label directions. Perform the slide or tube agglutination test using Brucella Abortus Antigen (Tube), Brucella Abortus (Slide), Brucella Suis (Slide), or Brucella Melitensis (Slide). Dilute both positive and negative controls in the same proportion as the patient's serum and process in the same manner, following appropriate procedure.

An antigen is considered satisfactory if it does not agglutinate with the negative control and yields a 2+ reaction at a titer of 1:80 or more with the positive control.

Precautions

1. For In Vitro Diagnostic Use.
2. **Brucella Abortus Antiserum**
   The Packaging of This Product Contains Dry Natural Rubber.
3. Observe universal blood and body fluid precautions in the handling and disposing of specimens.[13,14]
4. Brucella Antigens are not intended for use in the immunization of humans or animals.
5. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store Brucella Antigens (Slide) and (Tube) at 2-8°C.

Store lyophilized and rehydrated Brucella Abortus Antiserum at 2-8°C.

Store lyophilized and rehydrated Febrile Negative Control at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Brucella Abortus Antigen (Slide)
Brucella Suis Antigen (Slide)

Brucella Melitensis Antigen (Slide)
Brucella Abortus Antigen (Tube)
Brucella Abortus Antiserum
Febrile Negative Control

Materials Required But Not Provided

Slide Test

Agglutination slides with 5 squares
Applicator sticks
Sterile 0.85% NaCl solution
Serological pipettes, 0.2 ml
Distilled or deionized water

Tube Test

Culture tubes, 12 x 75 mm, and rack
Waterbath, 35-37°C
Serological pipettes, 1 ml and 5 ml
Sterile 0.85% NaCl solution
Distilled or deionized water

Reagent Preparation

Brucella Abortus Antigen (Slide) and (Tube), Brucella Suis Antigen (Slide) and Brucella Melitensis Antigen (Slide) are ready to use.

Equilibrate all materials to room temperature prior to performing the tests. Ensure that all glassware and pipettes are clean and free of detergent residues.

Brucella Abortus Antiserum: To rehydrate, add 3 ml sterile 0.85% NaCl solution and rotate gently to completely dissolve the contents. The rehydrated antiserum is considered a 1:2 working dilution.

Febrile Negative Control: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

Specimen Collection and Preparation

Collect a blood specimen by aseptic venipuncture. After the specimen has clotted, centrifuge to obtain the serum required for the test. Serum specimens must be clear, free of hemolysis and show no visible evidence of bacterial contamination (turbidity, hemolysis or particulate matter). Consult appropriate references for more information on the collection of specimens.[15,16]

Store serum specimens at room temperature for no longer than 4 hours; for prolonged storage, keep at 2-8°C for up to 5 days or maintain below -20°C. Serum specimens must not be heated; heat may inactivate or destroy certain antibodies.

Slide Test

Use the slide test only as a screening test. Confirm positive results with the tube test.

In some cases of brucellosis, sera may display a prozone reaction, the inability of an antigen to react at higher serum antibody concentrations. It is advisable to run all 5 serum dilutions of the rapid slide test, rather than just one dilution, to eliminate the possibility of missing positive reactions due to the prozone phenomenon.

1. **Test serum**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of each test serum into a row of squares on the agglutination slide.
2. **Positive control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Brucella Abortus Antiserum into a row of squares on the agglutination slide.
3. **Negative control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Febrile Negative Control into a row of squares on the agglutination slide.
4. **Antigen**: Shake the vial of Brucella Antigen (Slide) well to ensure a smooth, uniform suspension. Dispense 1 drop (35 µl) of antigen in each drop of test serum, positive control and negative control.
5. Mix the rows of test and control serum, using a separate applicator stick for each row. Start with the most dilute mixture (0.005 ml) and work to the most concentrated (0.08 ml).
6. Rotate the slide for 1 minute and read for agglutination.
7. The final dilutions in squares 1-5 correspond to tube dilutions of 1:20, 1:40, 1:80, 1:160 and 1:320, respectively.

Tube Test

1. In a rack, prepare a row of 8 culture tubes (12 x 75 ml) for each test serum, including a positive control row for the Brucella Abortus Antiserum and an antigen control row for the Febrile Negative Control Serum.
2. Dispense 0.9 ml of sterile 0.85% NaCl solution in the first tube of each row and 0.5 ml in the remaining tubes.
3. **Test serum**: Using a 1 ml serological pipette, dispense 0.1 ml of serum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Proceed in like manner for each serum to be tested. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
4. **Positive control**: Using a 1 ml serological pipette, dispense 0.1 ml of Brucella Abortus Antiserum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
5. **Antigen Control**: Shake the vial of Brucella Abortus Antigen (Tube) to ensure a smooth, uniform suspension. Add 0.5 ml of antigen to all 8 tubes in each row and shake the rack to mix the suspensions.
6. Final dilutions in tubes 1-7 are 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280, respectively.
7. Incubate in a waterbath at 35-37°C for 48 ± 3 hours.
8. Remove from the waterbath. Avoid excessive shaking before reading the reactions, either when the tubes are in the waterbath or when removing them from the waterbath.
9. Read and record results.

Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.

2. **Positive control**: Should produce 2+ or greater agglutination at a 1:80 dilution.

   Negative control–Rapid Slide Test, only: Should produce no agglutination.

   Antigen control–Macroscopic Tube Test, only: Should show no agglutination in tube #8 of each row.

   If results for either the positive or the negative control are not as specified, the test is invalid and results cannot be reported.

   Test serum: The titer is the highest dilution that shows 2+ agglutination.

   Refer to Table 1 and Table 2 for examples of test reactions.
3. The Rapid Slide Test is a screening test, only; results must be confirmed using the Macroscopic Tube Test.

Table 1. Sample Rapid Slide Test reactions.

SERUM (ml)	CORRELATED TUBE DILUTION	REACTIONS		
		SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
0.08	1:20	3+	4+	4+
0.04	1:40	2+	4+	3+
0.02	1:80	1+	3+	2+
0.01	1:160	–	3+	+
0.005	1:320	–	1+	–
Serum titer		**1:40**	**1:160**	**1:80**

Table 2. Sample Macroscopic Tube Test reactions.

SERUM DILUTION	REACTIONS		
	SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
1:20	4+	3+	4+
1:40	4+	2+	4+
1:80	3+	1+	4+
1:160	2+	–	4+
1:320	1+	–	3+
1:640	–	–	2+
1:1280	–	–	1+
Serum titer	**1:160**	**1:40**	**1:640**

Interpretation

For a single serum specimen, a titer of 1:80 is a weak positive that suggests infection, but not necessarily a recent infection.[3,17]

A two-dilution increase in the titer of paired serum specimens (from the acute to the convalescent serum) is significant and suggests infection. A one-dilution difference is within the limits of laboratory error.

Past history in the use of *Brucella* suspensions has produced a pattern of titers that are considered "significant". A titer of 1:80 is considered a weakly positive result while most patients with acute undulant fever demonstrate a titer of 1:160 or greater.

Limitations of the Procedure[18,19]

1. The slide test is intended for screening only and results should be confirmed by the tube test. Slide test dilutions are made to detect a prozone reaction and do not represent true quantitation of the antibody. A serum specimen with a prozone reaction shows no agglutination because of excessively high antibody concentrations. To avoid this occurrence, all 5 slide test serum dilutions should be run.

   The detection of antibodies in serum specimens may complete the clinical picture of brucellosis. However, isolation of the causative agent from patient specimens may be required. A definitive diagnosis must be made by a physician based on patient history, physical examination and data from all laboratory tests.
3. The accuracy and precision of the tests can be affected not only by test conditions but also by the subjectivity of the person reading the endpoint.
4. Cross-reactions may occur due to antigenic similarities to other organisms. A definite serological relationship exists between *Brucella* and *Francisella tularensis*. Cross-reactions may also occur between *Brucella*-positive sera and *Proteus* OX19 antigen, *Vibrio cholerae* or *Yersinia enterocolitica* serotype 9.[20]
5. While a single serum specimen showing a positive reaction at a 1:80 dilution suggests infection, it is not diagnostic. An antibody titer greater than 1:160 may occur in healthy individuals with a past history of the disease.
6. To test for a significant rise in antibody titer, at least two specimens are necessary, an acute specimen obtained at the time of initial symptoms and a convalescent specimen obtained 7 to 14 days later. A two-dilution increase in titer is significant and suggests infection.
7. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
8. Exposure to temperatures below 2°C can cause autoagglutination. Antigens must be smooth, uniform suspensions. Examine antigen vials for agglutination before use. Agglutinated suspensions are not usable and should be discarded.
9. Adhering to the recommended time and temperature of incubation is important when performing the tube test. For best results, locate the waterbath in an area free of mechanical vibration.
10. Serum specimens from patients suffering from acute brucellosis demonstrate little or no antibody titer during the first 10 days of the disease.
11. Serological interpretation of an agglutinin titer in vaccinated individuals should be avoided since antibody levels may persist for years.
12. Individuals who have recovered from brucellosis may demonstrate a nonspecific agglutinin response upon infection with an etiological agent of a heterologous febrile species.

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Packaging

Brucella Abortus Antigen (Slide)	5 ml	2909-56
Brucella Abortus Antigen (Tube)	25 ml	2466-65
Brucella Melitensis Antigen (Slide)	5 ml	2916-56
Brucella Suis Antigen (Slide)	5 ml	2915-56
Brucella Abortus Antiserum	3 ml	2871-47
Febrile Negative Control	3 ml	3239-56

Bacto® Candida Albicans Antiserum

Intended Use

Bacto Candida Albicans Antiserum is used in the slide agglutination test for identifying *Candida albicans.*

Summary and Explanation

Candida albicans is an opportunistic pathogen. Infection with this organism will usually arise from an endogenous source in a compromised host. Candidiasis caused by *C. albicans* presents as superficial infections of the skin, oral thrush, systemic and disseminated infections involving most internal organs, and mucocutaneous candidiasis.[1] Vaginitis caused by *C. albicans* is the most common type of yeast infection.

C. albicans, a saprophyte, appears in large numbers throughout the oral-gastrointestinal tract of many warm-blooded vertebrates.[1] It is rarely isolated from normal skin. Person-to-person transmission of candidiasis can occur.

Candida albicans appears to possess many virulence attributes that may promote successful parasitism. These attributes include rapid germination upon seeding tissue from the bloodstream,[3] protease production,[4] complement protein-binding receptor,[5,6] surface variation and hydrophobicity.[7]

Candida albicans will grow on Sabauroud Dextrose Agar as white to cream-colored, butyrous colonies. *C. albicans* can be isolated from blood agar as a colony with short marginal extensions. Microscopically, *C. albicans* produces budding yeast cells, pseudohyphae or true hyphae. The organism may be identified by the production of germ tubes or chlamydospores. Identification of *Candida albicans* includes both biochemical and serological confirmation.[8]

Principles of the Procedure

Serological confirmation requires that the microorganism (antigen) react with its corresponding antibody. This in vitro reaction produces macroscopic clumping called agglutination. The desired homologous reaction is rapid, does not dissociate (has high avidity), and bonds strongly (has high affinity).

Because a microorganism (antigen) may agglutinate with an antibody produced in response to another species, heterologous reactions are possible. These are weak in strength or slow in formation. Such unexpected and, perhaps, unpredictable reactions may lead to some confusion in serological identification. Therefore, a positive homologous agglutination reaction should support the morphological and biochemical identification of the microorganism.

Agglutination of the somatic antigen in the slide test appears as a firm granular clumping. Homologous reactions occur rapidly and are strong (3+). Heterologous reactions are slow and weak.

Reagents

Candida Albicans Antiserum is a lyophilized, polyclonal rabbit antiserum containing approximately 0.04% Thimerosal as a preservative.

When rehydrated and used as described, each 3 ml vial contains sufficient Candida Albicans Antiserum for 60 tests.

Precautions

1. For In Vitro Diagnostic Use.
2. The Packaging of This Product Contains Dry Natural Rubber.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated Candida Albicans Antiserum at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Candida Albicans Antiserum

Materials Required But Not Provided

Agglutination slides
Applicator sticks
0.85% Sodium Chloride
Inoculating loop

User Quality Control

Identity Specifications

Candida Albicans Antiserum

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated Appearance: Light gold to amber, clear liquid.

Performance Response

Rehydrate Candida Albicans Antiserum per label directions. Perform the slide agglutination test using appropriate positive and negative control cultures. The negative control should show no agglutination. Homologous cultures should give a 3+ or greater agglutination.

Reagent Preparation

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergents.

Candida Albicans Antiserum: To rehydrate, add 3 ml sterile 0.85% NaCl solution and rotate gently to dissolve the contents completely. The rehydrated antiserum is considered a 1:2 working dilution.

Specimen Collection and Preparation

Candida albicans can be recovered on Tryptic Soy Agar with 5% Sheep Blood, Sabouraud Dextrose Agar or Brain Heart Infusion Agar. For specific recommendations on isolation of *Candida albicans* from clinical specimens, consult appropriate references.[9,10,11]

Test Procedure

1. Culture the test organism on Sabouraud Dextrose Agar at room temperature.
2. **Candida Albicans Antiserum**: Dispense one drop at one end of a microscope slide.
3. **0.85% NaCl solution**: Dispense one drop at the other end of the same slide.
4. **Test organism**: Place a partial loopful of a smooth homologous culture between the drops of antiserum and NaCl solution but not in direct contact with either.
5. Using an applicator stick, suspend the culture in the drop of NaCl solution and check for autoagglutination. If there is no autoagglutination, mix the culture suspension with the drop of antiserum.
6. Rotate the slide by hand for one minute and read immediately for agglutination. Record results.

Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
3. **Negative control**: Should produce no agglutination. If agglutination occurs, the culture is rough and cannot be tested. Subculture to a non-inhibitory medium, incubate and test the organism again.
4. **Test isolate**: 3+ or greater agglutination is a positive result.
5. Partial (less than 3+) or delayed agglutination should be considered negative.

Limitations of the Procedure

1. Serological techniques employing Candida Albicans Antiserum for the identification of *Candida albicans* serve as corroborative evidence in the determination of the organism as the etiological agent of the disease. Final identification cannot be made without consideration of morphological, serological, and biochemical characterization.
2. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent making a smooth suspension of the microorganism or cause evaporation or precipitation of the test mixture. False-positive reactions may occur.

3. Rough culture isolates do occur and will agglutinate spontaneously, causing agglutination of the negative control (autoagglutination). Smooth colonies must be selected and tested in serological procedures.
4. Agglutination reactions of 3+ or greater in the slide test are interpreted as positive reactions. Cross-reactions resulting in a 1+ or 2+ agglutination are likely since somatic antigens are shared among such organisms as *Candida tropicalis*, *Candida kefyr* and *Candida stellatoidea*.
5. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
6. Discard any Candida Albicans Antiserum that is cloudy or has a precipitate after rehydration or storage.

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Packaging

Candida Albicans Antiserum	3 ml	2281-47

Bacto® Coagulase Plasma
Bacto Coagulase Plasma EDTA

Intended Use

Bacto Coagulase Plasma[1] and Bacto Coagulase Plasma EDTA[1-8] are used for detecting coagulase activity by staphylococci.

Bacto Coagulase Plasma is used for detecting the production of germ tubes by *Candida albicans*.[2]

Summary and Explanation

Coagulase Detection

Identification of staphylococci is based on microscopic examination, colonial morphology, and cultural and biochemical characteristics. Staphylococci associated with acute infection (*S. aureus* in humans and *S. intermedius* and *S. hyicus* in animals) can clot plasma. The most widely used and generally accepted criterion for identification of these pathogenic organisms is based on the presence of the enzyme coagulase.[1] The ability of *Staphylococcus* to produce coagulase was first reported by Loeb[9] in 1903. Coagulase binds plasma fibrinogen, causing the organisms to agglutinate or plasma to clot. Two different forms of coagulase can be produced, free and bound. Free coagulase is an extracellular enzyme produced when the organism is cultured in broth. Bound coagulase, also known as clumping factor, remains attached to the cell wall of the organism. The tube test can detect the presence of both bound and free coagulase. The slide test can detect only bound coagulase.[10] Isolates that do not produce clumping factor must be tested for the ability to produce extracellular coagulase (free coagulase).

The tube test has traditionally been the standard in determining coagulase activity. The slide test is unreliable in the identification of some strains of oxacillin-resistant *S. aureus*.[11,12] False-positive results are sometimes obtained with the slide test when testing *S. saprophyticus*,[13] *S. schleiferi*, *S. lugdunensis*, *S. intermedius*,[4] *S. hyicus*[3] and micrococci.[11,14] In addition, colonies used for testing must not be picked from media containing high concentrations of salt (for example, mannitol-salt agar), because autoagglutination and false-positive results may occur.[1] Slide tests must be read quickly, because false-positive results may appear with reaction times longer than 10 seconds. Isolates that autoagglutinate cannot be reliably tested with the slide coagulase method. Finally, 10-15% of *S. aureus* strains may yield a negative result, which requires that the isolates be reexamined by the tube test. Coagulase Plasma and Coagulase Plasma EDTA are recommended for performing the tube coagulase test. The inoculum used for testing must be pure because a contaminant may produce false results after prolonged incubation. For the coagulase test, Coagulase Plasma EDTA is superior to citrated plasma because citrate-utilizing organisms such as *Pseudomonas* species, *Serratia marcescens*, *Enterococcus faecalis* and strains of *Streptococcus* will clot citrated plasma in 18 hours.[15]

Germ Tube Development

C. albicans is usually associated with an animal host. It appears in large numbers as a saprophyte throughout the oral-gastrointestinal tract

of many warm-blooded vertebrates.[16] It is rarely isolated from normal skin. Person-to-person transmission of candidiasis can occur. Usually, candidiasis caused by *C. albicans* is endogenous in origin and develops with stress or debilitation of the host.[16]

C. albicans is the species most commonly isolated from patients with nearly all forms of candidiasis.[17] This organism is an opportunistic pathogen and appears to possess many virulence attributes that may promote successful parasitism. These attributes include rapid germination upon seeding tissue from the bloodstream,[18] protease production,[19] complement protein-binding receptor,[20,21] and surface variation and hydrophobicity.[22]

C. albicans will grow on Sabouraud Dextrose Agar as white to cream colored, creamy colonies. It can be isolated from blood agar as a colony with short marginal extensions. Microscopically, *C. albicans* produces budding yeast cells, pseudohyphae or true hyphae.

One of the simplest and most valuable tests for the rapid presumptive identification of *C. albicans* is the germ tube test.[23] Smith and Elliott recommended the use of rabbit coagulase plasma.[2] The test is considered presumptive because not all isolates of *C. albicans* will be germ tube-positive and false positives may be obtained despite well-trained staff.[24] Ferrigno, Ramirez and Robison recommended testing for germ tube production with citrated plasma.[25]

User Quality Control

Identity Specifications

Coagulase Plasma

Lyophilized Appearance: Off-white to cream colored, dried button or fluffy powder.

Rehydrated Appearance: Off-white to cream to light rose colored, opaque liquid.

Coagulase Plasma EDTA

Lyophilized Appearance: Off-white to cream colored, dried button or fluffy powder.

Rehydrated Appearance: Off-white to cream to light rose colored, opaque liquid.

Performance Response

Rehydrate Coagulase Plasma or Coagulase Plasma EDTA per label directions. Perform the Coagulase Test or the Germ Tube Test procedure as described (see Test Procedure).

ORGANISM	ATCC®	COAGULASE TEST	GERM TUBE DEVELOPMENT
Staphylococcus aureus	25923*	Clot in tube	–
Staphylococcus aureus	3647	Clot in tube	–
Staphylococcus epidermidis	12228*	No clot in tube	–
Staphylococcus saprophyticus	15305	No clot in tube	–
Candida albicans	18804	–	Germ tube development
Candida tropicalis	750	–	No germ tube development

The cultures listed are the minimum that should be used for performance testing.

*These cultures are available as Bactrol™ Disks and should be used as directed in Bactrol Disks Technical Information.

Principles of the Procedure

Coagulase Detection

S. aureus produces two types of coagulase, free and bound. Free coagulase is an extracellular enzyme produced when the organism is cultured in broth. Bound coagulase, also known as the clumping factor, remains attached to the cell wall of the organism.

In the tube test, free coagulase liberated from the cell acts on prothrombin in the coagulase plasma to give a thrombin-like product. This product then acts on fibrinogen to form a fibrin clot.[3]

The tube test is performed by mixing an overnight broth culture or colonies from a noninhibitory agar plate into a tube of rehydrated coagulase plasma. The tube is incubated at 37°C. The formation of a clot in the plasma indicates coagulase production.

Germ Tube Development

The germ tube test involves suspending suspected colonies of yeast in a tube of Coagulase Plasma. The tube is incubated at 37°C for 2-4 hours. The cells are then observed microscopically for short, hyphal extensions from the yeast cells called germ tubes. Germ tubes are easily differentiated from blastoconidial germination; germ tubes have no constriction at their juncture with the yeast cell while blastoconidial germination does produce a constriction. *C. albicans* usually produces germ tubes under specified test conditions within 2 hours. Other species of *Candida* do not produce germ tubes, except for an occasional isolate of *Candida tropicalis*.[4]

Reagents

Coagulase Plasma is lyophilized rabbit plasma to which sodium citrate has been added as the anticoagulant.

Coagulase Plasma EDTA is lyophilized rabbit plasma to which EDTA (ethylenediaminetetraacetic acid) has been added as the anticoagulant. EDTA is not utilized by bacteria. Coagulase Plasma EDTA does not give false-positive reactions with bacteria that utilize citrate.

Precautions

1. For In Vitro Diagnostic Use.
2. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store unopened Coagulase Plasma and Coagulase Plasma EDTA at 2-8°C.

Store reconstituted plasma at 2-8°C for up to 5 days, or aliquot in 0.5 ml amounts, freeze promptly and store at -20°C for up to 30 days. Do not thaw and refreeze.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Coagulase Plasma
Coagulase Plasma EDTA

Materials Required But Not Provided

Bacteriological inoculating loop
Sterile 1 ml pipettes
Sterile Pasteur pipettes
Sterile serological pipettes, 1, 5, and 10 ml
Incubator (37°C)
Sterile distilled or deionized water
Culture tubes, 12 x 75 mm
Timer
Waterbath (35-37°C)
BHI broth or noninhibitory agar (Coagulase Detection)
Sabouraud Dextrose Agar (Germ Tube Development)

Reagent Preparation

Rehydrate Coagulase Plasma and Coagulase Plasma EDTA by adding sterile distilled or deionized water to the vial as indicated below. Mix by gentle end-over-end rotation of the vial.

PRODUCT SIZE	STERILE DISTILLED WATER	APPROXIMATE NUMBER OF TESTS
3 ml	3 ml	6
15 ml	15 ml	30
25 ml	25 ml	50

Specimen Collection and Preparation

1. Collect specimens or samples in sterile containers or with sterile swabs and transport immediately to the laboratory according to recommended guidelines.[1,3-8]
2. Process each specimen using procedures appropriate for that sample.[1,3-8]

Coagulase Detection

1. Obtain a pure culture of the organism to be tested. Select well-isolated colonies.
2. Determine that the test culture has characteristics of *S. aureus* as listed below. Consult appropriate references for further identification of *S. aureus*.[1,3-8]

   Morphology (media dependent):

Blood Agar Base w/5% Sheep Blood	Opaque, yellow to orange, with hemolysis.
DNase Test Agar w/Methyl Green	Clearing of green dye.
Mannitol Salt Agar	Yellow to orange, surrounded by yellow zones.
Staphylococcus Medium 110	Yellow to orange.
Tellurite Glycine Agar	Black.
VJ Agar	Black, surrounded by yellow zones.
Baird Parker Agar	Grey to black shiny colonies surrounded by zones of clearing.
Gram Stain:	Gram-positive cocci occurring in grape-like clusters or, occasionally, in chains.
Catalase Test:	Positive.
Mannitol Fermentation:	Positive.

3. Using a bacteriological loop, transfer a well-isolated colony from a pure culture into a tube of sterile Brain Heart Infusion broth. Incubate for 18-24 hours or until a dense growth is observed. Alternatively, 2-4 colonies (1 loopful) taken directly from a noninhibitory agar plate may be used as an inoculum instead of a broth culture.

Germ Tube Development

1. Obtain a pure culture of the organism to be tested. Select well-isolated colonies grown on Sabouraud Dextrose Agar for 48-72 hours.

Test Procedure

Coagulase Test

1. Using a sterile 1 ml pipette, add 0.5 ml of rehydrated Coagulase Plasma or Coagulase Plasma EDTA to a 12 x 75 mm test tube supported in a rack.
2. Using a sterile 1 ml serological pipette, add 2 drops of the overnight broth culture of the test organism to the tube of plasma or, using a sterile bacteriological loop, thoroughly emulsify 2-4 colonies (1 loopful) from a noninhibitory agar plate in the tube of plasma.
3. Mix gently.
4. Incubate in a waterbath at 35-37°C for up to 4 hours.
5. Examine the tube for coagulation hourly until a clot is evident or until 4 hours have elapsed. If no clot has formed within 4 hours, reincubate and examine after 24 hours.

   Examine by gently tipping the tube. Avoid shaking or agitating the tube, which could cause breakdown of the clot and, consequently, doubtful or false-negative test results.
6. Record results.

Germ Tube Test

1. Using a sterile 1 ml pipette, add 0.5 ml of the rehydrated Coagulase Plasma (citrated) to a 12 x 75 mm test tube in a rack.
2. Touch the tip of a sterile Pasteur pipette to a yeast colony growing on a Sabouraud Dextrose Agar plate.
3. Gently emulsify the cells in the tube of rehydrated plasma.
4. Incubate the mixture in a waterbath at 37°C for 2-4 hours.
5. Examine 1 drop of the incubated mixture microscopically for germ tubes.
6. Record results.

Results

Coagulase Test

Any degree of clotting in Coagulase Plasma or Coagulase Plasma EDTA is considered a positive test.

Germ Tube Test

The development of short, lateral hyphal filaments (germ tubes) on the individual yeast cells with no constriction at the point of attachment is considered a positive test.

Limitations of the Procedure

1. The slide agglutination technique for determining the coagulase activity of staphylococci is not recommended because false-positive reactions may occur with some strains when animal plasmas are used. In addition, spontaneous agglutination may occur when rough cultures are used. Because 10-15% of *S. aureus* isolates may yield a negative result when this test is employed, all negative slide reactions must be confirmed by the tube test.

2. Some species of organisms utilize citrate in their metabolism and will yield false-positive reactions for coagulase activity. Normally, this would not cause problems since the coagulase test is performed almost exclusively on staphylococci. However, it is possible that bacteria which utilize citrate may contaminate *Staphylococcus* cultures on which the coagulase test is being performed. These contaminated cultures may, upon prolonged incubation, give false-positive results due to citrate utilization.[3]
3. When checking results of the Coagulase Test, observe tubes hourly during the first 4 hours of incubation. Some strains of *S. aureus* produce fibrinolysin, which may lyse clots. If the tubes are not read until 24 hours of incubation, reversion to a false-negative may occur.[26]
4. Do not use plasmas if a heavy precipitate or clot has formed before inoculation.

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Packaging

Coagulase Plasma	6 x 3 ml	0286-46
	6 x 15 ml	0286-86
	6 x 25 ml	0286-66
Coagulase Plasma EDTA	6 x 3 ml	0803-46
	6 x 15 ml	0803-86
	6 x 25 ml	0803-66

Bacto® E. Coli Antisera

E. Coli O Antiserum O157 · E. Coli H Antiserum H7

Intended Use

Bacto E. Coli O Antiserum O157 and E. Coli H Antiserum H7 are used for identifying *Escherichia coli* O157:H7.

Summary and Explanation

E. coli O157:H19 was described in 1972 as a causative agent of diarrhea in swine.[1] The H19 flagellar antigen was the common antigen for serogroup O157. An H7 variant of somatic group O157 has been incriminated in severe hemorrhagic colitis.

E. coli O157:H7 is a foodborne pathogen that can cause potentially fatal enteric-related disease in humans.[2,3,4,5,6,7,8] This disease is characterized by sudden onset of severe cramps and abdominal pain, followed by a watery stool that may become markedly bloody. A series of 107 outbreaks involving 387 persons was traced to imported Camembert cheese in the United States in 1971.[9] E. coli O157:H7 was recognized as a cause of hemorrhagic colitis in 1982[5], and hemolytic uremic syndrome in 1983.[10] The 1982 outbreak was derived from ingested hamburgers.[5,11]

The incidence of disease caused by this organism has increased significantly over the past decade.[4,12] The largest outbreak of *E. coli* O157:H7 disease occurred during January 1993, in Washington State, where more than 600 patients with hemorrhagic colitis were confirmed.[2] The source of the outbreak was identified as undercooked hamburger at multiple outlets of the same fast food restaurant chain.

E. coli O157:H7 is an enteric pathogen that requires only a low inoculum to cause disease. Transmission is usually via high volume food items whose preparation is not always under stringent control and is served to a target audience (children and the elderly) most at risk for complications of illness. The organism has been isolated from several foods, including undercooked hamburger, drinking water, new potatoes, turkey roll, raw milk and apple cider. Serotyping of the entero-hemorrhagic *E. coli* is useful in the epidemiological documentation of the spread of a particular strain in a foodborne outbreak.[12]

User Quality Control

Identity Specifications

E. Coli O Antiserum O157

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated Appearance: Light gold to amber, clear liquid.

E. Coli H Antiserum H7

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated Appearance: Straw colored, clear solution.

Cultural Response

Rehydrate E. Coli O Antiserum O157 and E. Coli H Antiserum H7 per label directions. Test as described (see Test Procedure). Known positive and negative control cultures must give appropriate reactions.

ORGANISM	ATCC®	REACTION
E. coli O157:H7	35150	Positive
E. coli O111:K58:H21	29552	Negative

These strains may be used for Quality Control. All cultures should be serologically validated before use.

Principles of the Procedure

E. Coli O Antiserum O157 is used in the tube technique for O antigen titration. E. Coli H Antiserum H7 is used in the tube agglutination technique for detecting H antigens. These antisera are used to confirm the presence of *E. coli* O157:H7 after selective isolation. For isolation of this organism from food, use MacConkey Sorbitol Agar.[13] MacConkey Sorbitol Agar has also been used successfully to isolate *E. coli* O157:H7 from stool specimens. However, high levels of contaminating coliform organisms will mask the O157 strains.[14]

Procedures for serological confirmation by E. Coli O Antiserum O157 and E. Coli H Antiserum H7 require a pure culture of the test organism isolated on Veal Infusion Agar or other enriched solid medium. The serological technique is based on the reaction of a specific antiserum with its homologous antigen. While the specificity of serological methods is not absolute, serotyping of *E. coli*, taken with biochemical characteristics, can provide an accurate identification of the etiological agent.

Reagents

E. Coli O Antiserum O157 and E. Coli H Antiserum H7 are lyophilized, polyclonal rabbit antisera containing approximately 0.04% Thimerosal as a preservative.

Precautions

1. For In Vitro Diagnostic Use.
2. The Packaging of This Product Contains Dry Natural Rubber.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated E. Coli Antisera at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

E. Coli O Antiserum O157
E. Coli H Antiserum H7

Materials Required But Not Provided

Veal Infusion Agar
Motility GI Medium

Test tubes (12 x 75 mm) or other suitable test tubes and rack
Sterile 0.85% NaCl solution
Formalin
1 ml serological pipettes
McFarland Standard No. 3
Waterbath (50 ± 2°C)

Method of Preparation

E. Coli Antiserum: To rehydrate, add 3 ml sterile 0.85% NaCl solution and rotate gently to dissolve contents completely. The rehydrated antiserum is considered a 1:2 working dilution.

Tube Technique for O Antigen Titration

1. To prepare pure cultures of the test organism, plate the organism on Veal Infusion Agar and incubate at 35 ± 2°C for 16-18 hours.
2. Suspend some growth from the solid medium in 0.85% NaCl solution to give a homogeneous suspension.
3. Heat the bacterial suspension in a boiling water bath for 30-60 minutes. The culture should be homogeneous. Precipitation indicates a rough culture and the suspension should be discarded.
4. Allow the suspension to cool; dilute with 0.85% NaCl solution to a density approximating that of a McFarland Barium Sulfate Standard No. 3.
5. Add formalin to a final concentration of 0.5% by volume.
6. In a rack, prepare a row of 8 culture tubes (12 x 75 mm) for each test suspension
7. Dispense 0.9 ml of 0.85% NaCl solution in the first tube of each row and 0.5 ml in the remaining tubes.
8. **E. Coli O Antiserum O157**: Prepare serial dilutions using the rehydrated antiserum, which is already at a 1:2 working dilution. Dispense 0.1 ml of antiserum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Proceed in like manner for each suspension to be tested. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.

   This procedure yields antiserum dilutions of 1:20-1:1280.
9. **Heated bacterial suspension**: Add 0.5 ml to each of the 8 tubes. Final antiserum dilutions are 1:40-1:2560.
8. Incubate in a waterbath at 50 ± 2°C for 18-20 hours. Read for agglutination.

Tube Agglutination Technique for H Antigen Detection

1. Prepare an actively motile culture of the suspect E. coli culture by several successive transfers in Motility GI Medium. At least 2-3 passages through Motility GI Medium are necessary before attempting to establish the presence and identity of H antigens. Fresh isolates of *E. coli* generally have poorly developed flagella.
2. Inoculate a loopful of the Motility GI Medium culture into a tube of Veal Infusion Broth. Incubate 6-8 hours at 35 ± 2°C or overnight, if necessary.
3. Inactivate the culture by adding formalin to a final concentration of 0.3% (0.3 parts formaldehyde per 100 parts of the Veal Infusion Broth culture). If necessary, adjust the density of the suspension with formalinized saline to approximate a McFarland Barium Sulfate Standard No. 3. This broth culture will be used as the test antigen in step 6.
4. **E. Coli H Antiserum H7**: Prepare a 1:500 dilution by adding 0.2 ml of rehydrated antiserum, which is already a 1:2 working dilution, to 49.8 ml of 0.85% NaCl solution.
5. Pipette 0.5 ml of the antiserum dilution into a test tube.
6. **Test antigen**: Add 0.5 ml to the above dilution and shake well. The resulting antiserum dilution will be 1:1,000.
7. Incubate the tube in a 50 ± 2°C waterbath for 1 hour and read for agglutination.

Results

Observe test results with indirect lighting against a dark background. Record as follows.

4+	100% agglutination of cells; supernatant fluid is clear to very slightly hazy.
3+	75% agglutination of cells; supernatant fluid is slightly cloudy.
2+	50% agglutination of cells; supernatant fluid is moderately cloudy.
1+	25% agglutination of cells; supernatant fluid is cloudy.
±	Less than 25% agglutination of cells.
–	No agglutination.

E. Coli O157: Cultures showing 2+ or greater agglutination at a dilution of 1:320 or greater are considered positive.

E. Coli H7: Tubes showing 2+ or greater agglutination are considered positive.

Limitations of the Procedure

1. Final identification of *E. coli* O157 is based on biochemical reactions and the presence of the O antigen.
2. The test organism must be identified to at least the genus level and, in some cases, to the species level biochemically before serotyping *E. coli*.
3. If the antiserum is cloudy after rehydration, check its bacterial purity and the pH of the saline. Discard any serum that is cloudy and/or has a precipitate unless it has been clarified and shown to react properly with known control cultures.
4. Adhere strictly to the time limitations in both tests.
5. Exposure of the organism or plate to heat from external sources (a hot bacteriological loop, burner flame, light source, etc.) may result in either a culture that cannot be suspended readily or evaporation and/or precipitation of the test mixture. These conditions may cause false-positive reactions.
6. The test culture must be checked in a saline control for smoothness. Stock cultures and, sometimes, isolated cultures may be rough and will agglutinate spontaneously in a normal serum. Therefore, it is necessary to select smooth colonies for serological testing.
7 In *E. coli* serology, as in any serological test, known positive and negative control cultures should be employed.
8. Antisera should not be subjected to repeated freezing and thawing. Such treatment is detrimental to antibody content.

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Packaging

E. Coli O Antiserum O157	3 ml	2970-47
E. Coli H Antiserum H7	3 ml	2159-47

Bacto® FA Bordetella Pertussis
Bacto FA Bordetella Parapertussis

Intended Use

Bacto FA Bordetella Pertussis and Bacto FA Bordetella Parapertussis are used for identifying *Bordetella pertussis* and *Bordetella parapertussis* by the direct fluorescent antibody technique.

User Quality Control

Identity Specifications

FA Bordetella Pertussis, FA Bordetella Parapertussis

Lyophilized appearance: Yellow button to powdered cake.

Rehydrated appearance: Yellow, clear solution.

Performance Response

Rehydrate FA Bordetella and FA Bordetella Parapertussis per label directions. Perform the fluorescent antibody staining procedure using appropriate known *Bordetella pertussis* and *Bordetella parapertussis* cultures as homologous and heterologous controls. The positive control should produce a 4+ reaction using the working dilution of the conjugate. The negative control should not exceed a 1+ reaction using the working dilution of the conjugate.

Summary and Explanation

All members of the genus *Bordetella* are respiratory pathogens of warm-blooded animals. *B. pertussis* and *B. parapertussis* are two uniquely human species. These organisms adhere to, multiply among and remain localized in the ciliated epithelial cells of the respiratory tract. *B. pertussis* is the major cause of whooping cough or pertussis. *Bordetella parapertussis* is associated with a milder, less frequently occurring form of the disease.[1] Person-to-person transmission occurs by the aerosol route.

Pertussis is a highly contagious disease that attacks unimmunized populations in more than 90% of cases.[2] Toxin production remains the major distinction of *B. pertussis*.

Classic pertussis caused by *B. pertussis* occurs in three stages. The first or catarrhal stage is characterized by nonspecific symptoms similar to a cold or viral infection. The disease is highly communicable during this stage, which lasts 1-2 weeks. In the second or paroxysmal stage, the cough increases in intensity and frequency. This stage is marked by sudden attacks of severe, repetitive coughing, often cumulating with the characteristic whoop that is caused by a rapid inspiration of air after the clearance of mucus-blocked airways.[3] This stage may last 1-4 weeks. The beginning of the convalescent stage is marked by a reduction in the frequency and severity of coughing spells. Complete recovery may require weeks or months.

Despite the availability of an effective whole-cell vaccine, pertussis remains a disease of worldwide distribution because many developing nations do not have the resources for vaccinating their populations.[4] Major outbreaks have occurred even in developed nations such as Great Britain and Sweden. Pertussis is endemic in the United States, with most disease occurring as isolated cases. A shift in the age group affected by the disease has occurred. In the past, children in the 1-5 year age group were more prone to pertussis. Children less than one year of age[2] have become more susceptible to the disease because of a decrease in passively transferred maternal antibodies, since adults do not receive booster vaccinations.

Bordetella are tiny gram-negative coccobacilli occurring singly or in pairs and they may exhibit a bipolar appearance. They are strict aerobes and some members are motile. *B. pertussis* and *B. parapertussis* are nonmotile and produce no acid from carbohydrates. *B. pertussis* will not grow on common blood agar bases or chocolate agar, whereas *B. parapertussis* will grow on blood agar and sometimes chocolate agar. Media for primary isolation must include starch, charcoal, ion-exchange resins or a high percentage of blood to inactivate inhibitory substances.[3] *B. pertussis* may be recovered from secretions collected from the posterior nasopharynx, bronchoalveolar lavage and transbronchial specimens.

The direct fluorescent antibody test (DFA) has long been used for the rapid, direct detection of *B. pertussis* and *B. parapertussis* in nasopharyngeal specimens with varying degrees of success.[5,6,7] Eldering, Eveland and Kendrick[8,9] and Holwerda and Eldering[10] showed the usefulness of the FA procedure, although a complete correlation between the agglutination method and FA technique was not obtained. Nonetheless, the FA procedure could detect both smooth and rough cultures of *B. pertussis* and *B. parapertussis* and could also be applied to direct specimens. Further data showed little or no cross reactions between conjugates prepared from *B. pertussis* and *B. parapertussis* cultures. The disadvantage of this procedure is that technical skill and experience are required for the technician to perform and read the test. The DFA should always be used with, not as a replacement for, culture.[11,12] It has been suggested that laboratories proficient in DFA and culture for pertussis should obtain a DFA sensitivity of 60% or greater and a specificity of at least 90% over time compared with culture.[12]

B. pertussis and *B. parapertussis* are slow growing organisms, developing in 3-4 days.

By employing the fluorescent antibody technique, the time required to detect these organisms can be significantly reduced. The FA procedure may be applied to direct nasopharyngeal smears or may be used to identify young cultures of *B. pertussis* or *B. parapertussis*.

Principles of The Procedure

The direct FA technique involves preparation of a smear from the clinical specimen on a glass slide. Nasopharyngeal swabs are obtained from the patient and inoculated into 0.5 ml of Casamino Acids solution.[12] Smears are made from this solution and stained with a specific antibody labeled with a fluorescent marker (fluorescein isothiocyanate or FITC) directed against *B. pertussis* or *B. parapertussis*. After incubation with the antibody preparation, smears are washed in phosphate-buffered saline, air dried, cover slipped with fluorescent-antibody mounting fluid, and examined under a fluorescent microscope.

Reagents

FA Bordetella Pertussis and **FA Bordetella Parapertussis** are lyophilized, polyclonal, fluorescein-conjugated chicken antisera. They have been prepared according to modifications of the methods of Eldering, Eveland and Kendrick[8,9] and Holwerda and Eldering.[1] Approximately 0.02% Thimerosal is added as a preservative.

Precautions

1. For In Vitro Diagnostic Use.
2. **FA Bordetella Pertussis**
   FA Bordetella Parapertussis
   The Packaging of This Product Contains Dry Natural Rubber.
3. Observe universal blood and body fluid precautions in the handling and disposing of specimens.[13,14]
4. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized FA Bordetella Pertussis and FA Bordetella Parapertussis at 2-8°C.

Aliquots of the titered conjugate may be prepared in small vials, frozen in the undiluted state and stored below -20°C for optimal stability. The conjugate should not be exposed to repeated freezing and thawing.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

FA Bordetella Pertussis
FA Bordetella Parapertussis

Materials Required But Not Provided

FA Buffer, Dried
FA Mounting Fluid pH 7.2
1% Casamino Acids
Staining Tray
Fluorescent microscope assembly:

Lamps:	HBO-50, HBO-100, HBO-200 or Xenon XBO-150; 6X 5A Tungsten
Excitation wavelength:	365 nm
Ocular:	10X
Objective:	10X, 40X (Fluorite)
Filters:	BG-12 or KP490, K515 or K530
Condenser:	Dark-field D1.20-1.40

Microscope slides
95% ethanol
Staining jar
Cover slips
McFarland Barium Sulfate Standard #3

Reagent Preparation

Equilibrate all materials to room temperature before performing the test. Ensure that all glassware and pipettes are clean and free of detergent residues.

FA Bordetella Pertussis and FA Bordetella Parapertussis: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

The working dilution of the conjugate should be determined shortly after rehydration. The titer of a conjugate varies with the technique used, the fluorescent microscope and filter used, and the age of the bulb.

The conjugate should be titrated using a known culture of *B. pertussis* or *B. parapertussis* homologous to the conjugate. (Dilutions of the conjugate are made in FA Buffer.) The titer is determined as follows:

DILUTION OF CONJUGATE	FLUORESCENCE
1:5	4+
1:10	4+
1:20	4+
1:40	4+
1:80	2+

In this example, the last 4+ fluorescence is found in a 1:40 dilution of the conjugate. One less dilution is chosen for a margin of safety. The working dilution in this case is, therefore, 1:20.

Specimen Collection and Preparation

Direct Nasopharyngeal Smears

1. Obtain a nasopharyngeal swab and emulsify it in 0.5 ml of sterile 1% Casamino Acids.[12]
2. Hold the specimen in Casamino Acids solution for no more than 2 hours.
3. Smear the emulsified specimen on a clean microscope slide.
4. Allow the smear to air dry and fix it by gentle heating or by a 1 minute immersion in 95% ethanol.

Culture Isolates

1. Isolation of *Bordetella* from clinical specimens requires the use of certain media such as Bordet-Gengou Agar. Colonies of *B. pertussis* on Bordet-Gengou Agar or Charcoal Agar are very small, white, opaque, convex and entire. For specific recommendations, consult appropriate references.[3,12] Determine that a pure culture of the microorganism has been obtained and that biochemical test reactions are consistent with the identification of the organism as a *Bordetella* species. After these criteria are met, serological identification can be performed.
2. Pick appropriate colonies and emulsify in approximately 2 ml sterile distilled or deionized water. Adjust cell density to approximate a McFarland Barium Sulfate Standard #3.
3. Smear the emulsified specimen on a clean microscope slide.
4. Allow the smear to air dry and fix it by a 1 minute immersion in 95% ethanol. Remove slides and allow them to air dry.

Control Slides

Prepare positive and negative control slides using appropriate homologous antigens, following the procedure listed under Culture Isolates.

Test Procedure

1. Add several drops (one drop equals ~35 µl) of the appropriate FA Bordetella conjugate to the fixed smear.
2. Spread the conjugate over the surface of the smear.
3. Place the slide in a Staining Tray or moisture chamber.
4. Incubate at room temperature for 30 minutes.
5. Remove excess conjugate and place the slide in a staining jar containing FA Buffer for 10 minutes with 2 changes of the buffer followed by 1 rinse in distilled water for 2 minutes.
6. Remove the slide; allow to drain and air dry or blot with bibulous paper.
7. Add a small drop (~35 µl) of FA Mounting Fluid pH 7.2 to the center of the stained area and mount with a cover slip.
8. Examine each smear using a fluorescent microscope with an excitation wavelength of 365 nm under a 40X or 100X objective. Record the absence or presence and degree of fluorescence.

Results

1. Read and record results based on the intensity of fluorescence, as follows:
   - 4+ Maximum fluorescence; brilliant yellow-green peripheral staining.
   - 3+ Bright yellow-green peripheral staining.
   - 2+ Definite, but dull, yellow-green peripheral staining.
   - 1+ Barely visible peripheral staining.
   - – Complete absence of yellow-green peripheral fluorescence.
2. **Positive control**: Should show a 4+ reaction using the working dilution of the conjugate.
   Negative control: Should not exceed a 1+ reaction using the working dilution of the conjugate.
   Test smears: A 2+ fluorescence should be considered a positive result.
3. If the positive control is less than 3+, or if the negative control exceeds 1+, the conjugate may have deteriorated or the pH of the FA Buffer or FA Mounting Fluid may have changed. Repeat the test with new reagents.

Limitations of the Procedure

1. At the Routine Test Dilution (RTD) of both FA Bordetella Pertussis and FA Bordetella Parapertussis, the reaction should be brilliant and specific with smooth strains of homologous cultures. Usually, there are no cross reactions with the heterologous strains at the RTD. With some heterologous strains, a 1+ reaction may occur. Such a minimal reaction should not interfere with the interpretation of the test results.

   Some strains of *Bordetella bronchiseptica*, on the other hand, may cross react with both conjugates in varying degrees, giving 1+ to 4+ reactions with a small population of the cells in the smear. The majority of the cell population, however, should not stain at more than a 2+ intensity.
2. Some experience is required to grade the intensity of the fluorescence and to ignore the occasional nonspecific staining of gram-negative diplococci, gram-positive cocci and diphtheroid-like rods.[12]
3. When testing cultural isolates, the density of the positive control should be adjusted to give 4+ fluorescence with the homologous conjugate. The density of culture isolates should be comparable to the positive control in order to standardize fluorescence.

4. All glassware employed in the preparation, testing and storage of these reagents must be free of detergents or other harmful residues.
5. The fluorescent antibody technique can provide only presumptive identification of *B. pertussis* or *B. parapertussis*. A negative result should not be considered conclusive as this type of reaction may occur when only a few organisms are present in the specimen. Final identification can be made only after consideration of cultural, morphological and serological characteristics.

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Packaging

FA Bordetella Pertussis	5 ml	2359-56
FA Bordetella Parapertussis	5 ml	2378-56

Bacto® FA Product Accessories and Reagents

FA Buffer, Dried · FA Mounting Fluid pH 7.2 · FA Mounting Fluid pH 9 · Staining Tray

Intended Use

Bacto FA Buffer, Dried is used in fluorescent antibody (FA) staining procedures.

Bacto FA Mounting Fluid pH 7.2 is used in FA procedures to mount specimens on slides at pH 7.2.

Bacto FA Mounting Fluid pH 9 is used in FA procedures to mount specimens on slides at pH 9.

The Staining Tray is used in FA staining procedures.

Summary and Explanation

FA Buffer, Dried is a phosphate buffer-NaCl mixture which, upon rehydration, yields a 0.85% NaCl solution buffered to pH 7.2. It is used for making dilutions of rehydrated FA Globulins for test purposes, as well as for washing slides in FA staining procedures. It is also recommended as a general purpose phosphate buffered saline.

FA Mounting Fluids are buffered glycerine preparations used in fluorescent antibody procedures as a semipermanent mounting medium for specimens on slides. With mounting media, the cover slip should be pressed down firmly to reduce hazy images. Add a very small drop of mounting fluid and avoid forming bubbles.

Mounting media may be adjusted to any pH compatible with the fluorescence of the fluorochrome to be used. For FITC procedures, the pH should not be lower than 7.0 because fluorescence decreases rapidly below this pH. FITC preparations may be mounted at pH of 9.0 to increase the intensity of fluorescence;[1] however, nonspecific staining is also increased.[2] The pH of a mounting fluid decreases with

time because of oxidation of the glycerol and absorption of CO_2 by the mounting fluid.[3]

The Staining Tray provides a moist, dark incubation chamber for FA conjugated slides.

Principles of the Procedure

Slides are stained using fluorescent antibody procedures. After removal of excess conjugate or serum, a drop of the appropriate FA Mounting Fluid pH 7.2 or FA Mounting Fluid pH 9 is added. A cover slip is applied, taking care not to form bubbles when the cover slip is added.

Reagents

FA Buffer, Dried is a phosphate buffer-NaCl mixture which, upon rehydration, yields a 0.85% NaCl solution buffered to pH 7.2.

FA Mounting Fluid pH 7.2 and FA Mounting Fluid pH 9 are standardized, reagent grade glycerin adjusted to pH 7.2 and 9.0, respectively.

Precautions

1. For In Vitro Diagnostic Use.
2. **FA Buffer, Dried**

   MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

User Quality Control

Identity Specifications

FA Buffer, Dried

Dehydrated Appearance:	White, free flowing, homogeneous.
Solution:	solution, soluble in distilled or deionized water. Solution is colorless, clear.
Reaction of 1% Solution at 25°C:	pH 7.2 ± 0.05

FA Mounting Fluid pH 7.2

Appearance:	Colorless, clear, free from lint.
pH at 25°C:	7.2 ± 0.1

FA Mounting Fluid pH 9

Appearance:	Colorless, clear, free from lint.
pH at 25°C:	9.0 ± 0.1

Performance Response

Perform the fluorescent antibody procedure using an appropriately titrated conjugate. Rinse off excess conjugate. Add a cover slip with an appropriate FA Mounting Fluid. Read smears with a fluorescent microscope. The FA Mounting Fluid pH 7.2 or FA Mounting Fluid pH 9 must not show "quenching" of fluorescence and must show 4+ fluorescence for all slides tested with the homologous antigen.

Storage

Store dehydrated FA Buffer, Dried below 30°C. Upon rehydration, store FA Buffer, Dried at 2-8°C.

Store FA Mounting Fluid pH 7.2 and FA Mounting Fluid pH 9 at 15-30°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

FA Buffer, Dried

Refer to appropriate procedures for FA Bordetella, Fluorescent Treponemal Antibody Testing (FTA-ABS), FA Streptococcus Group A or FA Rhodamine Counterstain.

FA Mounting Fluid pH 7.2

Refer to appropriate procedures for FA Bordetella or FTA-ABS.

FA Mounting Fluid pH 9

Refer to appropriate procedures for FA Streptococcus Group A or FA Rhodamine Counterstain.

Limitations of the Procedure

1. An acid pH will cause a marked decrease in fluorescence.
2. Fresh mounting fluid should be used. The fluid should be discarded if any color or turbidity appears.[2]

References

1. **Pital, A., and S. L. Janowitz.** 1963. Enhancement of staining intensity in the fluorescent-antibody reaction. J. Bacteriol. **86:**888-889.
2. **Cherry, W. B.** 1974. Immunofluorescence techniques, p. 29-44. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D.C.
3. **Anhalt, J. P.** 1981. Fluorescent antibody procedures and counterimmuno-electrophoresis, p. 249-277. *In* J. Washington (ed.), Laboratory procedures in clinical microbiology. Springer-Verlag Inc, New York, NY.

Packaging

FA Buffer, Dried	6x10 ml	2314-33
	100 g	2314-15
	10 kg	2314-08
FA Mounting Fluid pH 7.2	6x5 ml	2329-57
FA Mounting Fluid pH 9	6x5 ml	3340-57
Staining Tray	1 tray	5251-31

Bacto® FA Streptococcus Group A

Intended Use

Bacto FA Streptococcus Group A is used for identifying Group A Streptococcus by the direct fluorescent antibody technique.

Summary and Explanation

Streptococcus pyogenes (Group A streptococcus) is the most common cause of bacterial pharyngitis in children. Symptoms include fever, pharyngeal erythema and edema, tonsillar exudate and enlarged cervical lymph nodes. Physical findings alone cannot distinguish between Group A streptococcal pharyngitis and pharyngitis caused by other agents such as viruses or mycoplasma. Other infections caused by Group A streptococci include scarlet fever, impetigo and skin infections that range from mild to severe with toxic shock symptoms and tissue necrosis.

Streptococci are facultatively anaerobic gram-positive cocci. They are catalase negative and may be alpha, beta or non-hemolytic. Lancefield divided the streptococci into serological groups according to the group-specific somatic carbohydrate they possessed.[1,2,3] The Lancefield groups have quite different clinical significance. There may be biochemical and hemolytic differences within the same serological group.

Moody, Ellis and Updyke[4] showed that group-specific conjugates could be prepared from the antiserum used in the Lancefield precipitin test. This led to the development of the direct fluorescent antibody technique for the identification of *Streptococcus* groups. The Group A conjugate, prepared according to the method of Moody, Ellis and Updyke[4] and Moody, Siegel, Pittman and Winter[5] , is used for the detection and identification of group A *Streptococcus*.[6-8]

Principles of the Procedure

The direct FA technique involves the preparation of a smear from the clinical specimen on a glass slide. Smears are ethanol-fixed and stained with a specific antibody labeled with a fluorescent marker (fluorescein isothiocyanate or FITC) directed against Group A *Streptococcus*. The antigen-antibody reaction is then observed microscopically, using a suitable wave length of light compatible with the fluorescent marker employed.

User Quality Control

Identity Specifications

FA Streptococcus Group A

Lyophilized Appearance: Yellow button to powdered cake.

Rehydrated Appearance: Yellow, clear solution.

Performance Response

Rehydrate FA Streptococcus Group A per label directions. Perform the fluorescent antibody staining procedure using a known culture of Group A *Streptococcus* as the homologous control. The positive control should produce a 4+ reaction in the 1:20 or greater dilution of the conjugate with the homologous antigen.

Reagents

FA Streptococcus Group A conjugate has been cross-absorbed to remove cross reactivity known to exist with serogroups C and G. Normally occurring *Staphylococcus* agglutinins have been blocked by unconjugated normal rabbit serum or by an unconjugated *Staphylococcus* immune serum. Approximately 0.02% Thimerosal is used as a preservative.

The working dilution of the conjugate should be determined upon rehydration. The titer of a conjugate varies with the technique, the fluorescent microscope, the filter and the age of the bulb. The working dilution may vary from laboratory to laboratory. Dilutions of the conjugate are made in rehydrated FA Buffer. The titer is determined as follows:

DILUTION OF CONJUGATE	FLUORESCENCE
1:5	4+*
1:10	4+
1:20	4+
1:40	4+
1:80	2+

*4+ fluorescence is defined as brilliant yellow-green cocci with sharp cell outlines and nonstaining centers.

In this example, the last 4+ fluorescence is found in a 1:40 dilution of the conjugate. Use one dilution lower for a margin of safety. The working dilution, therefore, in this case is 1:20.

Precautions

1. For In Vitro Diagnostic Use.
2. The Packaging of This Product Contains Dry Natural Rubber.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.

Storage

Store lyophilized FA Streptococcus Group A at 2-8°C.

Aliquots of the titered conjugate should be prepared in small vials, frozen in the undiluted state and stored below -20°C for optimal stability. Prepare only a sufficient amount of diluted conjugate for each day's use.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

FA Streptococcus Group A

Materials Required but not Provided

FA Buffer, Dried

FA Mounting Fluid pH 9

Staining Tray

Fluorescent microscope assembly:

Lamps:	HBO-50, HBO-100, HBO-200 or Xenon XBO-150; 6X 5A Tungsten
Excitation Wavelength:	365 nm
Ocular:	10X
Objective:	10X, 40X (Fluorite)
Filters:	BG-12 or KP490, K515 or K530
Condenser:	Dark-field D1.20-1.40

95% Ethanol

McFarland Barium Sulfate Standard #3

Glass slides

Cover slips

Sterile swabs

Culture tubes, 12 x 75 mm

Coplin jars

Serological pipettes, 1 ml and 5 ml

Todd Hewitt Broth

Incubator, 35 ± 2°C

Reagent Preparation

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergent.

FA Streptococcus Group A: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

Specimen Collection and Preparation

Specimens submitted to the laboratory for the detection of streptococci must be obtained under proper medical guidance. Suitable specimens are those collected from the nose, nasopharyngeal area and throat, skin, wounds, pus, blood, cerebrospinal fluid and urine. When collecting a throat specimen, it is imperative that the sample is collected properly to obtain an adequate amount of material. Improperly obtained specimens will yield cultures that contain minimal numbers of streptococci. For specific information on specimen collection and preparation, consult appropriate references.[9,10]

Note: Specimens containing streptococci survive well at 4EC on tightly capped blood agar slants but survive poorly in a broth medium.

1. Place a swab having a throat or other specimen, excluding urine, into a tube containing 1 ml Todd Hewitt Broth and incubate at 35 ± 2°C for 2-5 hours. For urine samples, proceed to step #3.
2. Drain the swab against the side of the tube and place it into another sterile tube for storage in a refrigerator, if desired.
3. Centrifuge tubes containing known and unknown cultures for 10 minutes at 1,500-2,000 rpm to sediment cells.
4. Pour off supernatant liquid (autoclave before discarding) and resuspend the cells in 2 ml distilled or deionized water. Adjust density to approximately a McFarland Barium Sulfate Standard #3.
5. Prepare duplicate smears of the same culture on prepared microscope slides.
6. Allow the smear to air dry.
7. Fix the smear by placing the slide in 95% ethanol for 1 minute.
8. Remove the slide and allow to air dry.

Note: Smears may also be prepared from specimens grown on blood agar and suspected of being *Streptococcus* because of their growth characteristics. The isolation medium recommended for this species is any one of several blood agar bases containing 5% sterile, defibrinated sheep blood. Hemolytic reactions should be determined from a pure culture before serological examination. Sheep blood plates are recommended because they exhibit clear-cut reactions for streptococci. For antigen preparation, Todd Hewitt Broth is recommended.

Test Procedure

1. Add several drops of a predetermined working dilution of FA Streptococcus Group A conjugate to the smear on one end of a microscope slide. Distribute it evenly over the entire smear with an applicator stick so as not to disturb the smear.
2. Place the slide in a Staining Tray or moist chamber. Incubate at room temperature for 30 minutes.
3. Drain off excess conjugate and place in a Coplin jar containing FA Buffer solution. Let stand for 10 minutes with 2 changes of buffer and a final rinse of distilled water.
4. Remove the slide; allow it to drain and air dry.
5. Add one small drop of FA Mounting Fluid pH 9 to the slide and mount with a glass cover slip.
6. Examine using a fluorescent microscope with an excitation wavelength of 365 nm under a 40X or 100X objective.
7. Read and record the amount of fluorescence.

Results

1. Read and record results as follows:
   - 4+ Maximum fluorescence; brilliant yellow-green; clear-cut cell outline; sharply defined cell center.
   - 3+ 75% fluorescence; less brilliant yellow-green; clear-cut cell outline; sharply defined cell center.
   - 2+ 50% fluorescence; definite but dim; cell outline less well defined.
   - 1+ 25% agglutination; background is cloudy.
   - – Negligible or complete lack of fluorescence (negative).
2. A 4+ fluorescence in the unknown smear with the homologous conjugate is evidence that the unknown organism is homologous to Streptococcus Group A conjugate.

Limitations of the Procedure

1. Some experience is required to grade the intensity of the fluorescence.
2. Discard the conjugate if contaminated.
3. The conjugate should not be subjected to repeated freezing and thawing. Such treatment is detrimental to the antibody content.
4. All glassware employed in the preparation, testing and storage of these reagents must be free of detergents or other harmful residues.
5. FA Buffer showing turbidity or mold growth should be discarded.

References

1. **Lancefield, R. C.** 1933. A serological differentiation of human and other groups of haemolytic streptococci. J. Exp. Med. **57:**571-595.
2. **Lancefield, R. C.** 1928. The antigenic complex of *Streptococcus haemolyticus*. I. Demonstration of a type of specific substance in extracts *of Streptococcus haemolyticus*. J. Exp. Med. **47:**91-103.

3. **Lancefield, R. C.** 1938. A micro precipitin-technique for classifying hemolytic streptococci, and improved methods for producing antisera. Proc. Soc. Exp. Biol. Med. **38:**473-478.
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9. **Miller, J. M., and H. T. Holmes.** 1995. Specimen collection, transport, and storage, p. 19-32. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
10. **Isenberg, H. D. (ed.)** 1992. Clinical microbiology procedures handbook, vol. 2. American Society for Microbiology, Washington, D. C.

Packaging

FA Streptococcus Group A	5 ml	2318-56
FA Buffer, Dried	6 x 10 ml	2314-33
	100 g	2314-15
	10 kg	2314-08
FA Mounting Fluid pH 9	6 x 5 ml	3340-57
Staining Tray	1 tray	5251-31

Bacto® FTA-ABS Test Reagents

FTA Serum Reactive · FTA Antigen · FTA Serum Non-Reactive FTA Sorbent · FTA Sorbent Control · FA Human Globulin Antiglobulin (Rabbit) · Tween® 80 · FA Buffer, Dried FA Mounting Fluid pH 7.2

Intended Use

The FTA-ABS Test (Fluorescent Treponemal Antibody Absorption) is an indirect immunofluorescent procedure for detecting human antibody against *Treponema pallidum*, the causative agent of syphilis. The test uses the following reagents: FTA Antigen, FTA Serum Reactive, FTA Serum Non-Reactive, FTA Sorbent, FTA Sorbent Control, FA Human Globulin Antiglobulin (Rabbit), Tween® 80, FA Buffer, Dried and FA Mounting Fluid pH 7.2.

The persistent reactivity of the FTA-ABS Test to a treated case of syphilis, sometimes for life, minimizes its use for following the response to therapy as well as making it unreliable for detecting new untreated cases in epidemiological investigations.

Bacto FTA Reagents are not FDA cleared (approved) for use in testing (i.e., screening) blood or plasma donors.[1,2]

Summary and Explanation

Treponema pallidum is the causative agent of syphilis, a chronic infection with many clinical manifestations. These manifestations occur in distinct stages and detection of each stage requires different laboratory tests.

During the primary stage, treponemes present in the characteristic lesion, a chancre, are detectable by dark-field microscopy[1] or by the Direct Fluorescent Antibody Test for *Treponema pallidum* (DFA-TP).

During the secondary stage, most serology tests for syphilis are reactive and treponemes may be found in the lesions by using dark-field microscopy. The latent period, which is asymptomatic, may last for years. Serological tests are usually reactive in the early latent period but reactivity in non-treponemal tests decreases during the late latent period. Symptoms of the tertiary or late stage of syphilis may occur 10-20 years after initial infection. Approximately 71% of patients in the tertiary stage of syphilis have reactive non-treponemal tests.[2,3] In the tertiary stage, treponemal tests will usually be reactive and are the only basis for diagnosis. The lesions in tertiary syphilis will have few treponemes. Neurosyphilis is a complication of tertiary syphilis.

Since the clinical manifestations of syphilis can be confused with other infectious diseases or with noninfectious conditions that cause skin lesions, proper diagnosis must be based on microscopic examination of lesion material and serological test results.[2]

The FTA test was introduced in 1957 by Deacon, Falcone and Harris.[4] Certain difficulties were encountered with respect to sensitivity versus specificity. In its original form using a 1:5 dilution of patient serum, the test yielded many false-positive reactions. There seemed to be a cross reaction of the treponemal antigen with antibodies to group antigens that are common to all treponemes. The titer of sera containing the nonspecific group antibodies ranged from 1:5 to 1:100.

In 1960, Deacon, Freeman and Harris[5] introduced a modified procedure, the FTA-200 test, which used a 1:200 dilution of patient serum. By increasing the dilution of the serum, nonspecific antibodies were

diluted beyond their titer and could no longer interfere with the test. However, testing a highly diluted serum decreased the sensitivity of the test. Low antibody titer, which occurs during primary syphilis, was not detected.

Deacon and Hunter[6] showed that appropriate absorption could eliminate or block the reactivity of nonspecific antibodies. This absorption produced the FTA-ABS test, an improved test using a 1:5 serum dilution.[7]

The FTA-ABS test is a standard diagnostic test for syphilis as defined by the Centers for Disease Control and Prevention (CDC). Other standard treponemal tests include Fluorescent Treponemal Antibody-Absorption Double Staining Test (FTA-ABS DS) and the Micro Hemagglutination Assay for Antibodies to *Treponema pallidum* (MHA-TP).

Treponemal antigen tests, such as the FTA-ABS test, are used as confirmatory tests in diagnostic problem cases, such as with patients for whom the clinical, historical or epidemiological evidence of syphilis disagrees with nontreponemal tests. The FTA-ABS test is more sensitive than the VDRL test in primary, late latent and tertiary syphilis. However, the persistent reactivity of the FTA-ABS test to a treated case of syphilis, sometimes for life, minimizes its use for following response to therapy. Therefore, the FTA-ABS test is also unreliable in detecting new untreated cases in epidemiological investigations. The test should not be used as a routine screening procedure.[3,8]

The likelihood of obtaining a reactive FTA-ABS test result in various stages of untreated syphilis has been reported as follows:[2]

STAGE OF UNTREATED SYPHILIS	% REACTIVE
Primary	84
Secondary	100
Latent	100
Tertiary (Late)	96

User Quality Control

Identity Specifications

FTA Antigen
Lyophilized Appearance: White button to powdered cake.
Rehydrated Appearance: White to off-white, slightly opalescent liquid.

FTA Serum Reactive
Lyophilized Appearance: Off-white to light amber, button to powdered cake.
Rehydrated Appearance: Light gold to slightly amber liquid.

FTA Serum Non-Reactive
Lyophilized Appearance: Off-white to light amber, button to powdered cake.
Rehydrated Appearance: Light gold to slightly amber liquid.

FTA Sorbent
Lyophilized Appearance: Light amber to dark brown, button to powdered cake.
Rehydrated Appearance: Gold to brown liquid.

FTA Sorbent Control:
Lyophilized Appearance: Off-white to light amber, button to powdered cake.
Rehydrated Appearance: Light gold to slightly amber liquid.

FTA Human Globulin Antiglobulin (Rabbit)
Lyophilized Appearance: Light yellow to yellow-orange, button to powdered cake.
Rehydrated Appearance: Yellow-green to yellow-orange liquid.

Control Pattern

Rehydrate and dilute reagents per directions (see Reagent Preparation). Test as described. Tests failing to exhibit the following control results are unsatisfactory and should not be reported.[8,13]

SERUM TESTED	EXPECTED FLUORESCENCE	INTERPRETATION
Reactive Control Serum - Unabsorbed	4+	Reactive
Reactive Control Serum - Absorbed	3+ to 4+	Reactive
Minimally Reactive Control Serum	1+	Reactive
Nonreactive Control Serum	N	Nonreactive
Nonspecific Serum Control - Unabsorbed	2+ to 4+	Reactive
Nonspecific Serum Control - Absorbed	N to ±	Nonreactive
Nonspecific Staining Control - Unabsorbed	N	Nonreactive
Nonspecific Staining Control - Absorbed	N	Nonreactive

Principles of the Procedure

Patient serum is diluted 1:5 in sorbent and layered on a microscope slide fixed with *T. pallidum*. If the patient's serum contains antibodies, these antibodies will coat the treponemes on the slide. Fluorescein-labeled anti-human immunoglobulin is added. It combines with the patient antibodies already adhering to the *T. pallidum* and produces fluorescein-stained spirochetes that can be observed with a fluorescent microscope.[7,9]

Reagents

FTA Antigen (also known as *T. pallidum* antigen) is a lyophilized, standardized, killed suspension of *Treponema pallidum* (Nichols strain).

FTA Serum Reactive is lyophilized, standardized syphilitic human sera containing 0.02% Thimerosal as a preservative. It is used to make Reactive Control Serum (4+) - Unabsorbed, Reactive Control Serum (4+) - Absorbed, and Minimally Reactive Control Serum (1+). It is used as a positive control in the FTA-ABS test.

FTA Serum Non-Reactive is lyophilized, standardized, non-syphilitic human sera containing 0.02% Thimerosal as a preservative. It is used to make Nonreactive Control Serum (N). It is used as a negative control in the FTA-ABS test.

FTA Sorbent is a lyophilized, standardized extract of the nonpathogenic Reiters treponeme (*T. phagedenis*) prepared from broth culture. It is used to remove antibodies against nonpathogenic treponemes during preparation of the test specimen, Reactive Control Serum (4+) - Absorbed and Nonspecific Staining Control - Absorbed.

FTA Sorbent Control is lyophilized, standardized, non-syphilitic human sera containing 0.02% Thimerosal as a preservative. It is used to make Nonspecific Control Serum - Unabsorbed, which demonstrates at least 2+ nonspecific reactivity at a 1:5 dilution in FA Buffer, and Nonspecific Control Serum - Absorbed, which demonstrates essentially no reactivity at a 1:5 dilution in FTA Sorbent.

FA Human Globulin Antiglobulin (Rabbit) is lyophilized, fluorescein-conjugated (FITC) antihuman globulin containing 0.02% Thimerosal as a preservative. It is used to show the presence of human syphilitic antibodies on the treponemal antigen.

Tween® 80 is Polysorbate 80, U.S.P. It is used to prepare 2% Tween 80, which acts as a dispersing agent.

FA Buffer, Dried is phosphate buffered saline (PBS) which, upon rehydration, yields a 0.85% NaCl solution buffered to pH 7.2. FA Buffer is used in preparing Reactive Control Serum (4+) - Unabsorbed, Minimally Reactive Control Serum (1+), Nonreactive Control Serum (N) and Nonspecific Staining Control - Unabsorbed.

FA Mounting Fluid pH 7.2 is standardized, reagent grade glycerin adjusted to pH 7.2 for use in mounting specimens on slides to be viewed under the fluorescent microscope.

Precautions

1. For In Vitro Diagnostic Use.
2. **FTA Serum Reactive**
   FTA Serum Non-Reactive
   FTA Sorbent Control
   WARNING! POTENTIAL BIOHAZARDOUS REAGENTS. Each donor unit used in the preparation of these reagents was tested by an FDA-approved method for the presence of the antibody to human immunodeficiency virus (HIV) as well as for hepatitis B surface antigen and found to be negative (were not repeatedly reactive).

   Because no test method can offer complete assurance that HIV, hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen.[10,11]
3. **FTA Antigen**
   FTA Serum Reactive
   FTA Serum Non-Reactive
   FTA Sorbent
   FTA Sorbent Control
   FA Human Globulin Antiglobulin (Rabbit)
   The Packaging of This Product Contains Dry Natural Rubber.
4. **FA Buffer, Dried**
   MAY BE IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN. (US) Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
5. Observe universal blood and body fluid precautions in handling and disposing of specimens.
6. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store unopened products as specified below:

FTA Antigen	2-8°C
FTA Serum Reactive	2-8°C
FTA Serum Non-Reactive	2-8°C
FTA Sorbent	2-8°C
FTA Sorbent Control	2-8°C
FA Human Globulin Antiglobulin (Rabbit)	2-8°C in the dark
Tween® 80	15-30°C
FA Buffer, Dried	Below 30°C
FA Mounting Fluid pH 7.2	15-30°C

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Rehydrated FTA Antigen stored at 2-8°C is stable for 1 week.

Rehydrated FA Buffer showing turbidity or mold growth should be discarded.

Discard 2% Tween 80 that exhibits a precipitate or pH change.

Procedure

Materials Provided

FTA Antigen
FTA Serum Reactive
FTA Serum Non-Reactive
FTA Sorbent
FTA Sorbent Control
FA Human Globulin Antiglobulin (Rabbit)
Tween® 80
FA Buffer, Dried
FA Mounting Fluid, pH 7.2

Materials Required But Not Provided

Timer
Serological pipettes, 0.2 ml, 5 ml, 1 ml
Micropipettors delivering 10-200 µl
Test tubes, 12 x 75 mm
Water bath (56°C)
Vortex mixer
Platinum loop, 2 mm, 26 gauge
Slides, plain or frosted, 1 x 3 inch, 1 mm thick, inscribed with 2 x 1 cm circles
Staining dish with removable slide carriers
Slide board or holder
Moisture chamber
Acetone
Bibulous paper
Distilled water
Incubator, 35-37°C
Oil, Immersion
Cover slips, No. 1, 22 mm square
Fluorescent microscope assembly:

Lamps:	HBO-50, HBO-100, HBO-200 or Xenon XBO-150; 6X 5A Tungsten
Ocular:	10X
Objective:	10X, 40X (Fluorite)
Filters:	BG-12 or KP490, K515 or K530
Condenser:	Dark-field D1.20-1.40

Reagent Preparation

FTA Antigen: Rehydrate with 1 ml distilled or deionized water and rotate to completely dissolve the contents. This solution will yield

approximately 3.5 x 10^7 treponemes per ml. Mix thoroughly with a disposable pipette and rubber bulb, drawing the suspension into and expelling it from the pipette 8-10 times to break treponemal clumps and ensure an even distribution of treponemes. Confirm the even distribution by dark-field examination. Use FTA Antigen in its entirety to prepare antigen smears on the day it is rehydrated. Approximately 200-300 slides may be prepared with 1 ml of antigen.

To prepare FTA Antigen smears:

1. Wipe inscribed slides with clean gauze and, if necessary, alcohol to remove dust particles.
2. Using a platinum wire loop (2 mm, 26 gauge), smear 1 loopful of reconstituted FTA Antigen within the 2 circles. Air dry at room temperature for at least 15 minutes.
3. Immerse the dry slide into acetone for 10 minutes to fix the treponemal antigen smear to the slide; air dry. Fix no more than 50 slides per 200 ml of acetone.
4. Use slides immediately or store at or below -20°C after acetone fixation. Thaw before use; do not refreeze. Use within 1 year, but only if satisfactory results are obtained with test controls.

FTA Serum Reactive: Rehydrate with 5 ml distilled or deionized water and rotate gently to completely dissolve the contents. Aliquot in 0.4 ml amounts and store at or below -20°C. Do not refreeze thawed aliquot. Approximately 12 tests may be obtained per 5 ml vial. This serum should be heated at 56°C for 30 minutes before use.

FTA Serum Non-Reactive: Rehydrate with 5 ml distilled or deionized water and rotate gently to completely dissolve the contents. Aliquot in 0.4 ml amounts and store at or below -20°C. Approximately 90-100 tests may be obtained per 5 ml vial. This serum should be heated at 56°C for 30 minutes before use.

FTA Sorbent: Rehydrate with 5 ml distilled or deionized water and rotate gently to completely dissolve the contents. Store at 2-8°C or aliquot and store at -20°C. The quantity of FTA Sorbent used for each test sample or serum is 0.2 ml. The quantity of FTA Sorbent needed for 3 controls is 0.6 ml. Approximately 20-25 tests may be performed with 5 ml of FTA Sorbent.

FTA Sorbent Control: Rehydrate with 0.5 ml distilled or deionized water and rotate gently to completely dissolve the contents. Aliquot in 0.25 ml amounts and store at or below -20°C. For each test, 0.1 ml of FTA Sorbent Control is needed. Approximately 2 tests may be performed per 0.5 ml vial because of evaporation from heating. This serum should be heated at 56°C for 30 minutes before using.

FA Human Globulin Antiglobulin (Rabbit): Rehydrate with 1 ml or 5 ml distilled or deionized water, depending on label directions. Aliquot in 0.5 ml amounts and store at or below -20°C. Each lot is supplied with a dilution titer. Since conditions and equipment differ from one laboratory to another, it is necessary to titer and test a new lot of conjugate with the fluorescent microscope assembly currently in use.[3,8,13]

1. Prepare serial dilutions in 2% Tween 80, including the titer specified on the vial.
2. Test each dilution per the Test Procedure with Reactive Control Serum (4+) and Nonspecific Staining Control.
3. Test a known lot of reagent using the Reactive Control Serum (4+), Minimally Reactive Control Serum (1+) and Nonspecific Staining Control as controls of the reagents and test conditions.
4. During further testing, use the dilution that produces 1 doubling dilution lower than the 4+ endpoint. The 4+ endpoint is the highest dilution of conjugate yielding 4+ fluorescence with the Reactive Control Serum (4+).

FA Buffer, Dried: Dissolve 10 grams in 1 liter of distilled or deionized water and rotate gently to completely dissolve the contents. Store at 2-8°C. Use the solution if it is free of mold growth and turbidity.

Tween® 80: Heat the bottle of Tween 80 and a flask containing 98 ml FA Buffer to 56°C in a water bath. Add 2 ml of Tween 80 to the buffer and rinse the pipette thoroughly in the buffer. Check the pH and adjust to pH 7.2 with 1N NaOH. Discard if a precipitate develops or the pH changes.

Specimen Collection and Preparation

Test serum: Collect patient (test) serum according to recommended procedures.[2,3,8,9,13] Store specimens at room temperature for up to 4 hours or at 2-8°C for up to 5 days; serum specimens may be frozen at or below -20°C.

Test and control sera: Equilibrate the sera to room temperature, then heat at 56°C for 30 minutes. Reheat previously heated sera for 10 minutes on the day of testing. Cool to room temperature before testing. Bacterial contamination or excessive hemolysis may render a specimen unsuitable for testing. Such specimens should not be tested.

Test Procedure

This procedure conforms with those published by the U. S. Department of Health, Education and Welfare[14] and with subsequent procedures published by the American Public Health Association.[9,13]

1. **FTA Antigen smears**: Obtain previously prepared smears, thaw and dry if appropriate, and identify the frosted end of the slides to correspond with each test and control serum to be tested.
2. Prepare the following test and control sera in appropriately identified tubes no more than 30 minutes before testing and mix thoroughly (at least 8 times):

   Test Serum (1:5): Dilute 0.05 ml (50 µl) of heated (or reheated) test serum in 0.2 ml (200 µl) FTA Sorbent.

   Reactive Control Serum (4+) - Unabsorbed: Dilute 0.05 ml (50 µl) FTA Serum Reactive in 0.2 ml (200 µl) FA Buffer (PBS).

   Reactive Control Serum (4+) - Absorbed: Dilute 0.05 ml (50 µl) FTA Serum Reactive in 0.2 ml (200 µl) FTA Sorbent.

   Minimally Reactive Control Serum (1+): Dilute FTA Serum Reactive, as indicated on the label, in FA Buffer (PBS) to yield a 1+ fluorescence. The minimal degree of fluorescence that can be reported as reactive is 1+ fluorescence.

   Nonreactive Control Serum (N) (1:40): Prepare a 1:40 dilution of FTA Serum Non-Reactive by adding 0.05 ml (50 µl) of serum to 1.95 ml FA Buffer (PBS).

   Nonspecific Serum Control - Unabsorbed (2+ nonspecif ic reactivity): Dilute 0.05 ml (50 µl) FTA Sorbent Control in 0.2 ml (200 µl) FA Buffer (PBS).

   Nonspecific Serum Control - Absorbed (nonreactive, - to ±): Dilute 0.05 ml (50 µl) FTA Sorbent Control in 0.2 ml (200 µl) FTA Sorbent.

   Nonspecific Staining Control - Unabsorbed: Use 0.03 µl (30 ml) FA Buffer (PBS) undiluted.

   Nonspecific Staining Control - Absorbed: Use 0.03 ml (30 µl) FTA Sorbent undiluted.

3. **FTA Antigen smears**: Cover the previously identified FTA Antigen smears with 0.03 ml (30 µl) of the corresponding test or control serum prepared above, making certain that the entire smear is covered.
4. Place the slides in a moist chamber to prevent evaporation and incubate at 35-37°C for 30 minutes.
5. Place the slides in a slide carrier and rinse as follows:
   - Rinse in running FA Buffer for 5 seconds.
   - Soak in FA Buffer for 5 minutes.
   - Agitate by dipping in and out of the buffer 30 times.
   - Repeat the soaking and agitation in fresh buffer.
   - Rinse in running distilled water for 5 seconds.
   - Gently blot dry with bibulous paper.
6. **FA Human Globulin Antiglobulin (Rabbit)**: Dilute the antiglobulin to its working titer (determined above) using 2% Tween 80 in FA Buffer.
7. **FTA Antigen smears**: Cover each test and control smear with approximately 0.03 ml (30 µl) of diluted FA Human Globulin Antiglobulin (Rabbit). Spread uniformly to cover the entire smear.
8. Repeat steps 4 and 5.
9. Mount the slides immediately using a small drop of FA Mounting Fluid pH 7.2 and apply a cover slip, being careful not to trap air bubbles in the mounting fluid.
10. Immediately examine the slides microscopically for intensity of fluorescence using the microscope assembly described above. If it is necessary to delay reading, store the slides in the dark and read within 4 hours. Results are valid only if the quality control pattern is satisfactory.
11. Verify the presence of treponemes on the nonreactive control slides by dark-field microscopy.

Results

Using the 1+ serum control as a reading standard, record the intensity of fluorescence of the treponemes and report as follows. Retest all specimens with an initial test fluorescence of 1+. When a specimen initially read as 1+ yields a retest reading of 1+ or greater, it is reported as reactive. All other results are reported as nonreactive. Retesting nonreactive specimens is not necessary.

Without historical or clinical evidence of treponemal infection, equivocal test results (see below) suggest the need for testing a second specimen obtained 1-2 weeks after the initial specimen.

INTENSITY OF FLUORESCENCE	INITIAL TEST RESULT	RETEST RESULT	REPORT
Moderate to strong	2+ to 4+	NA	Reactive
Equivalent to 1+ control	1+	>1+	Reactive
	1+	1+	Reactive minimal*
	1+	<1+	Nonreactive
Visible staining but <1+	± to <1+	NA	Nonreactive
None or vaguely visible, not distinct	–	NA	Nonreactive
“Moth eaten” or “beaded”			Atypical

*Equivocal result.

Limitations of the Procedure

1. When the treponemal test results and the clinical opinion disagree, repeat the treponemal test and obtain additional clinical and historical information. If the disagreement persists, send the specimen to a reference laboratory such as the local state health department for additional confirmatory tests. The final diagnosis depends on the clinical judgment of a specialist very experienced in sexually transmitted diseases.[2,3]
2. The test should not be used to follow the response to therapy nor can it be relied on to detect new, untreated cases in epidemiological investigations.
3. “Atypical” fluorescence and false-positive results have been associated with patients having active systemic, discoid and drug-induced varieties of lupus erythematosus[13-17] and other autoimmune diseases.
4. Elderly patients may exhibit unexplained FTA-ABS reactions.
5. At times, deciding whether a reading is weak or vaguely visible may be difficult. The ability to make this distinction is critical, since a nonreactive (vaguely visible to none) serum is not retested.

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Packaging

FA Buffer, Dried	6 x 10 g	2314-33
	100 g	2314-15
FA Human Globulin Antiglobulin (Rabbit)	1 ml	2449-50
	5 ml	2449-56
FA Mounting Fluid pH 7.2	6 x 5 ml	2329-57
FTA Antigen	1 ml	2344-50
FTA Serum Non-Reactive	5 ml	2440-56
FTA Serum Reactive	5 ml	2439-56
FTA Sorbent	5 ml	3259-56
FTA Sorbent Control	6 x 0.5 ml	3266-49
Tween® 80	6 x 5 ml	3118-57

FTA-ABS Test Procedure

Abbreviated Schematic

STEP 1	STEP 2	STEP 3	STEP 4	STEP 5
Prepare sera and reagents.	Dilute sera.	Add test and control sera to appropriate FTA Antigen smears.	Add conjugate to the FTA Antigen smears.	Record reactions of test and control sera. Verify that control sera provided the expected results.

FTA Antigen	FTA Antigen Smear
Rehydrate with 1 ml distilled or deionized water. Prepare smears. Fix with acetone. Use as "FTA Antigen smear".	Thaw, dry and identify sufficient FTA Antigen smears to correspond with each of the test and control sera to be tested.

Test (Patient) Serum				
Heat at 56°C for 30 minutes or reheat previously heated serum for 10 minutes.	Dilute 1:5 by adding 0.05 ml serum to 0.2 ml in FTA Sorbent.	Apply 0.03 ml test serum to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	Dependent on antibody status of test serum.

FTA Serum Reactive	Reactive Control Serum (4+) – Unabsorbed			
Rehydrate with 5 ml distilled or deionized water. Heat at 56°C for 30 minutes.	Dilute 1:5 by adding 0.05 ml FTA Serum Reactive to 0.2 ml FA Buffer.	Apply 0.03 ml Reactive Control Serum – Unabsorbed to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	4+ Reactive
	Reactive Control Serum (4+) – Absorbed			
	Dilute 1:5 by adding 0.05 ml FTA Serum Reactive to 0.2 ml FTA Sorbent.	Apply 0.03 ml Reactive Control Serum – Absorbed to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	3+ to 4+ Reactive
	Minimally Reactive Control Serum (1+)			
	Dilute FTA Serum Reactive to 1+ fluorescence (labeled titer) in FA Buffer.	Apply 0.03 ml Minimally Reactive Control Serum to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	1+ Reactive

FTA Serum Nonreactive	Nonreactive Control Serum			
Rehydrate with 5 ml distilled or deionized water. Heat at 56°C for 30 minutes.	Dilute 1:40 by adding 0.05 ml FTA Serum Nonreactive to 1.95 ml FA Buffer.	Apply 0.03 ml Nonreactive Control Serum to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	N Nonreactive

FTA Sorbent Control	Nonspecific Serum Control – Unabsorbed			
Rehydrate with 0.5 ml distilled or deionized water. Heat at 56°C for 30 minutes.	Dilute 1:5 by adding 0.05 ml FTA Sorbent Control to 0.2 ml FA Buffer.	Apply 0.03 ml Nonspecific Serum Control - Unabsorbed to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	2+ to 4+ Reactive
	Nonspecific Serum Control – Absorbed			
	Dilute 1:5 by adding 0.05 ml FTA Sorbent Control to 0.2 ml FA Buffer.	Apply 0.03 ml Nonspecific Serum Control - Unabsorbed to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	N to ± Nonreactive

FA Buffer, Dried	Nonspecific Staining Control – Unabsorbed			
Dissolve 10 grams in 1 liter distilled or deionized water.	Use 0.03 ml FA Buffer 0.05 ml FTA Serum Nonreactive to 1.95 ml FA Buffer.	Apply 0.03 ml Nonspecific Staining Control - Unabsorbed to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	N Nonreactive

FTA Sorbent	Nonspecific Staining Control – Absorbed			
Rehydrate with 5 ml distilled or deionized water.	Use 0.03 ml FTA Sorbent undiluted as the diluent (above) and as the Nonspecific Staining Control - Absorbed	Apply 0.03 ml Nonspecific Staining Control -Absorbed to an FTA Antigen smear. Incubate smear. Rinse.	Apply 0.03 ml conjugate to the smear. Incubate, rinse and mount slide. Examine microscopically.	N Nonreactive

FA Human Globulin Antiglobulin (Rabbit)	
Rehydrate with 1 ml or 5 ml distilled or deionized water. Determine titer if a new lot.	Dilute to labeled titer with 2% Tween. Use as “Conjugate”.

Tween® 80
Heat Tween 80 and FA Buffer to 56°C. Add 2 ml Tween 80 to 98 ml FA Buffer. Adjust to pH 7.2.

Bacto® Febrile Antigen Set

Contains: Brucella Abortus Antigen (Slide) · Proteus OX19 Antigen (Slide) · Salmonella O Antigen Group D · Salmonella H Antigen a Salmonella H Antigen b · Salmonella H Antigen d · Febrile Positive Control Polyvalent · Febrile Negative Control

Intended Use

Bacto Febrile Antigen Set is used in the detection of febrile antibodies by the slide and tube agglutination tests.

Summary and Explanation

Agglutination tests have been used in diagnosing certain febrile illnesses since the early 1900's. Patients experiencing "febrile" symptoms, including fever, chills, malaise and fatigue, were considered likely to have typhoid fever, brucellosis, rickettsial infection (either typhus or spotted fever) or tularemia. The agents of these infections are difficult or unlikely to be isolated by routine laboratory methods but do cause detectable increases in antibody levels in the patient's serum.

User Quality Control

Identity Specifications

Brucella Abortus Antigen (Slide), Proteus OX19 Antigen (Slide), Salmonella O Antigen Group D, Salmonella H Antigen a, Salmonella H Antigen b, Salmonella H Antigen d

Appearance: Turquoise-blue-violet suspension.

Febrile Positive Control Polyvalent

Lyophilized appearance: Light gold to amber, button to powdered cake.

Rehydrated appearance: Light gold to amber, clear liquid.

Febrile Negative Control

Lyophilized appearance: Colorless to light gold, button to powdered cake.

Rehydrated appearance: Colorless to light gold, clear liquid.

Performance Response

Rehydrate Febrile Positive Control Polyvalent and Febrile Negative Control per label directions. Perform the slide or tube agglutination test using an appropriate Febrile Antigen and positive and negative controls diluted in the same proportion as a patient serum.

A Febrile Antigen is considered satisfactory if it does not agglutinate with the negative control and shows 2+ or greater agglutination with the positive control at the following dilution:

Brucella Abortus Antigen	1:80
Proteus OX19 Antigen	1:160
Salmonella O Antigen Group D	1:80
Salmonella H Antigen a	1:80
Salmonella H Antigen b	1:80
Salmonella H Antigen d	1:80

"Febrile Antigen" is a term generally referring to bacterial suspensions representative of many species of microorganisms pathogenic to man which are accompanied by a fever in the host. A battery of febrile antigens evolved as the "febrile antigen" or "febrile agglutinin" test. The febrile antigen test is based on the Widal test (*Salmonella* somatic O and flagellar H antigens), the Weil-Felix test (antigens of selected *Proteus* strains), and the *Brucella abortus* antigen test.[1,2,3] In some situations, the *Francisella tularensis* antigen test is included in the battery.

DISEASE	ASSOCIATED FEBRILE ANTIGEN
Brucellosis	Brucella abortus
Rocky Mountain spotted fever	Proteus OX19
Typhus	Proteus OX19
Typhoid fever	Salmonella O Antigen Group D
Typhoid fever	Salmonella H Antigen d
Paratyphoid fever	Salmonella H Antigen a
Paratyphoid fever	Salmonella H Antigen b

In 1896, Widal introduced techniques for testing patients' serum for antibodies in cases of typhoid fever.[1] The Widal test was used diagnostically in two ways. First, it was considered diagnostic when a single high titer of antibodies occurred during the first week of illness. In addition, it was diagnostic if a greater than fourfold titer rise existed in serum samples taken 1 to 2 weeks apart.[2,4,5,6] The Widal test was developed to include *Salmonella typhi* and other species of *Salmonella* detected by a variety of O and H antigens. *S. typhi* and *S. paratyphi* A and B are the major pathogens in this group that can produce clinically distinct systemic illness. The Widal test for antibodies to the O antigens of *Salmonella* serotypes most likely to cause typhoid fever (usually *S. typhi* and *S. paratyphi* A and B) can be useful in diagnosing typhoid fever when other methods have failed.[7]

The Weil-Felix test became popular in the 1920's after it was observed that certain strains of *Proteus* would agglutinate early convalescent-phase sera from patients with suspected rickettsial disease.[3] *Proteus* antigens (OX2, OX19 and OXK) will cross-react in predictable patterns, although the reactions are not highly sensitive or specific.

Diagnosis of the cause of febrile disease cannot be based solely on the analysis of serum samples for antibody response. Many factors may affect measurable antibody levels. For example, the patient's immune response can be affected by age, immune status, general state of health and previous immunizations.

Certain organisms may share cross-reacting antigens leading to the production of heterologous antibodies. These heterologous antibodies may react with one or more antigens in an antibody test procedure resulting in low-level antibody titers that may not, when used alone, suggest disease. Cross reactions can occur among species of *Francisella* and *Brucella*, among various species of *Salmonella*, and

between *Brucella* species and *Yersinia enterocolitica* or *Vibrio cholerae*. Antibodies produced in response to a *Proteus* infection can react with *Proteus* OX19 and be misinterpreted as rickettsial antibodies.

The rapid slide test is the most widely used procedure employing febrile antigens because of the simplicity with which the results may be reported. Negative slide test reactions can usually be reported as such if all five serum dilutions have been used. Although the slide test is not quantitative, running the series of dilutions is necessary to detect agglutinin content of a serum that might be overlooked for a "prozone phenomenon" where higher concentrations of the serum may yield negative results, but a dilution of the serum is positive. This often occurs in sera containing *Brucella* agglutinins and, to a lesser extent, typhoid agglutinins.

The macroscopic tube test[2] should be used to confirm the presence of antibodies demonstrated by the slide technique and to quantitate their titer in suspect sera. When quantitative determinations of *Rickettsia* or *Brucella* agglutinins are necessary, tube antigens are used.

Principles of The Procedure

Agglutination tests involving the use of febrile antigens determine the presence of antibodies that react with the test antigen. The serological procedure involves serially diluting the patient serum, then adding a standard volume of antigen. The end point of the test is the last dilution of the serum that shows a specific amount of agglutination. The end point converted to a dilution of the serum is called the patient's antibody "titer."

Reagents

Antigens

1. Febrile Antigens are ready-to-use, whole cell suspensions of the organisms listed below. Proteus OX19 Antigen (Slide) contains 20% glycerin.
   Brucella Abortus Antigen (Slide) - *Brucella abortus*
   Proteus OX19 Antigen (Slide) - *Proteus vulgaris* OX19
   Salmonella O Antigen Group D - *Salmonella typhi* O901
   Salmonella H Antigen a - *Salmonella paratyphi* A
   Salmonella H Antigen b - *Salmonella paratyphi* B
   Salmonella H Antigen d - *Salmonella typhi* H901
2. **Slide test**: The Febrile Antigens (Brucella Abortus Antigen (Slide), Proteus OX19 Antigen (Slide), Salmonella O Antigen Group D, Salmonella H Antigen a, Salmonella H Antigen b and *Salmonella typhi* H901) are used in the slide test and contain sufficient reagent for 20 slide tests.

   Tube test: Salmonella O and H Antigens may also be used in the tube test and contain sufficient reagent for 25 tube tests.

   Brucella Abortus Antigen (Slide) and Proteus OX19 Antigen (Slide) are used *only* in the slide test. When confirmation of the slide test and quantitation are required, Brucella Abortus Antigen (Tube) and Proteus OX19 (Tube) may be purchased as separate products.
3. **Antigen Density**: Salmonella O and H Antigens are adjusted to a density approximating 20 times a McFarland Barium Sulfate Standard No. 3 (1.8 x 10^{10} organisms per ml). These antigens are used undiluted for the slide test and diluted 1:20 for the tube test.

   Because antigen density may vary, it is adjusted for optimum performance when standardized with hyperimmune sera obtained from laboratory animals.

   Variation in antigen color intensity is normal and will not affect test performance.
4. Febrile Antigens contain the following preservatives:

   Brucella Abortus Antigen (Slide): 0.5% phenol, and approximately 0.002% crystal violet and 0.005% brilliant green.

   Proteus OX19 Antigen (Slide): 0.25% formaldehyde, and approximately 0.002% crystal and 0.005% brilliant green.

   Salmonella O Antigen Group D: 0.5% phenol, and approximately 0.002% crystal violet and 0.005% brilliant green.

   Salmonella H Antigens a: 0.5% formaldehyde, and approximately 0.002% crystal violet and 0.005% brilliant green.

   Salmonella H Antigens b: 0.5% formaldehyde, and approximately 0.002% crystal violet and 0.005% brilliant green.

   Salmonella H Antigens d: 0.5% formaldehyde, and approximately 0.002% crystal violet and 0.005% brilliant green.

Antisera

1. **Febrile Positive Control Polyvalent** is lyophilized, polyclonal, polyvalent goat antisera containing approximately 0.04% Thimerosal as a preservative. It contains antibodies for all of the components of the Febrile Antigen Set. Each vial contains sufficient reagent for 32 slide tests or 50 tube tests using single antigens or for approximately 5 slide tests when using all of the antigens in the set.
2. **Febrile Negative Control** is a lyophilized, standard protein solution containing approximately 0.02% Thimerosal as a preservative. Each vial contains sufficient reagent for 32 slide tests using single antigens or for approximately 5 slide tests using all of the antigens in the set.

Precautions

1. For In Vitro Diagnostic Use.
2. Observe universal blood and body fluid precautions in the handling and disposing of specimens.[8,9]
3. **Proteus OX19 Antigen (Slide)**
   Salmonella H Antigen a
   Salmonella H Antigen b
   Salmonella H Antigen d
   POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Eyes, Kidneys, Lungs, Skin.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
4. Follow proper established laboratory procedure in handling and disposing of infectious materials.
5. Febrile Antigens are not intended for use in the immunization of humans or animals.

Storage

Store Febrile Antigens at 2-8°C.

Store lyophilized and rehydrated Febrile Positive Control Polyvalent at 2-8°C.

Store lyophilized and rehydrated Febrile Negative Control at 2-8°C.

Expiration Date

The expiration date applies to a product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Febrile Antigen Set:

Brucella Abortus Antigen (Slide)
Proteus OX19 Antigen (Slide)
Salmonella O Antigen Group D
Salmonella H Antigen a
Salmonella H Antigen b
Salmonella H Antigen d
Febrile Positive Control Polyvalent
Febrile Negative Control

Materials Required But Not Provided

Slide Test

Agglutination slides, 5 squares, 1" each
Applicator sticks
Sterile deionized water or equivalent
Serological pipettes, 0.2 ml

Tube Test

Culture tubes 12 x 75 mm and rack
Waterbath, 35-37°C and 50 ± 2°C
Refrigerator, 2-8°C
Serological pipettes, 1 ml and 5 ml
Sterile 0.85% NaCl solution

Reagent Preparation

Febrile Antigens are ready to use.

Febrile Positive Control Polyvalent: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

Febrile Negative Control: To rehydrate, add 5 ml sterile deionized water, or equivalent, and rotate gently to completely dissolve the contents.

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergent.

Specimen Collection and Preparation

Collect a blood specimen by aseptic venipuncture. Serum is required for the test. Store serum specimens at room temperature for no longer than 4 hours; for prolonged storage, keep at 2-8°C for up to 5 days or maintain at or below -20°C. Serum specimens must be clear, free of hemolysis and show no visible evidence of bacterial contamination (turbidity, hemolysis or particulate matter). Refer to appropriate references for more information on collection of specimens.[10,11] Serum specimens must not be heated. Heat may inactivate or destroy certain antibodies.

An increase in titer over a period of time is the best indicator of active infection. The accuracy and precision of the tests can be affected not only by test conditions, but also by the subjectivity of the person reading the endpoint.

A preliminary test using either the rapid slide test and/or the macroscopic tube test may be performed on the initial serum specimen and reported to the physician at that time. An aliquot of the serum should be transferred to a sterile test tube, sealed tightly, and kept in the freezer. When the second serum is obtained, it should be run in parallel with the original specimen. In this manner, the original serum will serve as a control and any difference in titer will be more credible, since the bias associated with the performance of the test and determining the endpoint will be reduced.

Test Procedure

Slide Test

Use the slide test only as a screening test; confirm positive results with the tube test. Test each Febrile Antigen separately, repeating steps 1-6 for each Antigen.

1. **Test Serum**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of each test serum into a row of squares on the agglutination slide.
2. **Positive control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Febrile Positive Control Polyvalent into a row of squares on the agglutination slide.
3. **Negative control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Febrile Negative Control into a row of squares on the agglutination slide.
4. **Febrile Antigen**: Gently shake the vial of antigen to ensure a smooth, uniform suspension. Place one drop (35 µl) of antigen suspension in each drop of test serum, positive control and negative control.
5. Mix each row of test and control serum, using a separate applicator stick for each row. Start with the most dilute mixture (0.005 ml) and work to the most concentrated (0.08 ml).
6. Rotate the slide for 1 minute and read for agglutination.
7. The final dilutions in squares 1-5 correspond with tube dilutions of 1:20, 1:40, 1:80, 1:160, 1:320, respectively.

Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. **Positive control**: Should show 2+ or greater agglutination at the following dilutions:

Brucella Abortus Antigen	1:80
Proteus OX19 Antigen	1:160
Salmonella O Antigen Group D	1:80
Salmonella H Antigen a	1:80
Salmonella H Antigen b	1:80
Salmonella H Antigen d	1:80

3. **Negative control**: Should show no agglutination.
4. **Test specimens**: The serum titer is that dilution which shows 2+ or greater agglutination. See Table 1.

Table 1. Sample Rapid Slide Test reactions.

SERUM (ml)	CORRELATED TUBE DILUTION	REACTIONS		
		SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
0.08	1:20	3+	4+	4+
0.04	1:40	2+	4+	3+
0.02	1:80	1+	3+	2+
0.01	1:160	–	3+	+
0.005	1:320	–	1+	–
Serum titer		**1:40**	**1:160**	**1:80**

Tube Test

Salmonella O Antigen Group D and Salmonella H Antigens a, b and d in the Febrile Antigen Set are used for both slide and tube agglutination tests. Brucella Abortus Antigen (Slide) and Proteus OX19 Antigen (Slide) are intended only for slide tests. When confirmation of the slide test and quantitation is required, separate tube test antigens, Brucella Abortus Antigen (Tube) and Proteus OX19 (Tube), may be purchased separately.

Each Febrile Antigen must be tested separately. Repeat steps 1-10 for each antigen.

Prepare a 1:20 dilution of each antigen to be tested by adding 1 part of antigen to 19 parts of sterile NaCl solution.

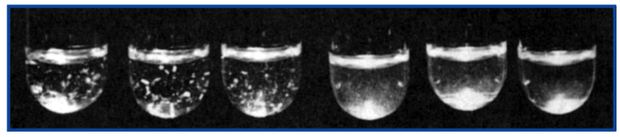
1. Prepare a row of 8 culture tubes (12 x 75 ml) for each test serum, including a row for the Febrile Positive Control Polyvalent.
2. **0.85% NaCl solution**: Dispense 0.9 ml in the first tube of each row and 0.5 ml in the remaining tubes.
3. **Test serum**: Using a 1 ml serological pipette, dispense 0.1 ml of test serum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
4. **Positive control**: Using a 1 ml serological pipette, dispense 0.1 ml of Febrile Positive Control Polyvalent in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
5. **Febrile Antigen**: Add 0.5 ml of the diluted antigen suspension to all 8 tubes in each row and shake the rack to mix.
6. The final dilutions in tubes 1-7 are 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280, respectively.
7. Incubate as specified (eg., in a waterbath or refrigerator):

Brucella Abortus Antigen:	35-37°C for 48 ± 3 hours.
Proteus OX19 Antigen:	35-37°C for 2 hours, then at 2-8°C for 22 ± 2 hours.
Salmonella O Antigen Group D:	50 ± 2°C for 17 ± 1 hours.
Salmonella H Antigens a:	50 ± 2°C for 1 hour.
Salmonella H Antigens b:	50 ± 2°C for 1 hour.
Salmonella H Antigens d:	50 ± 2°C for 1 hour.

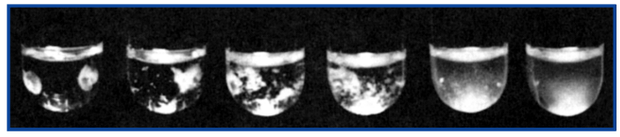
8. Remove from incubation. Avoid excessive shaking before reading the reactions, either when the tubes are incubating or when removing them from incubation.
9. Read and record results.

Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. Salmonella Antigens used in tube agglutination procedures detect antibodies to either O (somatic) antigens or H (flagellar) antigens and these antibodies give different reactions. An O antigen and the corresponding antibody give a coarse, compact agglutination that may be difficult to disperse. An H antigen and its corresponding antibody give a loose flocculent agglutination. Do not vigorously shake tubes containing H antigens. Characteristic O and H agglutination is illustrated below.



Somatic "O" Agglutination



Flagellar "H" Agglutination

3. **Positive control**: Should show a 2+ or greater agglutination at the following dilutions:

Brucella Abortus Antigen	1:80
Proteus OX19 Antigen	1:160
Salmonella O Antigen Group D	1:80
Salmonella H Antigens a, b and d	1:80

4. **Antigen control**: Tube 8 of each row should show no agglutination.
5. **Test serum**: The serum titer is that dilution which shows a 2+ or greater agglutination. See Table 2.

Table 2. Sample Macroscopic Tube Test reactions.

SERUM DILUTION	REACTIONS		
	SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
1:20	4+	3+	4+
1:40	4+	2+	4+
1:80	3+	1+	4+
1:160	2+	–	4+
1:320	1+	–	3+
1:640	–	–	2+
1:1280	–	–	1+
Serum titer	**1:160**	**1:40**	**1:640**

Interpretation

1. Compare results:

DISEASE	ASSOCIATED FEBRILE ANTIGEN	SIGNIFICANT TITER
Brucellosis	Brucella Abortus	1:160
Rocky Mountain spotted fever*	Proteus OX19	1:160
Typhus*	Proteus OX19	1:160
Typhoid fever	Salmonella O Antigen Group D**	1:80
Typhoid fever	Salmonella H Antigen d**	1:80
Paratyphoid fever	Salmonella H Antigen a**	1:80
Paratyphoid fever	Salmonella H Antigen b**	1:80

* Rocky Mountain spotted fever cannot be differentiated from typhus by this test.
** Antibodies produced in response to other *Salmonella* species can cross-react.

2. **Single serum specimen**: A significant titer suggests infection.
3. **Pair of serum specimens (acute and convalescent)**: A two-dilution increase in titer is significant and suggests infection. A one-dilution difference is within the limits of laboratory error.
4. **Positive control and antigen control**: If results are not as described, the test is invalid and results cannot be reported.

Limitations of the Procedure

1. The slide test is intended for screening only and should be confirmed by the tube test. Slide test dilutions are made to detect a prozone reaction and do not represent true quantitation of the antibody. A serum specimen with a prozone reaction shows no agglutination because of excessively high antibody concentrations. To avoid this occurrence, all 5 serum dilutions in the slide test should be run.
2. Detection of antibodies in serum specimens may complete the clinical picture of a patient having an infection. However, isolation of the causative agent from patient specimens may be required. A definitive diagnosis must be made by a physician based on patient history, physical examination and data from all laboratory tests.
3. Cross-reacting heterologous antibodies are responsible for many low-titer reactions. Infections with other organisms, vaccinations and history of disease may result in a low level of antibody titer. Antimicrobial therapy may suppress antibody production.

   Cross reactions between antigens and antibodies of *B. abortus* and *F. tularensis*, *Y. enterocolitica* or *V. cholerae* can occur.

   Rocky Mountain spotted fever and typhus cannot be differentiated by this test because species of *Rickettsia* cause cross-reacting antibodies.

   Infections with *Proteus* species can cause cross-reacting antibodies.

   Cross-reactions between antigens and antibodies of various *Salmonella* species can occur.

   Previous immunizations with typhoid vaccine or previous infection with *Salmonella* species sharing common antigens with *S. typhi* can cause elevated antibody titers for prolonged periods. Other non-typhoid febrile illnesses may cause elevation of cross-reacting antibodies.
4. While a single serum specimen showing a significant titer suggests infection, it is not diagnostic.
5. To test for a significant rise in antibody titer, at least two specimens are necessary: an acute specimen, obtained at the time of initial symptoms, and a convalescent specimen, obtained 7 to 14 days later. A two-dilution difference in the titers is a significant increase in antibody level and suggests infection.
6. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
7. Exposure to temperatures below 2°C can cause autoagglutination. Antigens must be smooth, uniform suspensions; before use, examine antigen vials for agglutination. Suspensions with agglutination are not usable and should be discarded.
8. Discard rehydrated Febrile Positive Control Polyvalent or Febrile Negative Control that is cloudy or has a precipitate anytime during its period of use.

References

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Packaging

Febrile Antigen Set	8 x 5 ml	2407-32
Contains:		
Brucella Abortus Antigen (Slide)		
Proteus OX19 Antigen (Slide)		
Salmonella O Antigen Group D		
Salmonella H Antigen a		
Salmonella H Antigen b		
Salmonella H Antigen d		
Febrile Positive Control Polyvalent		
Febrile Negative Control		

Available separately:		
Brucella Abortus Antigen (Slide)	5 ml	2909-56
Brucella Abortus Antigen (Tube)	25 ml	2466-65
Proteus OX19 Antigen (Slide)	5 ml	2234-56
Proteus OX19 Antigen (Tube)	25 ml	2247-65
Salmonella O Antigen Group D	5 ml	2842-56
Salmonella H Antigen a	5 ml	2844-56
Salmonella H Antigen b	5 ml	2845-56
Salmonella H Antigen d	5 ml	2847-56
Febrile Positive Control Polyvalent	5 ml	3238-56
Febrile Negative Control	5 ml	3239-56

Bacto® Francisella Tularensis Antigens and Antisera

Francisella Tularensis Antigen (Slide) · Francisella Tularensis Antigen (Tube) · Francisella Tularensis Antiserum Febrile Negative Control

Intended Use

Bacto Francisella Tularensis Antigens (Slide) and (Tube) are used in the detection of antibodies by the slide and tube agglutination tests.[1,2]

Bacto Francisella Tularensis Antiserum is used to demonstrate a positive quality control test reaction in the slide and tube agglutination tests.

Bacto Febrile Negative Control is used to demonstrate a negative quality control test reaction in the slide agglutination test.

Summary and Explanation

Two species of the genus *Francisella* exist, *Francisella tularensis* and *Francisella novicidia*.[3] The latter species occurs rarely and is not known to infect humans.

F. tularensis is the causative agent of tularemia in humans. The disease was first described in humans in 1907.[1] It is a zoonotic disease transmitted to humans by direct contact with wild animals or bites of insect vectors such as ticks and biting flies. Wild animals such as rabbits, beavers, muskrats, domestic mammals and birds are involved in disease transmission.

The organism directly invades the skin, conjunctiva or mucosa of the oropharynx from blood or tissue of the infected animal. Indirect transmission includes bites of insect vectors, inhalation of contaminated feces or soil, or ingestion of contaminated water or poorly cooked meat.

Patients experience a rapid onset of "febrile" symptoms including malaise, chills, fever and fatigue. Several forms of the infection occur, each with additional characteristic symptoms. *F. tularensis* is a pathogenic microorganism that, upon invasion, produces a fever in its host. Consequently, it is often called a "Febrile Antigen."

For growth on culture media, *F. tularensis* requires both blood and cystine or cysteine. Gram stains of cultural isolates aid in the identification of the organism. The organisms are gram negative, stain faintly, and have extremely small coccoid cells that are often hard to visualize even at 1,000X magnification.[2]

The human immune response to a particular microorganism results in measurable antibody production that can sometimes help in completing the patient's clinical diagnosis. In blood samples, the antibody titer during the initial (acute) phase of the infection is compared to the antibody titer 7-14 days later (convalescent). Antibody titers that are high initially in the acute phase (1:160) or an acute or con valescent pair of samples that shows an increase in antibody titer are helpful in the diagnosis of tularemia.[4,5,6]

Diagnosis of the cause of febrile disease cannot be based solely on the analysis of serum samples for antibody response. Many factors may affect measurable antibody levels. For example, the patient's immune response can be affected by age, immune status, general state of health and previous immunizations.

Certain organisms may share cross-reacting antigens, leading to the production of heterologous antibodies. These heterologous antibodies may react with one or more antigens in a febrile antibody test procedure, producing low-level antibody titers. A titer of less than 1:20 is not considered diagnostic because nonspecific cross-reactions are common at this level.[1] Cross-reactions between *Francisella* and *Brucella* can occur.

Principles of the Procedure

Agglutination tests involving the use of *Francisella* antigens detect the presence of antibodies that react with the test antigen. The serological procedure involves serially diluting the patient serum and then adding a standard volume of antigen. The endpoint of the test is the last dilution of the serum that shows a specific amount of agglutination. The end point, reported as a dilution of the serum, is called the patient's antibody "titer."

Reagents

Francisella Tularensis Antigen (Slide) is a ready-to-use suspension of *Francisella tularensis* containing 20% glycerin, as well as 0.5% phenol, approximately 0.2% crystal violet and approximately 0.5% brilliant green as preservatives. When used as described, each 5 ml vial contains sufficient reagent for 20 slide tests.

Francisella Tularensis Antigen (Tube) is a ready-to-use suspension of *Francisella tularensis* adjusted to a density approximating a McFarland Barium Sulfate Standard No. 3 (9×10^8 organisms per ml). Francisella Tularensis Antigen (Tube) contains 0.5% formalin but does not contain dye. When used as described, each 25 ml vial contains sufficient reagent for 6 tests.

Because antigen density may vary, density is adjusted to ensure optimum performance when the antigen is standardized with hyperimmune sera obtained from laboratory animals. Variation in antigen color intensity is normal and will not affect the outcome of the test.

Francisella Tularensis Antiserum is a lyophilized, polyclonal rabbit antiserum containing approximately 0.04% Thimerosal as a preservative. When rehydrated and used as described, each 3 ml vial contains sufficient reagent for 19 slide tests or 30 tube tests.

Febrile Negative Control is a standard protein solution containing approximately 0.02% Thimerosal as a preservative. When used as described, each 3 ml vial contains sufficient reagent for 32 slide tests.

User Quality Control

Identity Specifications

Francisella Tularensis Antigen (Slide)
Appearance: Blue-violet suspension.

Francisella Tularensis Antigen (Tube)
Appearance: Light gray to white suspension.

Francisella Tularensis Antiserum
Lyophilized Appearance: Light gold to amber, button to powdered cake.
Rehydrated Appearance: Light gold to amber, clear liquid.

Febrile Negative Control
Lyophilized Appearance: Colorless to light gold, button to powdered cake.
Rehydrated Appearance: Colorless to light gold, clear liquid.

Performance Response

Rehydrate Francisella Tularensis Antiserum and Febrile Negative Control per label directions. Perform the Rapid Slide Test using Francisella Tularensis Antigen (Slide) or the Macroscopic Tube Test using Francisella Tularensis Antigen (Tube). Dilute both positive and negative controls in the same proportion as a patient serum and process in the same manner, following appropriate procedure.

An antigen is considered satisfactory if it fails to agglutinate with the negative control and reacts to a titer of 1:160 or more with the positive control.

Precautions

1. For In Vitro Diagnostic Use.
2. **Francisella Tularensis Antigen (Tube)**

   POSSIBLE RISK OF IRREVERSIBLE EFFECTS. Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Target Organs: Eyes, Kidneys, Lungs, Skin.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
3. Observe universal blood and body fluid precautions in the handling and disposing of specimens.[7,8]
4. Biosafety level 2 precautions are recommended when handling specimens suspected of containing *F. tularensis*.[9]
5. Francisella Tularensis Antigens are not intended for use in the immunization of humans or animals.
6. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store Francisella Tularensis Antigens (Slide) and (Tube) at 2-8°C.

Store lyophilized and rehydrated Francisella Tularensis Antiserum at 2-8°C.

Store lyophilized and rehydrated Febrile Negative Control at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Francisella Tularensis Antigen (Slide)
Francisella Tularensis Antigen (Tube)
Francisella Tularensis Antiserum
Febrile Negative Control

Materials Required But Not Provided

Slide Test

Agglutination slides with five 1-inch squares
Applicator sticks
Sterile 0.85% NaCl solution
Serological pipettes, 0.2 ml
Distilled or deionized water

Tube Test

Culture tubes, 12 x 75 mm, and rack
Waterbath, 35-37°C
Serological pipettes, 1 ml and 5 ml
Sterile 0.85% NaCl solution
Distilled or deionized water

Reagent Preparation

Francisella Tularensis Antigen (Slide) and Francisella Tularensis Antigen (Tube) are ready to use.

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of detergent residues.

Francisella Tularensis Antiserum: To rehydrate, add 3 ml sterile 0.85% NaCl solution and rotate gently to dissolve the contents completely. The rehydrated antiserum is considered a 1:2 working dilution.

Febrile Negative Control: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to dissolve the contents completely.

Specimen Collection and Preparation

Collect a blood specimen by aseptic venipuncture. After the specimen has clotted, centrifuge to obtain the serum required for the test. Serum specimens must be clear, free of hemolysis and show no visible evidence of bacterial contamination (turbidity, hemolysis or particulate matter). Consult appropriate references for more information on collection of specimens.[2,10]

Store serum specimens at room temperature for no longer than 4 hours; for prolonged storage, keep at 2-8°C for up to 5 days or maintain below -20°C. Serum specimens must not be heated; heat may inactivate or destroy certain antibodies.

Slide Test

Use the slide test only as a screening test. Confirm positive results with the tube test.

1. **Test serum**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of each test serum into a row of squares on an agglutination slide.
2. **Positive control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Francisella Tularensis Antiserum into a row of squares on the agglutination slide.
3. **Negative control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Febrile Negative Control into a row of squares on the agglutination slide.
4. **Antigen**: Shake the vial of Francisella Tularensis Antigen (Slide) thoroughly to ensure a smooth, uniform suspension. Dispense 1 drop (35 µl) of antigen in each drop of test serum, positive control and negative control.
5. Mix each row of test serum and control serum, using a separate applicator stick for each row. Start with the most dilute mixture (0.005 ml) and work to the most concentrated (0.08 ml).
6. Rotate the slide for 1 minute and read for agglutination.
7. The final dilutions in squares 1-5 correspond approximately to tube dilutions of 1:20, 1:40, 1:80, 1:160 and 1:320, respectively.

Tube Test

1. In a rack, prepare a row of 8 culture tubes (12 x 75 mm) for each test serum and a positive control row for the Francisella Tularensis Antiserum.
2. Dispense 0.9 ml of sterile 0.85% NaCl solution in the first tube of each row and 0.5 ml in the remaining tubes.
3. **Test serum**: Using a 1 ml serological pipette, dispense 0.1 ml of serum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Proceed in like manner for each serum to be tested.
4. **Positive control**: Using a 1 ml serological pipette, dispense 0.1 ml of Francisella Tularensis Antiserum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing.
5. **Antigen control**: Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
6. **Antigen**: Shake the vial of Francisella Tularensis Antigen (Tube) to ensure a smooth, uniform suspension. Add 0.5 ml of antigen to all 8 tubes in each row and shake the rack to mix the suspensions.
7. Final dilutions in tubes 1-7 are 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280, respectively.
8. Incubate in a waterbath at 35-37°C for 22 ± 2 hours.
9. Remove from the waterbath. Avoid excessive shaking before reading the reactions, when the tubes are in the waterbath, or when removing them from the waterbath.

Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. **Positive control**: Should produce 2+ or greater agglutination at a 1:160 dilution.

   Negative control - Rapid Slide Test, only: Should produce no agglutination.

   Antigen control - Macroscopic Tube Test, only: Should produce no agglutination in tube #8 of each row.

   If results for either the positive or negative control are not as specified, the test is invalid and results cannot be reported.

   Test serum: The titer is the highest dilution that shows 2+ agglutination.

   Refer to Table 1 and Table 2[1] for examples of test reactions.
3. The Rapid Slide Test is a screening test, only; results must be confirmed using the Macroscopic Tube Test.

Table 1. Sample Rapid Slide Test reactions.

SERUM (ml)	CORRELATED TUBE DILUTION	REACTIONS		
		SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
0.08	1:20	3+	4+	4+
0.04	1:40	2+	4+	3+
0.02	1:80	1+	3+	2+
0.01	1:160	–	3+	+
0.005	1:320	–	1+	–
Serum titer		**1:40**	**1:160**	**1:80**

Table 2. Sample Macroscopic Tube Test reactions.

SERUM DILUTION	REACTIONS		
	SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
1:20	4+	3+	4+
1:40	4+	2+	4+
1:80	3+	1+	4+
1:160	2+	–	4+
1:320	1+	–	3+
1:640	–	–	2+
1:1280	–	–	1+
Serum titer	**1:160**	**1:40**	**1:640**

Interpretation

For a single serum specimen, a titer of 1:160 at 2+ or greater suggests infection.[1]

A 2-dilution increase in the titer of paired serum specimens (from the acute to the convalescent serum) is significant and suggests infection. A 1-dilution difference is within the limits of laboratory error.

Limitations of the Procedure

1. The slide test is intended for screening only and results should be confirmed by the tube test. Slide test dilutions are made to detect a prozone reaction and do not represent true quantitation of the antibody. A serum specimen with a prozone reaction shows no agglutination because of excessively high antibody concentrations. To avoid this occurrence, all five serum dilutions (slide test) should be run.
2. The detection of antibodies in serum specimens may complete the clinical picture of tularemia. However, isolation of the causative agent from patient specimens may be required. A definitive diagnosis must be made by a physician based on patient history, physical examination and data from all laboratory tests.
3. Cross-reacting heterologous antibodies are responsible for many low titer reactions. Cross-reactions between antigens and antibodies of *Brucella* species and *Francisella tularensis* can occur. Infections with other organisms, vaccinations and a history of disease may cause low antibody titers. Antimicrobial therapy may suppress antibody production.
4. While a single serum specimen showing a titer of 1:160 suggests infection, it is not diagnostic.
5. To test for a significant rise in antibody titer, at least two specimens are necessary, an acute specimen obtained at the time of initial symptoms and a convalescent specimen obtained 7 to 14 days later. A two-dilution increase in titer is significant and suggests infection.
6. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
7. Exposure to temperatures below 2°C can cause antigen autoagglutination. Antigens must be smooth, uniform suspensions. Examine antigen vials for agglutination before use. Agglutinated suspensions are not usable and should be discarded.
8. Adhering to the recommended time and temperature of incubation is important when performing this test. For best results, locate the waterbath in an area free of mechanical vibration.

References

1. **Stewart, S. J.** 1995. *Francisella*, p. 545-548. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
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10. **Miller, J. M., and H. T. Holmes.** 1995. Specimen collection, transport and storage. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.

Packaging

Francisella Tularensis Antigen (Slide)	5 ml	2240-56
Francisella Tularensis Antigen (Tube)	5 ml	2251-56
	25 ml	2251-65
Francisella Tularensis Antiserum	3 ml	2241-47
Febrile Negative Control	3 ml	3239-56

Bacto® Haemophilus Influenzae Antisera

Haemophilus Influenzae Antiserum Poly · Haemophilus Influenzae Antiserum Type a · Haemophilus Influenzae Antiserum Type b · Haemophilus Influenzae Antiserum Type c Haemophilus Influenzae Antiserum Type d · Haemophilus Influenzae Antiserum Type e · Haemophilus Influenzae Antiserum Type f

Intended Use

Bacto Haemophilus Influenzae Antisera are used in slide agglutination tests for serotyping *Haemophilus influenzae*.

Summary and Explanation

H. influenzae was first described by Pfeiffer[1] in 1892 from patients during an influenza pandemic. Pittman[2] described the six capsular serotypes of *H. influenzae* in 1931. He recognized that members of serotype b were most likely to cause invasive infections.

H. influenzae is part of the normal respiratory flora of humans and many animal species. Often, the organism becomes an opportunistic secondary invader, usually following viral influenza. This organism can cause a variety of diseases from chronic respiratory infections to meningitis. Most of the *H. influenzae* isolates associated with meningitis possess the serotype b capsule.[3] Serotype b is believed to cause more than 90% of all *Haemophilus* infections in children less than six years of age. Although the incidence of *H. influenzae* type b infections has been drastically reduced by the introduction of effective vaccines, *Haemophilus* species remain important causes of a wide range of human infections.

User Quality Control

Identity Specifications

Haemophilus Influenzae Antiserum Poly
Haemophilus Influenzae Antiserum Type a
Haemophilus Influenzae Antiserum Type b
Haemophilus Influenzae Antiserum Type c
Haemophilus Influenzae Antiserum Type d
Haemophilus Influenzae Antiserum Type e
Haemophilus Influenzae Antiserum Type f

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated Appearance: Light gold to amber liquid.

Performance Response

Rehydrate Haemophilus Influenzae Antisera per label directions. Test as described (see Test Procedure). Known positive and negative control cultures must give appropriate reactions.

H. influenzae is a nonmotile, facultative anaerobe requiring both factor X (hemin) and factor V (NAD) for in vitro growth. In microscopic morphology, the organism is a pleomorphic gram-negative coccobacillus and sometimes forms threads or filaments.

The presence of a polysaccharide capsule is a major virulence factor for strains of *H. influenzae* that cause systemic infection. *H. influenzae* is divided into serological groups a, b, c, d, e and f based on capsular polysaccharides. Most encapsulated strains that cause infection belong to serotype b.[1] The encapsulated strains are referred to as typeable strains. Nonencapsulated or non-typeable strains may also cause infection. Infections caused by nonencapsulated strains are usually related to the upper respiratory tract.

Antigenic similarities exist between *H. influenzae* and many unrelated bacteria. *H. influenzae* serotype b shares cross-reacting antigens with *Streptococcus pneumoniae* serotypes 6, 15a, 29 and 35a, *Escherichia coli*, and several species of *Staphylococcus*, *Streptococcus and Bacillus.*

The Quellung (swelling) reaction has also been used for recognition of encapsulated (typeable) strains of *H. influenzae*.[1,4] The principle of this antigen-antibody reaction is not agglutination as in the slide technique, but an apparent increase in capsular size due to deposition of antibody on the cell surface. If the Quellung reaction is performed, one must be aware that these organisms are often found in the nonencapsulated state, which are untypable. In addition, capsulated strains of type “e” generally possess small capsules. Such strains should be defined serologically employing the slide agglutination test, only. Consult an appropriate reference for details of the Quellung reaction.[4]

Principles of the Procedure

Identification of *H. influenzae* includes isolation of the microorganism, biochemical identification and serological confirmation.

Serological confirmation involves the reaction in which the microorganism (antigen) reacts with its corresponding antibody. This *in vitro* reaction produces macroscopic clumping called agglutination. The desired homologous reaction is rapid, does not dissociate (high avidity), and binds (high affinity).

Because a microorganism (antigen) may agglutinate with an antibody produced in response to other species, heterologous reactions are possible. These are weak in strength or slow in formation. Such unexpected and, perhaps, unpredictable reactions may lead to some confusion in serological identification. Therefore, a positive homologous

agglutination reaction should support the morphological and biochemical identification of the microorganism.

Homologous reactions are rapid and strong. Heterologous reactions are slow and weak.

Reagents

Haemophilus Influenzae Antisera are lyophilized, polyclonal rabbit antisera containing approximately 0.02% Thimerosal as a preservative.

When rehydrated and used as described, each 1 ml vial of Haemophilus Influenzae Antiserum contains sufficient reagent for 20 slide tests.

Precautions

1. For In Vitro Diagnostic Use.
2. The Packaging of This Product Contains Dry Natural Rubber.
3. Follow established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated Haemophilus Influenzae Antisera at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Haemophilus Influenzae Antiserum Poly
Haemophilus Influenzae Antiserum Type a
Haemophilus Influenzae Antiserum Type b
Haemophilus Influenzae Antiserum Type c
Haemophilus Influenzae Antiserum Type d
Haemophilus Influenzae Antiserum Type e
Haemophilus Influenzae Antiserum Type f

Materials Required but not Provided

Agglutination slides
Applicator sticks
Sterile distilled or deionized water
Sterile 0.85% NaCl solution

Reagent Preparation

Haemophilus Influenzae Antiserum: To rehydrate, add 1 ml sterile distilled or deionized water and rotate to completely dissolve the contents.

Equilibrate all materials to room temperature prior to performing the tests. Ensure that all glassware and pipettes are clean and free of detergent residues.

Specimen Collection and Preparation

H. influenzae can be recovered from clinical specimens on chocolate agar. For specific recommendations, consult appropriate references.[1,5] Determine that a pure culture of the microorganism has been obtained and that biochemical test reactions are consistent with the identification of the organism as *H. influenzae*. After these criteria are met, serological identification can be performed.

Testing the Isolate for Autoagglutination

1. From the test culture on chocolate agar, transfer a loopful of growth to a drop of sterile 0.85% NaCl solution on a clean slide and emulsify the organism.
2. Rotate the slide for one minute and then observe for agglutination.
3. If agglutination (autoagglutination) occurs, the culture is rough and cannot be tested. Subculture to chocolate agar, incubate, and test the organism again as described in steps 1 and 2.

   If no agglutination occurs, proceed with testing the organism.

Test Procedure

Test culture isolates with Haemophilus Influenzae Poly for presumptive identification, then test with monospecific antisera.

1. Dispense 1 drop of the Haemophilus Influenzae Antiserum to be tested on an agglutination slide.
2. Transfer a loopful of growth of the test organism to the drop of antiserum and mix thoroughly.
3. Rotate the slide for one minute and read for agglutination.
4. Repeat this procedure for known positive and negative control cultures.

Results

Observe test results and record agglutination as follows:

4+	100% agglutination; background is clear to slightly hazy.
3+	75% agglutination; background is slightly cloudy.
2+	50% agglutination; background is moderately cloudy.
1+	25% agglutination; background is cloudy.
–	No agglutination.

Positive control: Should produce 3+ or greater agglutination.

Negative control: Should produce no agglutination.

Positive test result: Agglutination of 3+ or greater within one minute.

Limitations of the Procedure

1. Correct interpretation of serological reactions depends on culture purity as well as morphological characteristics and biochemical reactions that are consistent with identification of the microorganism as *H. influenzae*.
2. Serological methods alone cannot identify the isolate as *H. influenzae*.
3. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent a smooth suspension of the microorganism or may cause evaporation or precipitation of the test mixture. False-positive reactions may occur.
4. Rough culture isolates occur and will agglutinate spontaneously causing agglutination of the negative control (autoagglutination). Smooth colonies must be selected and tested in serological procedures.
5. *H. influenzae* has antigenic similarities to several unrelated bacteria. Cross-reactions can occur between *H. influenzae* and strains of *S. pneumoniae*, *Escherichia coli* and several species of *Staphylococcus*, *Streptococcus* and *Bacillus*.
6. Haemophilus Influenzae Antisera have been tested using undiluted cultures taken from agar media. These antisera have not been tested using antigen suspensions in NaCl solution or other diluents. If the

user employs a variation of the recommended procedure, each lot of antiserum must be tested with known control cultures to verify that expected reactions are obtained under the modified procedure.

7. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
8. A rehydrated Haemophilus Influenzae Antiserum that is cloudy or develops a precipitate during use should be discarded.

References

1. **Campos, J. M.** 1995. *Haemophilus*, p. 556-565. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D. C.
2. **Pittman, M.** 1931. Variation and type specificity in the bacterial species *Hemophilus influenzae*. J. Exp. Med. **53:**471-495.
3. **Insel, R., and P. Anderson.** 1986. *Haemophilus influenzae* Type b: assays for the capsular polysaccharide and for antipolysaccharide antibody. *In* N. R. Rose, H. Friedman, and J. L. Fahey (ed.), Manual of clinical laboratory immunology, 3rd ed. American Society for Microbiology, Washington, D.C.
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Packaging

Haemophilus Influenzae Antiserum Poly	1 ml	2237-50
Haemophilus Influenzae Antiserum Type a	1 ml	2250-50
Haemophilus Influenzae Antiserum Type b	1 ml	2236-50
Haemophilus Influenzae Antiserum Type c	1 ml	2789-50
Haemophilus Influenzae Antiserum Type d	1 ml	2790-50
Haemophilus Influenzae Antiserum Type e	1 ml	2791-50
Haemophilus Influenzae Antiserum Type f	1 ml	2792-50

Bacto® Listeria Antigens and Antisera

Listeria O Antiserum Type 1 · Listeria O Antiserum Type 4 Listeria O Antiserum Poly · Listeria O Antigen Type 1 (Slide) Listeria O Antigen Type 1 (Tube) · Listeria O Antigen Type 4 (Slide) Listeria O Antigen Type 4 (Tube)

Intended Use

Bacto Listeria O Antisera Types 1, 4, and Poly are used for identifying *Listeria monocytogenes* in the macroscopic tube and rapid slide tests.

Bacto Listeria O Antigens Types 1 and 4 (Tube) and (Slide) are used as positive controls in the macroscopic tube and rapid slide tests, respectively.

Summary and Explanation

First described in 1926 by Murray, Webb and Swann,[1] *Listeria monocytogenes* is a widespread problem in public health and the food industries. This organism can cause human illness and death, particularly in immunocompromised individuals and pregnant women.[2] The first reported food-borne outbreak of listeriosis was in 1985[3] and, since then, microbiological and epidemiological evidence from both sporadic and epidemic cases of listeriosis has shown that the principal route of transmission is via the consumption of foodstuffs contaminated with *Listeria monocytogenes*.[4]

Implicated vehicles of transmission include turkey frankfurters,[5] coleslaw, pasteurized milk, Mexican-style cheese, paté and pickled pork tongue. The organism has been isolated from commercial dairy and other food processing plants and is ubiquitous in nature, being present in a wide range of unprocessed foods and in soil, sewage, silage and river water.[6]

Listeria species grow over a pH range of 5.0-9.6 and survive in food products with pH levels outside these parameters.[7] *Listeria* species are microaerophilic, gram-positive, asporogenous, non-encapsulated, non-branching, regular, short, motile rods. Motility is most pronounced at 20°C.

The most common contaminating bacteria found in food sources potentially containing *Listeria* are streptococci, enterococci, micrococci, *Bacillus* species, *Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*.[8]

Identification of *Listeria* is based on successful isolation of the organism, biochemical characterization and serological confirmation.

Strains of *Listeria* species are divided into serotypes based on cellular (O) and flagellar (H) antigens.[9] Thirteen serotypes of *L. monocytogenes* are known. Most human disease is caused by serotypes 1/2a, 1/2b and 4b.[10]

Principles of the Procedure

Identification of *Listeria monocytogenes* includes both biochemical and serological confirmation. Serological confirmation requires that the microorganism (antigen) react with its corresponding antibody. This in vitro reaction produces macroscopic clumping called agglutination. The desired homologous reaction is rapid, does not dissociate (high avidity) and bonds strongly (high affinity).

Because a microorganism (antigen) may agglutinate with an antibody produced in response to another species, heterologous reactions are possible. These are characterized as weak in strength or slow in formation. Such unexpected and perhaps unpredictable reactions may lead to some confusion in serological identification. A positive homologous agglutination reaction should support the morphological and biochemical identification of the microorganism.

Agglutination of the somatic antigen in the slide test appears as a firm granular clumping. Homologous reactions occur rapidly and are strong (3+). Heterologous reactions form slowly and are weak.

The agglutination of the somatic antigen in the tube tests appears as a loose flocculation that can easily be resuspended. Homologous reactions using Listeria O Antisera should exceed a titer of 2+ at 1:320.

Reagents

Listeria O Antisera Types 1, 4, and Poly are lyophilized, polyclonal rabbit antisera containing approximately 0.04% Thimerosal as a preservative. The antisera are prepared according to procedures recommended by Gray.[11] Listeria O Antisera Types 1 and 4 are specific for the respective serotypes of *L monocytogenes* while Listeria O Antiserum Poly contains agglutinins for *L. monocytogenes* serotypes 1 and 4.

Listeria O Antigens Types 1 and 4 (Tube) and (Slide) are suspensions of appropriate *L. monocytogenes* serotypes containing 0.3% formaldehyde as a preservative. When used according to the suggested procedure, the reagents will yield the following:

REAGENT	VIAL	NUMBER OF TESTS
Listeria O Antiserum	1 ml	10 tube tests, 400 slide tests
Listeria O Antigen (Slide)	5 ml	100 slide tests
Listeria O Antigen (Tube)	25 ml	5 tube tests

User Quality Control

Identity Specifications

Listeria O Antiserum Type 1
Listeria O Antiserum Type 4
Listeria O Antiserum Poly

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated Appearance: Light gold to amber, clear liquid.

Listeria O Antigen Type 1 (Slide)
Listeria O Antigen Type 1 (Tube)
Listeria O Antigen Type 4 (Slide)
Listeria O Antigen Type 4 (Tube)

Appearance: White, liquid suspension.

Performance Response

Rehydrate Listeria O Antiserum per label directions. Perform the slide or tube agglutination test using appropriate Listeria O Antigens (Slide) or (Tube).

Slide test: An antiserum is considered satisfactory if it demonstrates a 3+ or greater reaction at 1:80 with a 1:5 dilution of the homologous antigen.

Macroscopic tube test: An antiserum is considered satisfactory if it demonstrates a 3+ or greater reaction with the 1:320 dilution of the homologous antigen.

Precautions

1. For In Vitro Diagnostic Use.
2. **Listeria O Antiserum Type 1**
   Listeria O Antiserum Type 4
   Listeria O Antiserum Poly

   The Packaging of This Product Contains Dry Natural Rubber.
3. **Listeria O Antigen Type 1 (Slide)**
   Listeria O Antigen Type 1 (Tube)
   Listeria O Antigen Type 4 (Slide)
   Listeria O Antigen Type 4 (Tube)

   POSSIBLE RISK OF IRREVERSIBLE EFFECTS. (US) Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Eyes, Kidneys, Lungs, Skin.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
4. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated Listeria O Antisera at 2-8°C.

Store Listeria O Antigen (Slide) and (Tube) at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Listeria O Antiserum Type 1
Listeria O Antiserum Type 4
Listeria O Antiserum Poly
Listeria O Antigen Type 1 (Slide)
Listeria O Antigen Type 1 (Tube)
Listeria O Antigen Type 4 (Slide)
Listeria O Antigen Type 4 (Tube)

Materials Required But Not Provided

Rapid Slide Test
FA Buffer, Dried
Agglutination slides
Applicator sticks
Waterbath, 80-100°C
Formaldehyde
Droppers

Macroscopic Tube Test
FA Buffer, Dried
McFarland Barium Sulfate Standard No. 3
Culture tubes 12 x 75 mm and rack
Serological pipettes, 1 ml
Waterbath, 50°C
Refrigerator, 2-8°C
Formaldehyde

Reagent Preparation

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergents.

Listeria O Antisera: To rehydrate, add 1 ml sterile distilled or deionized water to each vial. Rotate gently to dissolve contents completely.

Listeria O Antigens (Slide) and (Tube) are ready to use.

Specimen Collection and Preparation

From clinical specimens, *Listeria* can be recovered on selective differential media such as McBride Listeria Agar, Oxford Agar, Modified Oxford Agar, LPM Agar or Palcam Medium. For specific recommendations on isolation of *Listeria* from clinical specimens, consult appropriate references.[10,12,13] Determine that a pure culture of the microorganism has been obtained and that biochemical test reactions are consistent with the identification of the organism as *Listeria monocytogenes*. After these criteria are met, serological identification can be performed.

From food or dairy samples, *Listeria* can be recovered when samples are processed to recover injured microorganisms and prevent overgrowth of competing microorganisms. Consult appropriate references for recommended procedures for the isolation of *Listeria* from foods.[7,14,16] Having followed an established protocol, isolate a pure culture of the microorganism and confirm that biochemical test reactions are consistent with the identification of the organism as *Listeria monocytogenes*. After these criteria are met the serological identification can be performed.

Test Procedure

Rapid Slide Test

1. **FA Buffer, Dried**: Rehydrate per label directions.
2. **Test isolate**: Suspend growth from a solid agar medium in FA Buffer.
3. Heat the organism suspension at 80-100°C (in a waterbath) for 1 hour.
4. Centrifuge the suspension and remove the bulk of the supernatant fluid.
5. Resuspend the organism in the remaining portion of liquid.
6. **Listeria Antiserum**: On an agglutination slide, dispense 2 separate drops of the desired antiserum diluted 1:20 in NaCl solution. The first drop will be used for the test isolate and the second for the negative control.
7. **Organism suspension**: Add 1 drop of heated organism to the first drop of antiserum.
8. **Negative control**: Dispense 1 drop of FA Buffer on the agglutination slide. Add one drop of organism suspension from step 5.
9. **Positive control**: Add one drop of homologous Listeria O Antigen (Slide) to the second drop of antiserum.
10. Rotate the slide for 1-2 minutes and read for agglutination.

Slide Test Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. **Positive control**: Should show a 3+ or greater agglutination.
3. **Negative control**: Should show no agglutination. If agglutination occurs, the culture is rough and cannot be tested. Subculture to a non-inhibitory medium, incubate and test the organism again.
4. **Test isolates**: 3+ or greater agglutination is a positive result.
5. A partial (less than 3+) or a delayed agglutination reaction should be considered negative.

Macroscopic Tube Test

1. **Test isolate**: Suspend growth of the test organism from a solid agar medium in FA Buffer. Adjust to a density approximating that of a McFarland Barium Sulfate Standard No. 3.
2. Prepare a row of 9 culture tubes (12 x 75 mm) for each serum suspension to be tested, including the positive control.
3. **Formalized FA Buffer**: Dispense 0.9 ml formalized FA Buffer (0.3 ml formaldehyde per 300 ml FA Buffer) to the first tube in each row and 0.5 ml to the remaining tubes.
4. **Listeria O Antiserum**: Using a 1 ml serological pipette, add 0.1 ml of the desired antiserum to tube 1 in each row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. In like manner, continue transferring 0.5 ml through tube 8, discarding 0.5 ml from tube 8 after mixing. Tube 9 is an antigen control tube. Upon addition of the test suspension, final dilutions will be 1:20 through 1:2560 for tubes 1 through 8, respectively.
5. **Test Suspension**: Add 0.5 ml of the test suspension to each of 9 tubes.
6. **Positive control**: Add 0.5 ml of an appropriate Listeria O Antigen to each of 9 tubes containing antiserum.
7. **Negative control**: Add 0.5 ml of the test suspension to a tube containing FA Buffer.
8. Shake the rack to mix. Incubate in a 50°C waterbath for 2 hours. Refrigerate overnight. Read for agglutination the following morning.

Tube Test Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. **Positive control**: Should show 2+ or greater agglutination at 1:320.
3. **Antigen control**: Tube 9 of each row should show no agglutination.

4. If results of the positive control or antigen control are not as described, the test is invalid and results cannot be read.
5. **Test serum**: The titer is that dilution which shows a 2+ or greater agglutination at 1:320.

Limitations of the Procedure

1. Serological techniques employing Listeria O Antisera serve as corroborative evidence for the identification of *Listeria monocytogenes*. Final identification cannot be made without consideration of morphological, serological and biochemical characterization.
2. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent making a smooth suspension of the microorganism or cause evaporation or precipitation of the test mixture. False-positive reactions may occur.
3. Rough culture isolates occur and will agglutinate spontaneously, causing agglutination of the negative control (autoagglutination). Smooth colonies must be selected and tested in serological procedures.
4. Agglutination reactions of 3+ or greater in the slide test are interpreted as positive reactions. Cross-reactions resulting in a 1+ or 2+ agglutination are likely since there are somatic antigens shared among different organisms such as staphylococci, enterococci and *Bacillus* species.[10]
5. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
6. Exposure of Listeria O Antigens to temperatures below 2°C can result in autoagglutination. Antigens must be smooth uniform suspensions; examine antigen vials for agglutination before use. Suspensions with agglutination are not usable and should be discarded.
7. It is important in this test to use the recommended time and temperature of incubation. Also, care should be taken to make certain that the waterbath is in a location free of mechanical vibration.
8. Discard any Listeria O Antiserum that is cloudy or has a precipitate after rehydration or storage.

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Packaging

Listeria O Antiserum Type 1	1 ml	2300-50
Listeria O Antiserum Type 4	1 ml	2301-50
Listeria O Antiserum Poly	1 ml	2302-50
Listeria O Antigen Type 1 (Slide)	5 ml	2303-56
Listeria O Antigen Type 1 (Tube)	25 ml	2305-65
Listeria O Antigen Type 4 (Slide)	5 ml	2304-56
Listeria O Antigen Type 4 (Tube)	25 ml	2306-65
FA Buffer, Dried	6 x 10 g	2314-33
	100 g	2314-15
	10 kg	2314-08

Bacto® Neisseria Meningitidis Antisera

Neisseria Meningitidis Antiserum Group A · Neisseria Meningitidis Antiserum Group B · Neisseria Meningitidis Antiserum Group C Neisseria Meningitidis Antiserum Group D · Neisseria Meningitidis Antiserum Group W135 · Neisseria Meningitidis Antiserum Group X · Neisseria Meningitidis Antiserum Group Y · Neisseria Meningitidis Antiserum Group Z · Neisseria Meningitidis Antiserum Group Z´ · Neisseria Meningitidis Antiserum Poly (Groups A, B, C, D) Neisseria Meningitidis Antiserum Poly 2 (Groups X, Y, Z)

Intended Use

Bacto Neisseria Meningitidis Antisera are used in the slide agglutination test for serotyping *Neisseria meningitidis*.

Summary and Explanation

Neisseria meningitidis is found in the oropharynx and nasopharynx of humans. Because the organism survives poorly in the environment, humans are the primary reservoir. In asymptomatic persons, the carrier state lasts for various periods, usually several weeks. The microorganism is transmitted from person-to-person by direct contact with respiratory secretions or airborne droplets.[1,2]

User Quality Control

Identity Specifications

Neisseria Meningitidis Antiserum Poly
Neisseria Meningitidis Antiserum Poly 2
Neisseria Meningitidis Antiserum A
Neisseria Meningitidis Antiserum B
Neisseria Meningitidis Antiserum C
Neisseria Meningitidis Antiserum D
Neisseria Meningitidis Antiserum X
Neisseria Meningitidis Antiserum Y
Neisseria Meningitidis Antiserum Z
Neisseria Meningitidis Antiserum Z´
Neisseria Meningitidis Antiserum W135

Lyophilized Appearance: Light gold to amber button to powdered cake.

Rehydrated Appearance: Light gold to amber liquid.

Performance Response

Rehydrate Neisseria Meningitidis Antisera per label directions. Test as described (see Test Procedure). Known positive and negative control cultures must give appropriate reactions.

In some colonized persons, the organism spreads from the nasopharynx through the bloodstream to produce meningococcemia, meningitis or both. Meningococcemia is characterized by a petechial or purpuric skin rash. In fulminating infections (Waterhouse-Friderichsen syndrome), widespread coagulation and fulminant sepsis occur, resulting in shock and, usually, death.[3] Persons with inherited complement deficiencies are at greater risk for acquiring systemic meningococcal infections and may experience repeated episodes.[2]

Typical human specimens for isolating the organism are cerebrospinal fluid (CSF), blood, skin lesions (in cases where petechiae occur) and nasopharyngeal swabs. *N. meningitidis* occurs in the cervix and vagina of females and can cause serious pelvic disease. Other sources for the organism are the anal canal and, in males, the urethra.

N. meningitidis is divided into serological groups based on the presence of either capsular or outer membrane protein antigens. Among the currently recognized groups are A, B, C, D, 29E, H, I, K, L, X, Y, Z, Z´ and W135. Groups A, B, C, Y, and W135 are most frequently implicated in systemic disease.[4] Classically, group A and C strains cause epidemic meningococcal disease.[3] Group B strains have been associated with sporadic infections. Other serogroups are sporadically isolated from carriers and patients with disease.

N. meningitidis are gram-negative cocci, usually occurring in pairs called diplococci. They are strict aerobes and produce the enzyme, cytochrome oxidase. The growth of *N. meningitidis* is enhanced by a CO_2-enriched atmosphere.

The Quellung reaction (capsular swelling) has been performed for serotyping *N. meningitidis*. However, capsules have not been demonstrated in strains of serogroup B organisms. In addition, the Quellung reaction is very nonspecific. *N. meningitidis* should be defined serologically by the slide agglutination test rather than by the Quellung reaction.

Principles of the Procedure

Identification of *N. meningitidis* includes isolation of the microorganism, biochemical identification and serological confirmation.

Serological confirmation involves the reaction in which the microorganism (antigen) reacts with its corresponding antibody. This *in vitro* reaction produces macroscopic clumping called agglutination. The desired homologous reaction is rapid, has at least a 3+ reaction, does not dissociate (high avidity), and binds (high affinity).

Because a microorganism (antigen) may agglutinate with an antibody produced in response to another species, heterologous reactions are possible. These are characterized as weak in strength or slow in formation. Such unexpected and, perhaps, unpredictable reactions may lead to some confusion in serological identification. Therefore, a positive homologous agglutination reaction should support the morphological and biochemical identification of the microorganism.

Homologous reactions are rapid and strong. Heterologous reactions are slow and weak.

Reagents

Neisseria Meningitidis Antisera are lyophilized, polyclonal rabbit antisera containing approximately 0.02% Thimerosal as a preservative. Neisseria Meningitidis Antisera Poly and Group D are absorbed for detection of Group D; Neisseria Meningitidis Antisera Poly 2, Z´, W135, A, B, C, X, Y and Z are not absorbed for detection of Group D, which is rarely isolated.

Neisseria Meningitidis Antisera detect the following antigenic groups:

ANTISERUM	ANTIGENIC GROUP(S) DETECTED
Poly	A, B, C, D
Poly 2	X, Y, Z
W135	W135
A	A
B	B
C	C
D	D
X	X
Y	Y
Z	Z, Z´
Z´	Z´

When rehydrated and used as described, each 1 ml vial of Neisseria Meningitidis Antiserum contains sufficient reagent for 20 slide tests.

Precautions

1. For In Vitro Diagnostic Use.
2. The Packaging of This Product Contains Dry Natural Rubber.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated Neisseria Meningitidis Antisera at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Neisseria Meningitidis Antiserum Poly
Neisseria Meningitidis Antiserum Poly 2
Neisseria Meningitidis Antiserum A
Neisseria Meningitidis Antiserum B
Neisseria Meningitidis Antiserum C
Neisseria Meningitidis Antiserum D
Neisseria Meningitidis Antiserum X
Neisseria Meningitidis Antiserum Y
Neisseria Meningitidis Antiserum Z
Neisseria Meningitidis Antiserum Z´
Neisseria Meningitidis Antiserum W135

Materials Required But Not Provided

Agglutination slides
Applicator sticks
Sterile distilled or deionized water
Sterile 0.85% NaCl solution

Reagent Preparation

Neisseria Meningitidis Antisera: To rehydrate, add 1 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

Equilibrate all materials to room temperature prior to performing the tests. Ensure that all glassware and pipettes are clean and free of detergent residues.

Specimen Collection and Preparation

N. meningitidis can be recovered on blood agar or chocolate agar. Determine that the test organism has the following characteristics of *N. meningitidis*:

Morphology:	grayish-white, opaque, smooth, butyrous, non-pigmented
Gram stain:	gram-negative diplococcus
Oxidase:	positive
Catalase:	positive
ONPG reaction:	negative
Nitrate reduction:	negative
Glucose:	positive
Maltose:	positive
Sucrose:	negative
Fructose:	negative
Lactose:	negative

Determine that a pure culture of the microorganism has been obtained and that biochemical test reactions are consistent with the identification of the organism as *N. meningitidis*. For more detailed information on the biochemical identification of *N. meningitidis*, consult appropriate references.[3,5] After these criteria are met, serological identification can proceed.

Testing the Isolate for Autoagglutination

1. From the test culture on chocolate agar, transfer a loopful of growth to a drop of sterile 0.85% NaCl solution on a clean slide and emulsify the organism.
2. Rotate the slide for one minute and then observe for agglutination.

   If agglutination (autoagglutination) occurs, the culture is rough and cannot be tested. Subculture to chocolate agar, incubate, and test the organism again as described in steps 1 and 2.

   If no agglutination occurs, proceed with testing the organism.

Choosing Antisera to Test

1. Test the organism first with Neisseria Meningitidis Antisera Poly, Poly 2 and Group W135.
2. Depending on the reaction, continue testing as follows.

If agglutination occurs with Neisseria Meningitidis Antiserum:	Test with Neisseria Meningitidis Antiserum:
Poly	Groups A, B, C, D
Poly 2	Groups X, Y, Z, Z´ (See NOTE.)
Group W135	No further testing is required.

NOTE: *N. meningitidis* Group Z´ organisms *may* agglutinate monospecific Neisseria Meningitidis Antiserum Group Z. However, *N. meningitidis* Group Z organisms *will not* agglutinate Neisseria Meningitidis Antiserum Group Z´. The expected agglutination reactions of Neisseria Meningitidis Antiserum Groups Z´ and Z with test organisms are:

	Neisseria Meningitidis Antiserum	
Test Organism	Group Z´	Group Z
N. meningitidis Group Z´	3+	+
N. meningitidis Group Z	–	3+

Test Procedure

1. **Neisseria Meningitidis Antiserum**: Dispense 1 drop of the antiserum to be tested on an agglutination slide.
2. **Test isolate**: Transfer a loopful of growth to the drop of antiserum and mix thoroughly.
3. Rotate the slide for one minute and read for agglutination.
4. Repeat this procedure for known positive and negative cultures.

Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. **Positive control**: Should produce 3+ or greater agglutination.

   Negative control: Should produce no agglutination.

   Test isolate: A positive test result is defined as agglutination of 3+ or greater within one minute.

Limitations of the Procedure

1. Correct interpretation of serological reactions depends on culture purity, as well as morphological characteristics and biochemical reactions that are consistent with identification of the microorganism as *N. meningitidis*.
2. Serological methods alone cannot identify the isolate as *N. meningitidis*. Organisms unrelated to *Neisseria*, yet capable of causing meningitis, and other species of *Neisseria* can cross-react with meningococcal antisera. Cultural isolation must precede serological examination.
3. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent a smooth suspension of the microorganism or may cause evaporation or precipitation of the test mixture. False-positive reactions may occur.
4. Rough culture isolates occur and will agglutinate spontaneously, causing agglutination of the negative control (autoagglutination). Smooth colonies must be selected and tested in serological procedures.
5. *N. meningitidis* Group A and *N. meningitidis* Group C may cross-react due to the presence of common capsular polysaccharides.
6. Group Z´ meningococci *may* agglutinate group Z antiserum. Group Z meningococci *will not* agglutinate group Z´ antiserum.
7. Neisseria Meningitidis Antisera have been tested using undiluted cultures taken from agar media. These antisera have not been tested using antigen suspensions in NaCl solution or other diluents. If the user employs a variation of the recommended procedure, each lot of antiserum must be tested with known control cultures to verify that expected reactions are obtained under the modified procedure.
8. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
9. A rehydrated Neisseria Meningitidis Antiserum that is cloudy or develops a precipitate during use should be discarded.

References

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5. **Pezzlo, M.** 1994. Aerobic bacteriology, p. 1.0.1-1.20.47. *In* H. D. Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.

Packaging

Neisseria Meningitidis Antiserum Poly	1 ml	2232-50
Neisseria Meningitidis Antiserum Poly 2	1 ml	2910-50
Neisseria Meningitidis Antiserum Group A	1 ml	2228-50
Neisseria Meningitidis Antiserum Group B	1 ml	2229-50
Neisseria Meningitidis Antiserum Group C	1 ml	2230-50
Neisseria Meningitidis Antiserum Group D	1 ml	2231-50
Neisseria Meningitidis Antiserum Group X	1 ml	2880-50
Neisseria Meningitidis Antiserum Group Y	1 ml	2881-50
Neisseria Meningitidis Antiserum Group Z	1 ml	2891-50
Neisseria Meningitidis Antiserum Group Z´	1 ml	2252-50
Neisseria Meningitidis Antiserum Group W135	1 ml	2253-50

Bacto® Proteus Antigens and Antisera · The Weil-Felix Test

Proteus OX2 Antigen (Slide) · Proteus OX2 Antigen (Tube)
Proteus OX19 Antigen (Slide) · Proteus OX19 Antigen (Tube)
Proteus OXK Antigen (Slide) · Proteus OXK Antigen (Tube)
Proteus OX2 Antiserum · Proteus OX19 Antiserum
Proteus OXK Antiserum · Febrile Negative Control

Intended Use

Bacto Proteus OX2, OX19 and OXK Antigens (Slide) and (Tube) are used for detecting antibodies by the slide and tube agglutination tests.

Bacto Proteus OX2, OX19 and OXK Antisera are used in the quality control testing of Proteus OX2, OX19 and OXK Antigens in slide and tube agglutination tests.

User Quality Control

Identity Specifications

Proteus OX2 Antigen (Slide)
Proteus OX19 Antigen (Slide)
Proteus OXK Antigen (Slide)
Appearance: Turquoise-blue-violet suspension.

Proteus OX2 Antigen (Tube)
Proteus OX19 Antigen (Tube)
Proteus OXK Antigen (Tube)
Appearance: Light gray to white suspension.

Proteus OX2 Antiserum
Proteus OX19 Antiserum
Proteus OXK Antiserum
Lyophilized Appearance: Light gold to amber, button to powdered cake.
Rehydrated Appearance: Light gold to amber, clear liquid.

Febrile Negative Control
Lyophilized Appearance: Colorless to light gold, button to powdered cake.
Rehydrated Appearance: Colorless to light gold, clear liquid.

Performance Response

Rehydrate Proteus OX Antisera and Febrile Negative Control per label directions. Perform the slide or tube agglutination test using Proteus OX Antigen (Slide) or (Tube). Both positive and negative controls are diluted in the same proportion as a patient serum and processed in the same manner following procedures for the rapid slide test or the macroscopic tube test (see Test Procedure).

An antigen is considered satisfactory if it does not agglutinate with the negative control, and if it reacts 2+ or greater at a titer of 1:160 or more with the positive control.

Summary and Explanation

Rickettsiae cause a variety of human diseases that share symptoms such as chills, fever, malaise and myalgia. These symptoms occur suddenly, usually within 3 to 14 days after exposure. Patients frequently have a rash and may have mild pulmonary symptoms. The rickettsiae are obligate intracellular bacteria and multiply within arthropods (lice, ticks, fleas, etc.) which may serve as the vectors of infection.

The spotted fevers are caused by species of *Rickettsia*, with Rocky Mountain spotted fever caused by *Rickettsia rickettsii* being well known. Epidemic and murine typhus are caused by *R. prowazekii* and *R. typhi*, respectively. Scrub typhus is caused by *Orientalis tsutsugamushi*. For a complete discussion of the rickettsiae, consult an appropriate reference.[1-5]

Because rickettsial diseases develop as a febrile illness, patient diagnosis has frequently involved measurements of antibody response. The Weil-Felix test became popular in the 1920's after it was observed that certain strains of *Proteus* would agglutinate early-convalescent-phase sera from patients with suspected rickettsial disease.[6] *Proteus* antigens (OX2, OX19 and OXK) will cross-react in predictable patterns, although the reactions are not highly sensitive or specific. *Rickettsiae* are pathogenic microorganisms that, upon invasion, produce a fever in their host. *Proteus* antigens are often called "Febrile Antigens" because they are used to detect the response to a rickettsial infection.

The human immune response to a particular microorganism results in measurable antibody production that in some cases can help in completing the patient's clinical diagnosis. In blood samples, the antibody titer during the initial phase of the infection (acute) is compared to the antibody titer 7 to 14 days later (convalescent). Antibody titers that are high initially in the acute phase or an acute or convalescent pair of samples that shows an increase in antibody titer are helpful in the diagnosis of disease.

Diagnosis of the cause of febrile disease cannot be based solely on analysis of serum samples for antibody response. Many factors may affect measurable antibody levels. For example, the patient's immune response can be affected by age, immune status, general state of health and previous immunizations.

Certain organisms may share cross-reacting antigens leading to the production of heterologous antibodies. These heterologous antibodies may react with one or more antigens in an antibody test procedure resulting in low-level antibody titers that may not, as a single result, suggest disease. The Weil-Felix test is not specific for rickettsial diseases.

Principles of the Procedure

Agglutination tests involving the use of *Proteus* antigens determine the presence of antibodies that react with the test antigen. The serological procedure involves serially diluting the patient serum and then adding a standard volume of an antigen. The end point of the test is the last dilution of the serum that shows a specific amount of agglutination. The end point converted to a dilution of the serum is called the patient's antibody "titer."

Reagents

Antigens

1. **Proteus Antigens** are ready to use, nonmotile strains of the organisms listed below. Proteus Antigen (Slide) contains 20% glycerin. Each vial of Proteus Antigen (Slide) contains sufficient reagent for 33 slide tests. Each vial of Proteus Antigen (Tube) contains sufficient reagent for 6 tube tests.

   Proteus OX2 Antigen (Slide) and (Tube) - *Proteus vulgaris* OX2

   Proteus OX19 Antigens (Slide) and (Tube) - *Proteus vulgaris* OX19

   Proteus OXK Antigen (Slide) and (Tube) - *Proteus mirabilis* OXK

2. **Concentration of Antigen**: Antigen density may vary because it is adjusted for optimum performance when standardized with hyperimmune sera obtained from laboratory animals.

   Variation in color intensity is normal and will not affect test performance.

3. Proteus antigens contain the following preservative(s):

   Proteus OX2, OX19 and OXK Antigens (Slide): 0.5% formaldehyde, and approximately 0.002% crystal violet and 0.005% brilliant green.

   Proteus OX2, OX19 and OXK Antigens (Tube): 0.25% formaldehyde.

Antisera

1. **Proteus Antisera** are lyophilized, polyclonal rabbit antisera containing approximately 0.04% Thimerosal as a preservative. Each vial contains sufficient reagent for 19 slide tests or 30 tube tests.
2. Febrile Negative Control is a standard protein solution containing 0.02% Thimerosal as a preservative. Each vial of Febrile Negative Control contains sufficient reagent for 32 slide tests.

Precautions

1. For In Vitro Diagnostic Use.
2. Observe universal blood and body fluid precautions in the handling and disposing of specimens.[7,8]
3. **Proteus OX2 Antigen (Slide)**
   Proteus OX2 Antigen (Tube)
   Proteus OX19 Antigen (Slide)
   Proteus OX19 Antigen (Tube)
   Proteus OXK Antigen (Slide)
   Proteus OXK Antigen (Tube)

   POSSIBLE RISK OF IRREVERSIBLE EFFECTS. (US) Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Eyes, Kidneys, Lungs, Skin.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
4. **Proteus OX2 Antiserum**
   Proteus OX19 Antiserum
   Proteus OXK Antiserum

   The Packaging of This Product Contains Dry Natural Rubber.
5. Follow proper established laboratory procedure in handling and disposing of infectious materials.
6. Proteus Antigens are not intended for use in the immunization of humans or animals.

Storage

Store Proteus OX2, OX19 and OXK Antigens (Slide) and (Tube) at 2-8°C.

Store lyophilized and rehydrated Proteus OX2, OX19 and OXK Antisera at 2-8°C.

Store lyophilized and rehydrated Febrile Negative Control at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Proteus OX2 Antigen (Slide)
Proteus OX2 Antigen (Tube)
Proteus OX19 Antigen (Slide)
Proteus OX19 Antigen (Tube)
Proteus OXK Antigen (Slide)
Proteus OXK Antigen (Tube)
Proteus OX2 Antiserum
Proteus OX19 Antiserum
Proteus OXK Antiserum
Febrile Negative Control

Materials Required But Not Provided

Slide test

Agglutination slides, 5 squares, 1" each
Applicator sticks
Sterile 0.85% NaCl solution
Sterile distilled or deionized water
Serological pipettes, 0.2 ml

Tube Test

Culture tubes 12 x 75 mm and rack
Waterbath, 35-37°C
Serological pipettes, 1 ml and 5 ml
Sterile 0.85% NaCl solution
Sterile distilled or deionized water

Reagent Preparation

Proteus OX2, OX19 and OXK Antigens (Slide) and (Tube) are ready to use.

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergent.

Proteus OX2, OX19 and OXK Antisera: To rehydrate, add 3 ml sterile 0.85% NaCl solution and rotate gently to completely dissolve the contents. The rehydrated antiserum is considered a 1:2 working dilution.

Febrile Negative Control: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

Specimen Collection and Preparation

Collect a blood specimen by aseptic venipuncture. Serum is required for the test. Store serum specimens at room temperature for no longer than 4 hours; for prolonged storage, keep at 2-8°C for up to 5 days or maintain below -20°C.

Serum specimens must be clear, free of hemolysis and show no visible evidence of bacterial contamination (turbidity, hemolysis or particulate matter). Consult appropriate references for more information on collection of specimens.[9,10] Serum specimens must not be heated. Heat may inactivate or destroy certain antibodies.

Test Procedure

Slide Test

Use the slide test only as a screening test. Confirm positive results with the tube test.

1. **Test serum**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of serum into a row of squares on the agglutination slide.
2. **Positive control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Proteus Antiserum into a row of squares on the agglutination slide.
3. **Negative control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Febrile Negative Control into a row of squares on the agglutination slide.
4. **Proteus Antigen**: Shake the vial of antigen well to ensure a smooth, uniform suspension. Add one drop (approximately 35 µl) of antigen to each drop of diluted test serum, positive control and negative control.
5. Mix each row of test sera and control sera, using a separate applicator stick for each row. Start with the most dilute mixture (0.005 ml) and work to the most concentrated (0.08 ml).
6. Rotate the slide for 1 minute and read for agglutination.
7. The final dilutions in squares 1-5 correspond with tube dilutions of 1:20, 1:40, 1:80, 1:160, 1:320, respectively.

Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. **Positive control**: Should show 2+ or greater agglutination at 1:160.
3. **Negative control**: Should show no agglutination.
4. If results for either the positive or negative controls are not as described, the test is invalid and results cannot be read.
5. **Test specimens**: The serum titer is that dilution which shows 2+ or greater agglutination.
6. The slide test is a screening test, only; results must be confirmed with the tube test.

Tube Test

1. Prepare a row of 8 culture tubes (12 x 75 ml) for each test serum, including a row for the appropriate Proteus Antiserum.
2. **Sterile 0.85% NaCl solution**: Dispense 0.9 ml in the first tube of each row and 0.5 ml in the remaining tubes.
3. **Test serum**: Using a 1 ml serological pipette, add 0.1 ml of the serum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. In like manner, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
4. **Positive control**: Using a 1 ml serological pipette, add 0.1 ml of the appropriate Proteus Antiserum to the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
5. **Proteus Antigen**: Shake the vial of antigen to ensure a smooth, uniform suspension. Add 0.5 ml of the antigen to each of the 8 tubes in each row and shake the rack to mix the suspensions. Final dilutions in tubes 1-7 are 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280, respectively.
6. Incubate in a waterbath at 35-37°C for 2 hours; then refrigerate at 2-8°C for 22 ± 2 hours.
7. Remove from incubation. Avoid excessive shaking before reading the reactions either when the tubes are incubated or when removing them from the incubation.
8. Read and record the results.

Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. **Positive control**: Should show a 2+ or greater agglutination at 1:160.
3. **Antigen control (tube 8 of each row)**: Should show no agglutination.
4. If results of the positive control or antigen control are not as described, the test is invalid and results cannot be read.
5. For each test serum, the serum titer is that dilution which shows 2+ or greater agglutination.

Interpretation[1]

Compare results:

DISEASE	AGENT	PROTEUS OX2	PROTEUS OX19	PROTEUS OXK
Epidemic Typhus*	*R. prowazekii*	+	+	–
Murine Typhus*	*R. typhi*	+	+	–
Scrub Typhus	*O. tsutsugamushi*	–	–	+
Rocky Mountain Spotted Fever**	*R. rickettsii*	+	+	–
Other Spotted Fevers**	*Rickettsia* sp.	+	+	–

*In cases of epidemic and murine typhus, the strength of the antibody agglutination with Proteus OX19 is usually stronger (4+) than the agglutination with Proteus OX2 (2+).

**In cases of spotted fevers, antibodies may agglutinate either or both strains of Proteus OX19 or OX2, and the strength of agglutination may vary from 1+ to 4+.

For a single serum specimen, a titer of 1:160 is suggestive of infection.

A pair of serum specimens (acute and convalescent) showing a two-dilution difference in the titers is a significant increase in antibody level and is suggestive of infection. A one dilution difference is within the limits of laboratory error.

Limitations of the Procedure

1. The slide test is for screening only and results should be confirmed by performing the tube test. The slide test dilutions are made to detect a prozone reaction and do not represent true quantitation of the antibody. A serum specimen with a prozone reaction shows no agglutination because of excessively high antibody concentrations. To avoid this occurrence, all 5 serum dilutions (slide test) should be run.
2. The detection of antibodies in serum specimens may complete the clinical picture of a patient having infection. However, the isolation of the causative agent from patient specimens may be required. A definitive diagnosis must be made by a physician based on patient history, physical examination and data from all laboratory tests.
3. Cross-reacting heterologous antibodies are responsible for many low titer reactions. Infections with other organisms, vaccinations and past history of disease may result in low level of antibody titers. Antimicrobial therapy may suppress antibody production.

   The Weil-Felix test is not specific for rickettsial diseases. *Rickettsia* species cause cross-reacting antibodies, and infections with *Proteus* species can also cause cross-reacting antibodies.
4. While a single serum specimen showing a titer of 1:160 suggests infection, it is not diagnostic.
5. To test for a significant rise in antibody titer, at least two specimens are necessary, an acute specimen obtained at time of initial symptoms and a convalescent specimen obtained 7 to 14 days later. A two-dilution difference in titer is a significant increase in antibody level and is suggestive of infection.
6. The Weil-Felix test does not differentiate between epidemic and murine typhus.
7. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
8. Exposure of the antigen reagents to temperatures below 2°C can result in autoagglutination. Antigens must be smooth, uniform suspensions. Examine antigen vials for agglutination before use. Suspensions with agglutination are not usable and should be discarded.
9. Rehydrated Proteus OX2, OX19 and OXK Antisera that is cloudy or has a precipitate during use should be discarded.

References

1. **Eisemann, C. S., and J. V. Osterman.** 1986. *Rickettsiae*, p. 593-599. *In* N. R. Rose, H. Friedman, and J. L. Fahey, (ed.), Manual of clinical laboratory immunology, 3rd ed. American Society for Microbiology, Washington, D.C.
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9. **Pezzlo, M.** 1992. Aerobic bacteriology, p. 1.0.1-1.20.47. *In* H. D. Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
10. **Miller, J. M., and H. T. Holmes.** 1995. Specimen collection, transport and storage. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.

Packaging

Proteus OX2 Antigen (Slide)	5 ml	2243-56
Proteus OX2 Antigen (Tube)	25 ml	2248-65
Proteus OX19 Antigen (Slide)	5 ml	2234-56
Proteus OX19 Antigen (Tube)	25 ml	2247-65
Proteus OXK Antigen (Slide)	5 ml	2244-56
Proteus OXK Antigen (Tube)	25 ml	2249-65
Proteus OX2 Antiserum	3 ml	2245-47
Proteus OX19 Antiserum	3 ml	2235-47
Proteus OXK Antiserum	3 ml	2246-47
Febrile Negative Control	5 ml	3239-56

Bacto® QC Antigens Salmonella

QC Antigen Salmonella O Group A · QC Antigen Salmonella O Group B · QC Antigen Salmonella O Group C_1 · QC Antigen Salmonella O Group C_2 · QC Antigen Salmonella O Group D QC Antigen Salmonella O Group E_1 · QC Antigen Salmonella O Group E_2 · QC Antigen Salmonella O Group E_4 · QC Antigen Salmonella O Group F · QC Antigen Salmonella O Group G_1 QC Antigen Salmonella O Group H · QC Antigen Salmonella O Group I · QC Antigen Salmonella Vi Febrile Negative Control

Intended Use

Bacto QC Antigens Salmonella are used in the quality control testing of Salmonella Antisera by the slide agglutination test.

User Quality Control

Identity Specifications

QC Antigens Salmonella O Groups A, B, C_1, C_2, D, E_1, E_2, E_4, F, G_1, H, I and Salmonella Vi

Appearance:	Liquid, light gray to white, may settle on standing.

Febrile Negative Control

Lyophilized appearance:	Colorless to light gold, button to powdered cake.
Rehydrated appearance:	Colorless to light gold, clear liquid.

Cultural Response

Rehydrate the Salmonella antiserum per label directions. Perform the slide agglutination test using an appropriate QC Antigen Salmonella as the homologous (positive) or heterologous (negative) control. The homologous control should produce 3+ or greater agglutination. The heterologous control should not produce agglutination. Infrequently, a +/- reaction will occur.

The following chart lists the identifying antigen(s) of various Salmonella Antisera and the recommended homologous QC Antigen(s) Salmonella (positive control). To demonstrate a heterologous (negative control) reaction, use a QC Antigen Salmonella that contains antigens unrelated to those in the homologous control.

continued on following page

Summary and Explanation

Salmonella species cause a variety of human diseases called salmonelloses. The range of disease is from mild self-limiting gastroenteritis to a more severe form, possibly with bacteremia to typhoid fever, which can be severe and life-threatening. Severe disease and bacteremia are associated primarily with *S. choleraesuis*, *S. paratyphi* A and *S. typhi*, while most of the other 2300 or more strains are associated with gastroenteritis. The severity of the diarrheal disease depends on the virulence of the strain and the condition of the human host.

Salmonellae are found in nature and occur in the intestinal tract of many animals, both wild and domestic. The microorganism can spread to man through environmental contact or from eating contaminated meat or vegetable food products.

The genus *Salmonella* is in the family *Enterobacteriaceae*. Salmonellae are facultatively anaerobic, gram-negative bacilli that typically are oxidase negative, lactose negative, H_2S positive and produce gas.

Serotypes of *Salmonella* are defined based on the antigenic structure of both the somatic or cell wall (O) antigens and the flagellar (H) antigens. The antigenic formula provides the O antigen(s) first, followed by the H antigen(s). In characterizing serotypes of *Salmonella*, the somatic O heat-stable antigens are identified first and are numbered 1-67 using Arabic numerals. The numbers are not completely continuous because certain strains were reclassified to other genera and the antigenic Arabic numbers were deleted from the scheme.

Serogroups, which represent the organization of the *Salmonella* strains based on the antigen(s) shared in common, are designated by the letters A-Z. After exhausting the alphabet, the serogroups were numbered beginning with the numeral 51 (the serogroup Z organism having antigen number 50). While one somatic antigen identifies each serogroup, certain other antigens may be shared among several serogroups.

The use of Salmonella antisera in the serological identification of *Salmonella* requires the use of quality control test suspensions to verify that the antisera are performing as expected. Most laboratories are required to test antisera with positive and negative controls prior to use.[1,2] QC Antigens Salmonella are designed as homologous controls for testing the efficacy of the *Salmonella* grouping antisera employed in routine laboratory procedures.

Principles of the Procedure

Serological procedures that confirm the identification of an organism are usually agglutination reactions. Agglutination reactions may be either homologous or heterologous. Homologous reactions occur between a microorganism (antigen) and the corresponding antibody. These reactions occur rapidly and are strong. Heterologous reactions occur when a microorganism (antigen) reacts with an antibody produced in response to some other species or serotype. These reactions occur slowly and are weak.

Heterologous reactions may be unexpected and unpredictable and may lead to confusion in serological identification. Therefore, only strongly positive homologous agglutination reactions should be regarded as significant.

User Quality Control cont.

SALMONELLA ANTISERUM	QC ANTIGEN SALMONELLA HOMOLOGOUS CONTROL(S)
Poly A-I & Vi	A, B, D, E_1, E_2, E_4, F, G_1, H, I, Vi
Poly A	A, B, D, E_1, E_2, E_4
Poly B	C_1, C_2, F, G_1, H
Poly C	I
Group A Factors 1, 2, 12	A
Group B Factors 1, 4, 5, 12	B
Group B Factors 1, 4, 12, 27	B
Group C_1 Factors 6, 7	C_1
Group C_2 Factors 6, 8	C_2
Group D_1 Factors 1, 9, 12	D
Group E Factors 1, 3, 10, 15, 19, 34	E_1, E_2, E_4
Group E_1 Factors 3, 10	E_1
Group E_2 Factors 3, 15	E_2
Group E_4 Factors 1, 3, 19	E_4
Group F Factor 11	F
Group G Factors 13, 22, 23, (36), (37)	G_1
Group G_1 Factors 13, 22, (36), (37)	G_1
Group H Factors 1, 6, 14, 24, 25	H
Group I Factor 16	I
Vi	Vi
Factor 2	A
Factor 4	B
Factors 4, 5	B
Factor 5	B
Factor 7	C_1
Factor 8	C_2
Factor 9	D
Factor 10	E_1
Factor 15	E_2
Factor 19	E_4
Factor 22	G_1
Factor 14	H

Note: Parentheses ( ) indicate that the antigen is poorly developed or agglutinates weakly. For a complete and current explanation of the classification of *Salmonella*, consult appropriate references.[3,4,5]

Reagents

QC ANTIGEN SALMONELLA	ORGANISM USED FOR ANTIGEN PREPARATION	HOMOLOGOUS IDENTIFYING ANTIGEN(S)
O Group A	*S. paratyphi* A var. Durazzo, Factors 2, 12	2
O Group B	*S. typhimurium* Factors 1, 4, [5], 12	4, 5
O Group C_1	*S. choleraesuis* factors 6, 7	7
O Group C_2	*S. newport* factors 6, 8	8
O Group D	*S. gallinarum* factors 1, 9, 12	9
O Group E_1	*S. anatum* factors 3, 10	10
O Group E_2	*S. newington* factors 3, 15	15
O Group E_4	*S. senftenberg* factors 1, 3, 19	19
O Group F	*S. rubislaw* factor 11	11
O Group G_1	*S. poona* factors [1], 13, 22, [36], [37]	22
O Group H	*S. carrau* factors 6, 14, 24	14
O Group I	*S. hvittingfoss* factor 16	16
Vi	*Citrobacter ballerup* O29	Vi

Note: Brackets [ ] indicate that the antigen may be absent.

Underlining indicates that the O antigen has been lysogenized in that strain.

These antigen suspensions are ready to use. QC Antigens Salmonella O are preserved with 0.5% phenol USP; QC Antigen Salmonella Vi contains 0.01% Thimerosal. When used as described, each vial of QC Antigen Salmonella has sufficient reagent for 20 slide tests.

Febrile Negative Control is a lyophilized standard protein solution, containing approximately 0.04% Thimerosal as a preservative. When used as described, each vial of Febrile Negative Control has sufficient reagent for 100 slide tests.

Precautions

1. For In Vitro Diagnostic use.
2. **QC Antigens Salmonella**
   The Packaging of This Product Contains Dry Natural Rubber.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.
4. QC Antigens Salmonella are not to be used for immunization of humans or animals.

Storage

Store QC Antigens Salmonella at 2-8°C.

Store lyophilized and rehydrated Febrile Negative Control at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

QC Antigens Salmonella
Febrile Negative Control

Materials Required But Not Provided

Agglutination slides
Applicator sticks
Sterile distilled or deionized water

Reagent Preparation

QC Antigens Salmonella are ready to use.

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergents.

Febrile Negative Control: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

Before using QC Antigens Salmonella, examine the Salmonella antisera (Poly, Group or Factor) chosen for use. The antisera must meet all product specifications.

Test Procedure

1. **Positive control**: Dispense 1 drop (35 Fl) of the Salmonella Antiserum to be tested on an agglutination slide. Add 1 drop of the QC Antigen Salmonella chosen as the positive control and mix thoroughly.
2. **Negative control**: Dispense 1 drop of Febrile Negative Control on the agglutination slide. Add 1 drop of the QC Antigen Salmonella chosen as the positive control and mix thoroughly.
3. Rotate the slide for 1 minute and read for agglutination. Results must be read within 1 minute.

Results

1. Read and record results as follows.
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. **Positive control**: Should show 3+ or greater agglutination.
3. **Negative control**: Should show no agglutination. Rarely, a +/- reaction is possible.

Limitations of the Procedure

1. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent a smooth suspension of the microorganism or cause evaporation or precipitation of the test mixture. False-positive reactions may occur.
2. QC Antigens Salmonella will react with their corresponding homologous Salmonella polyvalent, group or factor antiserum. Some single factor antisera may give weaker reactions than polyvalent or grouping antisera due to specificity of the single factor antiserum for the identifying antigen(s).
3. The density of QC Antigens Salmonella is adjusted so that they give negative reactions with heterologous Salmonella Group Antisera. However, an antiserum may have a particular avidity for a given factor and agglutinate with another QC Antigen Salmonella having the common factor. For example, Salmonella O Group E_1 Antiserum Factors 3, 10 will agglutinate QC Antigen Salmonella O Group E_1 (3, 10) but may also agglutinate QC Antigen Salmonella O Group E_2 (3, 15) if the antiserum is of high titer for factor 3.
4. Exposure to temperatures below 2°C can result in autoagglutination. Antigens must be smooth, uniform suspensions. Examine the antigen vial for agglutination before use. Suspensions with agglutination are not usable and should be discarded.

References

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Packaging

QC Antigen Salmonella O Group A	1 ml	2130-50
QC Antigen Salmonella O Group B	1 ml	2131-50
QC Antigen Salmonella O Group C_1	1 ml	2132-50
QC Antigen Salmonella O Group C_2	1 ml	2133-50
QC Antigen Salmonella O Group D	1 ml	2134-50
QC Antigen Salmonella O Group E_1	1 ml	2135-50
QC Antigen Salmonella O Group E_2	1 ml	2136-50
QC Antigen Salmonella O Group E_4	1 ml	2137-50
QC Antigen Salmonella O Group F	1 ml	2138-50
QC Antigen Salmonella O Group G_1	1 ml	2139-50
QC Antigen Salmonella O Group H	1 ml	2140-50
QC Antigen Salmonella O Group I	1 ml	2141-50
QC Antigen Salmonella Vi	1 ml	2142-50
Febrile Negative Control	5 ml	3239-56

Bacto® QC Antigens Shigella

QC Antigen Shigella Group A · QC Antigen Shigella Group A_1 QC Antigen Shigella Group B · QC Antigen Shigella Group C QC Antigen Shigella Group C_1 · QC Antigen Shigella Group C_2 QC Antigen Shigella Group D · QC Antigen Alkalescens-Dispar Group 1

Intended Use

Bacto QC Antigens Shigella and Bacto QC Antigen Alkalescens-Dispar Group 1 are used in the quality control testing of Shigella Antisera Poly and Alkalescens-Dispar Antiserum Poly by the slide agglutination test.

User Quality Control

Identity Specifications

QC Antigens Shigella

Appearance: Light gray to white suspension.

QC Antigen Alkalescens-Dispar Group 1

Appearance: Light gray to white suspension.

Febrile Negative Control

Lyophilized appearance: Colorless to light gold, button to powdered cake.

Rehydrated appearance: Colorless to light gold, clear liquid.

Performance Response

Rehydrate Shigella Antiserum Poly and Alkalescens-Dispar Antiserum Poly per label directions. Perform the slide agglutination test using an appropriate QC Antigens Shigella or Alkalescens-Dispar Group 1.

The following chart lists the QC Antigens Shigella or QC Antigen Alkalescens-Dispar Group 1 recommended as the homologous (positive) control antigen. The homologous control antigen has certain identifying antigen(s) in common with the antiserum.

ANTISERUM	QC ANTIGEN HOMOLOGOUS CONTROL
Shigella Antiserum Poly Group A	Shigella Group A
Shigella Antiserum Poly Group A_1	Shigella Group A_1
Shigella Antiserum Poly Group B	Shigella Group B
Shigella Antiserum Poly Group C	Shigella Group C
Shigella Antiserum Poly Group C_1	Shigella Group C_1
Shigella Antiserum Poly Group C_2	Shigella Group C_2
Shigella Antiserum Poly Group D	Shigella Group D
Alkalescens-Dispar Antiserum Poly	Alkalescens-Dispar Group 1

Summary and Explanation

Shigella species cause the diarrheal disease known as shigellosis (classic bacillary dysentery) in humans. The range of illness is from mild diarrhea to severe dysentery characterized by abdominal cramps and frequent passage of bloody, mucoid stools. While the disease is usually self-limiting, it can be life threatening to the young, the elderly and malnourished persons. *Shigella* species are carried primarily in humans and are not generally distributed in nature. While transmission is usually direct person-to-person or through contaminated water supplies, food borne outbreaks do occur.

The genus *Shigella* belongs to the family Enterobacteriaceae. *Shigella* are facultatively anaerobic, gram-negative bacilli that typically are oxidase negative, lactose negative, H_2S negative and do not produce gas. *Shigella* and *Escherichia* are genetically related. Certain strains of *E. coli* may resemble *Shigella* biochemically because both can be lactose negative, nonmotile and non-gas-producing. These anaerogenic, nonmotile types have historically been called the Alkalescens-Dispar group and are presently classified as *E. coli*.[1-6]

Serological testing with polyvalent and group specific antisera should be used to confirm the identification of isolates that are morphologically and biochemically identified as *Shigella* species. *Shigella* are nonmotile, so serological identification is based on somatic ("O") antigens. However, some strains have envelope antigens that prevent agglutination in somatic antisera. Heating the suspension at 100°C for 15-60 minutes destroys these interfering antigens. The four named species or serotypes of *Shigella* are *S. dysenteriae* (10 serovars), *S. flexneri* (six serovars), *S. boydii* (15 serovars) and *S. sonnei*. For a complete and current explanation of the classification of *Shigella*, consult appropriate references.[1]

Shigella Antisera Poly and Alkalescens-Dispar Antiserum Poly are used in the serological identification of *Shigella* species and the Alkalescens-Dispar (A-D) Group. QC Antigens Shigella and Alkalescens-Dispar Group 1 are designed as positive controls for testing the efficacy of the *Shigella* grouping antisera used in laboratory procedures.

QC Antigens Shigella and QC Antigen Alkalescens-Dispar Group 1 may also be used as negative controls by using a heterologous antigen (possessing no common antigen) with a given test serum. However, cross reactivity may occur. Consult appropriate references for further details on cross reactivity.[1]

Principles of the Procedure

Serological procedures that confirm the identification of an organism are usually agglutination reactions. Agglutination reactions may be either homologous or heterologous. Homologous reactions occur between a microorganism (antigen) and the corresponding antibody. These reactions occur rapidly and are strong. Heterologous reactions occur when a microorganism (antigen) reacts with an antibody produced in response to another species or serotype. These reactions occur slowly and are weak.

Heterologous reactions may be unexpected and unpredictable and may lead to confusion in serological identification. Therefore, only strongly positive homologous agglutination reactions should be regarded as significant.

Reagents

QC ANTIGEN	ORGANISM IDENTITY
Shigella Group A	*Shigella dysenteriae* type 1
Shigella Group A_1	*Shigella dysenteriae* type 8
Shigella Group B	*Shigella flexneri* type 6
Shigella Group C	*Shigella boydii* type 3
Shigella Group C_1	*Shigella boydii* type 8
Shigella Group C_2	*Shigella boydii* type 12
Shigella Group D	*Shigella sonnei*
Alkalescens-Dispar Group 1	*E. coli* A-D Group 1

QC Antigens Shigella and QC Antigen Alkalescens-Dispar Group 1 contain killed whole organisms preserved in 0.5% formaldehyde. They are ready to use.

When used as described, each vial of antigen contains sufficient reagent for 20 slide tests.

Precautions

1. For In Vitro Diagnostic Use.
2. **QC Antigen Shigella Group A**
   QC Antigen Shigella Group A_1
   QC Antigen Shigella Group B
   QC Antigen Shigella Group C
   QC Antigen Shigella Group C_1
   QC Antigen Shigella Group C_2
   QC Antigen Shigella Group D
   QC Antigen Alkalescens-Dispar Group 1

   POSSIBLE RISK OF IRREVERSIBLE EFFECTS. (US) Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Eyes, Kidneys, Lungs, Skin.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.

   The Packaging of This Product Contains Dry Natural Rubber.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.
4. QC Antigens Shigella and QC Antigen Alkalescens-Dispar Group 1 are not to be used for immunization of humans or animals.

Storage

Store QC Antigens Shigella and QC Antigen Alkalescens-Dispar Group 1 at 2-8°C. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

QC Antigens Shigella
QC Antigen Alkalescens-Dispar Group 1

Materials Required But Not Provided

Febrile Negative Control
Agglutination slides
Applicator sticks
Sterile 0.85% NaCl solution

Reagent Preparation

QC Antigens Shigella and QC Antigen Alkalescens-Dispar Group 1 are ready to use.

Equilibrate all materials to room temperature prior to performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergents.

Test Procedure

1. **Positive control**: Dispense 1 drop (35 µl) of the Shigella Antiserum or Alkalescens-Dispar Antiserum Poly to be tested on an agglutination slide. Add 1 drop of the appropriate QC Antigen Shigella or QC Antigen Alkalescens-Dispar Group 1 chosen as the positive control and mix thoroughly.
2. **Negative control**: Dispense 1 drop of sterile 0.85% NaCl solution or Febrile Negative Control on the agglutination slide. Add 1 drop of the appropriate QC Antigen Shigella or QC Antigen Alkalescens-Dispar Group 1 and mix thoroughly.
3. Rotate the slide for 1 minute and read for agglutination.

Results

1. Read and record results as follows:
   4+ 100% agglutination; background is clear to slightly hazy.
   3+ 75% agglutination; background is slightly cloudy.
   2+ 50% agglutination; background is moderately cloudy.
   1+ 25% agglutination; background is cloudy.
   – No agglutination.
2. **Positive control**: Should show 3+ or greater agglutination.
3. **Negative control**: Should show no agglutination. Rough reactions can occur. If so, repeat the test using Febrile Negative Control.

Limitations of the Procedure

1. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent a smooth suspension of the microorganism or may cause evaporation or precipitation of the test mixture. False-positive reactions can occur.
2. Exposure to temperatures below 2°C can cause autoagglutination. Antigens must be smooth uniform suspensions. Examine antigen vials for agglutination before use. Suspensions with agglutination are not usable and should be discarded.
3. Allow the QC Antigens, the antisera and all equipment used to be at room temperature at the time of testing. The test reagents, if cold, may cause false-negative reactions.
4. Shake the antigen well before use to suspend the organisms.

References

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5. **Andrews, W. H., G. A. June, and P. S. Sherrod.** 1995. Shigella, p. 6.01-6.06. *In* FDA Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **Smith, J. L.** 1992. *Shigella*, p. 423-431. *In* C. Vanderzant and D. F. Splittstoesser (eds.), Compendium of methods for the microbiological examination of foods, 3rd ed. American Public Health Association, Washington, D.C.

Packaging

QC Antigen Shigella Group A	1 ml	2100-50
QC Antigen Shigella Group A_1	1 ml	2101-50
QC Antigen Shigella Group B	1 ml	2102-50
QC Antigen Shigella Group C	1 ml	2103-50
QC Antigen Shigella Group C_1	1 ml	2104-50
QC Antigen Shigella Group C_2	1 ml	2105-50
QC Antigen Shigella Group D	1 ml	2106-50
QC Antigen Alkalescens-Dispar Group 1	1 ml	2116-50
Febrile Negative Control	5 ml	3239-56

Bacto® Salmonella Antisera

Salmonella O Antisera · Salmonella H Antisera · Salmonella H Antisera Spicer-Edwards

Intended Use

Bacto Salmonella O Antisera are used in agglutination tests for the identification of *Salmonella* by somatic (O) antigens.

Bacto Salmonella H Antisera are used in tube agglutination tests for the identification of *Salmonella* by flagellar (H) antigens.

User Quality Control

Identity Specifications

Salmonella O Antisera

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated appearance: Light gold to amber, clear liquid.

Salmonella H Antisera

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated appearance: Light gold to amber, clear liquid.

continued on following page

Bacto Salmonella H Antisera Spicer-Edwards are used in tube agglutination tests for screening and identifying the most commonly encountered salmonellae by flagellar (H) antigens.

Summary and Explanation

Salmonella species cause a variety of human diseases called salmonelloses. The range of disease is from mild self-limiting gastroenteritis to more severe forms, possibly with bacteremia or typhoid fever, which can be life-threatening. Severe disease and bacteremia are associated primarily with three serovars of *S. enterica* subsp. *enterica* (Cholerasuis, Paratyphi A and Typhi) while most of the other 2,300 or more strains are associated with gastroenteritis. The severity of the diarrheal disease depends upon the virulence of the strain and the condition of the human host.

Salmonella is found in nature and occurs in the intestinal tract of many animals, both wild and domestic. The microorganism can spread to man from contact with the environment or from eating meat or vegetable food products.

User Quality Control cont.

Performance Response

Rehydrate Salmonella O, Salmonella Vi, and Salmonella H Antisera per label directions. Perform the slide agglutination test using appropriate Salmonella O and Vi Antisera and QC Antigens Salmonella O Groups A -I and Vi.

The chart below includes the QC Antigens Salmonella O recommended as homologous (positive) control antigens. (The homologous control antigen has certain identifying antigen(s) in common with the antiserum.) For a negative (heterologous) antigen control, use a QC Antigen Salmonella containing antigens unrelated to those in the homologous control.

Homologous control: Should show 3+ or greater agglutination.

Negative control: Should show no agglutination. Rarely, a +/– reaction is possible.

For Salmonella H Antisera, maintain stock cultures of known serological identification, and prepare antigen positive and negative controls by using known serotypes and following the procedure described above in Tube Test Preparation.

SALMONELLA O ANTISERUM	QC ANTIGEN SALMONELLA O HOMOLOGOUS CONTROL
Poly A-I & Vi	Groups A,B,C_1,C_2,D,E_1,E_2, E_4,F,G_1,H,I,Vi
Poly A	Groups A,B,D,E_1,E_2,E_4
Poly B	Groups C_1,C_2,F,G_1,H
Poly C	Group I
Group A Factors 1,2,12	Group A
Group B Factors 1,4,5,12	Group B
Group B Factors 1,4,12,27	Group B
Group C1 Factors 6,7	Group C_1
Group C2 Factors 6,8	Group C_2
Group D1 Factors 1,9,12	Group D
Group E Factors 1,3,10,15,19,34	Groups E_1,E_2,E_4
Group E1 Factors 3,10	Group E_1
Group E2 Factors 3,15	Group E_2
Group E4 Factors 1,3,19	Group E_4
Group F Factor 11	Group F
Group G Factors 13,22,23,(36),(37)	Group G_1
Group G1 Factors 13,22,(36),(37)	Group G_1
Group H Factors 1,6,14,24,25	Group H
Group I Factor 16	Group I
Vi	Group Vi
Factor 2	Group A
Factor 4	Group B
Factors 4,5	Group B
Factor 5	Group B
Factor 7	Group C_1
Factor 8	Group C_2
Factor 9	Group D
Factor 10	Group E_1
Factor 15	Group E_2
Factor 19	Group E_4
Factor 22	Group G_1
Factor 14	Group H

Note: Parentheses ( ) enclosing the designation for an antigen indicate that the antigen is poorly developed or agglutinates weakly agglutinates. For a complete and current explanation of the classification of *Salmonella*, consult appropriate references.[1,2,3,8,11]

Table 1. Differentiation of the genus Salmonella from other genera.[1]

Test	*Salmonella*	*Citrobacter amolonaticus*	*Citrobacter diversus*	*Citrobacter freundii*	*Edwardsiella*
Indole production	–	+	+	–	d
Citrate, Simmons	+	[+]	+	+	–
H_2S production	+	–	–	[+]	d
Urea hydrolysis	–	[+]	d	d	–
Lysine decarboxylase	+	–	–	–	+
Ornithine decarboxylase	+	+	+	[–]	[+]
D-Adonitol, acid production	–	–	+	–	–
L-Arobinose, acid production	+	+	+	+	–
L-Rhamnose, acid production	+	+	+	+	–
D-Sorbitol, acid production	+	+	+	+	–
D-Xylose, acid production	+	+	+	+	–
Acetate utilization	+	[+]	[+]	[+]	–

\+ 90-100% positive
[+] 76-89% positive
d 26-75% positive
[–] 11-25% positive
– 0-10% positive

All *Salmonella* serovars belong to two species: *S. bongori*, which contains 18 serovars, and *S. enterica*, which contains the remaining 2,300 or more serovars divided among six subspecies.[2,3]

The six subspecies of *S. enterica* are:

S. enterica subsp. *enterica* (I or 1)
S. enterica subsp. *salamae* (II or 2)
S. enterica subsp. *arizonae* (IIIa or 3a)
S. enterica subsp. *diarizonae* (IIIb or 3b)
S. enterica subsp. *houtenae* (IV or 4)
S. enterica subsp. *indica* (VI or 6)

(The legitimate species name for *S. enterica* is *S. choleraesuis*. However, this name may be confused with the serotype named "choleraesuis." At the International Congress for Microbiology in 1986, the International Subcommittee for *Enterobacteriaceae* agreed to adopt the species name, *S. enterica*.[4] LeMinor and Popoff[5] published a request to the Judicial Commission to use *S. enterica* as a species name. The Commission ruled that *S. choleraesuis* is the legitimate name.[6,7] *S. enterica* is used in many countries and is favorably accepted as the species name.[8,9] The Centers for Disease Control has adopted this designation until the problem of naming this species is resolved.[2])

Nomenclature and classification of these bacteria are constantly changing.[1] *Salmonella* and the former *Arizona* should be considered a single genus, *Salmonella*.[9] It is recommended that laboratories report the names of *Salmonella* serovars for the subspecies *enterica*. The serovar names are no longer italicized and the first letter is capitalized. For example, the strain that used to be identified as *Salmonella typhimurium* is now known as *Salmonella* Typhimurium.

Serovars of other subspecies of *S. enterica* (except some in the subspecies *salamae* and *houtenae*) and those of *S. bongori* are not named and are designated by their antigenic formula. For the most recent information on nomenclature, consult appropriate references.[1,7,10-13]

Results are for 48-hours incubation. Tests were performed at 35-37°C.

Serotypes of *Salmonella* are defined based on the antigenic structure of both somatic or cell wall (O) antigens and flagellar (H) antigens. The antigenic formula lists the O antigen(s) first, followed by the H antigen(s). The major antigens are separated by colons and the components of the antigens separated by commas. For example, the antigenic formula for *Salmonella* Typhimurium is *Salmonella* 1,4,5,12:i:1,2. This means that the strain has O antigen factors 1,4,5 and 12, the flagella phase 1 antigen i, and flagella phase 2 antigens 1 and 2.

Complete identification of *Salmonella* requires cultural isolation, biochemical characterization and serotyping. However well-defined the serology of *Salmonella*, the use of serological procedures does not supersede cultural isolation and biochemical characterization. Any serological results obtained before biochemical identification must be considered as presumptive identification only. Consult to appropriate references for complete identification of *Salmonella*.[1,2,3,8,11-14]

Characterizing the Serotypes of *Salmonella*

Salmonella O Antigens: The somatic (O) heat-stable antigens are identified first. The O antigens are numbered 1-67 using Arabic numerals. The numbers are not completely continuous because certain strains were reclassified to other genera and the antigenic Arabic numbers were deleted from the schema.

Table 2a. Differentiation of *Salmonella* species, subspecies, and some serovars.[1,2]

Test	*Salmonella enterica*					
	Salmonella bongori	subsp. *arizonae*	subsp. *enterica*	subsp. *diazonae*	subsp. *houtenae*	subsp. *indica*
Citrate, Simmons	+	+	+	+	+	[+]
H_2S production	+	+	+	+	+	+
Lysine decarboxylase	+	+	+	+	+	+
Ornithine decarboxylase	+	+	+	+	+	+
Motility	+	+	+	+	+	+
KCN, growth	+	–	–	–	+	–
Malonate utilization	–	+	–	+	–	–
D-Glucose, gas	[+]	+	+	+	+	+
L-arobinose, acid	+	+	+	+	+	+
Dulcitol, acid	+	–	+	–	–	d
Lactose, acid	–	[–]	–	[+]	–	[–]
Maltose, acid	+	+	+	+	+	+
Melibiose, acid	[+]	+	+	+	+	[+]
L-Rhamnose, acid	+	+	+	+	+	+
D-Sorbitol	+	+	+	+	+	–
Trehalose, acid	+	+	+	+	+	+
D-Xylose, acid	+	+	+	+	+	+
Mucate, acid	+	+	+	d	–	+
Tartrate, Jordans	–	–	+	[–]	d	+
ONPG	+	+	–	+	–	D

Table 2b. Differentiation of *Salmonella* species, subspecies and some serovars.[1,2]

Test	*Salmonella enterica*					
	subsp. *salamae*	subsp. *enterica* serovar Choleraesuis	serovar Gallinarum	Serovar Paratyphi A	serovar Pullorum	Serovar Typhi
Citrate, Simmons	+	[–]	–	–	–	–
H_2S production	+	d	+	–	+	+
Lysine decarboxylase	+	+	+	–	+	+
Ornithine decarboxylase	+	+	–	+	+	–
Motility	+	+	–	+	–	+
KCN, growth	–	–	–	–	–	–
Malonate utilization	+	–	–	–	–	–
D-Glucose, gas	+	+	–	+	+	–
L-arobinose, acid	+	–	[+]	+	+	–
Dulcitol, acid	+	–	+	+	–	–
Lactose, acid	–	–	–	–	–	–
Maltose, acid	+	+	+	+	–	+
Melibiose, acid	–	d	–	+	–	+
L-Rhamnose, acid	+	+	–	+	+	–
D-Sorbitol	+	[+]	–	+	[–]	+
Trehalose, acid	+	–	d	+	[+]	+
D-Xylose, acid	+	+	d	–	[+]	[+]
Mucate, acid	+	–	d	–	–	–
Tartrate, Jordans	d	[+]	+	–	–	+
ONPG	[–]	–	–	–	–	–

\+ 90-100% positive
[+] 76-89% positive
d 26-75% positive
[–] 11-25% positive
0 10% positive

Serogroups represent the organization of *Salmonella* strains based on the antigen(s) shared in common and are designated by the letters A-Z. After exhausting the alphabet, the serogroups were numbered beginning with No. 51 (the serogroup Z organism having antigen No. 50). While one somatic antigen identifies each serogroup, certain other antigens may be shared among several serogroups.

Most organisms contain antigens in common that will cause cross-reactions in an unabsorbed or "partially absorbed" antiserum. One somatic antigen identifies a serogroup and is shared in common by all members of a given serogroup. For example, serogroup A is represented by three members, *Salmonella* Paratyphi A (somatic antigens 1,2,12), *Salmonella* Kiel (somatic antigens 1,2,12), and *Salmonella* Nitra (somatic antigens 2,12). All three members of this serogroup contain antigens 2 and 12 in common. Serogroup B is represented by many organisms consisting having different combinations of somatic antigens 1,4,5,12 and 27. Serogroup D organisms contain somatic antigens 1,9,12, etc.

In the above example, all three serogroups A, B, and D contain antigens 1 and 12. An antiserum prepared from a 1,2,12 culture, if not absorbed, will react with cultures of serogroups B and D in varying degrees depending on the concentration of the commonly shared 1 and 12 factors. This must be taken into consideration when choosing an antiserum to be used in the examination of the salmonellae.

Several different antisera are available. Some represent group antigens. Others are single factor sera, which should be used when testing for an identifiable antigen in a given serogroup. Such a single factor serum is not called a "group" serum, though it contains the group identifiable agglutinin. (It has been recommended by the CDC that the term "group" be applied only to those sera possessing all the major agglutinins found in that group.)

In unabsorbed antisera, cross-reactions occur if strains sharing some "like" antigens are tested, even when they are in separate serogroups based on the major group antigen(s) they possess. Unabsorbed antisera are available as group antisera containing all factors in that group.

In absorbed antisera, cross reactions are less likely and are weaker. Absorbed antisera are available as factor specific antisera.

Flagellar *Salmonella* H Antigens: The flagellar (H) antigens are heat labile and are usually associated with motility. Cultures are ordinarily flagellated and actively motile, although flagellated cultures can be nonmotile. H antigen characterization is done after the serogroup of the strain is determined. The H antigens of *Salmonella* are designated by letters of the alphabet, a-z, followed by z, z_1, z_2, etc., and by Arabic numerals. H antigens exist in 2 phases, phase 1 and phase 2. Phase 1 antigens are expressed in letters a-z, etc., and the phase 2 antigens are most often expressed in Arabic numerals. Older cultures may express both phases of a diphasic serotype, but recent clinical isolates more often express only one phase. Phase reversal may be necessary to isolate both phases of a diphasic culture. Consult an appropriate reference for more detailed information.[8]

A pure H antiserum cannot be prepared without some somatic content. However, since H antigens are highly antigenic, the serum derived from motile cultures may be used at a dilution that reduces somatic agglutination below the detection level.

***Salmonella* Vi Antigen**: The Vi Antigen is a heat-labile envelope antigen that may surround a cell wall and mask somatic antigen activity. Microorganisms having the Vi Antigen will not agglutinate in O antisera.

Using Salmonella Antisera

Salmonella O Antisera: The recommended serological Identification scheme begins with Salmonella O Antisera Poly A through Poly G, which contain the following:

SALMONELLA POLY GROUP ANTISERA	SOMATIC GROUPS PRESENT
Bacto Salmonella O Antiserum Poly A	A,B,D,E_1,(E_2,E_3),*E_4,L
Bacto Salmonella O Antiserum Poly B	C_1,C_2,F,G,H
Bacto Salmonella O Antiserum Poly C	I,J,K,M,N,O
Bacto Salmonella O Antiserum Poly D	P,Q,R,S,T,U
Bacto Salmonella O Antiserum Poly E	V,W,X,Y,Z
Bacto Salmonella O Antiserum Poly F	51–55
Bacto Salmonella O Antiserum Poly G	56–61

*Strains of groups E_2 and E_3 are lysogenized by phage 15, then by phage 34. These strains are now classified into group E_1.[3]

If agglutination occurs, use individual Salmonella O Group Factor Antisera to determine the specific serogroup to which the isolate belongs. For efficiency, test first with individual Salmonella O Group Factor Antisera.

If agglutination does not occur with Poly A or B, test the isolate with Salmonella O Antiserum Vi. If positive, heat and retest. If agglutination does not occur with Salmonella O Antiserum Vi, the isolate is not likely to be *Salmonella*. Results should be examined. If questions exist, the isolate should be sent to a reference laboratory.

If agglutination does not occur with Poly C, D, E, F, and G, the isolate is not likely to be *Salmonella*.

Table 3. Schema for using Salmonella O Antisera Poly Groups A, B, C, D, E, F and G.

Test with	Salmonella O antisera Poly Groups A, B, C, D, E, F and G			
Test Result	+	– with Poly A or B		– with Poly C, D, E, F and G
Test with	Individual Salmonella O Antisera	Vi Antiserum		
Test Result	+ with one Salmonella O Antiserum (required)	+	–	
Test Conclusion or Next Action	Determine the *Salmonella* H Antigen	Heat and retest with individual Salmonella O Antisera	Test isolate is not a *Salmonella*	Test isolate is not a *Salmonella*

Salmonella O Antiserum Poly A-I and Vi: This antiserum detects factors 1-16, 19, 22-25, 34 and Vi. This combination of factors represents the most frequently isolated Groups A-I and the Vi antigens and is used to screen possible *Salmonella* isolates.

A positive reaction indicates that further serological testing is needed to identify the isolate using Salmonella O Group Factor Antisera. The most common serogroups are B, D and C_1. For efficiency, first use the Salmonella O Group Factor Antisera for these serogroups.

If the isolate is positive with Salmonella O Antiserum Poly A-I and Vi but negative with Poly A-Poly G, test the isolate with Salmonella Vi Antiserum. If positive with Salmonella Vi Antiserum, heat and retest using individual Salmonella O Antisera. If negative with Salmonella

Vi Antiserum, the isolate is not likely to be Salmonella. Results should be examined. If questions exist, the isolate should be sent to a reference laboratory.

A negative reaction indicates the isolate is not in serogroups A-I. If the biochemical reactions are consistent with *Salmonella*, a serogroup other than A-I is possible. Further testing with antisera for other serogroup antigens is necessary.

Table 4. Schema for using Salmonella O Antiserum Poly A-I & Vi.

Test with	Salmonella O Antisera Poly A-I and Vi			
Test Result	+			–
Test with	Individual Salmonella O Antisera			
Test Result	+	–		
Test with		Salmonella Vi Antiserum		
Test Result		+	–	
Test Conclusion or Next Action	Determine the Salmonella H Antigen	Heat and retest with individual Salmonella O Antisera	Test isolate is not a *Salmonella*	*May* be a *Salmonella* detectable by use of Salmonella O Antisera Poly C, D, E, F or G

Salmonella O Group Factor Antisera and Single Factor Antisera: Use selected Salmonella O Group Factor Antisera. Cross reactions may occur between serogroups that share O antigens. Consider this partial list of Salmonella O Group Factor Antisera as an example:

Salmonella O Antiserum Group A Factors 1, 2, 12

Salmonella O Antiserum Group B Factors 1, 4, 5, 12

Salmonella O Antiserum Group B Factors 1, 4, 12, 27

Factors 1 and 12 occur in combination with other antigens and may cause cross-reactions. The strength of the reactions will help in interpretation. Rapidly forming 3+ or greater agglutination indicates a homologous reactions.

Use selected Salmonella O Factor Antisera. Absorbed antisera specific for an identifiable antigen in a given serogroup is used to identify the isolate further. In the example above, Salmonella O Factor Antisera could be used:

Salmonella O Antiserum Factor 2

Salmonella O Antiserum Factor 4

Salmonella O Antiserum Factors 4, 5

Salmonella O antiserum Factor 5

Polyvalent Salmonella H Antisera: Further identification of a *Salmonella* isolate includes the characterization of the flagellar antigens. Agglutination with the following Polyvalent H Antisera can be done:

SALMONELLA POLY GROUP ANTISERA	FLAGELLAR ANTIGENS PRESENT
Salmonella H Antiserum Poly a-z	Groups EN,G,L,Z_4, 1 complexes and a-k,r-z,z_6,z_{10},z_{29}
Salmonella H Antiserum Poly A	Groups a,b,c,d,i,z_{10},z_{29}
Salmonella H Antiserum Poly B	Groups eh,en,enx,enz$_{15}$, G complex
Salmonella H Antiserum Poly C	Groups k,l,r,y,z,z_4
Salmonella H Antiserum Poly D	Groups z_{35},z_{36},z_{37},z_{38},z_{39},z_{41},z_{42}
Salmonella H Antiserum Poly E	1 complex z_6

Absorbed H antisera specific for single antigens or a complex of antigens can be used to identify the isolate further.

Unabsorbed and Absorbed Salmonella H Antisera: Complete identification of a *Salmonella* isolate involves analysis of phase 1 and phase 2 antigens using H antisera. For the complex pattern of analysis and procedures, consult appropriate references.[8]

Salmonella H Antisera Spicer-Edwards: Salmonella H Antisera Spicer-Edwards is used for screening and identifying the most commonly encountered *Salmonella* using a combination of polyvalent and single complex antisera.

Table 5. Identification of *Salmonella* H using Salmonella H Antisera Spicer-Edwards.

	Salmonella H Antisera Spicer-Edwards			
H Antigen(s)	**1**	**2**	**3**	**4**
a	+	+	+	–
b	+	+	–	+
c	+	+	–	–
d	+	–	+	+
e,h	+	+	+	–
G Complex*	+	–	–	+
i	+	–	–	–
k	–	+	+	+
r	–	+	–	+
y	–	+	–	–
z	–	–	+	+
Z_4 Complex**	–	–	+	–
z_{10}	–	–	–	+
z_{29}	–	+	+	–

Table 6. Identification of *Salmonella* H using Salmonella H Antisera.

H Antigen(s)	Salmonella H Antisera
e,n,x, e,n,z_{15}	EN Complex
I,v I,w I,z_{13} I,z_{28}	L Complex
1,2 1,5 1,6 1,7	1 Complex

* The G complex component of Salmonella H Antisera Spicer-Edwards 1 and 4 reacts with antigens f,g; f,g,s; f,g,t; g,m; g,m,q; g,m,s; g,m,s,t; g,m,t; g,p; g,p,s; g,p,u; g,q; g,s,t; g,t; m,p,t,u; and m,t.

** The Z_4 Complex component reacts with z_4,z_{23}; z_4,z_{24}; and z_4, z_{32}.

Note that no antigen is positive with all four Salmonella H Antisera Spicer-Edwards Any antigen that reacts with all four sera should be checked for smoothness.

Extent of Serological Identification Necessary

Complete serological characterization of *Salmonella* is not required for successful detection of the microorganism when it occurs as a pathogen. The use of adequate isolation procedures and differential biochemical tests is of primary importance. Possible *Salmonella* isolates can be presumptively identified with a minimum of serological identification. Isolates can be sent to laboratories that perform the level of testing necessary to completely identify the microorganism.

For a further discussion of the serological identification of *Salmonella*, consult appropriate references.[1,2,3,8,11]

Principles of the Procedure

Identification of *Salmonella* species includes both biochemical and serological identification. Serological confirmation involves the procedure in which the microorganism (antigen), reacts with its corresponding antibody. This *in vitro* reaction produces macroscopic clumping called agglutination. The desired homologous reaction is rapid, does not dissociate (high avidity) and bonds strongly (high affinity).

Because a microorganism (antigen) may agglutinate with an antibody produced in response to another species, heterologous reactions are possible. These are characterized as weak in strength or slow in formation. Such unexpected and perhaps unpredictable reactions may lead to some confusion in serological identification. Therefore, a positive homologous agglutination reaction should support the morphological and biochemical identification of the microorganism.

Agglutination of the somatic antigen in the slide test appears as a firm granular clumping. Homologous reactions are rapid and strong (3+). Heterologous reactions are slow and weak.

Agglutination of the flagellar antigens in the tube test appears as a loose flocculation that can easily be resuspended.

Reagents

Salmonella O, H, and Vi Antisera are lyophilized, polyclonal rabbit antisera containing approximately 0.04% Thimerosal as a preservative.

Salmonella O Poly Antisera are polyvalent antisera. Each antiserum is specific for certain serogroup antigens. When properly rehydrated and used as recommended, each vial of Salmonella O or Vi Antisera contains sufficient reagent for 60 tests. Salmonella O Antisera Poly A-I and Vi is prepared with representative strains of these serogroups and is not absorbed. It may cross-react with other antisera because of shared common O antigens.

Salmonella O Group Antisera are specific for the major factors present in the serogroup. Salmonella O Factor Antisera are specific for the factors of the individual serogroups. When using Salmonella O Group Antisera, cross-reactions are possible because serogroups may share non-major group antigens. Salmonella O Factor Antisera are absorbed as necessary to render each antiserum as specific as practical without reducing the homologous reactions to an unsatisfactory level.

Salmonella H Poly Antisera are polyvalent antisera. Each antiserum is specific for certain flagellar antigens. Each vial of Salmonella H Antiserum contains sufficient reagent to perform between 150-1500 tests, depending on the antiserum used. Salmonella H Antisera are either absorbed or unabsorbed specifically for either phase 1 or phase 2 antigens. Salmonella H Antisera Spicer-Edwards are pooled, polyvalent antisera and additional adjunctive antisera to identify the more commonly occurring H antigens.

Precautions

1. For In Vitro Diagnostic Use.
2. The Packaging of This Product Contains Dry Natural Rubber.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated Salmonella O, H and Vi antisera at 2-8°C.

Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products. Discard any antiserum that becomes cloudy during storage.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Salmonella O Antisera
Salmonella H Antisera
Salmonella Vi Antiserum
(See Packaging.)

Materials Required But Not Provided

Slide Test

0.85% NaCl solution, sterile
Agglutination slides with 1 inch squares
Applicator sticks
Boiling waterbath
Centrifuge
QC Antigens Salmonella

Tube Test

0.85% NaCl solution, sterile
Culture tubes, 12 x 75 mm, and rack
Waterbath, 50 ± 2°C
Serological pipettes, 1 ml
Formaldehyde

Reagent Preparation

Equilibrate all materials to room temperature prior to performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergents.

Salmonella O, H and Vi Antisera: To rehydrate, add 3 ml of sterile 0.85% NaCl solution and rotate gently to completely dissolve the contents. Rehydrated antisera are considered a 1:2 dilution. Subsequent Salmonella O Antisera dilutions are based on this as a starting dilution. The H antisera are further diluted for use.

Specimen Collection and Preparation

Clinical specimens: *Salmonella* can be recovered from selective differential media such as Hektoen Enteric Agar or XLD agar. For specific recommendations, consult appropriate references.[11,12] Determine that a pure culture of the microorganism has been obtained and

that biochemical test reactions are consistent with the identification of the organism as a *Salmonella* species. After these criteria are met, serological identification can be performed.

Food samples: *Salmonella* can be recovered when samples are processed to recover injured microorganisms and prevent overgrowth of competing microorganisms. Consult appropriate references for recommended procedures for isolation of Salmonella from foods.[13,14] Determine that a pure culture of the microorganism has been obtained and that biochemical test reactions are consistent with the identification of the organism as a *Salmonella* species. After these criteria have been met, serological identification can be performed.

Slide Test Procedure

Salmonella O and Vi Antisera

Use this procedure to test the isolate with each selected antiserum.

1. **Salmonella Antiserum**: Add Dispense 1 drop (3 µl) of each antiserum to be tested on an agglutination slide.
2. **Negative control**: Dispense 1 drop of 0.85% sterile NaCl solution on an agglutination slide.
3. **Test isolate**: From a solid agar medium, transfer a portion of a loopful of an isolated colony to each of the two reaction areas above and mix thoroughly.
4. **Positive control**: Dispense 1 drop of each Salmonella O Antiserum to be tested on an agglutination slide. Add 1 drop of an appropriate QC Antigen Salmonella.
5. Rotate the slides for 1 minute and read for agglutination. Results must be read within 1 minute.

Slide Test Results

1. Read and record results as follows:

   4+ 100% agglutination; background is clear to slightly hazy.
   3+ 75% agglutination; background is slightly cloudy.
   2+ 50% agglutination; background is moderately cloudy.
   1+ 25% agglutination; background is cloudy.
   – No agglutination.

2. **Positive control**: Should show a 3+ or greater agglutination.
3. **Negative control**: Should show no agglutination. If agglutination occurs, the culture is rough and cannot be tested. Subculture to a non-inhibitory medium, incubate and test the organism again.
4. **Test isolates**: 3+ or greater agglutination is a positive result.
5. A partial (less than 3+) or delayed agglutination reaction should be considered negative.
6. If a positive reaction occurs with Salmonella Vi Antiserum, follow this procedure:
   - Prepare a dense suspension of the isolate from an agar medium in 3-5 ml of sterile 0.85% NaCl solution.
   - Heat in a boiling waterbath for 30-60 minutes and cool. The suspension should not precipitate after heating. If this occurs, select another colony for testing.
   - Centrifuge at 1,000 rpm for 10-15 minutes.
   - Aspirate and discard the supernatant.
   - Resuspend the sediment in 0.5 ml sterile 0.85% NaCl solution.
   - Retest a drop of the sediment with Salmonella O Group Antisera, as outlined.
7. If the heated culture continues to react with Salmonella Vi Antiserum and not with the Salmonella O Antisera, the isolate may not be *Salmonella*. Test the isolate further to determine if it is correctly identified.
8. If an H antigen identification is required, proceed to the next section.
9. When a negative reaction is obtained with Salmonella O Antiserum Poly A-I and Vi in the above procedure, the organism is presumptively negative for *Salmonella* that belong to serogroups A-I. Biochemical tests should be performed to confirm this negative result. If biochemical tests prove the organism to be a *Salmonella*, a serogroup beyond serogroup A-I is probably involved.

   If the organism reacts with Poly A-I and Vi but does not react with the specific somatic antisera, it should be checked with Salmonella Vi Antiserum by the above procedure.

Tube Test Preparation

1. **0.6% formalized saline**: Prepare by adding 6 ml formaldehyde per 1,000 ml of sterile 0.85% NaCl solution.
2. **Test organism**: It is often necessary to increase the motility of the test organism. To accomplish this, make several consecutive transfers in Motility GI Medium.
   - Inoculate the tube slightly below the surface of the medium using the stab method.
   - Incubate at 35-37°C for 18-20 hours.
   - Transfer only those organisms that have migrated to the bottom of the tube.
   - When the organism successfully travels 50-60 mm through the medium in 18-20 hours, it is ready for use.
   - An infusion broth such as Veal Infusion Broth is recommended for cultivating the motile *Salmonella* prior to testing. It should be inoculated and incubated at 35°C for 24 hours. Brain Heart Infusion Broth may be used with incubation at 35°C for 4-6 hours. If Tryptic Soy Broth is used, incubate at 35°C for 24 hours.
   - Prepare the test organism suspension by using equal volumes of broth culture and 0.6% formalized saline. The final density of this test suspension should be that of a McFarland Barium Sulfate Standard No. 3.
3. **Positive control**: Commercially prepared QC Salmonella H antigens are not available. The user must maintain stock cultures of known serological identification for use in quality control. Prepare the antigen by using known serotypes and following the procedure described above. (See Test organism, above.)
4. **Salmonella H Antisera**: Rehydrated antisera is considered a 1:2 working dilution. Prepare dilutions as follows and use on the day prepared. Discard any unused portion.
   - Most **Salmonella H Antisera**: A 1:1,000 final dilution is used. Prepare by adding 0.1 ml rehydrated antiserum to 25 ml of 0.85% NaCl solution.
   - **Salmonella H Antisera x, z_{13}, z_{15} and z_{28}**: A 1:500 final dilution is used. Prepare by adding 0.1 ml rehydrated antiserum to 12.5 ml of 0.85% NaCl solution.
   - **Salmonella H Antiserum Poly a-z**: A 1:100 final dilution is used. Prepare by adding 0.1 ml rehydrated antiserum to 2.5 ml of 0.85% NaCl solution.

Tube Test Procedure

Salmonella H Antiserum:

1. Prepare a 12 x 75 ml culture tube for each organism to be tested.
2. **Diluted antiserum**: Dispense Add 0.5 ml of diluted antiserum in each tube.
3. **Test isolate**: Add 0.5 ml to the appropriate tube.
4. **Positive control**: Add 0.5 ml of antigen positive control to a tube containing 0.5 ml of antiserum.
5. **Negative control**: Add 0.5 ml of 0.85% NaCl solution to a tube containing 0.5 ml of test isolate.
6. Incubate all tubes in a waterbath at 50 ± 2°C for 1 hour.
7. Read for flocculation (agglutination).
8. Repeat the Tube Test using a phase-reversed test organism. (See the procedure for phase reversal below.)

Phase Reversal:

1. Prepare Motility GI Medium phase reversal medium according to directions.
2. Prepare the antiserum opposite to the phase desired. For example, incubating *Salmonella* Typhimurium phase 1[i] in GI Motility Medium containing i antiserum allows the growth and spread of *S.* Typhimurium phase 2 [1,2].
3. Add 1 ml of a 1:10 dilution of antiserum to 25 ml of sterile GI Motility Medium and mix well. Pour into a sterile Petri dish and allow to solidify.
4. Inoculate by punching the edge of the solidified medium.
5. Incubate at 35-37°C for 24 hours.
6. Transfer growth from the spreading edge opposite the inoculation site to a liquid medium for testing according to steps under Tube Test Procedure – Salmonella H Antisera.
7. If motility is not acceptable, pass through GI Motility Medium again.

Tube Test Procedure

Salmonella H Antiserum Spicer-Edwards

1. Prepare the test organism and the 1:2 antiserum dilution as described above in Tube Test Preparation.
2. **Final 1:1,000 dilution of antiserum**: Prepare by adding 0.1 ml of rehydrated antiserum (1:2 working dilution) to 25 ml of 0.85% NaCl solution.
3. Prepare 4 culture tubes (12 x 75 ml) for each test organism.
4. **Salmonella H Antisera Spicer-Edwards 1-4**: Add 0.5 ml of the diluted antiserum to the culture tubes.
5. **Test organism**: Add 0.5 ml to each tube.
6. Incubate tubes in waterbath at 50 ± 2°C for 1 hour.
7. Remove from the waterbath. Avoid excessive shaking when the tubes are in the waterbath or when removing them from the waterbath prior to reading the reactions.
8. Read for flocculation (agglutination).

Tube Test Results

Compare results with the flocculation (agglutination) patterns for the Spicer-Edwards schemae. (Table 5).

Limitations of the Procedure

1. Complete O and H antigen characterization of a *Salmonella* isolate is required for final identification. Due to the complexity of the laboratory procedures, identification with polyvalent antisera may be sufficient for most laboratories.
2. Possible *Salmonella* isolates having inconsistencies in biochemical reactions and O and H antigen tests should be referred to a reference laboratory for further testing.
3. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent making a smooth suspension of the microorganism or cause evaporation or precipitation of the test mixture. False-positive reactions may occur.
4. Rough culture isolates do occur and will agglutinate spontaneously, causing agglutination of the negative control reaction (autoagglutination). Smooth colonies must be selected and tested in serological procedures.
5. In the slide agglutination procedure for O antigen testing, it is recommended that several colonies be tested and that unabsorbed polyvalent antisera be used followed by absorbed single factor antisera. For example, colonies of a 1,2,12 culture on an agar plate will have varying degrees of each antigen. A 1,2,12 antiserum absorbed of 1 and 12 antibodies will be highly specific but will show weak or no agglutination with colonies that have less of antigen 2 and more of antigens 1 and 12. Using unabsorbed Salmonella O Antiserum Group Factors 1,2,12 to test several suspicious colonies on a plate followed by testing with absorbed Salmonella O Antiserum Factor 2 gives the needed balance of sensitivity and specificity.
6. The slide agglutination antisera (Salmonella O Antisera) have been prepared for use in identifying cultures already defined biochemically. Such cultures are taken from an agar medium using a bacterial loop. A portion of the isolated colony is emulsified in a drop of antiserum. It is recommended that more than one colony be tested.

   The sera have been tested and absorbed using this method. They have not been tested employing an antigen suspension in NaCl solution or alcohol-treated cultures. If variations in the recommended procedures are to be used, the investigator is advised to test each lot of antiserum with known positive control cultures to ensure its proper homologous and heterologous reactions under their test conditions.
7. Agglutination reactions of 3+ or greater are interpreted as positive reactions. Cross-reactions resulting in a 1+ or 2+ agglutination are likely since there are somatic antigens shared among different groups as non-major group antigens.
8. The tube agglutination technique is recommended for H antigen testing because cross-reactions with somatic antigens may occur at the dilutions used in the slide technique.
9. No attempt has been made to absorb or test for O antibodies in H antisera.
10. In the tube test, make certain that the proper dilution is prepared for a given antiserum. Various dilutions are used for various sera. The information is given under Tube Test Preparation. Also, it is important in this test to use the recommended time and temperature of incubation. Make certain that the waterbath is in a location free of mechanical vibration.

11. There may exist common antigens between various "O" serogroups of Salmonella. As an example, Salmonella O Antiserum Poly A contains, among others, agglutinins for factor 1, since cultures possessing factor 1 were used in immunization. It may be expected that this polyvalent antiserum will react with cultures other than those contained in "O" serogroups A, B, D, E, and L due to the common 1 antigen (those organisms in Group G_1, G_2, H, R, T, etc., which contain factor 1).
12. Salmonella O Antiserum Poly A-I & Vi has been prepared with representative members of those somatic groups and has not been absorbed. It is obvious that this serum may and will react with higher O groups of Salmonella.

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Packaging

Salmonella H Antiserum a	3 ml	2820-47
Salmonella H Antiserum b	3 ml	2821-47
Salmonella H Antiserum c	3 ml	2822-47
Salmonella H Antiserum d	3 ml	2823-47
Salmonella H Antiserum eh	3 ml	2273-47
Salmonella H Antiserum f	3 ml	2544-47
Salmonella H Antiserum h	3 ml	2545-47
Salmonella H Antiserum I	3 ml	2824-47
Salmonella H Antiserum k	3 ml	2274-47
Salmonella H Antiserum m	3 ml	2546-47
Salmonella H Antiserum p	3 ml	2548-47
Salmonella H Antiserum r	3 ml	2275-47
Salmonella H Antiserum s	3 ml	2550-47
Salmonella H Antiserum t	3 ml	2551-47
Salmonella H Antiserum w	3 ml	2554-47
Salmonella H Antiserum x	3 ml	2555-47
Salmonella H Antiserum y	3 ml	2276-47
Salmonella H Antiserum z	3 ml	2277-47
Salmonella H Antiserum z_6	3 ml	2473-47
Salmonella H Antiserum z_{10}	3 ml	2279-47
Salmonella H Antiserum z_{13}	3 ml	2556-47
Salmonella H Antiserum z_{15}	3 ml	2557-47
Salmonella H Antiserum z_{23}	3 ml	2558-47
Salmonella H Antiserum z_{28}	3 ml	2561-47
Salmonella H Antiserum z_{29}	3 ml	2280-47
Salmonella H Antiserum z_{32}	3 ml	2562-47
Salmonella H Antiserum EN Complex	3 ml	2270-47
Salmonella H Antiserum G Complex	3 ml	2269-47
Salmonella H Antiserum L Complex	3 ml	2271-47
Salmonella H Antiserum Z_4 Complex	3 ml	2278-47
Salmonella H Antiserum Poly a-z	3 ml	2406-47
Salmonella H Antiserum Poly A (a,b,c,d,i,z_{10},z_{29})	3 ml	2539-47
Salmonella H Antiserum Poly B (eh,en,enx,enz$_{15}$, and G Complex)	3 ml	2540-47
Salmonella H Antiserum Poly C (k,l,r,y,z_1,z_4)	3 ml	2541-47
Salmonella H Antiserum Poly D (z_{35},z_{36},z_{37},z_{38},z_{39},z_{41},z_{42})	3 ml	2542-47
Salmonella H Antiserum Poly E (1 Complex, z_6)	3 ml	2543-47
Salmonella H Antiserum Single Factor 2	3 ml	2474-47

Salmonella H Antiserum Single Factor 5	3 ml	2475-47
Salmonella H Antiserum Single Factor 6	3 ml	2476-47
Salmonella H Antiserum Single Factor 7	3 ml	2477-47
Salmonella H Antiserum Spicer-Edwards 1	3 ml	2265-47
Salmonella H Antiserum Spicer-Edwards 2	3 ml	2266-47
Salmonella H Antiserum Spicer-Edwards 3	3 ml	2267-47
Salmonella H Antiserum Spicer-Edwards 4	3 ml	2268-47
Salmonella H Antiserum 1 Complex	3 ml	2272-47
Salmonella O Antiserum Factor 2	3 ml	2814-47
Salmonella O Antiserum Factor 4	3 ml	2659-47
Salmonella O Antiserum Factors 4,5	3 ml	2815-47
Salmonella O Antiserum Factor 5	3 ml	2660-47
Salmonella O Antiserum Factor 7	3 ml	2816-47
Salmonella O Antiserum Factor 8	3 ml	2817-47
Salmonella O Antiserum Factor 9	3 ml	2818-47
Salmonella O Antiserum Factor 10	3 ml	2257-47
Salmonella O Antiserum Factor 12	3 ml	2779-47
Salmonella O Antiserum Factor 14	3 ml	2661-47
Salmonella O Antiserum Factor 15	3 ml	2258-47
Salmonella O Antiserum Factor 19	3 ml	2259-47
Salmonella O Antiserum Factor 20	3 ml	2662-47
Salmonella O Antiserum Factor 22	3 ml	2663-47
Salmonella O Antiserum Factor 23	3 ml	2664-47
Salmonella O Antiserum Factor 25	3 ml	2666-47
Salmonella O Antiserum Factor 27	3 ml	2667-47
Salmonella O Antiserum Factor 34	3 ml	2512-47
Salmonella O Antiserum Group A Factors 1,2,12	3 ml	2947-47
Salmonella O Antiserum Group B Factors 1,4,5,12	3 ml	2948-47
Salmonella O Antiserum Group B Factors 1,4,12,27	3 ml	2973-47
Salmonella O Antiserum Group C_1 Factors 6,7	3 ml	2949-47
Salmonella O Antiserum Group C_2 Factors 6,8	3 ml	2950-47
Salmonella O Antiserum Group C_3 Factors (8), 20	3 ml	3016-47
Salmonella O Antiserum Group D_1 Factors 1,9,12	3 ml	2951-47
Salmonella O Antiserum Group D_2 Factors (9),46	3 ml	3017-47
Salmonella O Antiserum Group E Factors 1,3,10,15,19,34	3 ml	2819-47
Salmonella O Antiserum Group E_1 Factors 3,10	3 ml	2952-47
Salmonella O Antiserum Group E_2 Factors 3,15	3 ml	2954-47
Salmonella O Antiserum Group E_3 Factors (3),(15),34	3 ml	3018-47
Salmonella O Antiserum Group E_4 Factors 1,3,19	3 ml	3019-47
Salmonella O Antiserum Group F Factor 11	3 ml	2260-47
Salmonella O Antiserum Group G Factors 13,22,23,(36),(37)	3 ml	3029-47
Salmonella O Antiserum Group G_1 Factors 13,22,(36)	3 ml	2261-47
Salmonella O Antiserum Group G_2 Factors 1,13,23,(37)	3 ml	3020-47
Salmonella O Antiserum Group H Factors 1,6,14,24,25,47	3 ml	2262-47
Salmonella O Antiserum Group I Factor 16	3 ml	2263-47
Salmonella O Antiserum Group J Factor 17	3 ml	2517-47
Salmonella O Antiserum Group K Factor 18	3 ml	2518-47
Salmonella O Antiserum Group L Factor 21	3 ml	2519-47
Salmonella O Antiserum Group M Factor 28	3 ml	2520-47
Salmonella O Antiserum Group N Factor 30	3 ml	2521-47
Salmonella O Antiserum Group O Factor 35	3 ml	2522-47
Salmonella O Antiserum Poly A-I & Vi	3 ml	2264-47
Salmonella O Antiserum Poly A (A,B,D,E_1,E_2,E_3,E_4,& L)	3 ml	2534-47
Salmonella O Antiserum Poly B (C_1,C_2,F,G, & H)	3 ml	2535-47
Salmonella O Antiserum Poly C (I,J,K,M,N,& O)	3 ml	2536-47
Salmonella O Antiserum Poly D (P,Q,R,S,T,& U)	3 ml	2537-47
Salmonella O Antiserum Poly E (V,W,X,Y, & Z)	3 ml	2538-47
Salmonella O Antiserum Poly F (Groups 51-55)	3 ml	2645-47
Salmonella O Antiserum Poly G (Groups 56-61)	3 ml	2646-47
Salmonella O Antiserum Vi	3 ml	2827-47

Salmonella, Antigenic Scheme
Update of the Kauffmann-White Scheme

The Centers for Disease Control[1] has modified the Kauffmann-White[2] Antigenic Scheme originally proposed by Ewing.[3] The updated scheme is used with Difco *Salmonella* Antisera as an aid in the serological identification of *Salmonella*.

All of the *Salmonella* serovars belong to two species: *S. bongori* containing 18 serovars and *S. enterica* containing the remaining 2300+ serovars divided among 6 subspecies.[1,4]

The six subspecies of *S. enterica* are:

- *S. enterica* subsp. *enterica* (I or 1)
- *S. enterica* subsp. *salamae* (II or 2)
- *S. enterica* subsp. *arizonae* (IIIa or 3a)
- *S. enterica* subsp. *diarizonae* (IIIb or 3b)
- *S. enterica* subsp. *houtenae* (IV or 4)
- *S. enterica* subsp. *indica* (VI or 6)

The legitimate species name for the above subspecies is *S. choleraesuis*. However, this name may be confused with the serotype named "*choleraesuis*." At the International Congress for Microbiology in 1986, the International Subcommittee for *Enterobacteriaceae* agreed to adopt the species name "*S. enterica*."[5] LeMinor and Popoff[6] published a request for the use of *S. enterica* as a species name to the Judicial Commission. The Judicial Commission ruled that *S. choleraesuis* is the legitimate name.[7,8] *S. enterica* is used in many countries and is favorably accepted as the species name.[3,9] The Centers for Disease Control has adopted this designation until the problem of naming this species is resolved.[1]

Nomenclature and classification of these bacteria are ever changing.[13] *Salmonella* and the former *Arizona* should be considered a single genus - *Salmonella*.[9] It is recommended that laboratories report names of *Salmonella* serovars for the subspecies *enterica*. These serovar names are no longer italicized.[1,4] The first letter of the serovar name begins with a capital letter. For example, the strain that used to be identified as "*Salmonella typhimurium*" is now known as "*Salmonella* Typhimurium."

Serovars of other subspecies of *S. enterica* (except some in subspecies *salamae* and *houtenae*) and those of *S. bongori* are not named, and are designated by their antigenic formula. For the most recent information on nomenclature, consult appropriate references.[1,4,8,10-14]

Serotypes of *Salmonella* are defined based on the antigenic structure of both somatic or cell wall (O) antigens and flagellar (H) antigens. The O antigen groups were first designated by letters of the alphabet. Additional antigens were later delineated. Since each letter of the alphabet was already used to describe an O antigen group, numbers 51 to 67 were used to describe the next O antigen groups.

O ANTIGEN GROUP	O ANTIGEN FACTORS PRESENT
A	1,2,12
B	4,12; 1,4,5,12; or 1,4,12,27
C_1	6,7,[Vi] or 6,7,14
C_2	6,8
C_3	8; or 8,20
D_1	1,9,12
D_2	9,46
D_3	1,9,12,46,27
E_1	3,10
E_2	3,15
E_3	3,15,34
E_4	1,3,19
F	11
G	13,22 or 13,23
H	6,14; 6,14,24; or 1,6,14,25
I	16
J	17
K	18
L	21
M	28
N	30
O	35
P	38
Q	30
R	40
S	41
T	42
U	43
V	44
W	45
X	47
Y	48
Z	50
51	51
52	52
53	53
54	54
55	55
56	56
57	57
58	58
59	59
60	60
61	61
62	62
63	63
65	65
66	66
67	67

When writing an antigenic formula, list the O antigen(s) first followed by the H antigen(s). Separate the major antigens by colons and the components of the antigens by commas. For example, the antigenic formula for *Salmonella* Typhimurium is *Salmonella* 1,4,5,12:i:1,2. This means that the strain has O antigen factors 1,4,5 and 12, the flagella phase 1 antigen i, and flagellar phase 2 antigens 1 and 2.

Complete identification of *Salmonella* requires cultural isolation, biochemical characterization and serotyping. Any serological results obtained before biochemical identification must be considered as presumptive identification only. Refer to appropriate references for complete identification of *Salmonella*.[1,3,4,10,12-15]

The Kauffmann-White Scheme is presented in two forms. Appendix A contains a list of Salmonella Serotypes by O Group. Appendix B contains an alphabetical list of *Salmonella* Serotypes.

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Appendix A

Kauffmann-White Scheme

List of *Salmonella* Serotypes by O Group

(Updated 1994)

Appendix A contains a list of Salmonella Serotypes by O Group. The serotypes are sorted by O group first, then by Phase I and Phase 2 of the H antigens. The z antigens do not appear in the correct numerical order - z_4 will be listed after z_{10} because the 1 of 10 is read first, and will appear after z_{29}.

Key:

IP	Institut Pasteur. See reference #16 in Kauffmann-White Scheme monograph published by WHO Collaborating Centre for Reference and Research on Salmonella.
Ar.	"Arizona" antigenic formula
Rohde	Refer to reference #17 in Kauffmann-White Scheme monograph by R. Rohde. He incorporated all known Arizona serotypes into the Kauffmann-White Scheme.
Bergey	Refer to reference #18 in Kauffmann-White Scheme monograph by L. LeMinor for "Bergey's Manual of Systematic Bacteriology."
Underlined Numbers	Numbers that are underlined in a serotype represent somatic factors determined by phage conversion. They are present if the culture is lysogenized by the corresponding converting phage.
[ ]	O or H factors may be present or absent without relation to phage conversion.
( )	O or H factor is weakly agglutinable.
R phases	Abnormal specificities of H antigens that were described by Kauffmann. They are uncommon.

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	A	Paratyphi A	1,2,12	a	[1,5]	
I	A	Nitra	2,12	g,m	-	
I	A	Kiel	1,2,12	g,p	-	
I	A	Koessen	2,12	l,v	1,5	
II	B		4,12	–	1,6	
I	B	Abortusequi	4,12	–	e,n,x	
I	B	Kisangani	1,4,[5],12	a	1,2	
I	B	Fulica	4,[5],12	a	1,5	
I	B	Hessarek	4,12,27	a	1,5	
I	B	Arechavaleta	4,[5],12	a	[1,7]	
I	B	Bispejerg	1,4,[5],12	a	e,n,x	
II	B		1,4,[5],12,27	a	e,n,x	
II	B	Makoma	1,4,[5],12,27	a	[e,n,x]	
I	B	Tinda	1,4,5,27	a	e,n,z_{15}	
I	B	Huettwilen	1,4,12	a	l,w	
II	B		1,4,12,27	a	z_{39}	
I	B	Nakuru	1,4,12,27	a	z_6	
I	B	Schleissheim	4,12,27	b	–	
I	B	Java	1,4,5,12	b	[1,2], (tartrate +)	IP calls Java, Paratyphi B var. Java. Java is often monophasic in the U.S. May possess H phase Rz_{33}.
I	B	Limete	1,4,12,27	b	1,5	
II	B		4,12	b	1,5	
I	B	Canada	4,12,27	b	1,6	
I	B	Uppsala	4,12,27	b	1,7	
I	B	Abony	1,4,5,12	b	e,n,x	IP combined Sladun (1,4,12,27:b:e,n,x) with Abony to form Abony 1,4,[5],12,27:b:e,n,x (gelatin neg.).
I	B	Abortusbovis	1,4,12,27	b	e,n,x	Gelatin pos., mucate pos.[1-4 days.] Abortusbovis was combined with Abony (1,4,5,12:b:e,n,x), gelatin neg. The name Abortusbovis was dropped.
I	B	Sladun	1,4,12,27	b	e,n,x	IP combined Sladun with Abony (1,3,4,12: b:e,n,x) to form Abony 1,4,[5],12,27:b:e,n,x. Sladun is now called Abony var. O 27+. The name Sladun has been dropped.
II	B	Sofia	1,4,12,27	b	[e,n,x]	
I	B	Wagenia	1,4,12,27	b	e,n,z_{15}	
I	B	Wien	1,4,12,27	b	l,w	
I	B	Abortuscanis	4,5,12	b	Rz_5	Abortuscanis was combined with Paratyphi B (1,4,[5],12:b:1,2) in 1938 and the name Abortuscanis was dropped.
I	B	Tripoli	1,4,12,27	b	z_6	
I	B	Paratyphi B	1,4,[5],12	[b]	[1,2]	Paratyphi B is tartrate neg.; Paratyphi B var. Java (CDC calls this *S.* ser. Java) is often monophasic (1,4,5,12:b:-) and is tartrate pos. Paratyphi B and Java may possess H phase Rz_{33}.
I	B	Legon	1,4,12,27	c	1,5	
I	B	Abortusovis	4,12	c	1,6	
I	B	Altendorf	4,12	c	1,7	IP combined Womba (4,12,27:c:1,7) with Altendorf to form Altendorf 4,12,27:c:1,7.
I	B	Womba	4,12,27	c	1,7	IP combined Womba with Altendorf (4,12:c:1,7) to form Altendorf 4,12,27:c:1,7. Womba is now called Altendorf var. 027+. The name Womba has been dropped.

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	B	Bissau	4,12	c	e,n,x	
I	B	Jericho	1,4,12,27	c	e,n,z_{15}	
I	B	Hallfold	1,4,12,27	c	l,w	
I	B	Bury	4,12,27	c	z_6	
I	B	Cairo	1,4,12,27	d	1,2	IP combined Cairo with Stanley (4,5,12:d:1,2) to form Stanley 1,4,[5],12,27:d:1,2. The name Cairo has been dropped.
I	B	Stanley	4,5,12	d	1,2	IP combined Cairo (1,4,12,27:d:1,2) with Stanley to form Stanley 1,4,[5],12,27:d:1,2
I	B	Eppendorf	1,4,12,27	d	1,5	
I	B	Brezany	1,4,12,27	d	1,6	
I	B	Schwarzengrund	1,4,12,27	d	1,7	
I	B	Sarajane	1,4,[5],12,27	d	e,n,x	
II	B	Kluetjenfelde	4,12	d	e,n,x	
I	B	Duisburg	1,4,12,27	d	e,n,z_{15}	
I	B	Mons	1,4,12,27	d	l,w	
I	B	Ayinde	1,4,12,27	d	z_6	
I	B	Salinatis	4,12	d,e,h	d,e,n,z_{15}	IP states that Salinatis was combined with Duisburg (1,4,12,27:d:e,n,z_{15}). This is incorrect; IP should have stated that it was combined with Sandiego (4,[5],12:e,h:e,n,z_{15}), because Salinatis loses the d and becomes Sandiego.
I	B	Saintpaul	1,4,[5],12	e,h	1,2	
I	B	Reading	1,4,[5],12	e,h	[1,5]	
I	B	Eko	4,12	e,h	1,6	
I	B	Kaapstad	4,12	e,h	1,7	
I	B	Chester	1,4,[5],12	e,h	e,n,x	
I	B	Sandiego	4,[5],12	[e,h]	e,n,z_{15}	
II	B		4,12	e,n,x	1,2,7	
II	B	Makumira	1,4,12,27	e,n,x	1,[5],7	
II	B		4,12	(f),g	–	Not in IP book
I	B	Derby	1,4,[5],12	f,g	[1,2]	
I	B	Agona	1,4,[5],12	f,g,s	[1,2]	
II	B		4,[5],12	f,g,t	z_6,z_{42}	
I	B	Essen	4,12	g,m	–	
I	B	Hato	4,[5],12	g,m,s	–	
I	B	California	4,12	g,m,t	–	
II	B		4,12	g,m,t	–	IP calls this monophasic var. of Bechuana.
II	B		4,12	g,m,t	z_{39}	
II	B	Caledon	1,4,12,27	g,[m],[s],t	e,n,x	
II	B	Bechuana	1,4,12,27	g,[m],t	[1,5]	
I	B	Joenkoeping	4,5,12	g,s,t	–	IP combined Joenkoeping with Kingston (1,4,12,27:g,s,t:-) to form Kingston 1,4,[5],12,27:g,s,t:-. The name Joenkoeping has been dropped.
I	B	Kingston	1,4,12,27	g,s,t	[1,2]	IP combined Kingston with Joenkoeping (4,5,12:g,s,t:-) to form Kingston 1,4,[5],12,27:g,s,t:[1,2]. Kingston may possess H phase Rz_{27} or Rz_{43}.
I	B	Budapest	1,4,12,27	g,t	–	
I	B	Travis	4,[5],12	g,z_{51}	1,7	
I	B	Tennyson	4,5,12	g,z_{51}	e,n,z_{15}	
II	B		4,12	g,z_{62}	–	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	B	Typhimurium var. Copenhagen	1,4,12	i	1,2	
I	B	Typhimurium	1,4,5,12	i	1,2,[7]	
I	B	Lagos	1,4,[5],12	i	1,5	
I	B	Agama	4,12	i	1,6	
I	B	Farsta	4,12	i	e,n,x	
I	B	Tsevie	4,12	i	e,n,z_{15}	
I	B	Gloucester	1,4,12,27	i	l,w	
II	B		4,12,27	i	z_{35}	
I	B	Tumodi	1,4,12	i	z_6	
I	B	Massenya	1,4,12,27	k	1,5	
I	B	Neumuenster	1,4,12,27	k	1,6	
II	B		1,4,12,27	k	1,6	
I	B	Ljubljana	4,12,27	k	e,n,x	
I	B	Texas	4,[5],12	k	e,n,z_{15}	
I	B	Fyris	4,[5],12	l,v	1,2	
I	B	Azteca	4,[5],12,27	l,v	1,5	
I	B	Bredeney	1,4,12,27	l,v	1,7	Bredeney may possess H phase Rl,z_{40} instead of l,v.
I	B	Kimuenza	1,4,12,27	l,v	e,n,x	
II	B		1,4,12,27	l,v	e,n,x	
I	B	Brandenburg	1,4,[5],12,27	l,v	e,n,z_{15}	
II	B		1,4,12,27	l,v	z_{39}	
I	B	Clackamas	4,12	l,v,[z13]	1,6	IP does not get z_{13}
I	B	Mono	4,12	l,w	1,5	
I	B	Togo	4,12	l,w	1,6	
II	B	Kilwa	4,12	l,w	e,n,x	
I	B	Ayton	1,4,12,27	l,w	z_6	
I	B	Haduna	4,12	l,z13,[z28]	1,6	
I	B	Kubacha	1,4,12,27	l,z13,z28	1,7	
I	B	Kano	1,4,12,27	l,z13,z28	e,n,x	
I	B	Vom	1,4,12,27	l,[z13],[z28]	e,n,z_{15}	
I	B	Tyresoe	4,12	l,[z13],z28	1,5	
I	B	Kunduchi	1,4,[5],12,27	l,[z13],[z28]	[1,2]	
II	B		4,12	l,z28	–	
I	B	Reinickendorf	4,12	l,z28	e,n,x	
I	B	Banana	1,4,[5],12	m,t	[1,5]	Banana may possess H phase Rz_{45}.
I	B	Madras	4,[5],12	m,t	e,n,z_{15}	
I	B	Heidelberg	1,4,[5],12	r	1,2	
I	B	Bradford	4,12,27	r	1,5	
I	B	Winneba	4,12	r	1,6	
I	B	Remo	1,4,12,27	r	1,7	
I	B	Bochum	4,[5],12	r	l,w	
I	B	Southampton	1,4,12,27	r	z_6	
I	B	Africana	4,12	r,i	l,w	
I	B	Drogana	1,4,12,27	r,(i)	e,n,z_{15}	IP calls Drogana r,i.
I	B	Coeln	4,[5],12	y	1,2	
I	B	Trachau	4,12,27	y	1,5	
I	B	Finaghy	4,12	y	1,6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	B	Teddington	1,4,12,27	y	1,7	
I	B	Ball	1,4,12,27	y	e,n,x	IP combined Ball with Ruki (4,5,12:y:e,n,x) and Dalat (4,5,27:y:e,n,x) to form Ball 1,4,[5],12,27:y:e,n,x.
I	B	Ruki	4,5,12	y	e,n,x	IP combined Ruki with Ball (1,4,12,27:y:e,n,x) and Dalat (4,5,27:y:e,n,x) to form Ball 1,4,[5], 12,27:y:e,n,x. The name Ruki has been dropped.
I	B	Dalat	4,5,27	y	e,n,x	Dalat was combined with Ball. The name Dalat has been dropped.
I	B	Jos	1,4,12,27	y	e,n,z_{15}	
I	B	Kamoru	4,12,27	y	z_6	
I	B	Shubra	4,[5],12	z	1,2	
I	B	Kiambu	4,12	z	1,5	
II	B		1,4,12,27	z	1,5	
I	B	Loubomo	4,12	z	1,6	
I	B	Indiana	1,4,12	z	1,7	
II	B		4,12	z	1,7	
I	B	Neftenbach	4,12	z	e,n,x	
II	B	Nordenham	1,4,12,27	z	e,n,x	
I	B	Koenigstuhl	1,4,[5],12	z	e,n,z_{15}	
I	B	Preston	1,4,12	z	l,w	
II	B		4,12	z	z_{39}	
I	B	Entebbe	1,4,12,27	z	z_6	
I	B	Haifa	1,4,[5],12	z_{10}	1,2	
I	B	Ituri	1,4,12	z_{10}	1,5	
I	B	Tudu	4,12	z_{10}	1,6	
I	B	Albert	4,12	z_{10}	e,n,x	
I	B	Tokoin	4,12	z_{10}	e,n,z_{15}	
I	B	Mura	1,4,12	z_{10}	l,w	
I	B	Vellore	1,4,12,27	z_{10}	z_{35}	
I	B	Fortune	1,4,12,27	z_{10}	z_6	
I	B	Brancaster	1,4,12,27	z_{29}	–	
II	B	Helsinki	1,4,12	z_{29}	[e,n,x]	
I	B	Pasing	4,12	z_{35}	1,5	
I	B	Tafo	1,4,12,27	z_{35}	1,7	
I	B	Yaounde	1,4,12,27	z_{35}	e,n,z_{15}	
I	B	Sloterdijk	1,4,12,27	z_{35}	z_6	
I	B	Tejas	4,12	z_{36}	–	
I	B	Wilhelmsburg	1,4,[5],12,27	z_{38}	[e,n,z15]	
II	B	Durbanville	1,4,12,27	[z_{39}]	1,[5],7	
I	B	Jaja	4,12,27	z_4,z_{23}	–	IP combined Jaja with Stanleyville (1,4,[5],12:z_4,z_{23}:[1,5]) to form Stanleyville 1,4,[5],12,27:z4,z23:[1,5]. Jaja is now called Stanleyville var. O 27+. The name Jaja has been dropped.
I	B	Stanleyville	1,4,[5],12	z_4,z_{23}	[1,2]	IP combined Jaja (4,12,27:z_4,z_{23}:-) with Stanleyville to form Stanleyville 1,4,[5],12,27:z_4,z_{23}:[1,2].
I	B	Vuadens	4,12,27	z_4,z_{23}	z_6	
I	B	Kalamu	4,[5],12	z_4,z_{24}	[1,5]	
I	B	Thayngen	1,4,12,27	z_{41}	1,(2),5	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	B	Maska	1,4,12,27	z_{41}	e,n,z_{15}	
II	C_1		6,7	–	1,6	
I	C_1	Sanjuan	6,7	a	1,5	
II	C_1		6,7,14	a	1,5	
I	C_1	Umhlali	6,7	a	1,6	
I	C_1	Austin	6,7	a	1,7	
I	C_1	Oslo	6,7,14	a	e,n,x	
I	C_1	Denver	6,7	a	e,n,z_{15}	
I	C_1	Coleypark	6,7,14	a	l,w	
II	C_1	Calvinia	6,7	a	z_{42}	
I	C_1	Damman	6,7	a	z_6	
II	C_1		6,7	a	z_6	
I	C_1	Nissii	6,7,14	b	–	Nissii was combined with Nienstedten (6,7,14:b:l,w) as a monophasic variant of Nienstedten. Nienstedten is now called a variant of Ohio by IP. The name Nissii has been dropped.
I	C_1	Brazzaville	6,7	b	1,2	
I	C_1	Edinburg	6,7	b	1,5	
I	C_1	Adime	6,7	b	1,6	
I	C_1	Koumra	6,7	b	1,7	
I	C_1	Lockleaze	6,7,14	b	e,n,x	
II	C_1	Bloemfontein	6,7	b	[e,n,x]:z_{42}	
I	C_1	Georgia	6,7	b	e,n,z_{15}	
I	C_1	Ohio	6,7	b	l,w	IP combined Nienstedten (6,7,14:b:[l,w]) with Ohio to form Ohio 6,7,14:b:[l,w}. Ohio may possess H phase Rz_{59}.
I	C_1	Nienstedten	6,7,14	b	[l,w]	Nienstedten was combined with Nissii (6,7,14:b:-) and called Nienstedten; then IP combined Nienstedten with Ohio (6,7:b:l,w) to form Ohio (6,7,14:b:[l,w]). Nienstedten is now called Ohio var. O 14+ by IP.
I	C_1	Kotte	6,7	b	z_{35}	
II	C_1		6,7	b	z_{39}	
I	C_1	Leopoldville	6,7,14	b	z_6	
I	C_1	Hissar	6,7,14	c	1,2	
I	C_1	Choleraesuis	6,7	c	1,5	H_2S negative
I	C_1	Decatur	6,7	c	1,5	IP has dropped Decatur and calls it dulcitol positive, mucate positive variant of Choleraesuis.
I	C_1	Paratyphi C	6,7,[Vi]	c	1,5	
I	C_1	Typhisuis	6,7	c	1,5	Typhisuis is a bioserotype found in pigs. It is like Choleraesuis except tartrate negative.
I	C_1	Choleraesuis var. Kunzendorf		6,7	c	[1,5] H_2S positive
I	C_1	Birkenhead	6,7	c	1,6	
I	C_1	Schwabach	6,7	c	1,7	
I	C_1	Namibia	6,7	c	e,n,x	
I	C_1	Kaduna	6,7,14	c	e,n,z_{15}	
I	C_1	Kisii	6,7	d	1,2	
I	C_1	Kambole	6,7	d	1,[2],7	
I	C_1	Isangi	6,7,14	d	1,5	
I	C_1	Mission	6,7	d	1,5	Mission was combined with Isangi 6,7,14:d:1,5. The name Mission has been dropped.
I	C_1	Kivu	6,7	d	1,6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	C_1	Amersfoort	6,7	d	e,n,x	IP combined Omderman (6,7,14:d:e,n,x) with Amersfoort to form Amersfoort 6,7,14:d:e,n,x.
I	C_1	Omderman	6,7,14	d	e,n,x	IP combined Omderman with Amersfoort (6,7:d:e,n,x) to form Amersfoort 6,7,14:d:e,n,x. Omderman is now called Amersfoort var. O 14+ by IP.
I	C_1	Gombe	6,7,14	d	e,n,z_{15}	
I	C_1	Eimsbuettel	6,7,14	d	l,w	IP combined Eimsbuettel with Livingstone (6,7:d:l,w) to form Livingstone 6,7,14:d:l,w. Eimbuettel is now called Livingstone var. O 14+ by IP.
I	C_1	Livingstone	6,7	d	l,w	IP combined Eimsbuettel (6,7,14:d:l,w) with Livingstone to form Livingstone 6,7,14:d:l,w.
I	C_1	Wil	6,7	d	l,z_{13},z_{28}	
II	C_1		6,7	d	z_{42}	
I	C_1	Nieukerk	6,7,14	d	z_6	
I	C_1	Larochelle	6,7	e,h	1,2	
I	C_1	Lomita	6,7	e,h	1,5	
I	C_1	Norwich	6,7	e,h	1,6	
I	C_1	Nola	6,7	e,h	1,7	
I	C_1	Braenderup	6,7,14	e,h	e,n,z_{15}	
I	C_1	Ardwick	6,7,14	f,g	–	IP combined Ardwick with Rissen (6,7:f,g:-) to form Rissen 6,7,14:f,g:-. Ardwick is now called Rissen var. O 14+ by IP.
I	C_1	Rissen	6,7	f,g	–	IP combined Ardwick (6,7,14:f,g:-) with Rissen for form Rissen 6,7,14:f,g:-.
I	C_1	Eingedi	6,7	f,g,t	1,2,7	
I	C_1	Afula	6,7	f,g,t	e,n,x	
II	C_1		6,7	(g),m,[s],t	[1,5]	
I	C_1	Montevideo	6,7,14	g,m,[p],s	[1,2,7]	
II	C_1		6,7	g,m,[s],t	e,n,x	
I	C_1	Othmarschen	6,7,14	g,m,[t]	–	
II	C_1		6,7	g,[m],s,t	[z_{42}]	
I	C_1	Menston	6,7	g,s,[t]	[1,6]	
I	C_1	Riggil	6,7	g,t	–	
II	C_1		6,7	g,t	e,n,x:z_{42}	
IV	C_1		6,7	g,z51	–	
I	C_1	Alamo	6,7	g,z_{51}	1,5	
I	C_1	Augustenborg	6,7,14	i	1,2	
I	C_1	Oritamerin	6,7	i	1,5	
I	C_1	Garoli	6,7	i	1,6	
I	C_1	Lika	6,7	i	1,7	
I	C_1	Athinai	6,7	i	e,n,z_{15}	
I	C_1	Norton	6,7	i	l,w	
I	C_1	Stuttgart	6,7,14	i	z_6	
I	C_1	Galiema	6,7,14	k	1,2	
I	C_1	Daytona	6,7	k	1,6	
I	C_1	Baiboukoum	6,7	k	1,7	
I	C_1	Singapore	6,7	k	e,n,x	
I	C_1	Escanaba	6,7	k	e,n,z_{15}	
I	C_1	Cardiff	6,7	k	R1,10	IP combined Cardiff that contains H phase R1,10 with Thompson (6,7,14:k:1,5).

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	C_1		6,7	k	[z_6]	
IIIa	C_1		6,7	(k)	z:[z_{55}]	(Ar. 27:22:31:[37])
I	C_1	Thompson	6,7,14	[k]	[1,5]	IP combined Cardiff that contains H phase R1,10 (6,7:k:R1,10) with Thompson.
I	C_1	Concord	6,7	l,v	1,2	
I	C_1	Irumu	6,7	l,v	1,5	
I	C_1	Mkamba	6,7	l,v	1,6	
I	C_1	Kortrijk	6,7	l,v	1,7	
I	C_1	Bonn	6,7	l,v	e,n,x	
I	C_1	Potsdam	6,7,14	l,v	e,n,z_{15}	
I	C_1	Coromandel	6,7	l,v	z_{35}	
IIIb	C_1		6,7	l,v	z_{53}	(Ar. 27:23:25)
I	C_1	Gdansk	6,7	l,v	z_6	IP combined Gelsenkirchen (6,7,14:l,v:z_6) with Gdansk to form Gdansk 6,7,14:l,v:z_6.
I	C_1	Gelsenkirchen	6,7,14	l,v	z_6	IP combined Gelsenkirchen with Gdansk (6,7:l,v:z_6) to form Gdansk 6,7,14:l,v:z_6. Gelsenkirchen is now called Gdansk var. O 14+ by IP.
I	C_1	Gabon	6,7	l,w	1,2	
I	C_1	Colorado	6,7	l,w	1,5	
II	C_1		6,7	l,w	1,5,7	
II	C_1		6,7	l,w	z_{42}	
I	C_1	Nessziona	6,7	l,z13	1,5	
I	C_1	Kenya	6,7	l,z13	e,n,x	
I	C_1	Strathcona	6,7	l,z13,z28	1,7	
I	C_1	Makiso	6,7	l,z13,z28	z_6	
I	C_1	Neukoelin	6,7	l,z13,[z28]	e,n,z_{15}	
II	C_1	Heilbron	6,7	l,z28	1,5:[z_{42}]	
II	C_1		6,7	l,z28	e,n,x	
II	C_1		6,7	l,z28	z_6	
I	C_1	Haelsingborg	6,7	m,p,t,[u]	–	
I	C_1	Oranienburg	6,7	m,t	–	IP combined Theilallee (6,7,14:m,t:-) with Oranienburg to form Oranienburg 6,7,14:m,t:-. Oranienburg may possess H phase Rz$_{57}$.
I	C_1	Thielallee	6,7,14	m,t	–	IP combined Thielallee with Oranienburg (6,7:m,t:-) to form Oranienburg 6,7,14:m,t:-. Theilallee is now called Oranienburg var. O 14+ by IP.
II	C_1		6,7	m,t	–	
I	C_1	Winston	6,7	m,t	1,6	
I	C_1	Oakey	6,7	m,t	z_{64}	
I	C_1	Virchow	6,7	r	1,2	
I	C_1	Infantis	6,7,14	r	1,5	Infantis may possess H phase Rz$_{49}$.
I	C_1	Nigeria	6,7	r	1,6	
I	C_1	Colindale	6,7	r	1,7	
I	C_1	Papuana	6,7	r	e,n,z_{15}	
I	C_1	Grampian	6,7	r	l,w	
I	C_1	Richmond	6,7	y	1,2	
I	C_1	Bareilly	6,7,14	y	1,5	
I	C_1	Oyonnax	6,7	y	1,6	
I	C_1	Gatow	6,7	y	1,7	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	C_1	Hartford	6,7	y	e,n,x	Hartford may possess H phase Rz_{50} or Rz_{67}.
I	C_1	Mikawasima	6,7,14	y	e,n,z_{15}	Mikawasima may possess H phase Rz_{47} or Rz_{50}.
I	C_1	Chile	6,7	z	1,2	
I	C_1	Poitiers	6,7	z	1,5	
II	C_1	Tosamanga	6,7	z	1,5	
I	C_1	Oakland	6,7	z	1,6,[7]	
I	C_1	Cayar	6,7	z	e,n,x	
I	C_1	Businga	6,7	z	e,n,z_{15}	
I	C_1	Bruck	6,7	z	l,w	
II	C_1		6,7	z	z_{39}	
II	C_1	Oysterbeds	6,7	z	z_{42}	
II	C_1		6,7	z	z_6	
I	C_1	Menden	6,7	z_{10}	1,2	
I	C_1	Inganda	6,7	z_{10}	1,5	
I	C_1	Eschweiler	6,7	z_{10}	1,6	
I	C_1	Ngili	6,7	z_{10}	1,7	
I	C_1	Djugu	6,7	z_{10}	e,n,x	
I	C_1	Mbandaka	6,7,14	z_{10}	e,n,z_{15}	
I	C_1	Jerusalem	6,7,14	z_{10}	l,w	
I	C_1	Omuna	6,7	z_{10}	z_{35}	
II	C_1		6,7	z_{10}	z_{35}	
I	C_1	Redba	6,7	z_{10}	z_6	
II	C_1		6,7	z_{29}	–	
I	C_1	Tennessee	6,7,14	z_{29}	[1,2,7]	
I	C_1	Tienba	6,7	z_{35}	1,6	
I	C_1	Palime	6,7	z_{35}	e,n,z_{15}	
IV	C_1	Argentina	6,7	z_{36}	–	
I	C_1	Tampico	6,7	z_{36}	e,n,z_{15}	
II	C_1	Bacongo	6,7	z_{36}	z_{42}	
I	C_1	Bornum	6,7,14	z_{38}	–	IP combined Bornum with Lille (6,7:z_{38}:-) to form Lille 6,7,14:z_{38}:-. Bornum is now called Lille var. O 14+ by IP.
I	C_1	Lille	6,7	z_{38}	–	IP combined Lille with Bornum (6,7,14:z_{38}:-) to form Lille 6,7,14:z_{38}:-.
I	C_1	Rumford	6,7	z_{38}	1,2	
II	C_1	Gilbert	6,7	z_{39}	1,5,7	
IV	C_1	Roterberg	6,7	z_4,z_{23}	-	
I	C_1	Obogu	6,7	z_4,z_{23}	1,5	
I	C_1	Planckendael	6,7	z_4,z_{23}	1,6	
I	C_1	Goma	6,7	z_4,z_{23}	z_6	
I	C_1	Aequatoria	6,7	z_4,z_{23}	[e,n,z_{15}]	
I	C_1	Somone	6,7	z_4,z_{24}	–	
IV	C_1	Kralendyk	6,7	z_4,z_{24}	–	
II	C_1		6,7	z_4,z_{24}	z_{42}	
VI	C_1		6,7	z41	1,7	
I	C_1	Hillsborough	6,7	z41	l,w	
I	C_1	Tamilnadu	6,7	z_{41}	z_{35}	
II	C_1	Sullivan	6,7	z_{42}	1,7	
II	C_1		6,7	z_{42}	e,n,x:1,6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	C_1	Bulovka	6,7	z_{44}	–	
II	C_1	Cape	6,7	z_6	1,7	
I	C_2	Newport var. Puerto Rico	6,8	–	1,2	
II	C_2		6,8	–	1,5,7	
I	C_2	Valdosta	6,8	a	1,2	
I	C_2	Doncaster	6,8	a	1,5	
I	C_2	Curacao	6,8	a	1,6	
I	C_2	Nordufer	6,8	a	1,7	
I	C_2	Narashino	6,8	a	e,n,x	
II	C_2		6,8	a	e,n,x	
I	C_2	Leith	6,8	a	e,n,z_{15}	
II	C_2		6,8	a	z_{39}	
II	C_2	Tulear	6,8	a	z_{52}	
I	C_2	Be	8,20	a	[z_6]	
I	C_2	Djelfa	8	b	1,2	
I	C_2	Skansen	6,8	b	1,2	
I	C_2	Korbol	8,20	b	1,5	
I	C_2	Nagoya	6,8	b	1,5	
II	C_2		6,8	b	1,5	
I	C_2	Stourbridge	6,8	b	1,6	
I	C_2	Eboko	6,8	b	1,7	
I	C_2	Sanga	8	b	1,7	
I	C_2	Gatuni	6,8	b	e,n,x	
I	C_2	Konstanz	8	b	e,n,x	
I	C_2	Presov	6,8	b	e,n,z_{15}	
I	C_2	Shipley	8,20	b	e,n,z_{15}	
I	C_2	Bukuru	6,8	b	l,w	
I	C_2	Banalia	6,8	b	z_6	
I	C_2	Tounouma	8,20	b	z_6	
I	C_2	Wingrove	6,8	c	1,2	
I	C_2	Utah	6,8	c	1,5	
I	C_2	Bronx	6,8	c	1,6	
I	C_2	Belfast	6,8	c	1,7	
I	C_2	Belem	6,8	c	e,n,x	
I	C_2	Santiago	8,20	c	e,n,x	
I	C_2	Quiniela	6,8	c	e,n,z_{15}	
I	C_2	Alexanderpolder	8	c	l,w	
I	C_2	Tado	8,20	c	z_6	
I	C_2	Mexicana	6,8	d	1,2	Mexicana was combined with Muenchen. The name Mexicana has been dropped.
I	C_2	Muenchen	6,8	d	1,2	
I	C_2	Virginia	8	d	[1,2]	
I	C_2	Manhattan	6,8	d	1,5	
I	C_2	Yovokome	8,20	d	1,5	
I	C_2	Dunkwa	6,8	d	1,7	
I	C_2	Portanigra	8,20	d	1,7	
I	C_2	Sterrenbos	6,8	d	e,n,x	
I	C_2	Herston	6,8	d	e,n,z_{15}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	C_2	Labadi	8,20	d	z_6	
II	C_2		6,8	d	z_6:z_{42}	
I	C_2	Bardo	8	e,h	1,2	
I	C_2	Newport	6,8,20	e,h	1,2	Newport may possess H phase Rz_{50} or Rz_{58} or Rz_{78} or R1,12
I	C_2	Ferruch	8	e,h	1,5	
I	C_2	Kottbus	6,8	e,h	1,5	
I	C_2	Cremieu	6,8	e,h	1,[6]	
I	C_2	Atakpame	8,20	e,h	1,7	
I	C_2	Tshiongwe	6,8	e,h	e,n,z_{15}	
I	C_2	Rechovot	8,20	e,h	z_6	
I	C_2	Sandow	6,8	f,g	e,n,z_{15}	
II	C_2		6,8	f,g,m,t	[e,n,x]	
I	C_2	Emek	8,20	g,m,s	–	
I	C_2	Chincol	6,8	g,m,[s]	[e,n,x]	
I	C_2	Reubeuss	8,20	g,m,t	–	
II	C_2		6,8	g,m,t	1,7	
I	C_2	Alminko	8,20	g,s,t	–	
I	C_2	Nanergou	6,8	g,s,t	–	
I	C_2	Lindenburg	6,8	i	1,2	
I	C_2	Bargny	8,20	i	1,5	
I	C_2	Takoradi	6,8	i	1,5	
I	C_2	Warnow	6,8	i	1,6	
I	C_2	Malmoe	6,8	i	1,7	
I	C_2	Bonariensis	6,8	i	e,n,x	
I	C_2	Aba	6,8	i	e,n,z_{15}	
I	C_2	Cyprus	6,8	i	l,w	
I	C_2	Magherafelt	8,20	i	l,w	
I	C_2	Kentucky	8,20	i	z_6	
I	C_2	Kallo	6,8	k	1,2	
I	C_2	Blockley	6,8	k	1,5	Blockley may possess H phase Rz_{58}
I	C_2	Haardt	8	k	1,5	
I	C_2	Schwerin	6,8	k	e,n,x	
I	C_2	Charlottenburg	6,8	k	e,n,z_{15}	
I	C_2	Litchfield	6,8	l,v	1,2	
I	C_2	Pakistan	8	l,v	1,2	
I	C_2	Loanda	6,8	l,v	1,5	
I	C_2	Manchester	6,8	l,v	1,7	
I	C_2	Holcomb	6,8	l,v	e,n,x	
II	C_2		6,8	l,v	e,n,x	
I	C_2	Edmonton	6,8	l,v	e,n,z_{15}	
I	C_2	Amherstiana	8	l,(v)	1,6	
I	C_2	Fayed	6,8	l,w	1,2	
II	C_2		6,8	l,w	z_6:z_{42}	
I	C_2	Hiduddify	6,8	l,z_{13},z_{28}	1,5	
II	C_2		6,8	l,z_{28}	e,n,x	
I	C_2	Breukelen	6,8	l,z_{13},[z_{28}]	e,n,z_{15}	
I	C_2	Bassa	6,8	m,t	-	
I	C_2	Yokoe	8,20	m,t	-	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	C_2	Baragwanath	6,8	m,t	1,5	
II	C_2	Germiston	6,8	m,t	e,n,x	
I	C_2	Bsilla	6,8	r	1,2	
I	C_2	Hindmarsh	8,20	r	1,5	
I	C_2	Akanji	6,8	r	1,7	
I	C_2	Noya	8	r	1,7	
I	C_2	Goldcoast	6,8	r	l,w	
I	C_2	Pikine	8,20	r	z_6	Pikine was combined with Altona (8,20:r,[i]:z_6). The name Pikine has been dropped.
I	C_2	Cocody	8,20	r,i	e,n,z_{15}	
I	C_2	Hidalgo	6,8	r,i	e,n,z_{15}	
I	C_2	Bovismorbificans	6,8	r,[i]	1,5	
I	C_2	Brikama	8,20	r,[i]	l,w	
I	C_2	Altona	8,20	r,[i]	z_6	Pikine (8,20:r:z_6) was combined with Altona and called Altona.
I	C_2	Giza	8,20	y	1,2	
I	C_2	Brunei	8,20	y	1,5	
I	C_2	Tananarive	6,8	y	1,5	
I	C_2	Bulgaria	6,8	y	1,6	
II	C_2		6,8	y	1,6:z_{42}	
I	C_2	Alagbon	8	y	1,7	
I	C_2	Inchpark	6,8	y	1,7	
I	C_2	Daarle	6,8	y	e,n,x	
I	C_2	Sunnycove	8	y	e,n,x	
I	C_2	Praha	6,8	y	e,n,z_{15}	
I	C_2	Benue	6,8	y	l,w	
I	C_2	Sindelfingen	8,20	y	l,w	
I	C_2	Kralingen	8,20	y	z_6	
I	C_2	Mowanjum	6,8	z	1,5	
II	C_2		6,8	z	1,5	
I	C_2	Kalumburu	6,8	z	e,n,z_{15}	
I	C_2	Phaliron	8	z	e,n,z_{15}	
I	C_2	Kuru	6,8	z	l,w	
I	C_2	Daula	8,20	z	z_6	
I	C_2	Bazenheid	8,20	z_{10}	1,2	
I	C_2	Zerifin	6,8	z_{10}	1,2	
I	C_2	Mapo	6,8	z_{10}	1,5	
I	C_2	Paris	8,20	z_{10}	1,5	
I	C_2	Cleveland	6,8	z_{10}	1,7	
I	C_2	Hadar	6,8	z_{10}	e,n,x	
I	C_2	Istanbul	8	z_{10}	e,n,x	
I	C_2	Chomedey	8,20	z_{10}	e,n,z_{15}	
I	C_2	Glostrup	6,8	z_{10}	e,n,z_{15}	
I	C_2	Remiremont	8,20	z_{10}	l,w	
I	C_2	Molade	8,20	z_{10}	z_6	
I	C_2	Wippra	6,8	z_{10}	z_6	
II	C_2		6,8	z_{29}	1,5	
II	C_2		6,8	z_{29}	e,n,x	
II	C_2		8	z_{29}	e,n,x:z_{42}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	C_2	Tamale	8,20	z_{29}	[e,n,z_{15}]	
I	C_2	Uno	6,8	z_{29}	[e,n,z_{15}]	
I	C_2	Kolda	8,20	z_{35}	1,2	
I	C_2	Yarm	6,8	z_{35}	1,2	
I	C_2	Angers	8,20	z_{35}	z_6	
I	C_2	Apeyeme	8,20	z_{38}	–	
I	C_2	Bellevue	8	z_4,z_{23}	1,7	
I	C_2	Lezennes	6,8	z_4,z_{23}	1,7	
I	C_2	Breda	6,8	z_4,z_{23}	e,n,x	
I	C_2	Chailey	6,8	z_4,z_{23}	[e,n,z_{15}]	
I	C_2	Dabou	8,20	z_4,z_{23}	l,w	
I	C_2	Corvallis	8,20	z_4,z_{23}	[z_6]	
I	C_2	Albany	8,20	z_4,z_{24}	–	Albany may possess H phase Rz_{45}.
I	C_2	Duesseldorf	6,8	z_4,z_{24}	–	
I	C_2	Tallahassee	6,8	z_4,z_{32}	–	
I	C_2	Diogoye	8,20	z_{41}	z_6	
I	C_2	Aesch	6,8	z_{60}	1,2	
I	D_1	Gallinarum	1,9,12	–	–	Gallinarum must be identified biochemically.
I	D_1	Pullorum	1,9,12	–	–	IP combined Pullorum with Gallinarum (1,9,12:-:-). They must be identified biochemically.
I	D_1	Miami	1,9,12	a	1,5	Miami must be differentiated from Sendai with biochemical tests. Miami is pos. for H_2S, citrate, and tartrate; Sendai is neg.
I	D_1	Sendai	1,9,12	a	1,5	Sendai must be differentiated from Miami with biochemical tests. Sendai is neg. for H_2S, citrate, and tartrate; Miami is pos.
II	D_1		9,12	a	1,5	
I	D_1	Os	9,12	a	1,6	
I	D_1	Saarbruecken	1,9,12	a	1,7	
I	D_1	Lomalinda	1,9,12	a	e,n,x	
II	D_1		1,9,12	a	e,n,x	
I	D_1	Durban	9,12	a	e,n,z_{15}	
II	D_1		9,12	a	z_{39}	
II	D_1		1,9,12	a	z_{42}	
I	D_1	Onarimon	1,9,12	b	1,2	
I	D_1	Frintrop	1,9,12	b	1,5	
II	D_1	Mjimwema	1,9,12	b	e,n,x	
II	D_1	Suederelbe	1,9,12	b	z_{39}	
II	D_1	Blankenese	1,9,12	b	z_6	
I	D_1	Goeteborg	9,12	c	1,5	
I	D_1	Ipeko	9,12	c	1,6	
I	D_1	Elokate	9,12	c	1,7	
I	D_1	Alabama	9,12	c	e,n,z_{15}	
I	D_1	Ridge	9,12	c	z_6	
I	D_1	Typhi	9,12,[Vi]	d	–	Typhi may possess H phase Rj or Rz_{66}.
I	D_1	Ndolo	1,9,12	d	1,5	
I	D_1	Tarshyne	9,12	d	1,6	
I	D_1	Eschberg	9,12	d	1,7	
II	D_1	Rhodesiense	9,12	d	e,n,x	
I	D_1	Bangui	9,12	d	e,n,z_{15}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	D_1	Jaffna	1,9,12	d	z_{35}	
II	D_1		9,12	d	z_{39}	
I	D_1	Zega	9,12	d	z_6	
I	D_1	Bournemouth	9,12	e,h	1,2	
I	D_1	Eastbourne	1,9,12	e,h	1,5	
I	D_1	Westafrica	9,12	e,h	1,7	
I	D_1	Israel	9,12	e,h	e,n,z_{15}	
II	D_1	Lindrick	9,12	e,n,x	1,[5],7	
II	D_1		9,12	e,n,x	1,6	
I	D_1	Enteritidis	1,9,12	[f],g,m,[p],[t]	[1,7]	
I	D_1	Berta	1,9,12	[f],g,t	–	
I	D_1	Blegdam	9,12	g,m,q	–	
II	D_1	Muizenberg	9,12	g,m,s,t	1,5	IP combined Muizenberg with Hamburg (1,9,12:g,t:-) and Manica (1,9,12:g,m,s,t:z_{42}) to form *S.* II 1,9,12:g,[m],[s],t:[1,5,7]:[z_{42}].
II	D_1	Kuilsrivier	1,9,12	g,m,s,t	e,n,x	
II	D_1	Manica	1,9,12	g,m,s,t	z_{42}	IP combined Manica with Hamburg (1,9,12:g,t:-) and Muizenberg (9,12:g,m,s,t:1,5) to form *S.* II 1,9,12:g,[m],[s],t:[1,5,7]:[z_{42}].
II	D_1		1,9,12	g,m,[s],t	[1,5,7]:[z_{42}]	
I	D_1	Dublin	1,9,12,[Vi]	g,p	–	
I	D_1	Naestved	1,9,12	g,p,s	–	
I	D_1	Rostock	1,9,12	g,p,u	–	
I	D_1	Moscow	9,12	g,q	–	
II	D_1	Neasden	9,12	g,s,t	e,n,x	
II	D_1	Hamburg	1,9,12	g,t	–	IP combined Hamburg with Manica (1,9,12:g,m,s,t:z_{42}) and Muizenberg (9,12:g,m,s,t:1,5) to form *S.* II 1,9,12:g,m,[s],t:[1,5,7]:[z_{42}].
I	D_1	Newmexico	9,12	g,z_{51}	1,5	
II	D_1		1,9,12	g,z_{62}	–	
I	D_1	Antarctica	9,12	g,z_{63}	–	
I	D_1	Seremban	9,12	i	1,5	
I	D_1	Claibornei	1,9,12	k	1,5	
I	D_1	Goverdhan	9,12	k	1,6	
I	D_1	Mendoza	9,12	l,v	1,2	
I	D_1	Panama	1,9,12	l,v	1,5	Panama may possess H phase R1,11
I	D_1	Kapemba	9,12	l,v	1,7	
I	D_1	Zaiman	9,12	l,v	e,n,x	
II	D_1		9,12	l,v	e,n,x	
I	D_1	Goettingen	9,12	l,v	e,n,z_{15}	
I	D_1	Italiana	9,12	l,v	R1,11	IP combined Italiana that contains H phase R1,11 with Panama (1,9,12:l,v:1,5). The name Italiana has been dropped.
II	D_1		9,12	l,v	z_{39}	
I	D_1	Victoria	1,9,12	l,w	1,5	
II	D_1	Daressalaam	1,9,12	l,w	e,n,x	
I	D_1	Itami	9,12	l,z_{13}	1,5	
I	D_1	Miyazaki	9,12	l,z_{13}	1,7	
I	D_1	Napoli	1,9,12	l,z_{13}	e,n,x	
I	D_1	Javiana	1,9,12	l,z_{28}	1,5	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	D_1		9,12	l,z_{28}	1,5:[z_{42}]	
I	D_1	Kotu	9,12	l,z_{28}	1,6	
II	D_1		9,12	l,z_{28}	e,n,x	
II	D_1		9,12	m,t	–	
I	D_1	Pensacola	1,9,12	m,t	[1,2]	
II	D_1		1,9,12	m,t	1,5	
II	D_1		1,9,12	m,t	z_{39}	
I	D_1	Jamaica	9,12	r	1,5	
I	D_1	Camberwell	9,12	r	1,7	
I	D_1	Campinense	9,12	r	e,n,z_{15}	
I	D_1	Lome	9,12	r	z_6	
I	D_1	Powell	9,12	y	1,7	
I	D_1	Lawndale	1,9,12	z	1,5	
I	D_1	Kimpese	9,12	z	1,6	
II	D_1	Stellenbosch	1,9,12	z	1,7	
II	D_1	Hueningen	9,12	z	z_{39}	
II	D_1	Angola	1,9,12	z	z_6	
I	D_1	Portland	9,12	z_{10}	1,5	
I	D_1	Ruanda	9,12	z_{10}	e,n,z_{15}	
I	D_1	Treguier	1,9,12	z_{10}	z_6	
II	D_1	Canastel	9,12	z_{29}	[1,5]	
II	D_1		1,9,12	z_{29}	e,n,x	
I	D_1	Penarth	9,12	z_{35}	z_6	
I	D_1	Elomrane	1,9,12	z_{38}	–	
II	D_1	Wynberg	1,9,12	z_{39}	1,7	
I	D_1	Wangata	1,9,12	z_4,z_{23}	[1,7]	
I	D_1	Natal	9,12	z_4,z_{24}	–	
I	D_1	Ottawa	1,9,12	z_{41}	1,5	
II	D_1		1,9,12	z_{42}	1,[5],7	
I	D_1	Franken	1,9,12	z_{60}	z_{67}	
I	D_2	Baildon	9,46	a	e,n,x	
I	D_2	Doba	9,46	a	e,n,z_{15}	
I	D_2	Cheltenham	9,46	b	1,5	
I	D_2	Zadar	9,46	b	1,6	
I	D_2	Worb	9,46	b	e,n,x	
II	D_2	Lundby	9,46	b	e,n,x	
I	D_2	Bamboye	9,46	b	l,w	
I	D_2	Kolar	9,46	b	z_{35}	
I	D_2	Linguere	9,46	b	z_6	
I	D_2	Itutaba	9,46	c	z_6	
I	D_2	Ontario	9,46	d	1,5	
I	D_2	Quentin	9,46	d	1,6	
I	D_2	Strasbourg	9,46	d	1,7	
I	D_2	Olten	9,46	d	e,n,z_{15}	
I	D_2	Plymouth	9,46	d	z_6	
I	D_2	Bergedorf	9,46	e,h	1,2	
I	D_2	Waedenswil	9,46	e,h	1,5	
I	D_2	Guerin	9,46	e,h	z_6	
II	D_2		9,46	e,n,x	1,5,7	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	D_2	Wernigerode	9,46	f,g	–	
I	D_2	Hillingdon	9,46	g,m	–	
I	D_2	Macclesfield	9,46	g,m,s,t	1,(2),7	
II	D_2	Duivenhoks	9,46	g,[m],[s],t	[e,n,x]	
I	D_2	Gateshead	9,46	g,s,t	–	
II	D_2		9,46	g,z_{62}	–	
I	D_2	Mathura	9,46	i	e,n,z_{15}	
I	D_2	Potto	9,46	i	z_6	
I	D_2	Marylebone	9,46	k	1,2	
I	D_2	Cochin	9,46	k	1,5	
I	D_2	Ceyco	9,46	k	z_{35}	
I	D_2	India	9,46	l,v	1,5	
I	D_2	Geraldton	9,46	l,v	1,6	
I	D_2	Toronto	9,46	l,v	e,n,x;[z_{44}]	
I	D_2	Ackwepe	9,46	l,w	–	
I	D_2	Sangalkam	9,46	m,t	–	
II	D_2		9,46	m,t	e,n,x	
I	D_2	Deckstein	9,46	r	1,7	
I	D_2	Shoreditch	9,46	r	e,n,z_{15}	
I	D_2	Sokode	9,46	r	z_6	
I	D_2	Benin	9,46	y	1,7	
I	D_2	Irchel	9,46	y	e,n,x	
I	D_2	Nantes	9,46	y	l,w	
I	D_2	Mayday	9,46	y	z_6	
II	D_2		9,46	z	1,5	
II	D_2	Haarlem	9,46	z	e,n,x	
I	D_2	Bambylor	9,46	z	e,n,z_{15}	
I	D_2	Lishabi	9,46	z_{10}	1,7	
I	D_2	Inglis	9,46	z_{10}	e,n,x	
I	D_2	Mahina	9,46	z_{10}	e,n,z_{15}	
II	D_2		9,46	z_{10}	z_{39}	
I	D_2	Louisiana	9,46	z_{10}	z_6	
II	D_2		9,46	z_{10}	z_6	
I	D_2	Ouakam	9,46	z_{29}	-	
I	D_2	Hillegersberg	9,46	z_{35}	1,5	
I	D_2	Basingstoke	9,46	z_{35}	e,n,z_{15}	
I	D_2	Trimdon	9,46	z_{35}	z_6	
I	D_2	Fresno	9,46	z_{38}	–	
II	D_2		9,46	z_{39}	1,7	
I	D_2	Ekotedo	9,46	z_4,z_{23}	–	
I	D_2	Ngaparou	9,46	z_4,z_{24}	–	
II	D_2	Maarssen	9,46	z_4,z_{24}	z_{39}:z_{42}	
I	D_2	Wuppertal	9,46	z_{41}	–	
II	D_3	Zuerich	1,9,12,46,27	c	z_{39}	
II	D_3		9,12,46,27	g,t	e,n,x	
II	D_3		1,9,12,46,27	l,z_{13},z_{28}	z_{39}	
II	D_3		1,9,12,46,27	y	z_{39}	
II	D_3		1,9,12,46,27	z_{10}	1,5	
II	D_3		1,9,12,46,27	z_{10}	e,n,x	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	D_3		1,9,12,46,27	z_{10}	z_{39}	
II	D_3		1,9,12,46,27	z_4,z_{24}	[1,5]	
I	E_1	Aminatu	3,10	a	1,2	
I	E_1	Goelzau	3,10	a	1,5	IP combined Clichy (3,15:a:1,5) with Goelzau to form Goelzau 3,10,[15]:a:1,5.
I	E_1	Oxford	3,10	a	1,7	IP combined Khartoum (3,15,34:a:1,7) with Oxford to form Oxford 3,10,[15],[15,34]:a:1,7.
I	E_1	Masembe	3,10	a	e,n,x	Masembe may possess H phase Rz_5.
II	E_1	Matroosfontein	3,10	a	e,n,x	
I	E_1	Galil	3,10	a	e,n,z_{15}	
II	E_1		3,10	a	z_{39}	
I	E_1	Kalina	3,10	b	1,2	
I	E_1	Butantan	3,10	b	1,5	IP combined Rosenthal (3,15:b:1,5) and unnamed 3,15,34:b:1,5 with Butantan to form Butantan 3,10,[15],[15,34]:b:1,5.
I	E_1	Allerton	3,10	b	1,6	
I	E_1	Huvudsta	3,10	b	1,7	
I	E_1	Benfica	3,10	b	e,n,x	
II	E_1		3,10	b	e,n,x	
I	E_1	Yaba	3,10,[15]	b	e,n,z_{15}	
I	E_1	Epicrates	3,10	b	l,w	
I	E_1	Westminster	3,10,[15]	b	z_{35}	CDC has no 3,10:b:z_{35}.
II	E_1		3,10	b	z_{39}	
I	E_1	Wilmington	3,10	b	z_6	
I	E_1	Asylanta	3,10	c	1,2	
I	E_1	Gbadago	3,10,[15]	c	1,5	
I	E_1	Ikayi	3,10,[15]	c	1,6	Ikayi Var. O 15+ was described after E_1 and E_2 were combined.
I	E_1	Pramiso	3,10	c	1,7	
I	E_1	Agege	3,10	c	e,n,z_{15}	
I	E_1	Anderlecht	3,10	c	l,w	
I	E_1	Okefoko	3,10	c	z_6	
I	E_1	Stormont	3,10	d	1,2	
I	E_1	Shangani	3,10	d	1,5	IP combined Pankow (3,15:d:1,5) with Shangani to form Shangani 3,10,[15]:d:1,5.
I	E_1	Lekke	3,10	d	1,6	
I	E_1	Onireke	3,10	d	1,7	
I	E_1	Souza	3,10	d	e,n,x	IP combined Eschersheim (3,15:d:e,n,x) with Souza to form Souza 3,10,[15]:d:e,n,x.
II	E_1		3,10	d	e,n,x	
I	E_1	Madjorio	3,10	d	e,n,z_{15}	
I	E_1	Birmingham	3,10	d	l,w	
I	E_1	Maron	3,10	d	z_{35}	
I	E_1	Weybridge	3,10	d	z_6	
I	E_1	Vejle	3,10	e,h	1,2	IP combined Goerlitz (3,15:e,h:1,2) with Vejle to form Vejle 3,10,[15]:e,h:1,2.
I	E_1	Muenster	3,10	e,h	1,5	IP combined Muenster with Newhaw (3,15:e,h:1,5) and Arkansas (3,15,34:e,h:1,5) to form Muenster 3,10,[15],[15,34]:e,h:1,5.
I	E_1	Anatum	3,10	e,h	1,6	IP combined Newington (3,15:e,h:1,6) and Minneapolis (3,15,34:e,h:1,6) with Anatum to form Anatum 3,10,[15],[15,34]:e,h:1,6.

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_1	Nyborg	3,10	e,h	1,7	IP combined Selandia (3,15:e,h:1,5) with Nyborg to form Nyborg 3,10,[15]:e,h:1,7.
I	E_1	Newlands	3,10,[15,34]	e,h	e,n,x	
I	E_1	Lamberhurst	3,10	e,h	e,n,z_{15}	
I	E_1	Meleagridis	3,10	e,h	l,w	IP combined Meleagridis with Cambridge (3,15:e,h:l,w) and Wildwood (3,15,34:e,h:l,w) to form Meleagridis 3,10,[15],[15,34]:e,h:l,w.
I	E_1	Sekondi	3,10	e,h	z_6	
II	E_1	Chudleigh	3,10	e,n,x	1,7	
I	E_1	Alfort	3,10	f,g	e,n,x	
I	E_1	Regent	3,10	f,g,[s]	[1,6]	
I	E_1	Suberu	3,10	g,m	–	
I	E_1	Amsterdam	3,10	g,m,s	–	IP combined Drypool (3,15:g,m,s) and Drypool var. O 34+ with Amsterdam to form Amsterdam 3,10,[15],[15,34]:g,m,s:-.
II	E_1	Parow	3,10,[15]	g,m,s,t	–	
I	E_1	Westhampton	3,10	g,s,t	–	IP combined Halmstad (3,15:g,s,t:-) and Canoga (3,15,34:g,s,t:-) with Westhampton to form Westhampton 3,10,[15],[15,34]:g,s,t:-. Westhampton may possess H phase Rz_{37} or Rz_{43} or Rz_{45}.
II	E_1	Islington	3,10	g,t	–	
I	E_1	Bloomsbury	3,10	g,t	1,5	
I	E_1	Cuckmere	3,10	i	1,2	
I	E_1	Amounderness	3,10	i	1,5	
I	E_1	Truro	3,10	i	1,7	
I	E_1	Bessi	3,10	i	e,n,x	
I	E_1	Falkensee	3,10	i	e,n,z_{15}	
I	E_1	Hoboken	3,10	i	l,w	
I	E_1	Yeerongpilly	3,10	i	z_6	
I	E_1	Wimborne	3,10	k	1,2	
I	E_1	Zanzibar	3,10,[15]	k	1,5	
I	E_1	Serrekunda	3,10	k	1,7	
I	E_1	Yundum	3,10	k	e,n,x	
I	E_1	Marienthal	3,10	k	e,n,z_{15}	
I	E_1	Newrochelle	3,10	k	l,w	
I	E_1	Nchanga	3,10	l,v	1,2	IP combined Nchanga with Nancy (3,15:l,v:1,2) to form Nchanga 3,10,[15]:l,v:1,2.
I	E_1	Sinstorf	3,10	l,v	1,5	
I	E_1	London	3,10	l,v	1,6	IP combined London with Portsmouth (3,15:l,v:1,6) to form London 3,10,[15]:l,v:1,6.
I	E_1	Give	3,10	l,v	1,7	IP combined Newbrunswick (3,15:l,v:1,7) and Menhaden (3,15,34:l,v:1,7) with Give to form Give 3,10,[15],[15,34]:[d],l,v:[d],1,7. Give may possess H phase d; Rl,z_{40}; or Rz_{77}.
II	E_1		3,10	l,v	e,n,x	
I	E_1	Ruzizi	3,10	l,v	e,n,z_{15}	
I	E_1	Sinchew	3,10	l,v	z_{35}	
II	E_1	Fuhlsbuettel	3,10	l,v	z_6	
I	E_1	Assinie	3,10	l,w	z_6	Assinie may possess H phase Rz_{45}.
I	E_1	Freiburg	3,10	l,z_{13}	1,2	
I	E_1	Uganda	3,10,15	l,z_{13}	1,5	
I	E_1	Fallowfield	3,10	l,z_{13},z_{28}	e,n,z_{15}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_1	Hoghton	3,10	l,z_{13},z_{28}	z_6	
II	E_1		3,10	l,z_{28}	1,5	
I	E_1	Joal	3,10	l,z_{28}	1,7	
I	E_1	Lamin	3,10	l,z_{28}	e,n,x	
II	E_1	Westpark	3,10	l,z_{28}	e,n,x	
II	E_1		3,10	l,z_{28}	z_{39}	
II	E_1		3,10	m,t	1,5	
I	E_1	Southbank	3,10,15,34	m,t	[1,6]	
II	E_1	Stikland	3,10	m,t	e,n,x	
I	E_1	Ughelli	3,10	r	1,5	
I	E_1	Elisabethville	3,10	r	1,7	
I	E_1	Simi	3,10	r	e,n,z_{15}	
I	E_1	Weltevreden	3,10	r	z_6	IP combined Lanka 3,15:r:z_6 with Weltevreden to form Weltevreden 3,10,[15]:r:z_6.
I	E_1	Seegefeld	3,10	r,[i]	1,2	
I	E_1	Dumfries	3,10	r,[i]	1,6	
I	E_1	Rutgers	3,10	Rl,z_{40}	1,7	Rutgers has been dropped from the scheme and the H phase Rl,z_{40} is now considered an R phase of Give.
I	E_1	Amager	3,10	y	1,2	IP combined Tuebingen (3,15:y:1,2) with Amager to form Amager 3,10,[15]:y:1,2. Amager may possess H phase Rz_{45}.
I	E_1	Orion	3,10	y	1,5	IP combined Binza (3,15:y:1,5) and Thomasville (3,15,34:y:1,5) with Orion to form Orion 3,10,[15],[15,34]:y:1,5.
I	E_1	Mokola	3,10	y	1,7	
I	E_1	Ohlstedt	3,10	y	e,n,x	
I	E_1	Bolton	3,10	y	e,n,z_{15}	
I	E_1	Langensalza	3,10	y	l,w	
I	E_1	Stockholm	3,10	y	z_6	IP combined Tournai (3,15:y:z_6) with Stockholm to form Stockholm 3,10,[15]:y:z_6.
I	E_1	Fufu	3,10	z	1,5	
II	E_1	Alexander	3,10	z	1,5	
I	E_1	Harleystreet	3,10	z	1,6	
I	E_1	Huddinge	3,10	z	1,7	
II	E_1	Finchley	3,10	z	e,n,x	
I	E_1	Clerkenwell	3,10	z	l,w	
II	E_1	Tafelbaai	3,10	z	z_{39}	
I	E_1	Landwasser	3,10	z	z_6	
I	E_1	Okerara	3,10	z_{10}	1,2	
I	E_1	Lexington	3,10	z_{10}	1,5	IP combined Lexington with Manila (3,15:z_{10}:1,5) and Illinois (3,15,34:z_{10}:1,5) to form Lexington 3,10,[15],[15,34]:z_{10},1,5. Lexington may possess H phase Rz_{49}.
I	E_1	Harrisonburg	3,10,[15],[15,34]	z_{10}	1,6	
I	E_1	Coquilhatville	3,10	z_{10}	1,7	
I	E_1	Kristianstad	3,10	z_{10}	e,n,z_{15}	
I	E_1	Biafra	3,10	z_{10}	z_6	
I	E_1	Jedburgh	3,10,[15]	z_{29}	–	
II	E_1		3,10	z_{29}	–	
I	E_1	Everleigh	3,10	z_{29}	e,n,x	
II	E_1		3,10	z_{29}	e,n,x	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_1	Zongo	3,10	z_{35}	1,7	
I	E_1	Shannon	3,10	z_{35}	l,w	
I	E_1	Cairina	3,10	z_{35}	z_6	
I	E_1	Macallen	3,10	z_{36}	–	
II	E_1	Mpila	3,10	z_{38}	z_{42}	
I	E_1	Bolombo	3,10	z_{38}	[z_6]	
II	E_1	Winchester	3,10	z_{39}	1,[5],7	
I	E_1	Adabraka	3,10	z_4,z_{23}	[1,7]	
I	E_1	Wagadugu	3,10	z_4,z_{23}	z_6	
I	E_1	Florian	3,10,[15]	z_4,z_{24}	–	
II	E_1		3,10	z_4,z_{24}	–	
I	E_1	Pietersburg	3,10,[15,34]	z_{69}	1,7	
I	E_2	Clichy	3,15	a	1,5	IP combined Clichy with Goelzau (3,10:a:1,5) to form Goelzau 3,10,[15]:a:1,5. Clichy is now called Goelzau var. O 15+ by IP.
I	E_2	Rosenthal	3,15	b	1,5	IP combined Rosenthal and unnamed 3,15,34:b:1,5 with Butantan (3,10:b:1,5) to form Butantan 3,10,[15],[15,34]:b:1,5. Rosenthal is now called Butantan var. O 15+ by IP.
I	E_2	Pankow	3,15	d	1,5	IP combined Pankow with Shangani (3,10:d:1,5) to form Shangani 3,10,15:d:1,5. Pankow is now called Shangani var. O 15+ by IP.
I	E_2	Eschersheim	3,15	d	e,n,x	IP combined Eschersheim with Souza (3,10:d:e,n,x) to form Souza 3,10,[15]:d:e,n,x. Eschersheim is now called Souza var. O 15+ by IP.
I	E_2	Goerlitz	3,15	e,h	1,2	IP combined Goerlitz with Vejle (3,10:e,h:1,2) to form Vejle 3,10,15:e,h:1,2. Goerlitz is now called Vejle var. O 15+ by IP.
I	E_2	Newhaw	3,15	e,h	1,5	IP combined Newhaw and Arkansas (3,15,34:e,h:1,5) with Muenster (3,10:e,h:1,5) to form Muenster 3,10,[15],[15,34]:e,h:1,5. Newhaw is now called Muenster var. O 15+ by IP.
I	E_2	Newington	3,15	e,h	1,6	IP combined Newington and Minneapolis (3,15,34:e,h:1,6) with Anatum (3,10:e,h:1,6) to form Anatum 3,10,[15],[15,34]:e,h:1,6. Newington is now called Anatum var. O 15+ by IP.
I	E_2	Selandia	3,15	e,h	1,7	IP combined Selandia with Nyborg (3,10:e,h:1,7) to form Nyborg 3,10,[15]:e,h:1,7. Selandia is now called Nyborg var. O 15+ by IP.
I	E_2	Cambridge	3,15	e,h	l,w	IP combined Cambridge and Wildwood (3,15,34:e,h:l,w) with Meleagridis (3,10:e,h:l,w) to form Meleagridis 3,10,[15],[15,34]:e,h:l,w. Cambridge is now called Meleagridis var. O 15+ by IP.
I	E_2	Drypool	3,[15],[15,34]	g,m,s	–	IP combined Drypool 3,15:g,m,s:- and Drypool var. O34+ with Amsterdam (3,10:g,m,s:-) to form Amsterdam 3,10,[15],[15,34]:g,m,s:-. Drypool is now called Amsterdam var. O 15+ or O 15+, 34+ by IP.
I	E_2	Halmstad	3,15	g,s,t	–	IP combined Halmstad and Canoga (3,15,34:g,s,t:-) with Westhampton (3,10:g,s,t:-) to form Westhampton 3,10,[15],[15,34]:g,s,t:-. Halmstad is now called Westhampton var. O 15+ by IP.
I	E_2	Nancy	3,15	l,v	1,2	IP combined Nancy with Nchanga (3,10:l,v:1,2) to form Nchanga 3,10,[15]:l,v:1,2. Nancy is now called Nchanga var. O 15+ by IP.

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_2	Portsmouth	3,15	l,v	1,6	IP combined Portsmouth with London (3,10:l,v:1,6) to form London 3,10,[15]:l,v:1,6. Portsmouth is now called London var. O 15+ by IP.
I	E_2	Newbrunswick	3,15	l,v	1,7	IP combined Newbrunswick and Menhaden (3,15,34:l,v:1,7) with Give (3,10:l,v:1,7) to form Give 3,10,[15],[15,34]:[d],l,v:[d],1,7. Newbrunswick is now called Give var. O 15+ by IP.
I	E_2	Kinshasa	3,15	l,z_{13}	1,5	IP combined Kinshasa with Uganda (3,10:l,z13:1,5) to form Uganda 3,10,[15]:l,z13:1,5. Kinshasa is now called Uganda var. O 15+ by IP.
I	E_2	Lanka	3,15	r	z_6	IP combined Lanka with Weltevreden (3,10:r:z6) to form Weltevreden 3,10,[15]:r:z6. Lanka is now called Weltevreden var. O 15+ by IP.
I	E_2	Hamilton	3,15	Rz_{27}	–	IP combined Hamilton with Goerlitz (3,15:e,h:1,2) and Vejle (3,10:e,h:1,2) to form Vejle 3,10,15:e,h:1,2:Rz_{27}. Hamilton is now called Vejle var. Rz_{27}+. The name Hamilton has been dropped.
I	E_2	Tuebingen	3,15	y	1,2	IP combined Tuebingen with Amager (e,10:y:1,2) to form Amager 3,10,[15]:y:1,2. Tuebingen is now called Amager var. O 15+ by IP.
I	E_2	Binza	3,15	y	1,5	IP combined Binza and Thomasville (3,15,34:y:1,5) with Orion (3,10:y:1,5) to form Orion 3,10,[15],[15,34]:y:1,5. Binza is now called Orion var. O 15+ by IP.
I	E_2	Tournai	3,15	y	z_6	IP combined Tournai with Stockholm (3,10:y:z6) to form Stockholm 3,10,[15]:y:z6. Tournai is now called Stockholm var. O 15+ by IP.
I	E_2	Manila	3,15	z_{10}	1,5	IP combined Manila and Illinois (3,15,34:z10:1,5) with Lexington (3,10:z10:1,5) to form Lexington 3,10,[15],[15,34]:z10:1,5. Manila is now called Lexington var. O 15+ by IP.
I	E_3	Khartoum	3,15,34	a	1,7	IP combined Khartoum with Oxford (3,10:a:1,7) to form Oxford 3,10,[15],[15,34]:a:1,7. Khartoum is now called Oxford var. O 15+ by IP. CDC has no 3,15:a:1,7. Khartoum was found by IP with colonies containing O 3,15.
I	E_3	Arkansas	3,15,34	e,h	1,5	IP combined Arkansas and Newhaw (3,15:e,h:1,5) with Muenster (3,10:e,h:1,5) to form Muenster 3,10,[15],[15,34]:e,h:1,5. Arkansas is now callled Muenster var. O 15+, 34+ by IP.
I	E_3	Minneapolis	3,15,34	e,h	1,6	IP combined Minneapolis and Newington (3,15:e,h:1,6) with Anatum (3,10:e,h:1,6) to form Anatum 3,10,[15],[15,34]:e,h:1,6. Minneapolis is now called Anatum var. O 15+ by IP.
I	E_3	Wildwood	3,15,34	e,h	l,w	IP combined Wildwood and Cambridge (3,15:e,h:l,w) with Meleagridis (3,10:e,h:l,w) to form Meleagridis 3,10,[15],[15,34]:e,h:l,w. Wildwood is now called Meleagridis var. O 15+, 34+ by IP.
I	E_3	Drypool	3,15,34	g,m,s	–	IP combined Drypool (3,15:g,m,s:-) and Drypool var. O 34+ with Amsterdam (3,10:g,m,s:-) to form Amsterdam 3,10,[15],[15,34]:g,m,s:-. Drypool is now called Amsterdam var. O 15+ or var. O 15+,34+ by IP.
I	E_3	Canoga	3,15,34	g,s,t	-	IP combined Canoga and Halmstad (3,15:g,s,t:-) with Westhampton (3,10:g,s,t:-) to form Westhampton 3,10,[15],[15,34]:g,s,t:-. Canoga is now called Westhampton var. O 15+, 34+ by IP.

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_3	Menhaden	3,15,34	l,v	1,7	IP combined Menhaden with Give (3,10:l,v:1,7) and Newbrunswick (3,15:l,v:1,7) to form Give 3,10,[15],[15,34]:[d],l,v:1,7. Menhaden is now called Give var. O 15+, 34+ by IP.
I	E_3	Thomasville	3,15,34	y	1,5	IP combined Thomasville and Binza (3,15:y:1,5) with Orion (3,10:y:1,5) to form Orion 3,10,[15],[15,34]:y:1,5. Thomasville is now called Orion var. O 15+ by IP.
I	E_3	Illinois	3,15,34	z_{10}	1,5	IP combined Illinois and Manila (3,15:z_{10}:1,5) with Lexington (3,10:z10:1,5) to form Lexington 3,10,[15],[15,34]:z10:1,5. Illinois is now called Lexington var. O 15+, 34+ by IP.
I	E_4	Niumi	1,3,19	a	1,5	
I	E_4	Juba	1,3,19	a	1,7	
I	E_4	Gwoza	1,3,19	a	e,n,z_{15}	
I	E_4	Alkmaar	1,3,19	a	l,w	
I	E_4	Gnesta	1,3,19	b	1,5	
I	E_4	Visby	1,3,19	b	1,6	
I	E_4	Tambacounda	1,3,19	b	e,n,x	
I	E_4	Kande	1,3,19	b	e,n,z_{15}	
I	E_4	Broughton	1,3,19	b	l,w	
I	E_4	Chittagong	1,3,10,19	b	z_{35}	
I	E_4	Accra	1,3,19	b	z_6	
I	E_4	Eastglam	1,3,19	c	1,5	
I	E_4	Bida	1,3,19	c	1,6	
I	E_4	Madiago	1,3,19	c	1,7	
I	E_4	Ahmadi	1,3,19	d	1,5	
I	E_4	Liverpool	1,3,19	d	e,n,z_{15}	
I	E_4	Tilburg	1,3,19	d	l,w	Tilburg may possess H phase Rz_{49}.
I	E_4	Niloese	1,3,19	d	z_6	
I	E_4	Vilvoorde	1,3,19	e,h	1,5	
I	E_4	Hayindogo	1,3,19	e,h	1,6	
I	E_4	Sanktmarx	1,3,19	e,h	1,7	
I	E_4	Sao	1,3,19	e,h	e,n,z_{15}	
I	E_4	Calabar	1,3,19	e,h	l,w	
I	E_4	Rideau	1,3,19	f,g	-	
I	E_4	Bilu	(1),3,10,(19)	f,g,t	1,(2),7	
I	E_4	Petahtikva	1,3,19	f,g,t	1,7	
I	E_4	Maiduguri	1,3,19	f,g,t	e,n,z_{15}	
I	E_4	Kouka	1,3,19	g,m,[t]	–	
I	E_4	Dessau	1,3,15,19	g,s,t	–	
I	E_4	Senftenberg	1,3,19	g,[s],t		Senftenberg may possess H phase Rz_{37} or Rz_{43} or Rz_{45} or Rz_{46}. Simsbury (1,3,19:Rz_{27}:-) is now considered an H phase Rz_{27} of Senftenberg.
I	E_4	Stratford	1,3,19	i	1,2	
I	E_4	Chichester	1,3,19	i	1,6	
I	E_4	Machaga	1,3,19	i	e,n,x	
I	E_4	Avonmouth	1,3,19	i	e,n,z_{15}	
I	E_4	Zuilen	1,3,19	i	l,w	
I	E_4	Taksony	1,3,19	[i]	z_6	
I	E_4	Bethune	1,3,19	k	1,7	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_4	Ngor	1,3,19	l,v	1,5	
I	E_4	Parkroyal	1,3,19	l,v	1,7	
I	E_4	Svedvi	1,3,19	l,v	e,n,z_{15}	
I	E_4	Fulda	1,3,19	l,w	1,5	
I	E_4	Westerstede	1,3,19	l,z_{13}	[1,2]	
I	E_4	Winterthur	1,3,19	l,z_{13}	1,6	
I	E_4	Lokstedt	1,3,19	l,z_{13},z_{28}	1,2	
I	E_4	Stuivenberg	1,3,19	l,z_{13},z_{28}	1,5	
I	E_4	Bedford	1,3,19	l,z_{13},z_{28}	e,n,z_{15}	
I	E_4	Tomelilla	1,3,19	l,z_{28}	1,7	
I	E_4	Kindia	1,3,19	l,z_{28}	e,n,x	
I	E_4	Cannstatt	1,3,19	m,t	–	
I	E_4	Yalding	1,3,19	r	e,n,z_{15}	
I	E_4	Fareham	1,3,19	r,i	l,w	
I	E_4	Simsbury	1,3,19	Rz_{27}	–	IP combined Simsbury with Senftenberg 1,3,19:g,[s],t:-. Simsbury is now considered an R phase of Senftenberg. The name Simsbury has been dropped.
I	E_4	Gatineau	1,3,19	y	1,5	
I	E_4	Thies	1,3,19	y	1,7	
I	E_4	Cannonhill	1,3,15,19	y	e,n,x	
I	E_4	Kinson	1,3,19	y	e,n,x	
I	E_4	Slade	1,3,19	y	e,n,z_{15}	
I	E_4	Krefeld	1,3,19	y	l,w	
I	E_4	Korlebu	1,3,19	z	1,5	
I	E_4	Kainji	1,3,19	z	1,6	
I	E_4	Lerum	1,3,19	z	1,7	
I	E_4	Schoeneberg	1,3,19	z	e,n,z_{15}	
I	E_4	Carno	1,3,19	z	l,w	
I	E_4	Hongkong	1,3,19	z	z_6	
I	E_4	Dallgow	1,3,19	z_{10}	e,n,z_{15}	
I	E_4	Llandoff	1,3,19	z_{29}	[z_6]	
I	E_4	Ochiogu	1,3,19	z_{38}	[e,n,z_{15}]	
I	E_4	Ilugun	1,3,10,19	z_4,z_{23}	z_6	
I	E_4	Sambre	1,3,19	z_4,z_{24}	–	
II	F		11	–	1,5	
I	F	Gallen	11	a	1,2	
I	F	Marseille	11	a	1,5	
VI	F		11	a	1,5	
I	F	Toowong	11	a	1,7	
II	F	Montgomery	11	a,[d]	[d]:e,n,z15	
I	F	Luciana	11	a	e,n,z_{15}	
I	F	Epinay	11	a	l,z_{13},z_{28}	
II	F	Glencairn	11	a	z_6:z_{42}	
I	F	Atento	11	b	1,2	
I	F	Leeuwarden	11	b	1,5	
I	F	Wohlen	11	b	1,6	
VI	F		11	b	1,7	
VI	F	Srinagar	11	b	e,n,x	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	F	Pharr	11	b	e,n,z_{15}	
I	F	Chiredzi	11	c	1,5	
I	F	Woodinville	11	c	e,n,x	
II	F		11	c	e,n,z_{15}	
I	F	Ati	11	d	1,2	
I	F	Gustavia	11	d	1,5	
I	F	Chandans	11	d	e,n,x:[r]	
I	F	Pennsylvania	11	d	e,n,z_{15}	
I	F	Findorff	11	d	z_6	
I	F	Chingola	11	e,h	1,2	
I	F	Adamstua	11	e,h	1,6	
I	F	Redhill	11	e,h	l,z_{13},z_{28}	
II	F	Grabouw	11	g,[m],s,t	[z_{39}]	
I	F	Missouri	11	g,s,t	–	
IV	F	Mundsburg	11	g,z51	–	
I	F	Aberdeen	11	i	1,2	
I	F	Brijbhumi	11	i	1,5	
I	F	Heerlen	11	i	1,6	
I	F	Veneziana	11	i	e,n,x	
I	F	Pretoria	11	k	1,2	
I	F	Abaetetuba	11	k	1,5	
I	F	Sharon	11	k	1,6	
I	F	Colobane	11	k	1,7	
I	F	Kisarawe	11	k	e,n,x,[z_{15}]	
I	F	Mannheim	11	k	l,w	
I	F	Amba	11	k	l,z_{13},z_{28}	
IIIb	F		11	k	z_{53}	(Ar. 17:29:25)
I	F	Stendal	11	l,v	1,2	
I	F	Maracaibo	11	l,v	1,5	
I	F	Fann	11	l,v	e,n,x	
I	F	Bullbay	11	l,v	e,n,z_{15}	
IIIb	F		11	l,v	z_{53}	(Ar. 17:23:25)
IIIb	F		11	l,v	z	(Ar. 17:23:31). May possess H phase Rz$_{56}$ (Ar. 38).
I	F	Glidji	11	l,w	1,5	
I	F	Connecticut	11	l,z_{13},z_{28}	1,5	
I	F	Osnabrueck	11	l,z_{13},z_{28}	e,n,x	
II	F	Huila	11	l,z_{28}	e,n,x	
I	F	Moers	11	m,t	–	
II	F	Lincoln	11	m,t	e,n,x	
I	F	Senegal	11	r	1,5	
I	F	Rubislaw	11	r	[e,n,x]	
I	F	Volta	11	r	l,z_{13},z_{28}	
I	F	Euston	11	r,i	e,n,x,z_{15}	
I	F	Solt	11	y	1,5	
I	F	Jalisco	11	y	1,7	
I	F	Herzliya	11	y	e,n,x	
I	F	Crewe	11	z	1,5	
I	F	Maroua	11	z	1,7	
II	F		11	z	e,n,x	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	F	Soutpan	11	z	z_{39}	
I	F	Nyanza	11	z	z_6	
I	F	Wentworth	11	z_{10}	1,2	
I	F	Straengnaes	11	z_{10}	1,5	
I	F	Telhashomer	11	z_{10}	e,n,x	
I	F	Lene	11	z_{38}	–	
IIIa	F		11	z_4,z_{23}	–	(Ar. 17:1,2,5:-)
IV	F	Parera	11	z_4,z_{23}	–	
I	F	Remete	11	z_4,z_{23}	1,6	
I	F	Etterbeek	11	z_4,z_{23}	e,n,z_{15}	
I	F	Yehuda	11	z_4,z_{24}	–	
IV	F		11	z_4,z_{32}	–	
I	F	Maastricht	11	z_{41}	1,2	
II	G		13,23	–	1,6	
I	G	Chagoua	1,13,23	a	1,5	
II	G		1,13,23	a	1,5	
I	G	Mim	13,22	a	1,6	
II	G		13,22	a	e,n,x	
I	G	Wyldegreen	1,13,23	a	l,w	
I	G	Marshall	13,22	a	l,z_{13},z_{28}	
II	G	Tygerberg	1,13,23	a	z_{42}	
I	G	Atlanta	13,23	b	–	Atlanta was combined with Mississippi (1,13,23:b:1,5). The name Atlanta has been dropped.
I	G	Ibadan	13,22	b	1,5	
I	G	Mississippi	1,13,23	b	[1,5]	
II	G	Acres	1,13,23	b	$[1,5]:z_{42}$	
I	G	Bracknell	13,23	b	1,6	
I	G	Oudwijk	13,22	b	1,6	
I	G	Rottnest	1,13,22	b	1,7	
I	G	Ullevi	1,13,23	b	e,n,x	
I	G	Vaertan	13,22	b	e,n,x	
I	G	Bahati	13,22	b	e,n,z_{15}	
I	G	Durham	13,23	b	e,n,z_{15}	
II	G		1,13,22	b	z_{42}	
I	G	Haouaria	13,22	c	e,n,x,z_{15}	
I	G	Handen	1,13,23	d	1,2	
I	G	Mishmarhaemek	1,13,23	d	1,5	
I	G	Friedenau	13,22	d	1,6	
I	G	Wichita	1,13,23	d	1,6	Wichita may possess H phase Rz_{37}.
I	G	Grumpensis	1,13,23	d	1,7	
II	G		13,23	d	e,n,x	
I	G	Diguel	1,13,22	d	e,n,z_{15}	
I	G	Telelkebir	13,23	d	e,n,z_{15}	
II	G		1,13,23	d	e,n,z_{15}	
I	G	Putten	13,23	d	l,w	
I	G	Isuge	13,23	d	z_6	
I	G	Tschangu	1,13,23	e,h	1,5	
I	G	Willemstad	1,13,22	e,h	1,6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	G	Vridi	1,13,23	e,h	l,w	
II	G	Epping	1,13,23	e,n,x	1,[5],7	
I	G	Raus	13,22	f,g	e,n,x	
I	G	Havana	1,13,23	f,g,[s]	–	Havana may possess H phase Rz_{45} or Rz_{79}.
IIIa	G		1,13,23	g,51	–	(Ar. 18:13,14:-)
I	G	Bron	13,22	g,m	[e,n,z15]	
II	G		1,13,23	g,m,s,t	1,5	
II	G	Luanshya	1,13,23	g,m,s,t	[e,n,x]	IP combined Luanshya with Kraaifontein (1,13,23:g,m,t:[e,n,x]) to form Luanshya 1,13,23:g,m,[s],t:[e,n,x].
II	G		1,13,23	g,[s],t	z_{42}	
II	G	Limbe	1,13,22	g,m,t	[1,5]	
II	G	Kraaifontein	1,13,23	g,m,t	[e,n,x]	IP combined Kraaifontein with Luanshya (1,13,23:g,m,s,t:[e,n,x]) to form Luanshya 1,13,23:g,m,[s],t:[e,n,x]. The name Kraaifontein has been dropped.
I	G	Agbeni	1,13,23	g,m,[t]	–	
I	G	Congo	13,23	g,[m],[s],t	–	IP calls Congo 13,23:g,m,s,t:-.
I	G	Newyork	13,22	g,s,t	–	
I	G	Okatie	13,23	g,[s],t	–	
II	G		1,13,23	g,m,s,t	z_{42}	
II	G	Gojenberg	1,13,23	g,t	1,5	
II	G	Rotterdam	1,13,22	g,t	1,5	
V	G		1,13,22	i	–	
I	G	Idikan	1,13,23	i	1,5	
I	G	Jukestown	13,23	i	e,n,z_{15}	
I	G	Kedougou	1,13,23	i	l,w	
I	G	Marburg	13,23	k	–	
II	G		13,22	k	$1,5:z_{42}$	
II	G		13,23	k	z_{41}	
I	G	Lovelace	13,22	l,v	1,5	
IIIb	G		13,22	l,v	1,5,7	(Ar. 18:23:30)
I	G	Borbeck	13,22	l,v	1,6	
I	G	Nanga	1,13,23	l,v	e,n,z_{15}	
II	G		13,23	l,w	e,n,x	
II	G		13,22	l,z_{28}	1,5	
II	G		13,23	l,z_{28}	1,5	
II	G	Vredelust	1,13,23	l,z_{28}	z_{42}	
II	G		13,23	l,z_{28}	z_6	
I	G	Kintambo	1,13,23	m,t	–	
I	G	Washington	13,22	m,t	–	
II	G	Katesgrove	1,13,23	m,t	1,5	
II	G	Worcester	1,13,23	m,t	e,n,x	
II	G	Boulders	1,13,23	m,t	z_{42}	
II	G		13,22	m,t	$z_{42}:z_{39}$	
V	G		13,22	r	–	
I	G	Adjame	13,23	r	1,6	
I	G	Linton	13,23	r	e,n,z_{15}	
I	G	Tanger	1,13,22	y	1,6	
I	G	Yarrabah	13,23	y	1,7	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	G	Ordonez	1,13,23	y	l,w	
I	G	Tunis	1,13,23	y	z_6	
II	G		13,22	z	–	
II	G	Nachshonim	1,13,23	z	1,5	
I	G	Farmsen	13,23	z	1,6	
I	G	Poona	1,13,22	z	1,6	Poona may possess H phase Rz_{59}.
I	G	Bristol	13,22	z	1,7	
I	G	Tanzania	1,13,22	z	e,n,z_{15}	
I	G	Worthington	1,13,23	z	l,w	Worthington may possess H phase Rz_{45}.
II	G		1,13,23	z	z_{42}	
I	G	Roodepoort	1,13,22	z_{10}	1,5	
I	G	Demerara	13,23	z_{10}	l,w	
II	G		1,13,22	z_{10}	z_6	
I	G	Agoueve	13,22	z_{29}	–	
I	G	Cubana	1,13,23	z_{29}	–	Cubana may possess H phase Rz_{37} or Rz_{43}.
II	G	Clifton	13,22	z_{29}	1,5	
II	G		1,13,23	z_{29}	1,5	
II	G	Goodwood	13,22	z_{29}	e,n,x	
II	G		1,13,23	z_{29}	e,n,x	
I	G	Mampong	13,22	z_{35}	1,6	
I	G	Anna	13,23	z_{35}	e,n,z_{15}	
I	G	Nimes	13,22	z_{35}	e,n,z_{15}	
I	G	Fanti	13,23	z_{38}	–	
I	G	Leiden	13,22	z_{38}	-	
II	G		1,13,23	z_{39}	1,5,7	
II	G		13,22	z_{39}	1,7	
I	G	Ajiobo	13,23	z_4,z_{23}	–	
IIIa	G		13,22	z_4,z_{23}	–	(Ar. 18:1,2,5:-)
I	G	Ried	1,13,22	z_4,z_{23}	[e,n,z_{15}]	
IIIa	G		13,23	z_4,z_{23},z_{32}	–	(Ar. 18:1,6,7:-). CDC would call this 1,6,7,9.
I	G	Romanby	1,13,23	z_4,z_{24}	–	
IIIa	G		1,13,23	z_4,z_{24}	–	(Ar. 18:1,3,11:-)
II	G	Stevenage	1,13,23	[z_{42}]	1,[5],7	
I	H	Garba	1,6,14,25	a	1,5	
VI	H		[1],6,14	a	1,5	
VI	H	Ferlac	1,6,14,25	a	e,n,x	
I	H	Banjul	1,6,14,25	a	e,n,z_{15}	
I	H	Ndjamena	1,6,14,25	b	1,2	
I	H	Kuntair	1,6,14,25	b	1,5	
I	H	Tucson	[1],6,14,[25]	b	[1,7]	
IIIb	H		(6),14	b	e,n,x,z_{15}	(Ar. 7a,7c:43:28)
I	H	Blijdorp	1,6,14,25	c	1,5	
I	H	Kassberg	1,6,14,25	c	1,6	
I	H	Runby	1,6,14,25	c	e,n,x	
I	H	Minna	1,6,14,25	c	l,w	
I	H	Finkenwerder	[1],6,14,[25]	d	1,5	
I	H	Heves	6,14,[24]	d	1,5	
I	H	Woodhull	1,6,14,25	d	1,6	
I	H	Florida	[1],6,14,[25]	d	1,7	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	H	Midway	6,14,24	d	1,7	
I	H	Charity	[1],6,14,[25]	d	e,n,x	
I	H	Lindern	6,14,[24]	d	e,n,x	
I	H	Teko	1,6,14,25	d	e,n,z_{15}	
I	H	Encino	1,6,14,25	d	l,z13,z_{28}	
I	H	Albuquerque	1,6,14,24	d	z_6	
I	H	Bahrenfeld	6,14,24	e,h	1,5	
I	H	Onderstepoort	1,6,14,[25]	e,h	1,5	
I	H	Magumeri	1,6,14,25	e,h	1,6	
I	H	Beaudesert	[1],6,14,[25]	e,h	1,7	
I	H	Warragul	[1],6,14,[25]	g,m	–	
I	H	Caracas	[1],6,14,[25]	g,m,s	–	
I	H	Sylvania	[1],6,14,[25]	g,p	–	
I	H	Catanzaro	6,14	g,s,t	–	
I	H	Mampeza	1,6,14,25	i	1,5	
I	H	Buzu	[1],6,14,[25]	i	1,7	
I	H	Schalkwijk	6,14,[24]	i	e,n,..	
I	H	Moussoro	1,6,14,25	i	e,n,z_{15}	
I	H	Harburg	[1],6,14,[25]	k	1,5	
II	H		6,14,[24]	k	1,6	
II	H		6,14	k	[e,n,x]	
IIIb	H		(6),14	k	z	(Ar. 7a,7c:29:31)
IIIb	H		(6),14	k	z_{53}	(Ar. 7a,7c:29:25)
II	H		1,6,14	k	z_6:z_{42}	
I	H	Boecker	[1],6,14,[25]	l,v	1,7	
I	H	Horsham	1,6,14,[25]	l,v	e,n,x	
IIIb	H		(6),14	l,v	z	(Ar. 7a,7c:23:31)
IIIb	H		(6),14	l,v	z_{35}	(Ar. 7a,7c:23:21)
IIIb	H		(6),14	l,v	z_{53}	(Ar. 7a,7c:23:25)
I	H	Aflao	1,6,14,25	l,z_{28}	e,n,x	
I	H	Kaitaan	1,6,14,25	m,t	–	
II	H	Rooikrantz	1,6,14	m,t	1,5	
II	H	Emmerich	6,14	[m,t]	e,n,x	
IIIb	H		(6),14	r	z	(Ar. 7a,7c:24:31)
I	H	Istoria	1,6,14,25	r,i	1,5	
I	H	Surat	[1],6,14,[25]	[r],[i]	e,n,z_{15}	
I	H	Carrau	6,14,[24]	y	1,7	
I	H	Madelia	1,6,14,25	y	1,7	
I	H	Fischerkietz	1,6,14,25	y	e,n,x	
I	H	Mornington	1,6,14,25	y	e,n,z_{15}	
I	H	Homosassa	1,6,14,25	z	1,5	
I	H	Kanifing	1,6,14,25	z	1,6	
I	H	Soahanina	6,14,24	z	e,n,x	
I	H	Sundsvall	[1],6,14,[25]	z	e,n,x	
I	H	Royan	1,6,14,25	z	e,n,z_{15}	
I	H	Poano	1,6,14,25	z	l,z_{13},z_{28}	
I	H	Nessa	1,6,14,25	z_{10}	1,2	
VI	H	Bornheim	1,6,14,25	z_{10}	1,(2),7	Bornheim was formerly in Subspecies II.
II	H	Simonstown	1,6,14	z_{10}	1,5	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIb	H		(6),14	z_{10}	e,n,x,z_{15}	(Ar. 7a,7c:27:28)
IIIb	H		(6),14	z_{10}	z_{53}	(Ar. 7a,7c:27:25)
II	H	Slangkop	1,6,14	z_{10}	z_6:z_{42}	
IIIb	H		(6),14	z_{10}	z:[z_{53}]	(Ar. 7a,7c:27:31:[25])
I	H	Potosi	6,14	z_{36}	1,5	
I	H	Sara	1,6,14,25	z_{38}	[e,n,x]	
IV	H		6,14	z_4,z_{23}	–	
I	H	Arapahoe	1,6,14	z_4,z_{23}	1,5	
I	H	Bousso	1,6,14,25	z_4,z_{23}	[e,n,z_{15}]	
I	H	Chichiri	6,14,24	z_4,z_{24}	–	
I	H	Uzaramo	1,6,14,25	z_4,z_{24}	–	
II	H		1,6,14	z_{42}	1,6	
IIIb	H		(6),14	z_{52}	e,n,x,z_{15}	(Ar. 7a,7c:26:28)
IIIb	H		(6),14	z_{52}	z_{35}	(Ar. 7a,7c:26:21)
I	I	Hannover	16	a	1,2	
I	I	Brazil	16	a	1,5	
I	I	Amunigun	16	a	1,6	
I	I	Nyeko	16	a	1,7	
I	I	Togba	16	a	e,n,x	
I	I	Fischerhuette	16	a	e,n,z_{15}	
I	I	Heron	16	a	z_6	
I	I	Hull	16	b	1,2	
I	I	Wa	16	b	1,5	
I	I	Glasgow	16	b	1,6	
I	I	Hvittingfoss	16	b	e,n,x	
II	I		16	b	e,n,x	
I	I	Sangera	16	b	e,n,z_{15}	
I	I	Vegesack	16	b	l,w	
II	I		16	b	z_{39}	
II	I		16	b	z_{42}	
I	I	Malstatt	16	b	z_6	
I	I	Vancouver	16	c	1,5	
I	I	Gafsa	16	c	1,6	
I	I	Shamba	16	c	e,n,x	
I	I	Hithergreen	16	c	e,n,z_{15}	
I	I	Yoruba	16	c	l,w	
I	I	Oldenburg	16	d	1,2	
I	I	Sculcoates	16	d	1,5	
II	I		16	d	1,5	
I	I	Sherbrooke	16	d	1,6	
I	I	Gaminara	16	d	1,7	
I	I	Barranquilla	16	d	e,n,x	
I	I	Nottingham	16	d	e,n,z_{15}	
I	I	Caen	16	d	l,w	
I	I	Barmbek	16	d	z6	
I	I	Malakal	16	e,h	1,2	
I	I	Saboya	16	e,h	1,5	
I	I	Rhydyfelin	16	e,h	e,n,x	
I	I	Weston	16	e,h	z_6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	I	Bellville	16	e,n,x	1,(5),7	
II	I	Elsiesrivier	16	[e,n,x]	1,6:z_{42}	
I	I	Tees	16	f,g	–	
I	I	Nikolaifleet	16	g,m,s	–	
I	I	Adeoyo	16	g,m,[t]	–	
II	I	Mobeni	16	g,[m],[s],t	[e,n,x]	
II	I		16	g,[m],[s],t	z_{42}	
I	I	Cardoner	16	g,s,t	–	
II	I	Merseyside	16	g,t	[1,5]	
I	I	Amina	16	i	1,5	
I	I	Agbara	16	i	1,6	
I	I	Wisbech	16	i	1,7	
I	I	Frankfurt	16	i	e,n,z_{15}	
I	I	Pisa	16	i	l,w	
IIIb	I		16	i	z_{35}	(Ar. 25:33:21)
I	I	Abobo	16	i	z_6	
I	I	Szentes	16	k	1,2	
I	I	Nuatja	16	k	e,n,x	
I	I	Orientalis	16	k	e,n,z_{15}	
IIIb	I		16	k	z	(Ar. 25:29:31)
IIIb	I		16	k	z_{53}	(Ar. 25:29:25)
IIIb	I		16	(k)	z_{35}	(Ar. 25:22:21)
IIIb	I		16	l,v	1,5,7	(Ar. 25:23:30)
I	I	Shanghai	16	l,v	1,6	
I	I	Welikade	16	l,v	1,7	
I	I	Salford	16	l,v	e,n,x	
I	I	Burgas	16	l,v	e,n,z_{15}	
IIIb	I		16	l,v	z_{35}	(Ar. 25:23:21)
IIIb	I		16	l,v	z_{53}	(Ar. 25:23:25)
I	I	Losangeles	16	l,v	z_6	
IIIb	I		16	l,v	z:[z_{61}]	(Ar. 25:23:31:[41])
I	I	Zigong	16	l,w	1,5	
I	I	Westeinde	16	l,w	1,6	
I	I	Brooklyn	16	l,w	e,n,x	
I	I	Lomnava	16	l,w	e,n,z_{15}	
II	I	Noordhoek	16	l,w	z_6	
I	I	Mandera	16	l,z13	e,n,z_{15}	
I	I	Battle	16	l,z_{13},z_{28}	1,6	
I	I	Ablogame	16	l,z_{13},z_{28}	z_6	
I	I	Enugu	16	l,[z_{13}],z_{28}	[1,5]	
II	I	Sarepta	16	l,z_{28}	z_{42}	
I	I	Mpouto	16	m,t	–	
II	I		16	m,t	e,n,x	
II	I	Rowburton	16	m,t	[z_{42}]	
I	I	Ivory	16	r	1,6	
I	I	Brunflo	16	r	1,7	
I	I	Annedal	16	r,i	e,n,x	
I	I	Zwickau	16	r,i	e,n,z_{15}	
I	I	Rovaniemi	16	r,[i]	1,5	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	I	Saphra	16	y	1,5	
I	I	Akuafo	16	y	1,6	
I	I	Kikoma	16	y	e,n,x	
I	I	Avignon	16	y	e,n,z_{15}	
I	I	Fortlamy	16	z	1,6	
I	I	Lingwala	16	z	1,7	
II	I	Louwbester	16	z	[e,n,x]	
I	I	Brevik	16	z	e,n,[x],z_{15}	
II	I		16	z	z_{42}	
I	I	Bouake	16	z	z_6	
I	I	Badagry	16	z_{10}	1,5	
IIIb	I		16	z_{10}	1,5,7	(Ar. 25:27:30)
I	I	Lisboa	16	z_{10}	1,6	
IIIb	I		16	z_{10}	e,n,x,z_{15}	(Ar. 25:27:28)
I	I	Redlands	16	z_{10}	e,n,z_{15}	
I	I	Angouleme	16	z_{10}	z_6	
I	I	Saloniki	16	z_{29}	–	
II	I		16	z_{29}	1,5	
II	I	Jacksonville	16	z_{29}	[e,n,x]	
I	I	Trier	16	z_{35}	1,6	
I	I	Dakota	16	z_{35}	e,n,z15	
IV	I		16	z_{36}	–	
I	I	Naware	16	z_{38}	–	
I	I	Grancanaria	16	z_{39}	[1,6]	Grancanaria can be d-tartrate neg., dulcitol neg., ONPG pos., and anaerogenic.
II	I	Haddon	16	z_4,z_{23}	–	
IV	I	Ochsenzoll	16	z_4,z_{23}	–	
I	I	Kibi	16	z_4,z_{23}	[1,6]	
II	I		16	z_4,z_{24}	–	
IV	I	Chameleon	16	z_4,z_{32}	–	
II	I	Woodstock	16	z_{42}	1,[5],7	
IIIb	I		16	z_{52}	z_{35}	(Ar. 25:26:21)
II	I		16	z_6	1,6	
I	J	Bonames	17	a	1,2	
I	J	Jangwani	17	a	1,5	
I	J	Kinondoni	17	a	e,n,x	
I	J	Kirkee	17	b	1,2	
I	J	Dahra	17	b	1,5	
II	J	Hillbrow	17	b	e,n,x,z_{15}	
I	J	Bignona	17	b	e,n,z_{15}	
II	J		17	b	z6	
I	J	Victoriaborg	17	c	1,6	
II	J	Woerden	17	c	z_{39}	
I	J	Berlin	17	d	1,5	
I	J	Niamey	17	d	l,w	
I	J	Jubilee	17	e,h	1,2	
II	J		17	e,n,x,z_{15}	1,[5],7	
II	J	Verity	17	e,n,x,z_{15}	1,6	
II	J	Bleadon	17	(f),g,t	[e,n,x,z_{15}]	IP has dropped f.

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	J		17	g,m,s,t	–	
I	J	Lowestoft	17	g,s,t	–	
II	J		17	g,t	z_{39}	
I	J	Ahanou	17	i	1,7	
IIIb	J		17	i	z_{35}	(Ar. 12:33:21)
II	J		17	k	–	
I	J	Irenea	17	k	1,5	
I	J	Warri	17	k	1,7	
I	J	Matadi	17	k	e,n,x	
I	J	Zaria	17	k	e,n,z_{15}	
IIIb	J		17	k	z	(Ar. 12:29:32)
I	J	Morotai	17	l,v	1,2	
I	J	Michigan	17	l,v	1,5	
I	J	Lancaster	17	l,v	1,7	
I	J	Carmel	17	l,v	e,n,x	
IIIb	J		17	l,v	e,n,x,z_{15}	(Ar. 12:23:28)
IIIb	J		17	l,v	z_{35}	(Ar. 12:23:21)
I	J	Granlo	17	l,z_{28}	e,n,x	
I	J	Bama	17	m,t	–	
II	J		17	m,t	–	
I	J	Lode	17	r	1,2	
IIIb	J		17	r	z	(Ar. 12:24:31)
II	J		17	y	–	
I	J	Hadejia	17	y	e,n,z_{15}	
I	J	Gori	17	z	1,2	
I	J	Warengo	17	z	1,5	
I	J	Tchamba	17	z	e,n,z_{15}	
II	J	Constantia	17	z	l,w:z_{42}	
I	J	Djibouti	17	z_{10}	e,n,x	
IIIb	J		17	z_{10}	e,n,x,z_{15}	(Ar.12:27:28). May possess H phase Rz$_{56}$ (Ar. 38).
IIIb	J		17	z_{10}	z	(Ar. 12:27:31)
I	J	Kandla	17	z_{29}	–	
IIIa	J		17	z_{29}	–	(Ar. 12:16,17,18:-)
IV	J		17	z_{29}	–	
IIIa	J		17	z_{36}	–	(Ar. 12:17,20:-)
IV	J		17	z_{36}	–	
IIIa	J		17	z_4,z_{23}	–	(Ar. 12:1,2,5:- and 12:1,2,6:-)
IIIa	J		17	z_4,z_{23},z_{32}	–	(Ar. 12:1,6,7,9:-)
IIIa	J		17	z_4,z_{24}	–	(Ar. 12:1,3,11:-)
IIIa	J		17	z_4,z_{32}	–	(Ar. 12:1,6,7:- and 12:1,7,8:-)
I	K	Cotia	18	–	1,6	
I	K	Brazos	6,14,18	a	e,n,z_{15}	
I	K	Fluntern	6,14,18	b	1,5	
I	K	Rawash	6,14,18	c	e,n,x	
I	K	Groenekan	18	d	1,5	
I	K	Usumbura	6,14,18	d	1,7	
I	K	Pontypridd	18	g,m	–	
IIIa	K		18	g,z_{51}	–	(Ar. 7a,7b:13,14:-)
I	K	Memphis	18	k	1,5	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIb	K		18	(k)	z_{53}	(Ar. 7a,7b:22:25)
IIIb	K		18	(k)	z_{54}	(Ar. 7a,7b:22:34)
IIIb	K		18	l,v	e,n,x,z_{15}	(Ar. 7a,7b:23:28)
I	K	Orlando	18	l,v	e,n,z_{15}	
IIIb	K		18	l,v	z	(Ar. 7a,7b:23:31)
IIIb	K		18	l,v	z_{53}	(Ar. 7a,7b:23:25)
I	K	Toulon	18	l,w	e,n,z_{15}	
I	K	Langenhorn	18	m,t	–	
II	K		18	m,t	1,5	
IIIb	K		18	r	z	(Ar. 7a,7b:24,31)
II	K		18	y	e,n,x,z_{15}	
I	K	Potengi	18	z	–	
I	K	Leer	18	z_{10}	1,5	
IIIb	K		18	z_{10}	e,n,x,z_{15}	(Ar. 7a,7b:27:28)
I	K	Carnac	18	z_{10}	z_6	
II	K	Zeist	18	z_{10}	z_6	
II	K	Beloha	18	z_{36}	–	
IV	K		18	z_{36},z_{38}	–	
I	K	Sinthia	18	z_{38}	–	
II	K		18	z_4,z_{23}	–	
IIIa	K		18	z_4,z_{23}	–	(Ar. 7a,7b:1,2,5:- and 7a,7b:1,2,6:-)
I	K	Cerro	18	z_4,z_{23}	[1,5]	Cerro was combined with Siegburg (6,14,18:z_4,z_{23}:[1,5]) and called Cerro. Cerro may possess H phase Rz_{45}.
I	K	Siegburg	6,14,18	z_4,z_{23}	[1,5]	IP combined Siegburg with Cerro (18:z_4,z_{23}:[1,5]) to form Cerro 6,14,18:z_4,z_{23}:[1,5]. Siegburg is now called Cerro var. O 14+. The name Siegburg has been dropped.
I	K	Aarhus	18	z_4,z_{23}	z_{64}	
I	K	Blukwa	18	z_4,z_{24}	–	
II	K		18	z_4,z_{24}	–	
IIIa	K	Shomron	18	z_4,z_{32}	–	Shomron was formerly in Subspecies II, but is now combined with *Arizona* 7a,7b:1,7,8:-. The name Shomron has been dropped.
IIIa	K		18	z_4,z_{32}	–	(Ar. 7a,7b:1,6,7:- and 7a,7b:1,7,8:-)
I	K	Delmenhorst	18	z_{71}	–	
I	L	Assen	21	a	[1,5]	
II	L		21	b	1,5	
I	L	Ghana	21	b	1,6	
I	L	Minnesota	21	b	e,n,x	Minnesota may possess H phase Rz_{33} or Rz_{49}.
I	L	Hydra	21	c	1,6	
I	L	Rhone	21	c	e,n,x	
II	L		21	c	e,n,x	
IIIb	L		21	c	e,n,x,z_{15}	(Ar. 22:32:28)
I	L	Spartel	21	d	1,5	
I	L	Magwa	21	d	e,n,x	
I	L	Madison	21	d	z_6	
I	L	Good	21	f,g	e,n,x	
II	L		21	g,[m],[s],t	–	
IIIa	L		21	g,z_{51}	–	(Ar. 22:13,14:-)

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IV	L		21	g,z_{51}	–	
I	L	Diourbel	21	i	1,2	
IIIb	L		21	i	1,5,7	(Ar. 22:33:30)
IIIb	L		21	i	e,n,x,z_{15}	(Ar. 22:33:28)
IIIb	L		21	k	e,n,x,z_{15}	(Ar. 22:29:28)
IIIb	L		21	k	z	(Ar. 22:29:31)
IIIb	L		21	l,v	z	(Ar. 22:23:31)
IIIb	L		21	l,v	z_{57}	(Ar. 22:23:40)
I	L	Keve	21	l,w	–	
I	L	Jambur	21	l,z_{28}	e,n,z_{15}	
II	L		21	m,t	–	
I	L	Mountmagnet	21	r	–	
IIIb	L		21	r	z	(Ar. 22:24:31)
I	L	Ibaragi	21	y	1,2	
I	L	Ruiru	21	y	e,n,x	
II	L		21	z	–	CDC does not have this.
IIIb	L		21	z_{10}	e,n,x,z_{15}	(Ar. 22:27:28)
IIIb	L		21	z_{10}	z	(Ar. 22:27:31)
IIIb	L		21	z_{10}	z_{53}	(Ar. 22:27:25)
II	L	Wandsbek	21	z_{10}	[z_6]	
IIIa	L		21	z_{29}	–	(Ar. 22:16,17,18:-)
I	L	Gambaga	21	z_{35}	e,n,z_{15}	
IV	L		21	z_{36}	–	
I	L	Baguida	21	z_4,z_{23}	–	
IIIa	L		21	z_4,z_{23}	–	(Ar. 22:1,2,5:- and 22:1,2,6:-)
IV	L	Soesterberg	21	z_4,z_{23}	–	
II	L	Gwaai	21	z_4,z_{24}	–	
IIIa	L		21	z_4,z_{24}	–	(Ar. 22:1,3,11:-)
IV	L		21	z_4,z_{32}	–	
IIIb	L		21	z_{65}	e,n,x,z_{15}	(Ar. 22:32:28)
I	M	Solna	28	a	1,5	
I	M	Dakar	28	a	1,6	
I	M	Bakau	28	a	1,7	
I	M	Seattle	28	a	e,n,x	
II	M		28	a	e,n,x	
I	M	Honelis	28	a	e,n,z_{15}	
I	M	Dibra	28	a	z_6	
I	M	Moero	28	b	1,5	
I	M	Ashanti	28	b	1,6	
I	M	Bokanjac	28	b	1,7	
I	M	Soumbedioune	28	b	e,n,x	
II	M		28	b	e,n,x	
I	M	Langford	28	b	e,n,z_{15}	
II	M	Kaltenhausen	28	b	z_6	
I	M	Hermannswerder	28	c	1,5	
I	M	Eberswalde	28	c	1,6	
I	M	Halle	28	c	1,7	
I	M	Dresden	28	c	e,n,x	
I	M	Wedding	28	c	e,n,z_{15}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	M	Techimani	28	c	z_6	
I	M	Amoutive	28	d	1,5	
I	M	Hatfield	28	d	1,6	
I	M	Mundonobo	28	d	1,7	
I	M	Mocamedes	28	d	e,n,x	
I	M	Patience	28	d	e,n,z_{15}	
I	M	Cullingworth	28	d	l,w	
I	M	Kpeme	28	e,h	1,7	
I	M	Gozo	28	e,h	e,n,z_{15}	
II	M		28	e,n,x	1,7	
I	M	Friedrichsfelde	28	f,g	–	
I	M	Yardley	28	g,m	1,6	
I	M	Abadina	28	g,m	[e,n,z_{15}]	
I	M	Croft	28	g,m,s	[e,n,z_{15}]	
II	M		28	g,m,t	e,n,x	
II	M		28	g,m,t	z_{39}	
II	M	Llandudno	28	g,[m],[s],t	1,5	
I	M	Ona	28	g,s,t	-	
II	M		28	g,s,t	e,n,x	
I	M	Doorn	28	i	1,2	
I	M	Cotham	28	i	1,5	
I	M	Volksmarsdorf	28	i	1,6	
I	M	Dieuppeul	28	i	1,7	
I	M	Warnemuende	28	i	e,n,x	
I	M	Kuessel	28	i	e,n,z_{15}	
I	M	Douala	28	i	l,w	
I	M	Guildford	28	k	1,2	
I	M	Ilala	28	k	1,5	
I	M	Adamstown	28	k	1,6	
I	M	Ikeja	28	k	1,7	
I	M	Taunton	28	k	e,n,x	
I	M	Ank	28	k	e,n,z_{15}	
I	M	Leoben	28	l,v	1,5	
I	M	Vitkin	28	l,v	e,n,x	
I	M	Nashua	28	l,v	e,n,z_{15}	
I	M	Ramsey	28	l,w	1,6	
I	M	Catalunia	28	l,z_{13},z_{28}	1,5	
I	M	Penilla	28	l,z_{13},z_{28}	e,n,z_{15}	
II	M		28	l,z_{28}	1,5	
I	M	Fajara	28	l,z_{28}	e,n,x	
I	M	Morillons	28	m,t	1,6	
II	M		28	m,t	[e,n,x]	
I	M	Vinohrady	28	m,t	[e,n,z_{15}]	
I	M	Bassadji	28	r	1,6	
I	M	Kibusi	28	r	e,n,x	
II	M	Oevelgoenne	28	r	e,n,z_{15}	
I	M	Fairfield	28	r	l,w	
I	M	Banco	28	r,i	1,7	
I	M	Chicago	28	r,[i]	1,5	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	M	Sanktgeorg	28	r,[i]	e,n,z_{15}	
I	M	Oskarshamn	28	y	1,2	
I	M	Nima	28	y	1,5	
I	M	Pomona	28	y	1,7	Pomona may possess H phases Rz_{60}, Rz_{70} or Rz_{80}.
I	M	Kitenge	28	y	e,n,x	
I	M	Telaviv	28	y	e,n,z_{15}	
I	M	Shomolu	28	y	l,w	
I	M	Selby	28	y	z_6	
I	M	Vanier	28	z	1,5	
II	M		28	z	1,5	
I	M	Doel	28	z	1,6	
I	M	Ezra	28	z	1,7	
I	M	Brisbane	28	z	e,n,z_{15}	
II	M	Ceres	28	z	z_{39}	
I	M	Rogy	28	z_{10}	1,2	
I	M	Farakan	28	z_{10}	1,5	
I	M	Libreville	28	z_{10}	1,6	
I	M	Malaysia	28	z_{10}	1,7	
I	M	Umbilo	28	z_{10}	e,n,x	
I	M	Luckenwalde	28	z_{10}	e,n,z_{15}	
I	M	Moroto	28	z_{10}	l,w	
IIIb	M		28	z_{10}	z	(Ar. 35:27:31)
IIIb	M		28	z_{10}	z:[z_{57}]	(Ar. 35:27:31:[40])
I	M	Djermaia	28	z_{29}	–	
II	M		28	z_{29}	1,5	
II	M		28	z_{29}	e,n,x	
I	M	Konolfingen	28	z_{35}	1,6	
I	M	Babili	28	z_{35}	1,7	
I	M	Santander	28	z_{35}	e,n,z_{15}	
I	M	Aderike	28	z_{38}	[e,n,z_{15}]	
I	M	Cannobio	28	z_4,z_{23}	1,5	
I	M	Teltow	28	z_4,z_{23}	1,6	
I	M	Babelsberg	28	z_4,z_{23}	[e,n,z_{15}]	
I	N	Overvecht	30	a	1,2	
I	N	Zehlendorf	30	a	1,5	
I	N	Guarapiranga	30	a	e,n,x	
I	N	Doulassame	30	a	e,n,z_{15}	
II	N	Odijk	30	a	z_{39}	
I	N	Louga	30	b	1,2	
I	N	Aschersleben	30	b	1,5	
I	N	Urbana	30	b	e,n,x	
I	N	Neudorf	30	b	e,n,z_{15}	
II	N		30	b	z_6	
I	N	Zaire	30	c	1,7	
I	N	Morningside	30	c	e,n,z_{15}	
II	N		30	c	z_{39}	
I	N	Messina	30	d	1,5	
I	N	Livulu	30	e,h	1,2	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	N	Torhout	30	e,h	1,5	
I	N	Giessen	30	g,m,s	–	
II	N		30	g,m,s	e,n,x	
I	N	Godesberg	30	g,m,[t]	–	
I	N	Sternschanze	30	g,s,t	–	Sternschanze may possess H phase Rz_{59}.
II	N	Slatograd	30	g,t	–	
I	N	Wayne	30	g,z_{51}	–	
I	N	Landau	30	i	1,2	
I	N	Morehead	30	i	1,5	
I	N	Mjordan	30	i	e,n,z_{15}	
I	N	Soerenga	30	i	l,w	
I	N	Hilversum	30	k	1,2	
I	N	Ramatgan	30	k	1,5	
I	N	Aqua	30	k	1,6	
I	N	Angoda	30	k	e,n,x	
II	N		30	k	e,n,x,z_{15}	
I	N	Odozi	30	k	e,n,[x],z_{15}	
I	N	Ligeo	30	l,v	1,2	
I	N	Donna	30	l,v	1,5	
I	N	Ockenheim	30	l,z_{13},z_{28}	1,6	
I	N	Morocco	30	l,z_{13},z_{28}	e,n,z_{15}	
II	N		30	l,z_{28}	z_6	
II	N		30	m,t	–	
I	N	Grandhaven	30	r	1,2	
I	N	Gege	30	r	1,5	
I	N	Matopeni	30	y	1,2	
I	N	Bietri	30	y	1,5	
I	N	Steinplatz	30	y	1,6	
I	N	Baguirmi	30	y	e,n,x	
I	N	Nijmegen	30	y	e,n,z_{15}	
I	N	Sada	30	z_{10}	1,2	
I	N	Senneville	30	z_{10}	1,5	
I	N	Kumasi	30	z_{10}	e,n,z_{15}	
I	N	Aragua	30	z_{29}	–	
I	N	Kokoli	30	z_{35}	1,6	
I	N	Wuiti	30	z_{35}	e,n,z_{15}	
I	N	Ago	30	z_{38}	–	
II	N		30	z_{39}	1,7	
I	N	Stoneferry	30	z_4,z_{23}	–	
I	N	Bodjonegoro	30	z_4,z_{24}	–	
II	N		30	z_6	1,6	
I	O	Umhlatazana	35	a	e,n,z_{15}	
I	O	Tchad	35	b	–	
I	O	Gouloumbo	35	c	1,5	
I	O	Yolo	35	c	[e,n,z_{15}]	
II	O		35	d	1,5	
I	O	Dembe	35	d	l,w	
I	O	Gassi	35	e,h	z_6	
I	O	Adelaide	35	f,g	–	Adelaide may possess H phase Rz_{27}.

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	O	Ealing	35	g,m,s	–	
II	O		35	g,m,s,t	–	
I	O	Ebrie	35	g,m,t	–	
I	O	Anecho	35	g,s,t	–	
I	O	Agodi	35	g,t	–	
II	O		35	g,t	1,5	
II	O		35	g,t	z_{42}	
IIIa	O		35	g,z_{51}	–	(Ar. 20:13,14:-)
IIIb	O		35	i	e,n,x,z_{15}	(Ar. 20:33:28)
I	O	Gambia	35	i	e,n,z_{15}	
I	O	Bandia	35	i	l,w	
IIIb	O		35	i	z	(Ar. 20:33:31)
IIIb	O		35	i	z_{35}	(Ar. 20:33:21)
IIIb	O		35	i	z_{53}	(Ar. 20:33:25)
IIIb	O		35	k	e,n,x,z_{15}	(Ar. 20:29:28)
IIIb	O		35	k	z	(Ar. 20:29:31)
IIIb	O		35	k	z_{53}	(Ar. 20:29:25). May possess H phase Rz_{50} (Ar.42).
IIIb	O		35	(k)	z	(Ar. 20:22:31)
IIIb	O		35	(k)	z_{35}	(Ar. 20:22:21)
IIIb	O		35	l,v	1,5,7	(Ar. 20:23:30)
IIIb	O		35	l,v	e,n,x,z_{15}	(Ar. 20:23:28)
IIIb	O		35	l,v	z_{35}	(Ar. 20:23:21)
II	O		35	l,z_{28}	–	
I	O	Monschaui	35	m,t	-	
II	O		35	m,t	-	
IIIb	O		35	r	e,n,x,z_{15}	(Ar. 20:24:28)
I	O	Massakory	35	r	l,w	
IIIb	O		35	r	z	(Ar. 20:24:31)
IIIb	O		35	r	z_{35}	(Ar. 20:24:21)
IIIb	O		35	r	z_{61}	(Ar. 20:24:41)
I	O	Camberene	35	z_{10}	1,5	
I	O	Enschede	35	z_{10}	l,w	
IIIb	O		35	z_{10}	z_{35}	(Ar. 20:27:21)
I	O	Ligna	35	z_{10}	z_6	
I	O	Widemarsh	35	z_{29}	–	
IIIa	O		35	z_{29}	–	(Ar. 20:16,17,18:-)
II	O	Utbremen	35	z_{29}	e,n,x	
IIIa	O		35	z_{36}	–	(Ar. 20:17,20:-)
I	O	Haga	35	z_{38}	–	
I	O	Alachua	35	z_4,z_{23}	–	Alachua may possess H phase Rz_{37} or Rz_{45}.
IIIa	O		35	z_4,z_{23}	–	(Ar. 20:1,2,6:-)
I	O	Westphalia	35	z_4,z_{24}	–	
IIIa	O		35	z_4,z_{32}	–	(Ar. 20:1,7,8:-)
IIIb	O		35	z_{52}	1,5,7	(Ar. 20:26:30)
IIIb	O		35	z_{52}	e,n,x,z_{15}	(Ar. 20:26:28)
IIIb	O		35	z_{52}	z	(Ar. 20:26:31)
IIIb	O		35	z_{52}	z_{35}	(Ar. 20:26:21)
II	P		38	b	1,2	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	P	Rittersbach	38	b	e,n,z_{15}	
I	P	Sheffield	38	c	1,5	
I	P	Kidderminster	38	c	1,6	
II	P	Carletonville	38	d	[1,5]	
I	P	Thiaroye	38	e,h	1,2	
I	P	Kasenyi	38	e,h	1,5	
I	P	Korovi	38	g,m,[s]	–	
II	P	Foulpointe	38	g,t	–	
IIIa	P		38	g,z_{51}	–	(Ar. 16:13,14:-)
IV	P		38	g,z_{51}	–	
I	P	Mgulani	38	i	1,2	
I	P	Lansing	38	i	1,5	
IIIb	P		38	i	z	(Ar. 16:33:31)
IIIb	P		38	i	z_{53}	(Ar. 16:33:25)
I	P	Echa	38	k	1,2	
I	P	Mango	38	k	1,5	
I	P	Inverness	38	k	1,6	
I	P	Njala	38	k	e,n,x	
IIIb	P		38	k	e,n,x,z_{15}	(Ar. 16:29:28)
IIIb	P		38	k	z	(Ar. 16:29:31)
IIIb	P		38	k	z_{53}	(Ar. 16:29:25)
IIIb	P		38	(k)	1,5,7	(Ar. 16:22:30)
IIIb	P		38	(k)	z	(Ar. 16:22:31)
IIIb	P		38	(k)	z_{35}	(Ar. 16:22:21). May possess H phase Rz_{56} (Ar. 38).
IIIb	P		38	(k)	z_{54}	(Ar. 16:22:34)
IIIb	P		38	(k)	z_{55}	(Ar. 16:22:37)
I	P	Alger	38	l,v	1,2	
I	P	Kimberley	38	l,v	1,5	
I	P	Roan	38	l,v	e,n,x	
IIIb	P		38	l,v	z	(Ar. 16:23:31)
IIIb	P		38	l,v	z_{35}	(Ar. 16:23:21)
IIIb	P		38	l,v	z_{53}:[z_{54}]	(Ar. 16:23:25:[34])
I	P	Rothenburgsort	38	m,t	–	
I	P	Lindi	38	r	1,5	
IIIb	P		38	r	1,5,7	(Ar. 16:24:30)
I	P	Emmastad	38	r	1,6	
IIIb	P		38	r	e,n,x,z_{15}	(Ar. 16:24:28)
IIIb	P		38	r	z:[z57]	(Ar. 16:24:31:[40])
IIIb	P		38	r	z_{35}	(Ar. 16:24:21)
I	P	Freetown	38	y	1,5	
I	P	Colombo	38	y	1,6	
I	P	Perth	38	y	e,n,x	
I	P	Stachus	38	z	–	
I	P	Neunkirchen	38	z_{10}	–	
IIIb	P		38	z_{10}	z	(Ar. 16:27:31)
IIIb	P		38	z_{10}	z_{53}	(Ar. 16:27:25)
I	P	Klouto	38	z_{38}	–	
IIIa	P		38	z_4,z_{23}	–	(Ar. 16:1,2,6:-)
IV	P		38	z_4,z_{23}	–	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	P	Yoff	38	z_4,z_{23}	1,2	
I	P	Bangkok	38	z_4,z_{24}	–	
IIIb	P		38	z_{47}	z_{53}	(Ar. 16:39:25)
IIIb	P		38	z_{52}	z_{35}	(Ar. 16:26:21)
IIIb	P		38	z_{52}	z_{53}	(Ar. 16:26:25)
IIIb	P		38	z_{53}	–	(Ar. 16:25:-). May possess H phase Rz_{50} (Ar. 42) or Rz_{76} (Ar. Rz_{76}). CDC does not have monophasic.
IIIa	P		38	z_{61}	–	(Ar. 16:41:-)
IIIb	P		38	z_{61}	z_{53}	(Ar. 16:41:25)
II	Q		39	–	1,7	
II	Q		39	a	z_{39}	
I	Q	Wandsworth	39	b	1,2	
I	Q	Abidjan	39	b	l,w	
II	Q		39	c	e,n,x	
I	Q	Logone	39	d	1,5	
I	Q	Mara	39	e,h	[1,5]	
II	Q		39	e,n,x	1,7	
II	Q		39	g,m,t	–	
I	Q	Hofit	39	i	1,5	
I	Q	Cumberland	39	i	e,n,x	
I	Q	Champaign	39	k	1,5	Champaign may possess H phase Rz_{48}
II	Q		39	l,v	1,5	
I	Q	Kokomlemle	39	l,v	e,n,x	
I	Q	Oerlikon	39	l,v	e,n,z_{15}	
II	Q	Mondeor	39	l,z_{28}	e,n,x	
II	Q		39	l,z_{28}	z_{39}	
II	Q		39	m,t	e,n,x	
I	Q	Cook	39	Rz_{48}	1,5	IP combined Cook with Champaign (39:k:1,5). The name Cook has been dropped.
I	Q	Anfo	39	y	1,2	
I	Q	Windermere	39	y	1,5	
I	Q	Hegau	39	z_{10}	–	
I	R	Shikmonah	40	a	1,5	
II	R		1,40	a	1,5	
II	R	Springs	40	a	z_{39}	
I	R	Greiz	40	a	z_6	
II	R		1,40	a	z_6	
II	R		40	b	–	
I	R	Riogrande	40	b	1,5	
I	R	Saugus	40	b	1,7	
I	R	Johannesburg	1,40	b	e,n,x	
I	R	Duval	1,40	b	e,n,z_{15}	
I	R	Benguella	40	b	z_6	
II	R	Suarez	1,40	c	e,n,x,z_{15}	
II	R		1,40	c	z_{39}	
II	R	Ottershaw	40	d	–	
I	R	Driffield	1,40	d	1,5	
I	R	Tilene	1,40	e,h	1,2	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	R		1,40	e,n,x	1,[5],7	
II	R		1,40	e,n,x,z_{15}	1,6	
I	R	Bijlmer	1,40	g,m	–	
II	R	Alsterdorf	1,40	g,m,[s],t	[1,5]	
II	R	Boksburg	40	g,[m],s,[t]	e,n,x	
II	R		1,40	g,[m],[s],t	z_{42}	
II	R		1,40	g,t	[e,n,x]	
II	R		1,40	g,t	1,5	
II	R		1,40	g,t	e,n,x,z_{15}	
II	R		40	g,t	z_{39}	
IV	R	Seminole	1,40	g,z_{51}	–	
IIIb	R		40	g,z_{51}	[e,n,x,z15]	(Ar. 10a,10b:13,14:[28])
IIIb	R		40	i	1,5,7	(Ar. 10a,10b:33:30)
I	R	Goulfey	1,40	k	1,5	
I	R	Allandale	1,40	k	1,6	
I	R	Hann	40	k	e,n,x	
II	R	Sunnydale	1,40	k	e,n,x,z_{15}	
IIIb	R		40	k	z_{53}	(Ar. 10a,10b:29:25)
II	R		40	k	z_6	
IIIb	R		40	k	z:z_{57}	(Ar. 10a,10b:29:31:40)
I	R	Millesi	1,40	l,v	1,2	
I	R	Canary	40	l,v	1,6	
IIIb	R		40	l,v	z	(Ar. 10a,10b,(10c):23:31)
IIIb	R		40	l,v	z_{53}	(Ar. 10a,10b:23:25)
I	R	Overchurch	1,40	l,w	[1,2]	
I	R	Tiko	40	l,z_{13},z_{28}	1,2	
I	R	Bukavu	1,40	l,z_{28}	1,5	
II	R		1,40	l,z_{28}	1,5:z_{42}	
I	R	Santhiaba	40	l,z_{28}	1,6	
II	R		1,40	l,z_{28}	z_{39}	
IV	R		40	m,t	–	
II	R		40	m,t	z_{39}	
II	R		1,40	m,t	z_{42}	
I	R	Odienne	40	y	1,5	
II	R	Bulawayo	1,40	z	1,5	
I	R	Casamance	40	z	e,n,x	
II	R		1,40	z	z_{39}	
II	R		40	z	z_{42}	
I	R	Nowawes	40	z	z_6	
II	R		1,40	z	z_6	
IIIb	R		40	z_{10}	z_{35}	(Ar. 10a,10b:27:21)
I	R	Trotha	40	z_{10}	z_6	
I	R	Omifisan	40	z_{29}	–	
IIIa	R		40	z_{29}	–	(Ar. 10a,10b:16,18:-)
V	R		1,40	z_{35}	–	
II	R	Fandran	1,40	z_{35}	e,n,x,z_{15}	
I	R	Yekepa	1,40	z_{35}	e,n,z_{15}	
IIIa	R		40	z_{35}	–	(Ar. 10a,10b:17,20:-)
II	R		40	z_{39}	1,5:z_{42}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	R	Grunty	1,40	z_{39}	1,6	
II	R		40	z_{39}	1,7	
IIIa	R		40	z_4,z_{23}	–	(Ar. 10a,10b:1,2,5:-; 10a,10b:1,2,5,6:-; and 10a,10b:1,2,6:-)
IV	R	Sachsenwald	1,40	z_4,z_{23}	–	
IIIa	R		40	z_4,z_{24}	–	(Ar. 10a,10b:1,3,11:-)
IV	R	Degania var. subsp. IV	40	z_4,z_{24}	–	
II	R	Degania	40	z_4,z_{24}	[z_{39}]	
IIIa	R		40	z_4,z_{32}	–	(Ar. 10a,10b:1,2,10:-; 10a,10c:1,2,10:-; and 10a,10b:1,7,8:-)
IIIa	R		40	z_4,z_{32}	–	
IV	R	Bern	1,40	z_4,z_{32}	–	
I	R	Karamoja	40	z_{41}	1,2	
II	R		1,40	z_{42}	1,6	
II	R		1,40	[z_{42}]	1,(5),7	
II	R		1,40	z_6	1,5	
V	R		40	z_{81}	–	H z_{81} was formerly H a in *S. bongori*.
II	S		41	–	1,6	
I	S	Burundi	41	a	–	
II	S	Vietnam var. subsp. II	41	b	–	
II	S		41	b	[1,5]	
I	S	Vaugirard	41	b	1,6	
VI	S		41	b	1,7	
I	S	Sica	41	b	e,n,z_{15}	
I	S	Vietnam	41	b	z_6	
IIIb	S		41	c	e,n,x,z_{15}	(Ar. 13:32:28)
II	S		41	c	[z_6]	
I	S	Egusi	41	d	[1,5]	
II	S	Hennepin	41	d	z_6	
II	S		41	g,m,s,t	z_6	
II	S	Lethe	41	g,t	–	
IIIa	S		41	g,z_{51}	–	(Ar. 13:13,14:-)
I	S	Samaru	41	i	1,5	
I	S	Verona	41	i	1,6	
I	S	Ferlo	41	k	1,6	
II	S		41	k	1,6	
II	S		41	k	[z_6]	
IIIb	S		41	(k)	[z_{35}]	(Ar. 13:22:[21])
II	S		41	l,z_{13},z_{28}	e,n,x,z_{15}	
I	S	Leatherhead	41	m,t	1,6	
I	S	Lubumbashi	41	r	1,5	
II	S	Dubrovnik	41	z	1,5	
II	S	Negev	41	z_{10}	1,2	
I	S	Leipzig	41	z_{10}	1,5	
I	S	Landala	41	z_{10}	1,6	
I	S	Inpraw	41	z_{10}	e,n,x	
II	S	Lurup	41	z_{10}	e,n,x,z_{15}	
II	S	Lichtenberg	41	z_{10}	[z_6]	
I	S	Lodz	41	z_{29}	–	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIa	S		41	z_{29}	–	(Ar. 13:16,17,18:-)
IV	S		41	z_{29}	–	
I	S	Ahoutoue	41	z_{35}	1,6	
IIIa	S		41	z_{36}	–	(Ar. 13:17,20:-)
I	S	Offa	41	z_{38}	–	
IIIa	S		41	z_4,z_{23}	–	(Ar. 13:1,2,5:- and 13:1,2,6:-)
IV	S	Waycross var. subsp. IV	41	z_4,z_{23}	–	
I	S	Waycross	41	z_4,z_{23}	[e,n,z_{15}]	
IIIa	S		41	z_4,z_{23},z_{32}	–	(Ar. 13:1,6,7,9:-)
IIIa	S		41	z_4,z_{24}	–	(Ar. 13:1,3,11:-)
I	S	Ipswich	41	z_4,z_{24}	[1,5]	
IIIa	S		41	z_4,z_{32}	–	(Ar. 13:1,6,7:- and 13:1,7,8:-)
IV	S		41	z_{52}	–	
I	T	Faji	1,42	a	e,n,z_{15}	
II	T	Chinovum	42	b	1,5	
I	T	Orbe	42	b	1,6	
II	T	Uphill	42	b	e,n,x,z_{15}	
I	T	Tomegbe	1,42	b	e,n,z_{15}	
I	T	Egusitoo	1,42	b	z_6	
II	T		42	b	z_6	
I	T	Antwerpen	1,42	c	e,n,z_{15}	
I	T	Kampala	1,42	c	z_6	
II	T		42	d	z_6	
II	T		42	[e,n,x]	1,6	
II	T	Fremantle	42	(f),g,t	–	
IIIa	T		42	g,z_{51}	–	(Ar. 15:13,14:-)
IV	T		1,42	g,z_{51}	–	
I	T	Maricopa	1,42	g,z_{51}	1,5	
I	T	Borromea	42	i	1,6	
I	T	Kaneshie	1,42	i	l,w	
I	T	Middlesbrough	1,42	i	z_6	
IIIb	T		42	k	–	(Ar. 15:29:-)
I	T	Haferbreite	42	k	[1,6]	
IIIb	T		42	k	e,n,x,z15	(Ar. 15:29:28)
IIIb	T		42	k	z	(Ar. 15:29:31)
IIIb	T		42	k	z_{35}	(Ar. 15:29:21)
I	T	Gwale	1,42	k	z_6	
IIIb	T		42	(k)	z_{35}	(Ar. 15:22:21)
IIIb	T		42	l,v	1,5,7	(Ar. 15:23:30)
II	T	Portbech	42	l,v	e,n,x,z_{15}	
IIIb	T		42	l,v	e,n,x,z_{15}	(Ar. 15:23:28)
I	T	Coogee	42	l,v	e,n,z_{15}	
IIIb	T		42	l,v	z	(Ar. 15:23:31)
IIIb	T		42	l,v	z_{53}	(Ar. 15:23:25)
II	T		1,42	l,w	e,n,x	
II	T		42	l,[z_{13}],z_{28}	[z_6]	
I	T	Waral	1,42	m,t	–	
II	T		42	m,t	[e,n,x,z_{15}]	
II	T	Nairobi	42	r	–	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIb	T		42	r	–	(Ar. 15:24:-). May possess H phase Rz_{50} (Ar. 42).
I	T	Sipane	1,42	r	e,n,z_{15}	
I	T	Brive	1,42	r	l,w	
IIIb	T		42	r	z	(Ar. 15:24:31)
IIIb	T		42	r	z_{53}	(Ar. 15:24:25)
I	T	Spalentor	1,42	y	e,n,z_{15}	
I	T	Harvestehude	1,42	y	z_6	
II	T	Detroit	42	z	1,5	
I	T	Ursenbach	1,42	z	1,6	
II	T	Rand	42	z	e,n,x,z_{15}	
I	T	Melbourne	42	z	e,n,z_{15}	
II	T	Nuernberg	42	z	z_6	
IIIb	T		42	z_{10}	–	(Ar. 15:27:-). May possess H phase Rz_{56} (Ar. 38) and Rz_{50} (Ar. 42).
II	T		42	z_{10}	1,2	
II	T		42	z_{10}	e,n,x,z_{15}	
IIIb	T		42	z_{10}	e,n,x,z_{15}	(Ar. 15:27:28)
IIIb	T		42	z_{10}	z	(Ar. 15:27:31)
IIIb	T		42	z_{10}	z_{35}	(Ar. 15:27:21)
I	T	Loenga	1,42	z_{10}	z_6	
II	T		42	z_{10}	z_6	
IIIb	T		42	z_{10}	z_{67}	(Ar. 15:27:46)
I	T	Djama	1,42	z_{29}	[1,5]	
I	T	Kahla	1,42	z_{35}	1,6	
I	T	Tema	1,42	z_{35}	z_6	
I	T	Weslaco	42	z_{36}	–	
IV	T		42	z_{36}	–	
I	T	Vogan	1,42	z_{38}	z_6	
IIIa	T		42	z_4,z_{23}	–	(Ar. 15:1,2,5:- and 15:1,2,6:-)
I	T	Gera	1,42	z_4,z_{23}	[1,6]	
I	T	Broc	42	z_4,z_{23}	e,n,z_{15}	
I	T	Toricada	1,42	z_4,z_{24}	–	
IIIa	T		42	z_4,z_{24}	–	(Ar. 15:1,3,11:-)
IV	T		1,42	z_4,z_{24}	–	
I	T	Taset	1,42	z_{41}	–	
IIIb	T		42	z_{52}	z	(Ar. 15:26:31)
II	T		42	z_6	1,6	
I	U	Graz	43	a	1,2	
I	U	Berkeley	43	a	1,5	
II	U		43	a	1,5	
II	U		43	a	z_6	
II	U	Kommetje	43	b	z_{42}	
I	U	Montreal	43	c	1,5	
I	U	Orleans	43	d	1,5	
II	U		43	d	e,n,x,z_{15}	
II	U		43	d	z_{39}	
II	U		43	d	z_{42}	
II	U		43	e,n,x,z_{15}	1,(5),7	
II	U		43	e,n,x,z_{15}	1,6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	U	Milwaukee	43	f,g,[t]	–	
II	U	Mosselbay	43	g,m,[s],t	[z_{42}]	
II	U	Veddel	43	g,t	–	
II	U		43	g,t	1,5	
IIIa	U		43	g,z_{51}	–	(Ar. 21:13,14:-)
IV	U		43	g,z_{51}	–	
II	U		43	g,z_{62}	e,n,x	
I	U	Mbao	43	i	1,2	
I	U	Voulte	43	i	e,n,x	
I	U	Thetford	43	k	1,2	
I	U	Ahuza	43	k	1,5	
IIIb	U		43	k	z	(Ar. 21:29:31)
IIIb	U		43	l,v	z_{53}:[Rz_{56}]	(Ar. 21:23:25:[38])
I	U	Sudan	43	l,z_{13}	–	
II	U		43	l,z_{13},z_{28}	1,5	
IIIb	U		43	r	e,n,x,z_{15}	(Ar. 21:24:28)
IIIb	U		43	r	z	(Ar. 21:24:31)
IIIb	U		43	r	z_{53}	(Ar. 21:24:25)
I	U	Farcha	43	y	1,2	
I	U	Kingabwa	43	y	1,5	
I	U	Ogbete	43	z	1,5	
II	U		43	z	1,5	
I	U	Arusha	43	z	e,n,z_{15}	
I	U	Adana	43	z_{10}	1,5	
I	U	Makiling	43	z_{29}	–	
IV	U		43	z_{29}	–	
II	U		43	z_{29}	e,n,x	
II	U		43	z_{29}	z_{42}	
I	U	Ahepe	43	z_{35}	1,6	
IIIa	U		43	z_{36}	–	(Ar. 21:17,20:-)
IV	U	Volksdorf	43	z_{36},z_{38}	–	
I	U	Irigny	43	z_{38}	–	
IIIa	U		43	z_4,z_{23}	–	(Ar. 21:1,2,5:- and 21:1,2,6:-)
IV	U	Houten	43	z_4,z_{23}	–	
IV	U		43	z_4,z_{23}	–	
IIIa	U		43	z_4,z_{24}	–	(Ar. 21:1,3,11:-)
IV	U		43	z_4,z_{24}	–	
IV	U	Tuindorp	43	z_4,z_{32}	–	
II	U	Bunnik	43	z_{42}	[1,5,7]	
IIIb	U		43	z_{52}	z_{53}	(Ar. 21:26:25)
I	V	Niakhar	44	a	1,5	
I	V	Tiergarten	44	a	e,n,x	
I	V	Niarembe	44	a	l,w	
I	V	Sedgwick	44	b	e,n,z_{15}	
I	V	Madigan	44	c	1,5	
I	V	Quebec	44	c	e,n,z_{15}	
I	V	Bobo	44	d	1,5	
I	V	Kermel	44	d	e,n,x	
I	V	Fischerstrasse	44	d	e,n,z_{15}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	V	Palamaner	1,44	d	z_{35}	
II	V		1,44	e,n,x	1,6	
I	V	Vleuten	44	f,g	–	
I	V	Gamaba	1,44	g,m,[s]	–	
I	V	Splott	44	g,s,t	–	
II	V		44	g,t	z_{42}	
I	V	Carswell	44	g,z_{51}	–	
IV	V		44	g,z_{51}	–	
I	V	Maritzburg	1,44	i	e,n,z_{15}	
I	V	Lawra	44	k	e,n,z_{15}	
I	V	Malika	44	l,z_{28}	1,5	
I	V	Muguga	44	m,t	–	
V	V	Camdeni	44	r	–	
I	V	Brefet	44	r	e,n,z_{15}	
I	V	Bolama	44	z	e,n,x	
I	V	Uhlenhorst	44	z	l,w	
I	V	Guinea	1,44	z_{10}	[1,7]	
I	V	Llobregat	1,44	z_{10}	e,n,x	
I	V	Zinder	44	z_{29}	–	
IV	V		44	z_{29}	–	
II	V		44	z_{29}	e,n,x:z_{42}	
IV	V		44	z_{36},[z_{38}]	–	
I	V	Koketime	44	z_{38}	–	
V	V		44	z_{39}	–	
II	V	Clovelly	1,44	z_{39}	[e,n,x,z_{15}]	
I	V	Kua	44	z_4,z_{23}	–	
II	V		44	z_4,z_{23}	–	
IIIa	V		44	z_4,z_{23}	–	(Ar. 1,3:1,2,5:- and 1,3:1,2,6:-)
IV	V		44	z_4,z_{23}	–	
I	V	Ploufragan	1,44	z_4,z_{23}	e,n,z_{15}	
IIIa	V		44	z_4,z_{23},z_{32}	–	(Ar. 1,3:1,6,7,9:-)
I	V	Christiansborg	44	z_4,z_{24}	–	
IIIa	V		44	z_4,z_{24}	–	(Ar. 1,3:1,3,11:-)
IV	V		44	z_4,z_{24}	–	
IIIa	V		44	z_4,z_{32}	–	(Ar. 1,3:1,2,10:- and 1,3:1,7,8:-). IP calls z_4,z_{23},z_{32}, Ar. 1,2,10.
IV	V	Lohbruegge	44	z_4,z_{32}	–	
VI	W	Vrindaban	45	a	e,n,x	
I	W	Meekatharra	45	a	e,n,z_{15}	
II	W	Ejeda	45	a	z_{10}	
I	W	Riverside	45	b	1,5	
I	W	Fomeco	45	b	e,n,z_{15}	
I	W	Deversoir	45	c	e,n,x	
I	W	Dugbe	45	d	1,6	
I	W	Karachi	45	d	e,n,x	
I	W	Warmsen	45	d	e,n,z_{15}	
I	W	Suelldorf	45	f,g	–	
II	W	Windhoek	45	g,m,s,t	1,5	
II	W	Bremen	45	g,m,s,t	e,n,x	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	W	Tornow	45	g,m,[s],[t]	–	
II	W	Perinet	45	g,m,t	e,n,x,z_{15}	
I	W	Binningen	45	g,s,t	–	
IIIa	W		45	g,z_{51}	–	(Ar. 11:13,14:-)
IV	W		45	g,z_{51}	–	
I	W	Verviers	45	k	1,5	
I	W	Casablanca	45	k	1,7	
I	W	Cairns	45	k	e,n,z_{15}	
I	W	Imo	45	l,v	[e,n,z_{15}]	
I	W	Apapa	45	m,t	–	
II	W		45	m,t	1,5	
I	W	Kofandoka	45	r	e,n,z_{15}	
II	W		45	z	1,5	
I	W	Yopougon	45	z	e,n,z_{15}	
II	W	Klapmuts	45	z	z_{39}	
I	W	Jodhpur	45	z_{29}	–	
IIIa	W		45	z_{29}	–	(Ar. 11:16,18:-)
II	W		45	z_{29}	1,5	
II	W		45	z_{29}	e,n,x	
II	W		45	z_{29}	z_{42}	
I	W	Lattenkamp	45	z_{35}	1,5	
I	W	Balcones	45	z_{36}	–	
IV	W		45	z_{36},z_{38}	–	
IIIa	W		45	z_4,z_{23}	–	(Ar. 11:1,2,5:-)
IV	W		45	z_4,z_{23}	–	
I	W	Transvaal	45	z_4,z_{24}	–	
IIIa	W		45	z_4,z_{24}	–	(Ar. 11:1,3,11:-)
IIIa	W		45	z_4,z_{32}	–	(Ar. 11:1,7,8:-)
II	X	Bilthoven	47	a	[1,5]	
II	X		47	a	e,n,x,z_{15}	
I	X	Saka	47	b	–	IP combined Saka with Sya (47:b:z_6) and called it Sya.
I	X	Wenatchee	47	b	1,2	
II	X	Phoenix	47	b	1,5	
II	X	Khami	47	b	[e,n,x,z_{15}]	
I	X	Sya	47	b	z_6	
II	X		47	b	z_6	
IIIb	X		47	c	1,5,7	(Ar. 28:32:30)
I	X	Kodjovi	47	c	[1,6]	Kodjovi may possess H phase Rz_{78}.
IIIb	X		47	c	e,n,x,z_{15}:[z_{57}]	(Ar. 23:32:28 and 28:32:28:[40])
IIIb	X		47	c	z	(Ar. 28:32:31)
IIIb	X		47	c	z_{35}	(Ar. 28:32:21)
II	X		47	d	e,n,x,z_{15}	
I	X	Stellingen	47	d	[e,n,x]	
II	X	Quimbamba	47	d	z_{39}	
II	X		47	e,n,x,z_{15}	1,6	
I	X	Sljeme	1,47	f,g	–	
I	X	Anie	47	(g),m,t	–	IP combined Anie with Mesbit (47:m,t:[e,n,z_{15}]) and called it Mesbit.

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	X	Luke	1,47	g,m	–	
II	X		47	g,t	e,n,x	
IIIa	X		47	g,z_{51}	–	(Ar. 28:13,14)
IIIb	X		47	i	e,n,x,z_{15}	(Ar. 23:33:28). May possess H phase Rz_{50} (Ar. 42).
I	X	Bergen	47	i	e,n,z_{15}	
IIIb	X		47	i	z	(Ar. 28:33:31)
IIIb	X		47	i	z_{35}	(Ar. 23:33:21 and 28:33:21)
IIIb	X		47	i	z_{53}:[z_{57}]	(Ar. 23:33:25 and 28:33:25:[40])
I	X	Staoueli	47	k	1,2	
I	X	Bootle	47	k	1,5	
IIIb	X		47	k	1,5,7	(Ar. 28:29:30)
I	X	Dahomey	47	k	1,6	Dahomey may possess H phase Rz_{58}.
IIIb	X		47	k	e,n,x,z_{15}	(Ar. 28:29:28)
I	X	Lyon	47	k	e,n,z_{15}	
IIIb	X		47	k	z	(Ar. 28:29:31)
IIIb	X		47	k	z_{35}	(Ar. 23:29:21)
IIIb	X		47	k	z_{53}	(Ar. 23:29:25)
IV	X		47	l,v	–	
IIIb	X		47	l,v	1,5,(7)	(Ar. 23:23:30). May possess H phase Rz_{50} (Ar. 42).
IIIa	X		47	l,v	e,n,x,z_{15}	(Ar. 28:23:28)
IIIb	X		47	l,v	z	(Ar. 23:23:31)
IIIb	X		47	l,v	z_{35}	(Ar. 28:23:21)
IIIb	X		47	l,v	z_{53}	(Ar. 28:23:25)
IIIb	X		47	l,v	z_{57}	(Ar. 28:23:40)
I	X	Teshie	1,47	l,z_{13},z_{28}	e,n,z_{15}	
I	X	Mesbit	47	m,t	[e,n,z_{15}]	
IIIa	X		47	r	–	(Ar. 23:24:-). CDC does not have this.
I	X	Dapango	47	r	1,2	
IIIb	X		47	r	1,5,7	(Ar. 23:24:30)
IIIb	X		47	r	z	(Ar. 23:24:31)
IIIb	X		47	r	z_{35}	(Ar. 23:24:21 and 28:24:21)
IIIb	X		47	r	z_{53}	(Ar. 23:24:25). May possess H phase Rz_{74} (Ar. Rz_{74})
IIIb	X		47	r	z_{53}:Rz_{50}:z_{60}	(Ar. 28:24:25:42:44). Not in IP book.
IIIb	X		47	r	z_{53}:[z_{60}]	(Ar. 23:24:25:[44]). May possess H phase Rz_{70} and Rz_{72} (Ar. Rz_{70} or Rz_{72}).
I	X	Moualine	47	y	1,6	
I	X	Blitta	47	y	e,n,x	
I	X	Mountpleasant	47	z	1,5	
I	X	Kaolack	47	z	1,6	
II	X		47	z	e,n,x,z_{15}	
II	X	Chersina	47	z	z_6	
IIIb	X		47	z_{10}	1,5,7	(Ar. 28:27:30)
IIIb	X		47	z_{10}	z	(Ar. 28:27:31)
IIIb	X		47	z_{10}	z_{35}	(Ar. 28:27:21)
I	X	Ekpoui	47	z_{29}	–	
IIIa	X		47	z_{29}	–	(Ar. 28:16,18:-)

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	X		47	z_{29}	e,n,x,z_{15}	
I	X	Bingerville	47	z_{35}	e,n,z_{15}	
IV	X		47	z_{36}	–	
I	X	Alexanderplatz	47	z_{38}	–	
IIIa	X		47	z_4,z_{23}	–	(Ar. 28:1,2,5:-)
I	X	Tabligbo	47	z_4,z_{23}	[e,n,z_{15}]	
I	X	Binche	47	z_4,z_{23}	l,w	
I	X	Bere	47	z_4,z_{23}	[z_6]	Bere may possess H phase Rz45.
I	X	Tamberma	47	z_4,z_{24}	–	
I	X	Quinhon	47	z_{44}	–	
IIIb	X		47	z_{52}	1,5,7	(Ar. 28:26:30)
IIIb	X		47	z_{52}	e,n,x,z_{15}	(Ar. 28:26:28)
IIIb	X		47	z_{52}	z	(Ar. 28:26:31)
IIIb	X		47	z_{52}	z_{35}	(Ar. 28:26:21)
II	X		47	z_6	1,6	
I	Y	Hisingen	48	a	1,5,7	
IIIb	Y		48	a	z_{35}	(Ar. 5:35:21). Not in 1992 IP, but is in Bergey.
II	Y		48	a	z_{39}	
II	Y		48	a	z_6	
V	Y		48	b	–	
II	Y		48	b	[z_6]	
IIIb	Y		48	c	z	(Ar. 29:32:31)
II	Y	Etosha	48	d	1,11	Etosha was not considered a new serotype by Kauffmann and is not used.
II	Y		48	d	1,2	
II	Y	Hagenbeck	48	d	z_6	
I	Y	Fitzroy	48	e,h	1,5	
II	Y	Hammonia	48	e,n,x,z_{15}	z_6	
II	Y	Erlangen	48	g,m,t	–	
IIIa	Y		48	g,z_{51}	–	(Ar. 5:13,14:-)
IV	Y	Marina	48	g,z_{51}	–	
IIIb	Y	Sydney	48	i	z	Sydney was formerly in subspecies II, but it it now combined with *Arizona* 5:33:31. The name Sydney has been dropped.
IIIb	Y		48	i	z_{35}:[z_{57}]	(Ar. 29:33:21:[40])
IIIb	Y		48	i	z_{53}	(Ar. 5:33:25)
IIIb	Y		48	i	z_{61}	(Ar. 5,29:33:41)
IIIb	Y		48	i	z:[z_{72}]	(Ar. 5,29:33:31:[z_{72}]). CDC does not have z_{72} strain.
IIIb	Y		48	k	1,5,(7)	(Ar. 5:29:30)
II	Y		48	k	e,n,x,z_{15}	
IIIb	Y		48	k	e,n,x,z_{15}	(Ar. 5:29:28)
I	Y	Dahlem	48	k	e,n,z_{15}	
IIIb	Y		48	k	z	(Ar. 5,29:29:31)
IIIb	Y		48	k	z_{35}:[Rz_{75}]	(Ar. [5:29:21:Rz_{75}]). CDC does not have Rz_{75}.
IIIb	Y		48	k	z_{53}	(Ar. 5,29:29:25)
IIIb	Y		48	(k)	z_{53}	(Ar. 5:22:25 and Ar. 5,29:22:25). Called 5:22:25 by IP.
II	Y	Sakaraha	48	[k]	z_{39}	
I	Y	Australia	48	l,v	1,5	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIb	Y		48	l,v	1,5,(7)	(Ar. 5:23:30). May possess H phase Rz_{47} or Rz_{50} (Ar. 39 or 42).
IIIb	Y		48	l,v	z	(Ar. 5,29:23:31)
IIIb	Y		48	r	e,n,x,z_{15}	(Ar. 5:24:28)
IIIb	Y		48	r	z	(Ar. 5,29:24:31)
I	Y	Toucra	48	z	1,5	Toucra may possess H phase Rz_{58}.
II	Y		48	z	1,5	
VI	Y		48	z_{10}	1,5	
II	Y	Ngozi	48	z_{10}	[1,5]	
I	Y	Isaszeg	48	z_{10}	e,n,x	
IIIb	Y		48	z_{10}	e,n,x,z_{15}	(Ar. 5:27:28)
IIIb	Y		48	z_{10}	z	(Ar. 5,29:27:31)
II	Y		48	z_{29}	–	
IIIa	Y		48	z_{29}	–	(Ar. 5:16,18). This is not in IP book, but is on Rohde's list.
IV	Y		48	z_{29}	–	
V	Y	Bongor	48	z_{35}	–	
IIIb	Y		48	z_{35}	z_{52}	(Ar. 5:21:26)
IIIa	Y		48	z_{36}	–	(Ar. 5,29:17,20:-)
IV	Y		48	z_{36},[z_{38}]	–	
V	Y		48	z_{39}	–	
IIIa	Y		48	z_4,z_{23}	–	(Ar. 5:1,2,5:-; 5:1,2,5,6:-; and 5:1,6:-)
IV	Y		48	z_4,z_{23}	–	
IIIa	Y		48	z_4,z_{23},z_{32}	–	(Ar. 5:1,6,7,9:-). IP calls this 5:1,6,7:-.
I	Y	Djakarta	48	z_4,z_{24}	–	
IIIa	Y		48	z_4,z_{24}	–	(Ar. 5:1,3,11:-)
IIIa	Y		48	z_4,z_{32}	–	(Ar. 5:1,6,7:-; 5:1,7,8:-; and Ar. 5:1,2,10:-). IP calls z_4,z_{32}, Ar. 1,7,8; and would call z_4,z_{23},z_{32}, Ar. 1,2,10.
IV	Y		48	z_4,z_{32}	–	
V	Y	Balboa	48	z_{41}	–	
IIIb	Y		48	z_{52}	e,n,x,z_{15}	(Ar. 29:26:28)
IIIb	Y		48	z_{52}	z	(Ar. 5:26:31)
V	Y		48	z_{65}	–	
V	Y		48	z_{81}	–	
IV	Z		50	a	–	
IV	Z		50	b	–	
I	Z	Rochdale	50	b	e,n,x	
II	Z		50	b	z_6	
IV	Z		50	d	-	
I	Z	Hemingford	50	d	1,5	Hemingford may possess H phase Rz_{82}.
II	Z	Krugersdorp	50	e,n,x	1,7	
II	Z	Namib	50	g,[m],s,t	[1,5]	
IV	Z	Wassenaar	50	g,z_{51}	-	
II	Z		50	g,z_{62}	e,n,x	
IIIb	Z		50	i	1,5,7	(Ar. 9a,9c:33:30)
IIIb	Z		50	i	e,n,x,z_{15}	(Ar. 9a,9c:33:28)
IIIb	Z		50	i	z	(Ar. 9a,9c:33:31)
IIIb	Z		50	k	1,5,7	(Ar. 9a,9c:29:30)
IIIb	Z		50	k	e,n,x,z_{15}	(Ar. 9a,9c:29:28)

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	Z		50	k	e,n,x:z_{42}	
IIIb	Z		50	k	z	(Ar. 9a,9b:29:31 and 9a,9c:29:31). Ar. 9a,9b may possess H phase Rz_{50} (Ar. 42).
IIIb	Z		50	k	z_{35}	(Ar. 9a,9b:29:21)
IIIb	Z		50	k	z_{53}	(Ar. 9a,9b:29:25 and 9a,9c:29:25). IP and Rohde only list the 9a,9c.
II	Z	Seaforth	50	k	z_6	
IIIb	Z		50	(k)	z	(Ar. 9a,9b:22:31)
IIIb	Z		50	(k)	z_{35}	(Ar. 9a,9b:22:21)
I	Z	Fass	50	l,v	1,2	
IIIb	Z		50	l,v	e,n,x,z_{15}	(Ar. 9a,9b:23:28)
IIIb	Z		50	l,v	z	(Ar. 9a,9b:23:31 and 9a,9c:23:31). IP only lists 9a,9c.
IIIb	Z		50	l,v	z_{35}	(Ar. 9a,9c:23:21)
II	Z		50	l,w	e,n,x,z_{15}:z_{42}	
II	Z		50	l,z_{28}	z_{42}	
II	Z	Atra	50	m,t	z_6:z_{42}	
IIIb	Z		50	r	1,5,(7)	(Ar. 9a,9b:24:30)
IIIb	Z		50	r	e,n,x,z_{15}	(Ar. 9a,9c:24:28)
IIIb	Z		50	r	z	(Ar. 9a,9b:24:31 and 9a,9c:24:31).
IIIb	Z		50	r	z_{35}	(Ar. 9a,9b:24:21). May possess H phase Rz_{58} (Ar. Rz58). This is not in IP book, but is on Rohde's list.
IIIb	Z		50	r	z_{53}	(Ar. 9a,9b:24:25). May possess H phase Rz_{50} (Ar. 42). This is not in IP book, but is on Rohde's list.
I	Z	Dougi	50	y	1,6	
II	Z	Greenside	50	z	e,n,x	
IIIb	Z		50	z_{10}	z	(Ar. 9a,9c:27:31). May possess H phase Rz_{56} (Ar. 38).
IIIb	Z		50	z_{10}	z_{53}	(Ar. 9a,9c:27:25)
II	Z	Hooggraven	50	z_{10}	z_6:z_{42}	
I	Z	Ivorycoast	50	z_{29}	–	
IIIa	Z		50	z_{29}	–	(Ar. 9a,9b:16,18:-)
IIIa	Z		50	z_{36}	–	(Ar. 9a,9b:17,20:-)
IIIa	Z		50	z_4,z_{23}	–	(Ar. 9a,9b:1,2,5:- and 9a,9b:1,2,6:-)
IV	Z	Flint	50	z_4,z_{23}	–	
IIIa	Z		50	z_4,z_{23},z_{32}	–	(Ar. 9a,9b:1,2,10:-). Called 9a,9b:1,6,7:- by IP and Rohde.
IIIa	Z		50	z_4,z_{24}	–	(Ar. 9a,9b:1,3,11:-)
IV	Z		50	z_4,z_{24}	–	
IIIa	Z		50	z_4,z_{32}	–	(Ar. 9a,9b:1,2,10; 9a,9b:1,6,7:-; and 9a,9b:1,7,8:-). 9a,9b:1,2,10:- and 9a,9b:1,7,8:- used by IP and Rohde.
IV	Z	Bonaire	50	z_4,z_{32}	–	
II	Z	Faure	50	z_{42}	1,7	
IIIb	Z		50	z_{52}	1,5,7	(Ar. 9a,9b:26:30 and 9a,9c:26:30)
IIIb	Z		50	z_{52}	z	(Ar. 9a,9b:26:31 and 9a,9c:26:31)
IIIb	Z		50	z_{52}	z_{35}	(Ar. 9a,9b:26:21 and 9a,9c:26:21)
IIIb	Z		50	z_{52}	z_{53}	(Ar. 9a,9b:26:25 and 9a,9c:26:25)
II	51	Roggeveld	51	–	1,7	
I	51	Tione	51	a	e,n,x	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IV	51		51	b	–	
I	51	Karaya	51	b	1,5	
II	51		51	c	–	
I	51	Gokul	1,51	d	[1,5]	
I	51	Meskin	51	e,h	1,2	
II	51		51	g,s,t	e,n,x	
IIIa	51		51	g,z_{51}	–	(Ar. 1,2:13,14:-)
I	51	Kabete	51	i	1,5	
I	51	Dan	51	k	e,n,z_{15}	
IIIb	51		51	k	z_{35}	(Ar. 1,2:29:21)
I	51	Harcourt	51	l,v	1,2	
I	51	Overschie	51	l,v	1,5	
I	51	Dadzie	51	l,v	e,n,x	
IIIb	51		51	l,v	z	(Ar. 1,2:23:31)
I	51	Moundou	51	l,z_{28}	1,5	
II	51	Askraal	51	l,z_{28}	[z_6]	
I	51	Lutetia	51	r,i	l,z_{13},z_{28}	
I	51	Antsalova	51	z	1,5	
I	51	Treforest	1,51	z	1,6	
I	51	Lechler	51	z	e,n,z_{15}	
I	51	Bergues	51	z_{10}	1,5	
II	51		51	z_{29}	e,n,x,z_{15}	
IIIa	51		51	z_4,z_{23}	–	(Ar. 1,2:1,2,5:- and 1,2:1,2,6:-)
IV	51	Harmelen	51	z_4,z_{23}	–	
IIIa	51		51	z_4,z_{24}	–	(Ar. 1,2:1,3,11:-)
IIIa	51		51	z_4,z_{32}	–	(Ar. 1,2:1,7,8:-)
I	52	Uithof	52	a	1,5	
I	52	Ord	52	a	e,n,z_{15}	
I	52	Molesey	52	b	1,5	
I	51	Flottbek	52	b	[e,n,x]	
II	52		52	c	k	
IIIb	52		52	c	k	(Ar. 31:32:29). This is not in IP book, but is on Rohde's list.
I	52	Utrecht	52	d	1,5	
II	52		52	d	e,n,x,z_{15}	
II	52		52	d	z_{39}	CDC does not have this.
I	52	Butare	52	e,h	1,6	
I	52	Derkle	52	e,h	1,7	
I	52	Saintemarie	52	g,t	–	
II	52		52	g,t	–	
I	52	Bordeaux	52	k	1,5	
IIIb	52		52	k	z_{35}	(Ar. 31:29:21)
IIIb	52		52	k	z_{53}	(Ar. 31:29:25)
IIIb	52		52	(k)	z_{35}	(Ar. 31:22:21)
IIIb	52		52	l,v	z_{53}	(Ar. 31:23:25)
II	52		52	z	z_{39}	
II	52	Wilhemstrasse	52	z_{44}	1,5	IP combined Wilhemstrasse with Lobatsi (52:z44:1,5,7). The name Wilhemstrasse has been dropped.

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	52	Lobatsi	52	z_{44}	1,5,7	
IIIb	52		52	z_{52}	z	(Ar. 31:26:31)
II	53		53	c	1,5	
II	53		53	d	1,5	
II	53		1,53	d	z_{39}	
II	53		53	d	z_{42}	
IIIa	53		53	g,z_{51}	–	(Ar. 1,4:13,14:-)
IV	53		1,53	g,z_{51}	–	
IIIb	53		53	i	z	(Ar. 1,4:33:31)
IIIb	53		53	k	e,n,x,z_{15}	(Ar. 1,4:29:28)
IIIb	53		53	k	z	(Ar. 1,4:29:31)
IIIb	53		53	(k)	z	(Ar. 1,4:22:31)
IIIb	53		53	(k)	z_{35}	(Ar. 1,4:22:21)
IIIb	53		53	l,v	e,n,x,z_{15}	(Ar. 1,4:23:28)
IIIb	53		53	l,v	z_{35}	(Ar. 1,4:23:21)
II	53		53	l,z_{28}	e,n,x	
II	53	Midhurst	53	l,z_{28}	z_{39}	
II	53		53	l,z_{28}	z_6	
IIIb	53		53	r	z	(Ar. 1,4:24:31)
IIIb	53		53	r	z_{35}	(Ar. 1,4:24:21)
IIIb	53		53	r	z_{68}	(Ar. 1,4:24:47). This was formerly called z_{56} (Ar. 38), but was changed to z_{68} (Ar. 47).
II	53		53	z	1,5	
IIIb	53		53	z	1,5,(7)	(Ar. 1,4:30:31)
II	53		53	z	z_6	
IIIb	53		53	z_{10}	z_{35}	(Ar. 1,4:27:21)
IIIa	53		53	z_{29}	–	(Ar. 1,4:16,18:-)
IV	53	Bockenheim	1,53	z_{36},z_{38}	–	
IIIa	53		53	z_4,z_{23}	–	(Ar. 1,4:1,2,5:- and 1,4:1,2,6:-)
IV	53		53	z_4,z_{23}	–	
IIIa	53		53	z_4,z_{23},z_{32}	–	(Ar. 1,4:1,6,7:- and 1,4:1,6,7,9:-)
II	53	Humber	53	z_4,z_{24}	–	
IIIa	53		53	z_4,z_{24}	–	(Ar. 1,4:1,3,11:-)
IIIa	53		53	z_4,z_{32}	–	(Ar. 1,4:1,6,7:-). IP combined this with 53:z4,z23,z32:- (Ar. 1,4:1,6,7,9:-).
IIIb	53		53	z_{52}	z_{35}	(Ar. 1,4:26:21)
IIIb	53		53	z_{52}	z_{53}	(Ar. 1,4:26:25)
I	54	Tonev	21,54	b	e,n,x	
I	54	Winnipeg	54	e,h	1,5	
I	54	Rossleben	54	e,h	1,6	
I	54	Borreze	54	f,g,s	–	
I	54	Uccle	3,54	g,s,t	–	
I	54	Poeseldorf	8,20,54	i	z_6	
I	54	Ochsenwerder	6,7,54	k	1,5	
I	54	Newholland	4,12,54	m,t	–	
I	54	Czernyring	54	r	1,5	
I	54	Steinwerder	3,15,54	y	1,5	
I	54	Canton	54	z_{10}	e,n,x	
I	54	Barry	54	z_{10}	e,n,z_{15}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	54	Yerba	54	z_4,z_{23}	–	
II	55	Tranoroa	55	k	z_{39}	
II	56	Artis	56	b	–	
II	56		56	d	–	
II	56		56	e,n,x	1,7	
II	56		56	l,v	z_{39}	
II	56		56	l,z_{28}	–	
II	56		56	z	z_6	
II	56		56	z_{10}	e,n,x	
IIIa	56		56	z_{29}	–	(Ar. 14:16,18:-)
IIIa	56		56	z_4,z_{23}	–	(Ar. 14:1,2,5:- and 14:1,2,6:-)
IIIa	56		56	z_4,z_{23},z_{32}	-	(Ar. 14:1,6,7,9:-)
II	57		57	a	z_{42}	
I	57	Antonio	57	a	z_6	
I	57	Maryland	57	b	1,7	
I	57	Batonrouge	57	b	e,n,z_{15}	
IIIb	57		57	c	z:[z_{60}]	(Ar. 34:32:31:[44])
II	57		57	d	1,5	
II	57		57	g,[m],s,t	z_{42}	
II	57		57	g,t	-	
IIIb	57		57	i	e,n,x,z_{15}	(Ar. 34:33:28)
IIIb	57		57	i	z	(Ar. 34:33:31)
IIIb	57		57	k	e,n,x,z_{15}	(Ar. 34:29:28). CDC does not have this and not on Rohde's list.
IIIb	57		57	z_{10}	z	(Ar. 34:27:31)
II	57	Locarno	57	z_{29}	z_{42}	
II	57	Manombo	57	z_{39}	e,n,x,z_{15}	
IV	57		57	z_4,z_{23}	-	
II	57	Tokai	57	z_{42}	1,6:z_{53}	
II	58		58	a	z_6	
II	58		58	b	1,5	
II	58		58	c	z_6	
II	58		58	d	z_6	
IIIb	58		58	i	e,n,x,z_{15}	(Ar. 1,33:33:28)
IIIb	58		58	k	z	(Ar. 1,33:29:31)
IIIb	58		58	l,v	e,n,x,z_{15}	(Ar. 1,33:23:28)
IIIb	58		58	l,v	z_{35}	(Ar. 1,33:23:21)
II	58	Basel	58	l,z_{13},z_{28}	1,5	
II	58		58	l,z_{13},z_{28}	z_6	
IIIb	58		58	r	e,n,x,z_{15}	(Ar. 1,33:24:28)
IIIb	58		58	r	z	(Ar. 1,33:24:31)
IIIb	58		58	r	z_{53}	(Ar. 1,33:24:25). May possess H phase Rz_{47} (Ar. 39) or Rz_{57} (Ar. 40) or Rz_{70} (Ar. Rz_{70}).
II	58		58	z_{10}	1,6	
IIIb	58		58	z_{10}	e,n,x,z_{15}	(Ar. 1,33:27:28)
IIIb	58		58	z_{10}	z_{53}	(Ar. 1,33:27:25). May possess H phase Rz_{50} (Ar. 42).
II	58		58	z_{10}	z_6	
II	58		58	z_{39}	e,n,x,z_{15}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIb	58		58	z_{52}	z	(Ar. 1,33:26:31)
IIIb	58		58	z_{52}	z_{35}	(Ar. 1,33:26:21)
II	58		58	z_6	1,6	
IIIb	59		59	c	e,n,x,z_{15}	(Ar. 19:32:28)
IIIb	59		59	i	e,n,x,z_{15}	(Ar. 19:33:28)
IIIb	59		59	i	z	(Ar. 19:33:31)
IIIb	59		59	i	z_{35}	(Ar. 19:33:21)
II	59	Betioky	59	k	(z)	
IIIb	59		59	k	z_{53}	(Ar. 19:29:25)
IIIb	59		59	(k)	e,n,x,z_{15}	(Ar. 19:22:28)
IIIb	59		59	(k)	z	(Ar. 19:22:31)
IIIb	59		59	(k)	z_{35}	(Ar. 19:22:21)
IIIb	59		59	l,v	z	(Ar. 19:23:31)
IIIb	59		59	l,v	z_{53}	(Ar. 19:23:25)
IIIb	59		59	r	z_{35}	(Ar. 19:24:21)
II	59		1,59	z	z_6	
IIIb	59		59	z_{10}	z_{53}	(Ar. 19:27:25)
IIIb	59		59	z_{10}	z_{57}	(Ar. 19:27:40)
IIIa	59		59	z_{29}	–	(Ar. 19:16,18:-)
IIIa	59		59	z_{36}	–	(Ar. 19:17,20:-)
IIIa	59		59	z_4,z_{23}	–	(Ar. 19:1,2,5:- and 19:1,2,6:-)
IIIb	59		59	z_{52}	[z_{53}]	(Ar. 19:26:[25])
II	60		60	b	[1,16]	
II	60	Setubal	60	g,m,t	z_6	
IIIb	60		60	i	–	(Ar. 24:33:-). May possess H phase Rz_{50} (Ar. 42).
IIIb	60		60	i	e,n,x,z_{15}	(Ar. 24:33:28)
IIIb	60		60	i	z_{35}	(Ar. 24:33:21)
IIIb	60		60	k	z	(Ar. 24:29:31)
IIIb	60		60	k	z_{35}	(Ar. 24:29:21)
IIIb	60		60	(k)	z_{53}	(Ar. 24:22:25)
IIIb	60		60	l,v	z	(Ar. 24:23:31)
IIIb	60		60	r	e,n,x,z_{15}	(Ar. 24:24:28)
IIIb	60		60	r	z	(Ar. 24:24:31)
IIIb	60		60	r	z_{35}	(Ar. 24:24:21)
IIIb	60		60	r	z_{53}	(Ar. 24:24:25)
II	60	Luton	60	z	e,n,x	
IIIb	60		60	z_{10}	z	(Ar. 24:27:31)
IIIb	60		60	z_{10}	z_{35}	(Ar. 24:27:21)
IIIb	60		60	z_{10}	z_{53}	(Ar. 24:27:25)
II	60		60	z_{29}	e,n,x	
V	60		60	z_{41}	–	
IIIb	60		60	z_{52}	1,5,[7]	(Ar. 24:26:30)
IIIb	60		60	z_{52}	z	(Ar. 24:26:31)
IIIb	60		60	z_{52}	z_{35}	(Ar. 24:26:21)
IIIb	60		60	z_{52}	z_{53}	(Ar. 24:26:25)
IIIb	61		61	c	1,5,(7)	(Ar. 26:32:30)
IIIb	61		61	c	z_{35}	(Ar. 26:32:21)
IIIb	61		61	i	e,n,x,z_{15}	(Ar. 26:33:28)

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIb	61	Eilbeck	61	i	z	Eilbeck was formerly in subspecies II, but is now combined with *Arizona* 26:33:31. The name Eilbeck has been dropped.
IIIb	61		61	i	z	(Ar. 26:33:31)
IIIb	61		61	i	z_{35}	(Ar. 26:33:21)
IIIb	61		61	i	z_{53}	(Ar. 26:33:25)
IIIb	61		61	k	1,5,(7)	(Ar. 26:29:30)
IIIb	61		61	k	z_{35}	(Ar. 26:29:21). CDC does not have this.
IIIb	61		61	(k)	z53	(Ar. 26:22:25)
IIIb	61		61	l,v	1,5,7:[z_{57}]	(Ar. 26:23:30:[40])
IIIb	61		61	l,v	z	(Ar. 26:23:31)
IIIb	61		61	l,v	z_{35}	(Ar. 26:23:21)
IIIb	61		61	r	1,5,7	(Ar. 26:24:30)
IIIb	61		61	r	z	(Ar. 26:24:31)
IIIb	61		61	r	z_{35}	(Ar. 26:24:21)
IIIb	61		61	r	z_{53}	(Ar. 26:24:25). May possess H phase Rz_{47} (Ar. 39).
IIIb	61		61	z_{10}	z_{35}	(Ar. 26:27:21)
V	61		61	z_{35}	–	
IIIb	61		61	z_{52}	1,5,7	(Ar. 26:26:30)
IIIb	61		61	z_{52}	z	(Ar. 26:26:31)
IIIb	61		61	z_{52}	z_{35}	(Ar. 26:26:21)
IIIb	61		61	z_{52}	z_{53}	(Ar. 26:26:25)
IIIa	62		62	g,z_{51}	–	(Ar. 6:13,14:-)
IIIa	62		62	z_{29}	–	(Ar. 6:17,18:-)
IIIa	62		62	z_{36}	–	(Ar. 6:17,20:-)
IIIa	62		62	z_4,z_{23}	–	(Ar. 6:1,2,5:-)
IIIa	62		62	z_4,z_{32}	–	(Ar. 6:1,7,8:-)
IIIa	63		63	g,z_{51}	–	(Ar. 8:13,14:-)
IIIa	63		63	z_{36}	–	(Ar. 8:17,20:-)
IIIa	63		63	z_4,z_{23}	–	(Ar. 8:1,2,5:-)
IIIa	63		63	z_4,z_{32}	–	(Ar. 8:1,7,8:-)
II	65		65	–	1,6	
IIIb	65		65	c	1,5,7	(Ar. 30:32:30)
IIIb	65		65	c	z	(Ar. 30:32:31)
IIIb	65		65	c	z_{53}	(Ar. 30:32:25)
II	65		65	g,t	-	
IIIb	65		65	i	e,n,x,z_{15}	(Ar. 30:33:28)
IIIb	65		65	(k)	z	(Ar. 30:22:31)
IIIb	65		65	(k)	z_{35}	(Ar. 30:22:21)
IIIb	65		65	(k)	z_{53}	(Ar. 30:22:25)
IIIb	65		65	l,v	e,n,x,z_{15}	(Ar. 30:23:28)
IIIb	65		65	l,v	z	(Ar. 30:23:31)
IIIb	65		65	l,v	z_{35}	(Ar. 30:23:21)
IIIb	65		65	l,v	z_{53}	(Ar. 30:23:25)
IIIb	65		65	r	z_{35}	(Ar. 30:24:21)
IIIb	65		65	z_{10}	e,n,x,z_{15}	(Ar. 30:27:28)
IIIb	65		65	z_{10}	z	(Ar. 30:27:31)
IIIb	65		65	z_{52}	e,n,x,z_{15}	(Ar. 30:26:28)

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIb	65		65	z_{52}	z	(Ar. 30:26:31)
IIIb	65		65	z_{52}	z_{35}	(Ar. 30:26:21)
IIIb	65		65	z_{52}	z_{53}	(Ar. 30:26:25)
V	66	Maregrosso	66	z_{35}	–	
V	66		66	z_{39}	–	
V	66	Brookfield	66	z_{41}	–	
V	66	Malawi	66	z_{65}	–	
V	66		66	z_{81}	–	
I	67	Crossness	67	r	1,2	

Appendix B

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	K	Aarhus	18	z_4,z_{23}	z_{64}	
I	C_2	Aba	6,8	i	e,n,z_{15}	
I	M	Abadina	28	g,m	[e,n,z_{15}]	
I	F	Abaetetuba	11	k	1,5	
I	F	Aberdeen	11	i	1,2	
I	Q	Abidjan	39	b	l,w	
I	I	Ablogame	16	l,z_{13},z_{28}	z_6	
I	I	Abobo	16	i	z_6	
I	B	Abony	1,4,5,12	b	e,n,x	IP combined Sladun (1,4,12,27:b:e,n,x) with Abony to form Abony 1,4,[5],12,27:b:e,n,x (gelatin neg.).
I	B	Abortusbovis	1,4,12,27	b	e,n,x	Gelatin pos., mucate pos.1-4 days. Abortusbovis was combined with Abony (1,4,5,12:b:e,n,x), gelatin neg. The name Abortusbovis was dropped.
I	B	Abortuscanis	4,5,12	b	Rz_5	Abortuscanis was combined with Paratyphi B (1,4,[5],12:b:1,2) in 1938 and the name Abortuscanis was dropped.
I	B	Abortusequi	4,12	–	e,n,x	
I	B	Abortusovis	4,12	c	1,6	
I	E_4	Accra	1,3,19	b	z6	
I	D_2	Ackwepe	9,46	l,w	–	
II	G	Acres	1,13,23	b	[1,5]:z_{42}	
I	E_1	Adabraka	3,10	z_4,z_{23}	[1,7]	
I	M	Adamstown	28	k	1,6	
I	F	Adamstua	11	e,h	1,6	
I	U	Adana	43	z_{10}	1,5	
I	O	Adelaide	35	f,g	–	Adelaide may possess H phase Rz_{27}.
I	I	Adeoyo	16	g,m,[t]	–	
I	M	Aderike	28	z_{38}	[e,n,z_{15}]	
I	C_1	Adime	6,7	b	1,6	
I	G	Adjame	13,23	r	1,6	
I	C_1	Aequatoria	6,7	z_4,z_{23}	[e,n,z_{15}]	
I	C_2	Aesch	6,8	z_{60}	1,2	
I	H	Aflao	1,6,14,25	l,z_{28}	e,n,x	
I	B	Africana	4,12	r,i	l,w	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	C_1	Afula	6,7	f,g,t	e,n,x	
I	B	Agama	4,12	i	1,6	
I	I	Agbara	16	i	1,6	
I	G	Agbeni	1,13,23	g,m,[t]	–	
I	E_1	Agege	3,10	c	e,n,z_{15}	
I	N	Ago	30	z_{38}	–	
I	O	Agodi	35	g,t	–	
I	B	Agona	1,4,[5],12	f,g,s	[1,2]	
I	G	Agoueve	13,22	z_{29}	–	
I	J	Ahanou	17	i	1,7	
I	U	Ahepe	43	z_{35}	1,6	
I	E_4	Ahmadi	1,3,19	d	1,5	
I	S	Ahoutoue	41	z_{35}	1,6	
I	U	Ahuza	43	k	1,5	
I	G	Ajiobo	13,23	z_4,z_{23}	–	
I	C_2	Akanji	6,8	r	1,7	
I	I	Akuafo	16	y	1,6	
I	D_1	Alabama	9,12	c	e,n,z_{15}	
I	O	Alachua	35	z_4,z_{23}	–	Alachua may possess H phase Rz_{37} or Rz_{45}.
I	C_2	Alagbon	8	y	1,7	
I	C_1	Alamo	6,7	g,z_{51}	1,5	
I	C_2	Albany	8,20	z_4,z_{24}	–	Albany may possess H phase Rz_{45}.
I	B	Albert	4,12	z_{10}	e,n,x	
I	H	Albuquerque	1,6,14,24	d	z_6	
II	E_1	Alexander	3,10	z	1,5	
I	X	Alexanderplatz	47	z_{38}	–	
I	C_2	Alexanderpolder	8	c	l,w	
I	E_1	Alfort	3,10	f,g	e,n,x	
I	P	Alger	38	l,v	1,2	
I	E_4	Alkmaar	1,3,19	a	l,w	
I	R	Allandale	1,40	k	1,6	
I	E_1	Allerton	3,10	b	1,6	
I	C_2	Alminko	8,20	g,s,t	–	
II	R	Alsterdorf	1,40	g,m,[s],t	[1,5]	
I	B	Altendorf	4,12	c	1,7	IP combined Womba (4,12,27:c:1,7) with Altendorf to form Altendorf 4,12,27:c:1,7.
I	C_2	Altona	8,20	r,[i]	z_6	Pikine (8,20:r:z_6) was combined with Altona and called Altona.
I	E_1	Amager	3,10	y	1,2	IP combined Tuebingen (3,15:y:1,2) with Amager to form Amager 3,10,[15]:y:1,2. Amager may possess H phase Rz_{45}.
I	F	Amba	11	k	l,z_{13},z_{28}	
I	C_1	Amersfoort	6,7	d	e,n,x	IP combined Omderman (6,7,14:d:e,n,x) with Amersfoort to form Amersfoort 6,7,14:d:e,n,x.
I	C_2	Amherstiana	8	l,(v)	1,6	
I	I	Amina	16	i	1,5	
I	E_1	Aminatu	3,10	a	1,2	
I	E_1	Amounderness	3,10	i	1,5	
I	M	Amoutive	28	d	1,5	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_1	Amsterdam	3,10	g,m,s	–	IP combined Drypool (3,15:g,m,s) and Drypool var. O 34+ with Amsterdam to form Amsterdam 3,10,[15],[15,34]:g,m,s:-.
I	I	Amunigun	16	a	1,6	
I	E_1	Anatum	3,10	e,h	1,6	IP combined Newington (3,15:e,h:1,6) and Minneapolis (3,15,34:e,h:1,6) with Anatum to form Anatum 3,10,[15],[15,34]:e,h:1,6.
I	E_1	Anderlecht	3,10	c	l,w	
I	O	Anecho	35	g,s,t	–	
I	Q	Anfo	39	y	1,2	
I	C_2	Angers	8,20	z_{35}	z_6	
I	N	Angoda	30	k	e,n,x	
II	D_1	Angola	1,9,12	z	z_6	
I	I	Angouleme	16	z_{10}	z_6	
I	X	Anie	47	(g),m,t	–	IP combined Anie with Mesbit (47:m,t:[e,n,z_{15}]) and called it Mesbit.
I	M	Ank	28	k	e,n,z_{15}	
I	G	Anna	13,23	z_{35}	e,n,z_{15}	
I	I	Annedal	16	r,i	e,n,x	
I	D_1	Antarctica	9,12	g,z_{63}	–	
I	57	Antonio	57	a	z_6	
I	51	Antsalova	51	z	1,5	
I	T	Antwerpen	1,42	c	e,n,z_{15}	
I	W	Apapa	45	m,t	–	
I	C_2	Apeyeme	8,20	z_{38}	–	
I	N	Aqua	30	k	1,6	
I	N	Aragua	30	z_{29}	–	
I	H	Arapahoe	1,6,14	z_4,z_{23}	1,5	
I	C_1	Ardwick	6,7,14	f,g	–	IP combined Ardwick with Rissen (6,7:f,g:-) to form Rissen 6,7,14:f,g:-. Ardwick is now called Rissen var. O 14+ by IP.
I	B	Arechavaleta	4,[5],12	a	[1,7]	
IV	C_1	Argentina	6,7	z_{36}	–	
I	E_3	Arkansas	3,15,34	e,h	1,5	IP combined Arkansas and Newhaw (3,15:e,h:1,5) with Muenster (3,10:e,h:1,5) to form Muenster 3,10,[15],[15,34]:e,h:1,5. Arkansas is now callled Muenster var. O 15+, 34+ by IP.
II	56	Artis	56	b	–	
I	U	Arusha	43	z	e,n,z_{15}	
I	N	Aschersleben	30	b	1,5	
I	M	Ashanti	28	b	1,6	
II	51	Askraal	51	l,z_{28}	[z_6]	
I	L	Assen	21	a	[1,5]	
I	E_1	Assinie	3,10	l,w	z_6	Assinie may possess H phase Rz$_{45}$.
I	E_1	Asylanta	3,10	c	1,2	
I	C_2	Atakpame	8,20	e,h	1,7	
I	F	Atento	11	b	1,2	
I	C_1	Athinai	6,7	i	e,n,z_{15}	
I	F	Ati	11	d	1,2	
I	G	Atlanta	13,23	b	–	Atlanta was combined with Mississippi (1,13,23:b:1,5). The name Atlanta has been dropped.

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	Z	Atra	50	m,t	z_6:z_{42}	
I	C_1	Augustenborg	6,7,14	i	1,2	
I	C_1	Austin	6,7	a	1,7	
I	Y	Australia	48	l,v	1,5	
I	I	Avignon	16	y	e,n,z_{15}	
I	E_4	Avonmouth	1,3,19	i	e,n,z_{15}	
I	B	Ayinde	1,4,12,27	d	z_6	
I	B	Ayton	1,4,12,27	l,w	z_6	
I	B	Azteca	4,[5],12,27	l,v	1,5	
I	M	Babelsberg	28	z_4,z_{23}	[e,n,z_{15}]	
I	M	Babili	28	z_{35}	1,7	
II	C_1	Bacongo	6,7	z_{36}	z_{42}	
I	I	Badagry	16	z_{10}	1,5	
I	L	Baguida	21	z_4,z_{23}	–	
I	N	Baguirmi	30	y	e,n,x	
I	G	Bahati	13,22	b	e,n,z_{15}	
I	H	Bahrenfeld	6,14,24	e,h	1,5	
I	C_1	Baiboukoum	6,7	k	1,7	
I	D_2	Baildon	9,46	a	e,n,x	
I	M	Bakau	28	a	1,7	
V	Y	Balboa	48	z_{41}	–	
I	W	Balcones	45	z_{36}	–	
I	B	Ball	1,4,12,27	y	e,n,x	IP combined Ball with Ruki (4,5,12:y:e,n,x) and Dalat (4,5,27:y:e,n,x) to form Ball 1,4,[5],12,27:y:e,n,x.
I	J	Bama	17	m,t	–	
I	D_2	Bamboye	9,46	b	l,w	
I	D_2	Bambylor	9,46	z	e,n,z_{15}	
I	C_2	Banalia	6,8	b	z_6	
I	B	Banana	1,4,[5],12	m,t	[1,5]	Banana may possess H phase Rz_{45}.
I	M	Banco	28	r,i	1,7	
I	O	Bandia	35	i	l,w	
I	P	Bangkok	38	z_4,z_{24}	–	
I	D_1	Bangui	9,12	d	e,n,z_{15}	
I	H	Banjul	1,6,14,25	a	e,n,z_{15}	
II	C_2	Baragwanath	6,8	m,t	1,5	
I	C_2	Bardo	8	e,h	1,2	
I	C_1	Bareilly	6,7,14	y	1,5	
I	C_2	Bargny	8,20	i	1,5	
I	I	Barmbek	16	d	z_6	
I	I	Barranquilla	16	d	e,n,x	
I	54	Barry	54	z_{10}	e,n,z_{15}	
II	58	Basel	58	l,z_{13},z_{28}	1,5	
I	D	Basingstoke	9,46	z_{35}	e,n,z_{15}	
I	C_2	Bassa	6,8	m,t	–	
I	M	Bassadji	28	r	1,6	
I	57	Batonrouge	57	b	e,n,z_{15}	
I	I	Battle	16	l,z_{13},z_{28}	1,6	
I	C_2	Bazenheid	8,20	z_{10}	1,2	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	C_2	Be	8,20	a	[z_6]	
I	H	Beaudesert	[1],6,14,[25]	e,h	1,7	
II	B	Bechuana	1,4,12,27	g,[m],t	[1,5]	
I	E_4	Bedford	1,3,19	l,z_{13},z_{28}	e,n,z_{15}	
I	C_2	Belem	6,8	c	e,n,x	
I	C_2	Belfast	6,8	c	1,7	
I	C_2	Bellevue	8	z_4,z_{23}	1,7	
I	I	Bellville	16	e,n,x	1,(5),7	
II	K	Beloha	18	z_{36}	–	
I	E_1	Benfica	3,10	b	e,n,x	
I	R	Benguella	40	b	z_6	
I	D_2	Benin	9,46	y	1,7	
I	C_2	Benue	6,8	y	l,w	
I	X	Bere	47	z_4,z_{23}	[z_6]	Bere may possess H phase Rz_{45}.
I	D_2	Bergedorf	9,46	e,h	1,2	
I	X	Bergen	47	i	e,n,z_{15}	
I	51	Bergues	51	z_{10}	1,5	
I	U	Berkeley	43	a	1,5	
I	J	Berlin	17	d	1,5	
IV	R	Bern	1,40	z_4,z_{32}	–	
I	D_1	Berta	1,9,12	[f],g,t	–	
I	E_1	Bessi	3,10	i	e,n,x	
I	E_4	Bethune	1,3,19	k	1,7	
II	59	Betioky	59	k	(z)	
I	E_1	Biafra	3,10	z_{10}	z_6	
I	E_4	Bida	1,3,19	c	1,6	
I	N	Bietri	30	y	1,5	
I	J	Bignona	17	b	e,n,z_{15}	
I	R	Bijlmer	1,40	g,m	–	
II	X	Bilthoven	47	a	[1,5]	
I	E_4	Bilu	(1),3,10,(19)	f,g,t	1,(2),7	
I	X	Binche	47	z_4,z_{23}	l,w	
I	X	Bingerville	47	z_{35}	e,n,z_{15}	
I	W	Binningen	45	g,s,t	–	
I	E_2	Binza	3,15	y	1,5	IP combined Binza and Thomasville (3,15,34:y:1,5) with Orion (3,10:y:1,5) to form Orion 3,10,[15],[15,34]:y:1,5. Binza is now called Orion var. O 15+ by IP.
I	C_1	Birkenhead	6,7	c	1,6	
I	E_1	Birmingham	3,10	d	l,w	
I	B	Bispebjerg	1,4,[5],12	a	e,n,x	
I	B	Bissau	4,12	c	e,n,x	
II	D_1	Blankenese	1,9,12	b	z_6	
II	J	Bleadon	17	(f),g,t	[e,n,x,z_{15}]	IP has dropped f.
I	D_1	Blegdam	9,12	g,m,q	–	
I	H	Blijdorp	1,6,14,25	c	1,5	
I	X	Blitta	47	y	e,n,x	
I	C_2	Blockley	6,8	k	1,5	Blockley may possess H phase Rz_{58}
II	C_1	Bloemfontein	6,7	b	[e,n,x]:z_{42}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_1	Bloomsbury	3,10	g,t	1,5	
I	K	Blukwa	18	z_4,z_{24}	–	
I	V	Bobo	44	d	1,5	
I	B	Bochum	4,[5],12	r	l,w	
IV	53	Bockenheim	1,53	z_{36},z_{38}	–	
I	N	Bodjonegoro	30	z_4,z_{24}	–	
I	H	Boecker	[1],6,14,[25]	l,v	1,7	
I	M	Bokanjac	28	b	1,7	
II	R	Boksburg	40	g,[m],s,[t]	e,n,x	
I	V	Bolama	44	z	e,n,x	
I	E_1	Bolombo	3,10	z_{38}	$[z_6]$	
I	E_1	Bolton	3,10	y	e,n,z_{15}	
IV	Z	Bonaire	50	z_4,z_{32}	–	
I	J	Bonames	17	a	1,2	
I	C_2	Bonariensis	6,8	i	e,n,x	
V	Y	Bongor	48	z_{35}	–	
I	C_1	Bonn	6,7	l,v	e,n,x	
I	X	Bootle	47	k	1,5	
I	G	Borbeck	13,22	l,v	1,6	
I	52	Bordeaux	52	k	1,5	
VI	H	Bornheim	1,6,14,25	z_{10}	1,(2),7	Bornheim was formerly in Subspecies II.
I	C_1	Bornum	6,7,14	z_{38}	–	IP combined Bornum with Lille (6,7:z_{38}:-) to form Lille 6,7,14:z_{38}:-. Bornum is now called Lille var. O 14+ by IP.
I	54	Borreze	54	f,g,s	–	
I	T	Borromea	42	i	1,6	
I	I	Bouake	16	z	z_6	
II	G	Boulders	1,13,23	m,t	z_{42}	
I	D_1	Bournemouth	9,12	e,h	1,2	
I	H	Bousso	1,6,14,25	z_4,z_{23}	$[e,n,z_{15}]$	
I	C_2	Bovismorbificans	6,8	r,[i]	1,5	
I	G	Bracknell	13,23	b	1,6	
I	B	Bradford	4,12,27	r	1,5	
I	C_1	Braenderup	6,7,14	e,h	e,n,z_{15}	
I	B	Brancaster	1,4,12,27	z_{29}	–	
I	B	Brandenburg	1,4,[5],12,27	l,v	e,n,z_{15}	
I	I	Brazil	16	a	1,5	
I	K	Brazos	6,14,18	a	e,n,z_{15}	
I	C_1	Brazzaville	6,7	b	1,2	
I	C2	Breda	6,8	z_4,z_{23}	e,n,x	
I	B	Bredeney	1,4,12,27	l,v	1,7	Bredeney may possess H phase Rl,z_{40} instead of l,v.
I	V	Brefet	44	r	e,n,z_{15}	
II	W	Bremen	45	g,m,s,t	e,n,x	
I	C_2	Breukelen	6,8	$l,z_{13},[z_{28}]$	e,n,z_{15}	
I	I	Brevik	16	z	$e,n,[x],z_{15}$	
I	B	Brezany	1,4,12,27	d	1,6	
I	F	Brijbhumi	11	i	1,5	
I	C_2	Brikama	8,20	r,[i]	l,w	
I	M	Brisbane	28	z	e,n,z_{15}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	G	Bristol	13,22	z	1,7	
I	T	Brive	1,42	r	l,w	
I	T	Broc	42	z_4,z_{23}	e,n,z_{15}	
I	G	Bron	13,22	g,m	[e,n,z_{15}]	
I	C_2	Bronx	6,8	c	1,6	
V	66	Brookfield	66	z_{41}	–	
I	I	Brooklyn	16	l,w	e,n,x	
I	E_4	Broughton	1,3,19	b	l,w	
I		Broxbourne				Combined with Wien. The name Broxbourne has been dropped.
I	C_1	Bruck	6,7	z	l,w	
I	C_2	Brunei	8,20	y	1,5	
I	I	Brunflo	16	r	1,7	
I	C_2	Bsilla	6,8	r	1,2	
I	B	Budapest	1,4,12,27	g,t	–	
I	R	Bukavu	1,40	l,z_{28}	1,5	
I	C_2	Bukuru	6,8	b	l,w	
II	R	Bulawayo	1,40	z	1,5	
I	C_2	Bulgaria	6,8	y	1,6	
I	F	Bullbay	11	l,v	e,n,z15	
I	C_1	Bulovka	6,7	z_{44}	–	
II	U	Bunnik	43	z_{42}	[1,5,7]	
I	I	Burgas	16	l,v	e,n,z_{15}	
I	S	Burundi	41	a	–	
I	B	Bury	4,12,27	c	z_6	
I	C_1	Businga	6,7	z	e,n,z_{15}	
I	E_1	Butantan	3,10	b	1,5	IP combined Rosenthal (3,15:b:1,5) and unnamed 3,15,34:b:1,5 with Butantan to form Butantan 3,10,[15],[15,34]:b:1,5.
I	52	Butare	52	e,h	1,6	
I	H	Buzu	[1],6,14,[25]	i	1,7	
I	I	Caen	16	d	l,w	
I	E_1	Cairina	3,10	z_{35}	z_6	
I	W	Cairns	45	k	e,n,z_{15}	
I	B	Cairo	1,4,12,27	d	1,2	IP combined Cairo with Stanley (4,5,12:d:1,2) to form Stanley 1,4,[5],12,27:d:1,2. The name Cairo has been dropped.
I	E_4	Calabar	1,3,19	e,h	l,w	
II	B	Caledon	1,4,12,27	g,[m],[s],t	e,n,x	
I	B	California	4,12	g,m,t	–	
II	C_1	Calvinia	6,7	a	z_{42}	
I	O	Camberene	35	z_{10}	1,5	
I	D_1	Camberwell	9,12	r	1,7	
I	E_2	Cambridge	3,15	e,h	l,w	IP combined Cambridge and Wildwood (3,15,34:e,h:l,w) with Meleagridis (3,10:e,h:l,w) to form Meleagridis 3,10,[15],[15,34]:e,h:l,w. Cambridge is now called Meleagridis var. O 15+ by IP.
V	V	Camdeni	44	r	–	
I	D_1	Campinense	9,12	r	e,n,z_{15}	
I	B	Canada	4,12,27	b	1,6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	R	Canary	40	l,v	1,6	
II	D_1	Canastel	9,12	z_{29}	[1,5]	
I	M	Cannobio	28	z_4,z_{23}	1,5	
I	E_4	Cannonhill	1,3,15,19	y	e,n,x	
I	E_4	Cannstatt	1,3,19	m,t	–	
I	E_3	Canoga	3,15,34	g,s,t	–	IP combined Canoga and Halmstad (3,15:g,s,t:-) with Westhampton (3,10:g,s,t:-) to form Westhampton 3,10,[15],[15,34]:g,s,t:-. Canoga is now called Westhampton var. O 15+, 34+ by IP.
I	54	Canton	54	z_{10}	e,n,x	
II	C_1	Cape	6,7	z_6	1,7	
I	H	Caracas	[1],6,14,[25]	g,m,s	–	
I	C_1	Cardiff	6,7	k	R1,10	IP combined Cardiff that contains H phase R1,10 with Thompson (6,7,14:k:1,5).
I	I	Cardoner	16	g,s,t	–	
II	P	Carletonville	38	d	[1,5]	
I	J	Carmel	17	l,v	e,n,x	
I	K	Carnac	18	z_{10}	z_6	
I	E_4	Carno	1,3,19	z	l,w	
I	H	Carrau	6,14,[24]	y	1,7	
I	V	Carswell	44	g,z_{51}	–	
I	W	Casablanca	45	k	1,7	
I	R	Casamance	40	z	e,n,x	
I	M	Catalunia	28	l,z_{13},z_{28}	1,5	
I	H	Catanzaro	6,14	g,s,t	–	
I	C_1	Cayar	6,7	z	e,n,x	
II	M	Ceres	28	z	z_{39}	
I	K	Cerro	18	z_4,z_{23}	[1,5]	Cerro was combined with Siegburg (6,14,18:z_4,z_{23}:[1,5]) and called Cerro. Cerro may possess H phase Rz_{45}.
I	D_2	Ceyco	9,46	k	z_{35}	
I	G	Chagoua	1,13,23	a	1,5	
I	C_2	Chailey	6,8	z_4,z_{23}	$[e,n,z_{15}]$	
IV	I	Chameleon	16	z_4,z_{32}	–	
I	Q	Champaign	39	k	1,5	Champaign may possess H phase Rz_{48}
I	F	Chandans	11	d	e,n,x:[r]	
I	H	Charity	[1],6,14,[25]	d	e,n,x	
I	C_2	Charlottenburg	6,8	k	e,n,z_{15}	
I	D_2	Cheltenham	9,46	b	1,5	
II	X	Chersina	47	z	z_6	
I	B	Chester	1,4,[5],12	e,h	e,n,x	
I	M	Chicago	28	r,[i]	1,5	
I	E_4	Chichester	1,3,19	i	1,6	
I	H	Chichiri	6,14,24	z_4,z_{24}	–	
I	C_1	Chile	6,7	z	1,2	
I	C_2	Chincol	6,8	g,m,[s]	[e,n,x]	
I	F	Chingola	11	e,h	1,2	
II	T	Chinovum	42	b	1,5	
I	F	Chiredzi	11	c	1,5	
I	E4	Chittagong	1,3,10,19	b	z_{35}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	C_1	Choleraesuis	6,7	c	1,5	H_2S negative
I	C_1	Choleraesuis var. Kunzendorf		6,7	c	[1,5] H_2S positive
I	C_2	Chomedey	8,20	z_{10}	e,n,z_{15}	
I	V	Christiansborg	44	z_4,z_{24}	–	
II	E_1	Chudleigh	3,10	e,n,x	1,7	
I	B	Clackamas	4,12	l,v,[z13]	1,6	IP does not get z_{13}
I	D_1	Claibornei	1,9,12	k	1,5	
I	E_1	Clerkenwell	3,10	z	l,w	
I	C_2	Cleveland	6,8	z_{10}	1,7	
I	E_2	Clichy	3,15	a	1,5	IP combined Clichy with Goelzau (3,10:a:1,5) to form Goelzau 3,10,[15]:a:1,5. Clichy is now called Goelzau var. O 15+ by IP.
II	G	Clifton	13,22	z_{29}	1,5	
II	V	Clovelly	1,44	z_{39}	[e,n,x,z_{15}]	
I	D_2	Cochin	9,46	k	1,5	
I	C_2	Cocody	8,20	r,i	e,n,z_{15}	
I	B	Coeln	4,[5],12	y	1,2	
I	C_1	Coleypark	6,7,14	a	l,w	
I	C_1	Colindale	6,7	r	1,7	
I	F	Colobane	11	k	1,7	
I	P	Colombo	38	y	1,6	
I	C_1	Colorado	6,7	l,w	1,5	
I	C_1	Concord	6,7	l,v	1,2	
I	G	Congo	13,23	g,[m],[s],t	–	IP calls Congo 13,23:g,m,s,t:-.
I	F	Connecticut	11	l,z_{13},z_{28}	1,5	
II	J	Constantia	17	z	l,w:z_{42}	
I	T	Coogee	42	l,v	e,n,z_{15}	
I	Q	Cook	39	Rz_{48}	1,5	IP combined Cook with Champaign (39:k:1,5). The name Cook has been dropped.
I	E_1	Coquilhatville	3,10	z_{10}	1,7	
I	C_1	Coromandel	6,7	l,v	z_{35}	
I	C_2	Corvallis	8,20	z_4,z_{23}	[z_6]	
I	M	Cotham	28	i	1,5	
I	K	Cotia	18	–	1,6	
I	C_2	Cremieu	6,8	e,h	1,[6]	
I	F	Crewe	11	z	1,5	
I	M	Croft	28	g,m,s	[e,n,z_{15}]	
I	67	Crossness	67	r	1,2	
I	G	Cubana	1,13,23	z_{29}	–	Cubana may possess H phase Rz_{37} or Rz_{43}.
I	E_1	Cuckmere	3,10	i	1,2	
I	M	Cullingworth	28	d	l,w	
I	Q	Cumberland	39	i	e,n,x	
I	C_2	Curacao	6,8	a	1,6	
I	C_2	Cyprus	6,8	i	l,w	
I	54	Czernyring	54	r	1,5	
I	C_2	Daarle	6,8	y	e,n,x	
I	C_2	Dabou	8,20	z_4,z_{23}	l,w	
I	51	Dadzie	51	l,v	e,n,x	
I	Y	Dahlem	48	k	e,n,z_{15}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	X	Dahomey	47	k	1,6	Dahomey may possess H phase Rz_{58}.
I	J	Dahra	17	b	1,5	
I	M	Dakar	28	a	1,6	
I	I	Dakota	16	z_{35}	e,n,z_{15}	
I	B	Dalat	4,5,27	y	e,n,x	Dalat was combined with Ball. The name Dalat has been dropped.
I	E_4	Dallgow	1,3,19	z_{10}	e,n,z_{15}	
I	C_1	Damman	6,7	a	z_6	
I	51	Dan	51	k	e,n,z_{15}	
I	X	Dapango	47	r	1,2	
II	D_1	Daressalaam	1,9,12	l,w	e,n,x	
I	C_2	Daula	8,20	z	z_6	
I	C_1	Daytona	6,7	k	1,6	
I	C_1	Decatur	6,7	c	1,5	IP has dropped Decatur and calls it dulcitol positive, mucate positive variant of Choleraesuis.
I	D_2	Deckstein	9,46	r	1,7	
II	R	Degania	40	z_4,z_{24}	[z_{39}]	
IV	R	Degania var. subsp. IV	40	z_4,z_{24}	–	
I	K	Delmenhorst	18	z_{71}	–	
I	O	Dembe	35	d	l,w	
I	G	Demerara	13,23	z_{10}	l,w	
I	C_1	Denver	6,7	a	e,n,z_{15}	
I	B	Derby	1,4,[5],12	f,g	[1,2]	
I	52	Derkle	52	e,h	1,7	
I	E_4	Dessau	1,3,15,19	g,s,t	–	
II	T	Detroit	42	z	1,5	
I	W	Deversoir	45	c	e,n,x	
I	M	Dibra	28	a	z_6	
I	M	Dieuppeul	28	i	1,7	
I	G	Diguel	1,13,22	d	e,n,z_{15}	
I	C_2	Diogoye	8,20	z_{41}	z_6	
I	L	Diourbel	21	i	1,2	
I	Y	Djakarta	48	z_4,z_{24}	–	
I	T	Djama	1,42	z_{29}	[1,5]	
I	C_2	Djelfa	8	b	1,2	
I	M	Djermaia	28	z_{29}	–	
I	J	Djibouti	17	z_{10}	e,n,x	
I	C_1	Djugu	6,7	z_{10}	e,n,x	
I	D_2	Doba	9,46	a	e,n,z_{15}	
I	M	Doel	28	z	1,6	
I	C_2	Doncaster	6,8	a	1,5	
I	N	Donna	30	l,v	1,5	
I	M	Doorn	28	i	1,2	
I	M	Douala	28	i	l,w	
I	Z	Dougi	50	y	1,6	
I	N	Doulassame	30	a	e,n,z_{15}	
I	M	Dresden	28	c	e,n,x	
I	R	Driffield	1,40	d	1,5	
I	B	Drogana	1,4,12,27	r,(i)	e,n,z_{15}	IP calls Drogana r,i.

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_2	Drypool	3,[15],[15,34]	g,m,s	–	IP combined Drypool 3,15:g,m,s:- and Drypool var. O34+ with Amsterdam (3,10:g,m,s:-) to form Amsterdam 3,10,[15],[15,34]:g,m,s:-. Drypool is now called Amsterdam var. O 15+ or O 15+, 34+ by IP.
I	D_1	Dublin	1,9,12,[Vi]	g,p	–	
II	S	Dubrovnik	41	z	1,5	
I	C_2	Duesseldorf	6,8	z_4,z_{24}	–	
I	W	Dugbe	45	d	1,6	
I	B	Duisburg	1,4,12,27	d	e,n,z_{15}	
II	D_2	Duivenhoks	9,46	g,[m],[s],t	[e,n,x]	
I	E_1	Dumfries	3,10	r,[i]	1,6	
I	C_2	Dunkwa	6,8	d	1,7	
I	D_1	Durban	9,12	a	e,n,z_{15}	
II	B	Durbanville	1,4,12,27	$[z_{39}]$	1,[5],7	
I	G	Durham	13,23	b	e,n,z_{15}	
I	R	Duval	1,40	b	e,n,z_{15}	
I	O	Ealing	35	g,m,s	–	
I	D_1	Eastbourne	1,9,12	e,h	1,5	
I	E_4	Eastglam	1,3,19	c	1,5	
I	M	Eberswalde	28	c	1,6	
I	C_2	Eboko	6,8	b	1,7	
I	O	Ebrie	35	g,m,t	–	
I	P	Echa	38	k	1,2	
I	C_1	Edinburg	6,7	b	1,5	
I	C_2	Edmonton	6,8	l,v	e,n,z_{15}	
I	S	Egusi	41	d	[1,5]	
I	T	Egusitoo	1,42	b	z_6	
IIIb	61	Eilbeck	61	i	z	Eilbeck was formerly in subspecies II, but is now combined with *Arizona* 26:33:31. The name Eilbeck has been dropped.
I	C_1	Eimsbuettel	6,7,14	d	l,w	IP combined Eimsbuettel with Livingstone (6,7:d:l,w) to form Livingstone 6,7,14:d:l,w. Eimbuettel is now called Livingstone var. O 14+ by IP.
I	C_1	Eingedi	6,7	f,g,t	1,2,7	
II	W	Ejeda	45	a	z_{10}	
I	B	Eko	4,12	e,h	1,6	
I	D_2	Ekotedo	9,46	z_4,z_{23}	–	
I	X	Ekpoui	47	z_{29}	–	
I	E_1	Elisabethville	3,10	r	1,7	
I	D_1	Elokate	9,12	c	1,7	
I	D_1	Elomrane	1,9,12	z38	–	
II	I	Elsiesrivier	16	[e,n,x]	$1,6:z_{42}$	
I	C_2	Emek	8,20	g,m,s	–	
I	P	Emmastad	38	r	1,6	
II	H	Emmerich	6,14	[m,t]	e,n,x	
	I	H	Encino	1,6,14,25	d	l,z_{13},z_{28}
I	O	Enschede	35	z_{10}	l,w	
I	B	Entebbe	1,4,12,27	z	z_6	
I	D_1	Enteritidis	1,9,12	[f],g,m,[p],[t]	[1,7]	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	I	Enugu	16	l,[z13],z_{28}	[1,5]	
I	E_1	Epicrates	3,10	b	l,w	
I	F	Epinay	11	a	l,z_{13},z_{28}	
I	B	Eppendorf	1,4,12,27	d	1,5	
II	G	Epping	1,13,23	e,n,x	1,[5],7	
II	Y	Erlangen	48	g,m,t	–	
I	C_1	Escanaba	6,7	k	e,n,z_{15}	
I	D_1	Eschberg	9,12	d	1,7	
I	E_2	Eschersheim	3,15	d	e,n,x	IP combined Eschersheim with Souza (3,10:d:e,n,x) to form Souza 3,10,[15]:d:e,n,x. Eschersheim is now called Souza var. O 15+ by IP.
I	C_1	Eschweiler	6,7	z_{10}	1,6	
I	B	Essen	4,12	g,m	–	
II	Y	Etosha	48	d	1,11	Etosha was not considered a new serotype by Kauffmann and is not used.
I	F	Etterbeek	11	z_4,z_{23}	e,n,z_{15}	
I	F	Euston	11	r,i	e,n,x,z_{15}	
I	E_1	Everleigh	3,10	z_{29}	e,n,x	
I	M	Ezra	28	z	1,7	
I	M	Fairfield	28	r	l,w	
I	M	Fajara	28	l,z_{28}	e,n,x	
I	T	Faji	1,42	a	e,n,z_{15}	
I	E_1	Falkensee	3,10	i	e,n,z_{15}	
I	E_1	Fallowfield	3,10	l,z_{13},z_{28}	e,n,z_{15}	
II	R	Fandran	1,40	z_{35}	e,n,x,z_{15}	
I	F	Fann	11	l,v	e,n,x	
I	G	Fanti	13,23	z_{38}	–	
I	M	Farakan	28	z_{10}	1,5	
I	U	Farcha	43	y	1,2	
I	E_4	Fareham	1,3,19	r,i	l,w	
I	G	Farmsen	13,23	z	1,6	
I	B	Farsta	4,12	i	e,n,x	
I	Z	Fass	50	l,v	1,2	
II	Z	Faure	50	z_{42}	1,7	
I	C_2	Fayed	6,8	l,w	1,2	
VI	H	Ferlac	1,6,14,25	a	e,n,x	
I	S	Ferlo	41	k	1,6	
I	C_2	Ferruch	8	e,h	1,5	
I	B	Finaghy	4,12	y	1,6	
II	E_1	Finchley	3,10	z	e,n,x	
I	F	Findorff	11	d	z_6	
I	H	Finkenwerder	[1],6,14,[25]	d	1,5	
I	I	Fischerhuette	16	a	e,n,z_{15}	
I	H	Fischerkietz	1,6,14,25	y	e,n,x	
I	V	Fischerstrasse	44	d	e,n,z_{15}	
I	Y	Fitzroy	48	e,h	1,5	
IV	Z	Flint	50	z_4,z_{23}	–	
I	E_1	Florian	3,10,[15]	z4,z24	–	
I	H	Florida	[1],6,14,[25]	d	1,7	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	51	Flottbek	52	b	[e,n,x]	
I	K	Fluntern	6,14,18	b	1,5	
I	W	Fomeco	45	b	e,n,z_{15}	
I	I	Fortlamy	16	z	1,6	
I	B	Fortune	1,4,12,27	z_{10}	z6	
II	P	Foulpointe	38	g,t	–	
I	D_1	Franken	1,9,12	z_{60}	z_{67}	
I	I	Frankfurt	16	i	e,n,z_{15}	
I	P	Freetown	38	y	1,5	
I	E_1	Freiburg	3,10	l,z_{13}	1,2	
II	T	Fremantle	42	(f),g,t	–	
I	D_2	Fresno	9,46	z_{38}	–	
I	G	Friedenau	13,22	d	1,6	
I	M	Friedrichsfelde	28	f,g	–	
I	D_1	Frintrop	1,9,12	b	1,5	
I	E_1	Fufu	3,10	z	1,5	
II	E_1	Fuhlsbuettel	3,10	l,v	z_6	
I	E_4	Fulda	1,3,19	l,w	1,5	
I	B	Fulica	4,[5],12	a	1,5	
I	B	Fyris	4,[5],12	l,v	1,2	
I	C_1	Gabon	6,7	l,w	1,2	
I	I	Gafsa	16	c	1,6	
I	C_1	Galiema	6,7,14	k	1,2	
I	E_1	Galil	3,10	a	e,n,z_{15}	
I	F	Gallen	11	a	1,2	
I	D_1	Gallinarum	1,9,12	–	–	Gallinarum must be identified biochemically.
I	V	Gamaba	1,44	g,m,[s]	–	
I	L	Gambaga	21	z_{35}	e,n,z_{15}	
I	O	Gambia	35	i	e,n,z_{15}	
I	I	Gaminara	16	d	1,7	
I	H	Garba	1,6,14,25	a	1,5	
I	C_1	Garoli	6,7	i	1,6	
I	O	Gassi	35	e,h	z_6	
I	D_2	Gateshead	9,46	g,s,t	–	
I	E_4	Gatineau	1,3,19	y	1,5	
I	C_1	Gatow	6,7	y	1,7	
I	C_2	Gatuni	6,8	b	e,n,x	
I	E_1	Gbadago	3,10,[15]	c	1,5	
I	C_1	Gdansk	6,7	l,v	z_6	IP combined Gelsenkirchen (6,7,14:l,v:z_6) with Gdansk to form Gdansk 6,7,14:l,v:z_6.
I	N	Gege	30	r	1,5	
I	C_1	Gelsenkirchen	6,7,14	l,v	z_6	IP combined Gelsenkirchen with Gdansk (6,7:l,v:z_6) to form Gdansk 6,7,14:l,v:z_6. Gelsenkirchen is now called Gdansk var. O 14+ by IP.
I	C_1	Georgia	6,7	b	e,n,z_{15}	
I	T	Gera	1,42	z_4,z_{23}	[1,6]	
I	D_2	Geraldton	9,46	l,v	1,6	
II	C_2	Germiston	6,8	m,t	e,n,x	
I	L	Ghana	21	b	1,6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	N	Giessen	30	g,m,s	–	
II	C_1	Gilbert	6,7	z_{39}	1,5,7	
I	E_1	Give	3,10	l,v	1,7	IP combined Newbrunswick (3,15:l,v:1,7) and Menhaden (3,15,34:l,v:1,7) with Give to form Give 3,10,[15],[15,34]:[d],l,v:[d],1,7. Give may possess H phase d; Rl,z_{40}; or Rz_{77}.
I	C_2	Giza	8,20	y	1,2	
I	I	Glasgow	16	b	1,6	
II	F	Glencairn	11	a	z_6:z_{42}	
I	F	Glidji	11	l,w	1,5	
I	C_2	Glostrup	6,8	z_{10}	e,n,z_{15}	
I	B	Gloucester	1,4,12,27	i	l,w	
I	E_4	Gnesta	1,3,19	b	1,5	
I	N	Godesberg	30	g,m,[t]	–	
I	E_1	Goelzau	3,10	a	1,5	IP combined Clichy (3,15:a:1,5) with Goelzau to form Goelzau 3,10,[15]:a:1,5.
I	E_2	Goerlitz	3,15	e,h	1,2	IP combined Goerlitz with Vejle (3,10:e,h:1,2) to form Vejle 3,10,15:e,h:1,2. Goerlitz is now called Vejle var. O 15+ by IP.
I	D_1	Goeteborg	9,12	c	1,5	
I	D_1	Goettingen	9,12	l,v	e,n,z_{15}	
II	G	Gojenberg	1,13,23	g,t	1,5	
I	51	Gokul	1,51	d	[1,5]	
I	C_2	Goldcoast	6,8	r	l,w	
I	C_1	Goma	6,7	z_4,z_{23}	z_6	
I	C_1	Gombe	6,7,14	d	e,n,z_{15}	
I	M	Good	21	f,g	e,n,x	
II	G	Goodwood	13,22	z_{29}	e,n,x	
I	J	Gori	17	z	1,2	
I	R	Goulfey	1,40	k	1,5	
I	O	Gouloumbo	35	c	1,5	
I	D_1	Goverdhan	9,12	k	1,6	
I	M	Gozo	28	e,h	e,n,z_{15}	
II	F	Grabouw	11	g,[m],s,t	[z_{39}]	
I	C_1	Grampian	6,7	r	l,w	
I	I	Grancanaria	16	z_{39}	[1,6]	Grancanaria can be d-tartrate neg., dulcitol neg., ONPG pos., and anaerogenic.
I	N	Grandhaven	30	r	1,2	
I	J	Granlo	17	l,z_{28}	e,n,x	
I	U	Graz	43	a	1,2	
II	Z	Greenside	50	z	e,n,x	
I	R	Greiz	40	a	z_6	
I	K	Groenekan	18	d	1,5	
		Group A	1,2,12			
		Group B	4,12; 1,4,5,12; or 1,4,12,27			
		Group C1	6,7,[Vi] or 6,7,14			
		Group C2	6,8			IP combined C_2 and C_3.
		Group C3	8; or 8,20			IP combined with C_2.
		Group D1	1,9,12			
		Group D2	9,46			

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
		Group D3	1,9,12,46,27			
		Group E1	3,10			
		Group E2	3,15			IP combined E_2 and E_3 with E_1.
		Group E3	3,15,34			IP combined E_2 and E_3 with E_1.
		Group E4	1,3,19			
		Group F	11			
		Group G	13,22 or 13,23			
		Group H	6,14; 6,14,24; or 1,6,14,25			
		Group I	16			
		Group J	17			
		Group K	18			
		Group L	21			
		Group M	28			
		Group N	30			
		Group O	35			
		Group P	38			
		Group Q	39			
		Group R	40			
		Group S	41			
		Group T	42			
		Group U	43			
		Group V	44			
		Group W	45			
		Group X	47			
		Group Y	48			
		Group Z	50			
		Group 51	51			
		Group 52	52			
		Group 53	53			
		Group 54	54			
		Group 55	55			
		Group 56	56			
		Group 57	57			
		Group 58	58			
		Group 59	59			
		Group 60	60			
		Group 61	61			
		Group 62	62			
		Group 63	63			
		Group 65	65			
		Group 66	66			
		Group 67	67			
I	G	Grumpensis	1,13,23	d	1,7	
II	R	Grunty	1,40	z_{39}	1,6	
I	N	Guarapiranga	30	a	e,n,x	
I	D_2	Guerin	9,46	e,h	z_6	
I	M	Guildford	28	k	1,2	
I	V	Guinea	1,44	z_{10}	[1,7]	
I	F	Gustavia	11	d	1,5	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	L	Gwaai	21	z_4,z_{24}	–	
I	T	Gwale	1,42	k	z_6	
I	E_4	Gwoza	1,3,19	a	e,n,z_{15}	
I	C_2	Haardt	8	k	1,5	
II	D_2	Haarlem	9,46	z	e,n,x	
I	C_2	Hadar	6,8	z_{10}	e,n,x	
II	I	Haddon	16	z_4,z_{23}	–	
I	J	Hadejia	17	y	e,n,z_{15}	
I	B	Haduna	4,12	l,z_{13},[z_{28}]	1,6	
I	C_1	Haelsingborg	6,7	m,p,t,[u]	–	
I	T	Haferbreite	42	k	[1,6]	
I	O	Haga	35	z_{38}	–	
II	Y	Hagenbeck	48	d	z_6	
I	B	Haifa	1,4,[5],12	z_{10}	1,2	
I	M	Halle	28	c	1,7	
I	B	Hallfold	1,4,12,27	c	l,w	
I	E_2	Halmstad	3,15	g,s,t	–	IP combined Halmstad and Canoga (3,15,34:g,s,t:-) with Westhampton (3,10:g,s,t:-) to form Westhampton 3,10,[15],[15,34]:g,s,t:-. Halmstad is now called Westhampton var. O 15+ by IP.
II	D_1	Hamburg	1,9,12	g,t	–	IP combined Hamburg with Manica (1,9,12:g,m,s,t:z42) and Muizenberg (9,12:g,m,s,t:1,5) to form *S.* II 1,9,12:g,m,[s],t:[1,5,7]:[z_{42}].
I	E_2	Hamilton	3,15	Rz_{27}	–	IP combined Hamilton with Goerlitz (3,15:e,h:1,2) and Vejle (3,10:e,h:1,2) to form Vejle 3,10,15:e,h:1,2:Rz_{27}. Hamilton is now called Vejle var. Rz_{27}+. The name Hamilton has been dropped.
II	Y	Hammonia	48	e,n,x,z15	z_6	
I	G	Handen	1,13,23	d	1,2	
I	R	Hann	40	k	e,n,x	
I	I	Hannover	16	a	1,2	
I	G	Haouaria	13,22	c	e,n,x,z_{15}	
I	H	Harburg	[1],6,14,[25]	k	1,5	
I	51	Harcourt	51	l,v	1,2	
I	E_1	Harleystreet	3,10	z	1,6	
IV	51	Harmelen	51	z_4,z_{23}	–	
I	E_1	Harrisonburg	3,10,[15],[15,34]	z_{10}	1,6	
I	C_1	Hartford	6,7	y	e,n,x	Hartford may possess H phase Rz_{50} or Rz_{67}.
I	T	Harvestehude	1,42	y	z6	
I	M	Hatfield	28	d	1,6	
I	B	Hato	4,[5],12	g,m,s	–	
I	G	Havana	1,13,23	f,g,[s]	–	Havana may possess H phase Rz_{45} or Rz_{79}.
I	E_4	Hayindogo	1,3,19	e,h	1,6	
I	F	Heerlen	11	i	1,6	
I	Q	Hegau	39	z_{10}	–	
I	B	Heidelberg	1,4,[5],12	r	1,2	
II	C_1	Heilbron	6,7	l,z_{28}	1,5:[z_{42}]	
II	B	Helsinki	1,4,12	z_{29}	[e,n,x]	
I	Z	Hemingford	50	d	1,5	Hemingford may possess H phase Rz_{82}.

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	S	Hennepin	41	d	z_6	
I	M	Hermannswerder	28	c	1,5	
I	I	Heron	16	a	z_6	
I	C_2	Herston	6,8	d	e,n,z_{15}	
I	F	Herzliya	11	y	e,n,x	
I	B	Hessarek	4,12,27	a	1,5	
I	H	Heves	6,14,[24]	d	1,5	
I	C_2	Hidalgo	6,8	r,i	e,n,z_{15}	
I	C_2	Hiduddify	6,8	l,z_{13},z_{28}	1,5	
II	J	Hillbrow	17	b	e,n,x,z_{15}	
I	D_2	Hillegersberg	9,46	z_{35}	1,5	
I	D_2	Hillingdon	9,46	g,m	–	
I	C_1	Hillsborough	6,7	z_{41}	l,w	
I	N	Hilversum	30	k	1,2	
I	C_2	Hindmarsh	8,20	r	1,5	
I	Y	Hisingen	48	a	1,5,7	
I	C_1	Hissar	6,7,14	c	1,2	
I	I	Hithergreen	16	c	e,n,z_{15}	
I	E_1	Hoboken	3,10	i	l,w	
I	Q	Hofit	39	i	1,5	
I	E_1	Hoghton	3,10	l,z_{13},z_{28}	z_6	
I	C_2	Holcomb	6,8	l,v	e,n,x	
I	H	Homosassa	1,6,14,25	z	1,5	
I	M	Honelis	28	a	e,n,z_{15}	
I	E_4	Hongkong	1,3,19	z	z_6	
II	Z	Hooggraven	50	z_{10}	z_6:z_{42}	
I	H	Horsham	1,6,14,[25]	l,v	e,n,x	
IV	U	Houten	43	z_4,z_{23}	–	
I	E_1	Huddinge	3,10	z	1,7	
II	D_1	Hueningen	9,12	z	z_{39}	
I	B	Huettwilen	1,4,12	a	l,w	
II	F	Huila	11	l,z_{28}	e,n,x	
I	I	Hull	16	b	1,2	
II	53	Humber	53	z_4,z_{24}	–	
I	E_1	Huvudsta	3,10	b	1,7	
I	I	Hvittingfoss	16	b	e,n,x	
I	L	Hydra	21	c	1,6	
I	G	Ibadan	13,22	b	1,5	
I	L	Ibaragi	21	y	1,2	
I	G	Idikan	1,13,23	i	1,5	
I	E_1	Ikayi	3,10,[15]	c	1,6	Ikayi Var. O 15+ was described after E_1 and E_2 were combined.
I	M	Ikeja	28	k	1,7	
I	M	Ilala	28	k	1,5	
I	E_3	Illinois	3,15,34	z_{10}	1,5	IP combined Illinois and Manila (3,15:z_{10}:1,5) with Lexington (3,10:z10:1,5) to form Lexington 3,10,[15],[15,34]:z_{10}:1,5. Illinois is now called Lexington var. O 15+, 34+ by IP.
I	E_4	Ilugun	1,3,10,19	z_4,z_{23}	z_6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	W	Imo	45	l,v	[e,n,z_{15}]	
I	C_2	Inchpark	6,8	y	1,7	
I	D_2	India	9,46	l,v	1,5	
I	B	Indiana	1,4,12	z	1,7	
I	C_1	Infantis	6,7,14	r	1,5	Infantis may possess H phase Rz_{49}.
I	C_1	Inganda	6,7	z_{10}	1,5	
I	D_2	Inglis	9,46	z_{10}	e,n,x	
I	S	Inpraw	41	z_{10}	e,n,x	
I	P	Inverness	38	k	1,6	
I	D_1	Ipeko	9,12	c	1,6	
I	S	Ipswich	41	z_4,z_{24}	[1,5]	
I	D_2	Irchel	9,46	y	e,n,x	
I	J	Irenea	17	k	1,5	
I	U	Irigny	43	z_{38}	–	
I	C_1	Irumu	6,7	l,v	1,5	
I	C_1	Isangi	6,7,14	d	1,5	
I	Y	Isaszeg	48	z_{10}	e,n,x	
II	E_1	Islington	3,10	g,t	–	
I	D_1	Israel	9,12	e,h	e,n,z_{15}	
I	C_2	Istanbul	8	z_{10}	e,n,x	
I	H	Istoria	1,6,14,25	r,i	1,5	
I	G	Isuge	13,23	d	z_6	
I	D_1	Italiana	9,12	l,v	R1,11	IP combined Italiana that contains H phase R1,11 with Panama (1,9,12:l,v:1,5). The name Italiana has been dropped.
I	D_1	Itami	9,12	l,z_{13}	1,5	
I	B	Ituri	1,4,12	z_{10}	1,5	
I	D_2	Itutaba	9,46	c	z_6	
I	I	Ivory	16	r	1,6	
I	Z	Ivorycoast	50	z_{29}	–	
II	I	Jacksonville	16	z_{29}	[e,n,x]	
I	D_1	Jaffna	1,9,12	d	z_{35}	
I	B	Jaja	4,12,27	z_4,z_{23}	–	IP combined Jaja with Stanleyville (1,4,[5],12:z_4,z_{23}:[1,5]) to form Stanleyville 1,4,[5],12,27:z_4,z_{23}:[1,5]. Jaja is now called Stanleyville var. O 27+. The name Jaja has been dropped.
I	F	Jalisco	11	y	1,7	
I	D_1	Jamaica	9,12	r	1,5	
I	L	Jambur	21	l,z_{28}	e,n,z_{15}	
I	J	Jangwani	17	a	1,5	
I	B	Java	1,4,5,12	b	[1,2], (tartrate +)	IP calls Java, Paratyphi B var. Java. Java is often monophasic in the U.S. May possess H phase Rz_{33}.
I	D_1	Javiana	1,9,12	l,z_{28}	1,5	
I	E_1	Jedburgh	3,10,[15]	z_{29}	–	
I	B	Jericho	1,4,12,27	c	e,n,z_{15}	
I	C_1	Jerusalem	6,7,14	z_{10}	l,w	
I	E_1	Joal	3,10	l,z_{28}	1,7	
I	W	Jodhpur	45	z_{29}	–	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	B	Joenkoeping	4,5,12	g,s,t	–	IP combined Joenkoeping with Kingston (1,4,12,27:g,s,t:-) to form Kingston 1,4,[5],12,27:g,s,t:-. The name Joenkoeping has been dropped.
I	R	Johannesburg	1,40	b	e,n,x	
I	B	Jos	1,4,12,27	y	e,n,z_{15}	
I	E_4	Juba	1,3,19	a	1,7	
I	J	Jubilee	17	e,h	1,2	
I	G	Jukestown	13,23	i	e,n,z_{15}	
I	B	Kaapstad	4,12	e,h	1,7	
I	51	Kabete	51	i	1,5	
I	C_1	Kaduna	6,7,14	c	e,n,z_{15}	
I	T	Kahla	1,42	z_{35}	1,6	
I	E_4	Kainji	1,3,19	z	1,6	
I	H	Kaitaan	1,6,14,25	m,t	–	
I	B	Kalamu	4,[5],12	z_4,z_{24}	[1,5]	
I	E_1	Kalina	3,10	b	1,2	
I	C_2	Kallo	6,8	k	1,2	
II	M	Kaltenhausen	28	b	z_6	
I	C_2	Kalumburu	6,8	z	e,n,z_{15}	
I	C_1	Kambole	6,7	d	1,[2],7	
I	B	Kamoru	4,12,27	y	z_6	
I	T	Kampala	1,42	c	z_6	
I	E_4	Kande	1,3,19	b	e,n,z_{15}	
I	J	Kandla	17	z_{29}	–	
I	T	Kaneshie	1,42	i	l,w	
I	H	Kanifing	1,6,14,25	z	1,6	
I	B	Kano	1,4,12,27	l,z_{13},z_{28}	e,n,x	
I	X	Kaolack	47	z	1,6	
I	D_1	Kapemba	9,12	l,v	1,7	
I	W	Karachi	45	d	e,n,x	
I	R	Karamoja	40	z_{41}	1,2	
I	51	Karaya	51	b	1,5	
I	P	Kasenyi	38	e,h	1,5	
I	H	Kassberg	1,6,14,25	c	1,6	
II	G	Katesgrove	1,13,23	m,t	1,5	
I	G	Kedougou	1,13,23	i	l,w	
I	C_2	Kentucky	8,20	i	z6	
I	C_1	Kenya	6,7	l,z13	e,n,x	
I	V	Kermel	44	d	e,n,x	
I	L	Keve	21	l,w	–	
II	X	Khami	47	b	[e,n,x,z_{15}]	
I	E_3	Khartoum	3,15,34	a	1,7	IP combined Khartoum with Oxford (3,10:a:1,7) to form Oxford 3,10,[15],[15,34]:a:1,7. Khartoum is now called Oxford var. O 15+ by IP. CDC has no 3,15:a:1,7. Khartoum was found by IP with colonies containing O 3,15.
I	B	Kiambu	4,12	z	1,5	
I	I	Kibi	16	z_4,z_{23}	[1,6]	
I	M	Kibusi	28	r	e,n,x	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	P	Kidderminster	38	c	1,6	
I	A	Kiel	1,2,12	g,p	–	
I	I	Kikoma	16	y	e,n,x	
II	B	Kilwa	4,12	l,w	e,n,x	
I	P	Kimberley	38	l,v	1,5	
I	D_1	Kimpese	9,12	z	1,6	
I	B	Kimuenza	1,4,12,27	l,v	e,n,x	
I	E_4	Kindia	1,3,19	l,z_{28}	e,n,x	
I	U	Kingabwa	43	y	1,5	
I	B	Kingston	1,4,12,27	g,s,t	[1,2]	IP combined Kingston with Joenkoeping (4,5,12:g,s,t:-) to form Kingston 1,4,[5],12,27:g,s,t:[1,2]. Kingston may possess H phase Rz_{27} or Rz_{43}.
I	J	Kinondoni	17	a	e,n,x	
I	E_2	Kinshasa	3,15	l,z_{13}	1,5	IP combined Kinshasa with Uganda (3,10:l,z_{13}:1,5) to form Uganda 3,10,[15]:l,z_{13}:1,5. Kinshasa is now called Uganda var. O 15+ by IP.
I	E_4	Kinson	1,3,19	y	e,n,x	
I	G	Kintambo	1,13,23	m,t	–	
I	J	Kirkee	17	b	1,2	
I	B	Kisangani	1,4,[5],12	a	1,2	
I	F	Kisarawe	11	k	e,n,x,[z_{15}]	
I	C_1	Kisii	6,7	d	1,2	
I	M	Kitenge	28	y	e,n,x	
I	C_1	Kivu	6,7	d	1,6	
II	W	Klapmuts	45	z	z_{39}	
I	P	Klouto	38	z_{38}	–	
II	B	Kluetjenfelde	4,12	d	e,n,x	
I	X	Kodjovi	47	c	[1,6]	Kodjovi may possess H phase Rz_{78}.
I	B	Koenigstuhl	1,4,[5],12	z	e,n,z_{15}	
I	A	Koessen	2,12	l,v	1,5	
I	W	Kofandoka	45	r	e,n,z_{15}	
I	V	Koketime	44	z_{38}	–	
I	N	Kokoli	30	z_{35}	1,6	
I	Q	Kokomlemle	39	l,v	e,n,x	
I	D_2	Kolar	9,46	b	z_{35}	
I	C_2	Kolda	8,20	z_{35}	1,2	
II	U	Kommetje	43	b	z_{42}	
I	M	Konolfingen	28	z_{35}	1,6	
I	C_2	Konstanz	8	b	e,n,x	
I	C_2	Korbol	8,20	b	1,5	
I	E_4	Korlebu	1,3,19	z	1,5	
I	P	Korovi	38	g,m,[s]	–	
I	C_1	Kortrijk	6,7	l,v	1,7	
I	C_2	Kottbus	6,8	e,h	1,5	
I	C_1	Kotte	6,7	b	z_{35}	
I	D_1	Kotu	9,12	l,z_{28}	1,6	
I	E_4	Kouka	1,3,19	g,m,[t]	–	
I	C_1	Koumra	6,7	b	1,7	
I	M	Kpeme	28	e,h	1,7	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	G	Kraaifontein	1,13,23	g,m,t	[e,n,x]	IP combined Kraaifontein with Luanshya (1,13,23:g,m,s,t:[e,n,x]) to form Luanshya 1,13,23:g,m,[s],t:[e,n,x]. The name Kraaifontein has been dropped.
IV	C_1	Kralendyk	6,7	z_4,z_{24}	–	
I	C_2	Kralingen	8,20	y	z6	
I	E_4	Krefeld	1,3,19	y	l,w	
I	E_1	Kristianstad	3,10	z_{10}	e,n,z_{15}	
II	Z	Krugersdorp	50	e,n,x	1,7	
I	V	Kua	44	z_4,z_{23}	–	
I	B	Kubacha	1,4,12,27	l,z_{13},z_{28}	1,7	
I	M	Kuessel	28	i	e,n,z_{15}	
II	D_1	Kuilsrivier	1,9,12	g,m,s,t	e,n,x	
I	N	Kumasi	30	z_{10}	e,n,z_{15}	
I	B	Kunduchi	1,4,[5],12,27	l,[z13],[z28]	[1,2]	
I	H	Kuntair	1,6,14,25	b	1,5	
I	C_2	Kuru	6,8	z	l,w	
I	C_2	Labadi	8,20	d	z_6	
I	B	Lagos	1,4,[5],12	i	1,5	
I	E_1	Lamberhurst	3,10	e,h	e,n,z_{15}	
I	E_1	Lamin	3,10	l,z_{28}	e,n,x	
I	J	Lancaster	17	l,v	1,7	
I	S	Landala	41	z_{10}	1,6	
I	N	Landau	30	i	1,2	
I	E_1	Landwasser	3,10	z	z_6	
I	K	Langenhorn	18	m,t	–	
I	E_1	Langensalza	3,10	y	l,w	
I	M	Langford	28	b	e,n,z_{15}	
I	E_2	Lanka	3,15	r	z_6	IP combined Lanka with Weltevreden (3,10:r:z_6) to form Weltevreden 3,10,[15]:r:z_6. Lanka is now called Weltevreden var. O 15+ by IP.
I	P	Lansing	38	i	1,5	
I	C_1	Larochelle	6,7	e,h	1,2	
I	W	Lattenkamp	45	z_{35}	1,5	
I	D_1	Lawndale	1,9,12	z	1,5	
I	V	Lawra	44	k	e,n,z_{15}	
I	S	Leatherhead	41	m,t	1,6	
I	51	Lechler	51	z	e,n,z_{15}	
I	K	Leer	18	z_{10}	1,5	
I	F	Leeuwarden	11	b	1,5	
I	B	Legon	1,4,12,27	c	1,5	
I	G	Leiden	13,22	z_{38}	–	
I	S	Leipzig	41	z_{10}	1,5	
I	C_2	Leith	6,8	a	e,n,z_{15}	
I	E_1	Lekke	3,10	d	1,6	
I	F	Lene	11	z_{38}	–	
I	M	Leoben	28	l,v	1,5	
I	C_1	Leopoldville	6,7,14	b	z_6	
I	E_4	Lerum	1,3,19	z	1,7	
II	S	Lethe	41	g,t	–	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_1	Lexington	3,10	z_{10}	1,5	IP combined Lexington with Manila (3,15:z_{10}:1,5) and Illinois (3,15,34:z10:1,5) to form Lexington 3,10,[15],[15,34]:z10,1,5. Lexington may possess H phase Rz_{49}.
I	C_2	Lezennes	6,8	z_4,z_{23}	1,7	
I	M	Libreville	28	z_{10}	1,6	
II	S	Lichtenberg	41	z_{10}	[z_6]	
I	N	Ligeo	30	l,v	1,2	
I	O	Ligna	35	z_{10}	z_6	
I	C_1	Lika	6,7	i	1,7	
I	C_1	Lille	6,7	z_{38}	–	IP combined Lille with Bornum (6,7,14:z_{38}:-) to form Lille 6,7,14:z_{38}:-.
II	G	Limbe	1,13,22	g,m,t	[1,5]	
I	B	Limete	1,4,12,27	b	1,5	
II	F	Lincoln	11	m,t	e,n,x	
I	C_2	Lindenburg	6,8	i	1,2	
I	H	Lindern	6,14,[24]	d	e,n,x	
I	P	Lindi	38	r	1,5	
II	D_1	Lindrick	9,12	e,n,x	1,[5],7	
I	D_2	Linguere	9,46	b	z_6	
I	I	Lingwala	16	z	1,7	
I	G	Linton	13,23	r	e,n,z_{15}	
I	I	Lisboa	16	z_{10}	1,6	
I	D_2	Lishabi	9,46	z_{10}	1,7	
I	C_2	Litchfield	6,8	l,v	1,2	
I	E_4	Liverpool	1,3,19	d	e,n,z_{15}	
I	C_1	Livingstone	6,7	d	l,w	IP combined Eimsbuettel (6,7,14:d:l,w) with Livingstone to form Livingstone 6,7,14:d:l,w.
I	N	Livulu	30	e,h	1,2	
I	B	Ljubljana	4,12,27	k	e,n,x	
I	E_4	Llandoff	1,3,19	z_{29}	[z_6]	
II	M	Llandudno	28	g,[m],[s],t	1,5	
I	V	Llobregat	1,44	z_{10}	e,n,x	
I	C_2	Loanda	6,8	l,v	1,5	
II	52	Lobatsi	52	z_{44}	1,5,7	
II	57	Locarno	57	z_{29}	z_{42}	
I	C_1	Lockleaze	6,7,14	b	e,n,x	
I	J	Lode	17	r	1,2	
I	S	Lodz	41	z_{29}	–	
I	T	Loenga	1,42	z_{10}	z_6	
I	Q	Logone	39	d	1,5	
IV	V	Lohbruegge	44	z_4,z_{32}	–	
I	E_4	Lokstedt	1,3,19	l,z_{13},z_{28}	1,2	
I	D_1	Lomalinda	1,9,12	a	e,n,x	
I	D_1	Lome	9,12	r	z_6	
I	C_1	Lomita	6,7	e,h	1,5	
I	I	Lomnava	16	l,w	e,n,z_{15}	
I	E_1	London	3,10	l,v	1,6	IP combined London with Portsmouth (3,15:l,v:1,6) to form London 3,10,[15]:l,v:1,6.
I	I	Losangeles	16	l,v	z_6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	B	Loubomo	4,12	z	1,6	
I	N	Louga	30	b	1,2	
I	D_2	Louisiana	9,46	z_{10}	z_6	
II	I	Louwbester	16	z	[e,n,x]	
I	G	Lovelace	13,22	l,v	1,5	
I	J	Lowestoft	17	g,s,t	–	
II	G	Luanshya	1,13,23	g,m,s,t	[e,n,x]	IP combined Luanshya with Kraaifontein (1,13,23:g,m,t:[e,n,x]) to form Luanshya 1,13,23:g,m,[s],t:[e,n,x].
I	S	Lubumbashi	41	r	1,5	
I	F	Luciana	11	a	e,n,z_{15}	
I	M	Luckenwalde	28	z_{10}	e,n,z_{15}	
I	X	Luke	1,47	g,m	–	
II	D_2	Lundby	9,46	b	e,n,x	
II	S	Lurup	41	z_{10}	e,n,x,z_{15}	
I	51	Lutetia	51	r,i	l,z_{13},z_{28}	
II	60	Luton	60	z	e,n,x	
I	X	Lyon	47	k	e,n,z_{15}	
II	D_2	Maarssen	9,46	z_4,z_{24}	z_{39}:z_{42}	
I	F	Maastricht	11	z_{41}	1,2	
I	E_1	Macallen	3,10	z_{36}	–	
I	D_2	Macclesfield	9,46	g,m,s,t	1,(2),7	
I	E_4	Machaga	1,3,19	i	e,n,x	
I	H	Madelia	1,6,14,25	y	1,7	
I	E_4	Madiago	1,3,19	c	1,7	
I	V	Madigan	44	c	1,5	
I	L	Madison	21	d	z_6	
I	E_1	Madjorio	3,10	d	e,n,z_{15}	
I	B	Madras	4,[5],12	m,t	e,n,z_{15}	
I	C_2	Magherafelt	8,20	i	l,w	
I	H	Magumeri	1,6,14,25	e,h	1,6	
I	L	Magwa	21	d	e,n,x	
I	D_2	Mahina	9,46	z_{10}	e,n,z_{15}	
I	E_4	Maiduguri	1,3,19	f,g,t	e,n,z_{15}	
I	U	Makiling	43	z_{29}	–	
I	C_1	Makiso	6,7	l,z_{13},z_{28}	z_6	
II	B	Makoma	1,4,[5],12,27	a	[e,n,x]	
II	B	Makumira	1,4,12,27	e,n,x	1,[5],7	
I	I	Malakal	16	e,h	1,2	
V	66	Malawi	66	z_{65}	–	
I	M	Malaysia	28	z_{10}	1,7	
I	V	Malika	44	l,z_{28}	1,5	
I	C_2	Malmoe	6,8	i	1,7	
I	I	Malstatt	16	b	z_6	
I	H	Mampeza	1,6,14,25	i	1,5	
I	G	Mampong	13,22	z_{35}	1,6	
I	C_2	Manchester	6,8	l,v	1,7	
I	I	Mandera	16	l,z_{13}	e,n,z_{15}	
I	P	Mango	38	k	1,5	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	C_2	Manhattan	6,8	d	1,5	
II	D_1	Manica	1,9,12	g,m,s,t	z_{42}	IP combined Manica with Hamburg (1,9,12:g,t:-) and Muizenberg (9,12:g,m,s,t:1,5) to form *S.* II 1,9,12:g,[m],[s],t:[1,5,7]:[z_{42}].
I	E_2	Manila	3,15	z_{10}	1,5	IP combined Manila and Illinois (3,15,34:z_{10}:1,5) with Lexington (3,10:z_{10}:1,5) to form Lexington 3,10,[15],[15,34]:z_{10}:1,5. Manila is now called Lexington var. O 15+ by IP.
I	F	Mannheim	11	k	l,w	
II	57	Manombo	57	z_{39}	e,n,x,z_{15}	
I	C_2	Mapo	6,8	z_{10}	1,5	
I	Q	Mara	39	e,h	[1,5]	
I	F	Maracaibo	11	l,v	1,5	
I	G	Marburg	13,23	k	–	
V	66	Maregrosso	66	z_{35}	–	
I	T	Maricopa	1,42	g,z_{51}	1,5	
I	E_1	Marienthal	3,10	k	e,n,z_{15}	
IV	Y	Marina	48	g,z_{51}	–	
I	V	Maritzburg	1,44	i	e,n,z_{15}	
I	E_1	Maron	3,10	d	z_{35}	
I	F	Maroua	11	z	1,7	
I	F	Marseille	11	a	1,5	
I	G	Marshall	13,22	a	l,z_{13},z_{28}	
I	57	Maryland	57	b	1,7	
I	D_2	Marylebone	9,46	k	1,2	
I	E_1	Masembe	3,10	a	e,n,x	Masembe may possess H phase Rz_5.
I	B	Maska	1,4,12,27	z_{41}	e,n,z_{15}	
I	O	Massakory	35	r	l,w	
I	B	Massenya	1,4,12,27	k	1,5	
I	J	Matadi	17	k	e,n,x	
I	D_2	Mathura	9,46	i	e,n,z_{15}	
I	N	Matopeni	30	y	1,2	
II	E_1	Matroosfontein	3,10	a	e,n,x	
I	D_2	Mayday	9,46	y	z_6	
I	C_1	Mbandaka	6,7,14	z_{10}	e,n,z_{15}	
I	U	Mbao	43	i	1,2	
I	W	Meekatharra	45	a	e,n,z_{15}	
I	T	Melbourne	42	z	e,n,z_{15}	
I	E_1	Meleagridis	3,10	e,h	l,w	IP combined Meleagridis with Cambridge (3,15:e,h:l,w) and Wildwood (3,15,34:e,h:l,w) to form Meleagridis 3,10,[15],[15,34]:e,h:l,w.
I	K	Memphis	18	k	1,5	
I	C_1	Menden	6,7	z_{10}	1,2	
I	D_1	Mendoza	9,12	l,v	1,2	
I	E_3	Menhaden	3,15,34	l,v	1,7	IP combined Menhaden with Give (3,10:l,v:1,7) and Newbrunswick (3,15:l,v:1,7) to form Give 3,10,[15],[15,34]:[d],l,v:1,7. Menhaden is now called Give var. O 15+, 34+ by IP.
I	C_1	Menston	6,7	g,s,[t]	[1,6]	
II	I	Merseyside	16	g,t	[1,5]	
I	X	Mesbit	47	m,t	[e,n,z_{15}]	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	51	Meskin	51	e,h	1,2	
I	N	Messina	30	d	1,5	
I	C_2	Mexicana	6,8	d	1,2	Mexicana was combined with Muenchen. The name Mexicana has been dropped.
I	P	Mgulani	38	i	1,2	
I	D_1	Miami	1,9,12	a	1,5	Miami must be differentiated from Sendai with biochemical tests. Miami is pos. for H_2S, citrate, and tartrate; Sendai is neg.
I	J	Michigan	17	l,v	1,5	
I	T	Middlesbrough	1,42	i	z_6	
II	53	Midhurst	53	l,z_{28}	z_{39}	
I	H	Midway	6,14,24	d	1,7	
I	C_1	Mikawasima	6,7,14	y	e,n,z_{15}	Mikawasima may possess H phase Rz_{47} or Rz_{50}.
I	R	Millesi	1,40	l,v	1,2	
I	U	Milwaukee	43	f,g,[t]	-	
I	G	Mim	13,22	a	1,6	
I	H	Minna	1,6,14,25	c	l,w	
I	E_3	Minneapolis	3,15,34	e,h	1,6	IP combined Minneapolis and Newington (3,15:e,h:1,6) with Anatum (3,10:e,h:1,6) to form Anatum 3,10,[15],[15,34]:e,h:1,6. Minneapolis is now called Anatum var. O 15+ by IP.
I	L	Minnesota	21	b	e,n,x	Minnesota may possess H phase Rz_{33} or Rz_{49}.
I	G	Mishmarhaemek	1,13,23	d	1,5	
I	C_1	Mission	6,7	d	1,5	Mission was combined with Isangi 6,7,14:d:1,5. The name Mission has been dropped.
I	G	Mississippi	1,13,23	b	[1,5]	
I	F	Missouri	11	g,s,t	–	
I	D_1	Miyazaki	9,12	l,z_{13}	1,7	
II	D_1	Mjimwema	1,9,12	b	e,n,x	
I	N	Mjordan	30	i	e,n,z_{15}	
I	C_1	Mkamba	6,7	l,v	1,6	
II	I	Mobeni	16	g,[m],[s],t	[e,n,x]	
I	M	Mocamedes	28	d	e,n,x	
I	M	Moero	28	b	1,5	
I	F	Moers	11	m,t	–	
I	E_1	Mokola	3,10	y	1,7	
I	C_2	Molade	8,20	z_{10}	z6	
I	52	Molesey	52	b	1,5	
II	Q	Mondeor	39	l,z_{28}	e,n,x	
I	B	Mono	4,12	l,w	1,5	
I	B	Mons	1,4,12,27	d	l,w	
I	O	Monschaui	35	m,t	–	
I	C_1	Montevideo	6,7,14	g,m,[p],s	[1,2,7]	
II	F	Montgomery	11	a,[d]	$[d]:e,n,z_{15}$	
I	U	Montreal	43	c	1,5	
I	N	Morehead	30	i	1,5	
I	M	Morillons	28	m,t	1,6	
I	N	Morningside	30	c	e,n,z_{15}	
I	H	Mornington	1,6,14,25	y	e,n,z_{15}	
I	N	Morocco	30	l,z_{13},z_{28}	e,n,z_{15}	
I	J	Morotai	17	l,v	1,2	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	M	Moroto	28	z_{10}	l,w	
I	D_1	Moscow	9,12	g,q	–	
II	U	Mosselbay	43	g,m,[s],t	[z_{42}]	
I	X	Moualine	47	y	1,6	
I	51	Moundou	51	l,z_{28}	1,5	
I	L	Mountmagnet	21	r	–	
I	X	Mountpleasant	47	z	1,5	
I	H	Moussoro	1,6,14,25	i	e,n,z_{15}	
I	C_2	Mowanjum	6,8	z	1,5	
II	E_1	Mpila	3,10	z_{38}	z_{42}	
I	I	Mpouto	16	m,t	–	
I	C_2	Muenchen	6,8	d	1,2	
I	E_1	Muenster	3,10	e,h	1,5	IP combined Muenster with Newhaw (3,15:e,h:1,5) and Arkansas (3,15,34:e,h:1,5) to form Muenster 3,10,[15],[15,34]:e,h:1,5.
I	V	Muguga	44	m,t	–	
II	D_1	Muizenberg	9,12	g,m,s,t	1,5	IP combined Muizenberg with Hamburg (1,9,12:g,t:-) and Manica (1,9,12:g,m,s,t:z_{42}) to form *S.* II 1,9,12:g,[m],[s],t:[1,5,7]:[z_{42}].
I	M	Mundonobo	28	d	1,7	
IV	F	Mundsburg	11	g,z_{51}	–	
I	B	Mura	1,4,12	z_{10}	l,w	
II	G	Nachshonim	1,13,23	z	1,5	
I	D_1	Naestved	1,9,12	g,p,s	–	
I	C_2	Nagoya	6,8	b	1,5	
II	T	Nairobi	42	r	–	
I	B	Nakuru	1,4,12,27	a	z_6	
II	Z	Namib	50	g,[m],s,t	[1,5]	
I	C_1	Namibia	6,7	c	e,n,x	
I	E_2	Nancy	3,15	l,v	1,2	IP combined Nancy with Nchanga (3,10:l,v:1,2) to form Nchanga 3,10,[15]:l,v:1,2. Nancy is now called Nchanga var. O 15+ by IP.
I	C_2	Nanergou	6,8	g,s,t	–	
I	G	Nanga	1,13,23	l,v	e,n,z_{15}	
I	D_2	Nantes	9,46	y	l,w	
I	D_1	Napoli	1,9,12	l,z_{13}	e,n,x	
I	C_2	Narashino	6,8	a	e,n,x	
I	M	Nashua	28	l,v	e,n,z_{15}	
I	D_1	Natal	9,12	z_4,z_{24}	–	
I	I	Naware	16	z_{38}	–	
I	E_1	Nchanga	3,10	l,v	1,2	IP combined Nchanga with Nancy (3,15:l,v:1,2) to form Nchanga 3,10,[15]:l,v:1,2.
I	H	Ndjamena	1,6,14,25	b	1,2	
I	D_1	Ndolo	1,9,12	d	1,5	
II	D_1	Neasden	9,12	g,s,t	e,n,x	
I	B	Neftenbach	4,12	z	e,n,x	
II	S	Negev	41	z_{10}	1,2	
I	H	Nessa	1,6,14,25	z_{10}	1,2	
I	C_1	Nessziona	6,7	l,z_{13}	1,5	
I	N	Neudorf	30	b	e,n,z_{15}	
I	C_1	Neukoelin	6,7	l,z_{13},[z_{28}]	e,n,z_{15}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	B	Neumuenster	1,4,12,27	k	1,6	
I	P	Neunkirchen	38	z_{10}	-	
I	E_2	Newbrunswick	3,15	l,v	1,7	IP combined Newbrunswick and Menhaden (3,15,34:l,v:1,7) with Give (3,10:l,v:1,7) to form Give 3,10,[15],[15,34]:[d],l,v:[d],1,7. Newbrunswick is now called Give var. O 15+ by IP.
I	E_2	Newhaw	3,15	e,h	1,5	IP combined Newhaw and Arkansas (3,15,34:e,h:1,5) with Muenster (3,10:e,h:1,5) to form Muenster 3,10,[15],[15,34]:e,h:1,5. Newhaw is now called Muenster var. O 15+ by IP.
I	54	Newholland	4,12,54	m,t	–	
I	E_2	Newington	3,15	e,h	1,6	IP combined Newington and Minneapolis (3,15,34:e,h:1,6) with Anatum (3,10:e,h:1,6) to form Anatum 3,10,[15],[15,34]:e,h:1,6. Newington is now called Anatum var. O 15+ by IP.
I	E_1	Newlands	3,10,[15,34]	e,h	e,n,x	
I	D_1	Newmexico	9,12	g,z_{51}	1,5	
I	C_2	Newport	6,8,20	e,h	1,2	Newport may possess H phase Rz_{50} or Rz_{58} or Rz_{78} or R1,12
I	C_2	Newport var. Puerto Rico	6,8	–	1,2	
I	E_1	Newrochelle	3,10	k	l,w	
I	G	Newyork	13,22	g,s,t	–	
I	D_2	Ngaparou	9,46	z_4,z_{24}	–	
I	C_1	Ngili	6,7	z10	1,7	
I	E_4	Ngor	1,3,19	l,v	1,5	
II	Y	Ngozi	48	z_{10}	[1,5]	
I	V	Niakhar	44	a	1,5	
I	J	Niamey	17	d	l,w	
I	V	Niarembe	44	a	l,w	
I	C_1	Nienstedten	6,7,14	b	[l,w]	Nienstedten was combined with Nissii (6,7,14:b:-) and called Nienstedten; then IP combined Nienstedten with Ohio (6,7:b:l,w) to form Ohio (6,7,14:b:[l,w]). Nienstedten is now called Ohio var.O 14+ by IP.
I	C_1	Nieukerk	6,7,14	d	z_6	
I	C_1	Nigeria	6,7	r	1,6	
I	N	Nijmegen	30	y	e,n,z_{15}	
I	I	Nikolaifleet	16	g,m,s	–	
I	E_4	Niloese	1,3,19	d	z_6	
I	M	Nima	28	y	1,5	
I	G	Nimes	13,22	z_{35}	e,n,z_{15}	
I	C_1	Nissii	6,7,14	b	–	Nissii was combined with Nienstedten (6,7,14:b:l,w) as a monophasic variant of Nienstedten. Nienstedten is now called a variant of Ohio by IP. The name Nissii has been dropped.
I	A	Nitra	2,12	g,m	–	
I	E_4	Niumi	1,3,19	a	1,5	
I	P	Njala	38	k	e,n,x	
I	C_1	Nola	6,7	e,h	1,7	
II	I	Noordhoek	16	l,w	z_6	
II	B	Nordenham	1,4,12,27	z	e,n,x	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	C_2	Nordufer	6,8	a	1,7	
I	C_1	Norton	6,7	i	l,w	
I	C_1	Norwich	6,7	e,h	1,6	
I	I	Nottingham	16	d	e,n,z_{15}	
I	R	Nowawes	40	z	z_6	
I	C_2	Noya	8	r	1,7	
I	I	Nuatja	16	k	e,n,x	
II	T	Nuernberg	42	z	z_6	
I	F	Nyanza	11	z	z_6	
I	E_1	Nyborg	3,10	e,h	1,7	IP combined Selandia (3,15:e,h:1,5) with Nyborg to form Nyborg 3,10,[15]:e,h:1,7.
I	I	Nyeko	16	a	1,7	
I	C_1	Oakey	6,7	m,t	z_{64}	
I	C_1	Oakland	6,7	z	1,6,[7]	
I	C_1	Obogu	6,7	z_4,z_{23}	1,5	
I	E_4	Ochiogu	1,3,19	z_{38}	[e,n,z_{15}]	
I	54	Ochsenwerder	6,7,54	k	1,5	
IV	I	Ochsenzoll	16	z_4,z_{23}	–	
I	N	Ockenheim	30	l,z_{13},z_{28}	1,6	
I	R	Odienne	40	y	1,5	
II	N	Odijk	30	a	z_{39}	
I	N	Odozi	30	k	e,n,[x],z_{15}	
I	Q	Oerlikon	39	l,v	e,n,z_{15}	
II	M	Oevelgoenne	28	r	e,n,z_{15}	
I	S	Offa	41	z_{38}	–	
I	U	Ogbete	43	z	1,5	
I	C_1	Ohio	6,7	b	l,w	IP combined Nienstedten (6,7,14:b:[l,w]) with Ohio to form Ohio 6,7,14:b:[l,w}. Ohio may possess H phase Rz_{59}.
I	E_1	Ohlstedt	3,10	y	e,n,x	
I	G	Okatie	13,23	g,[s],t	–	
I	E_1	Okefoko	3,10	c	z_6	
I	E_1	Okerara	3,10	z_{10}	1,2	
I	I	Oldenburg	16	d	1,2	
I	D_2	Olten	9,46	d	e,n,z_{15}	
I	C_1	Omderman	6,7,14	d	e,n,x	IP combined Omderman with Amersfoort (6,7:d:e,n,x) to form Amersfoort 6,7,14:d:e,n,x. Omderman is now called Amersfoort var. O 14+ by IP.
I	R	Omifisan	40	z_{29}	–	
I	C_1	Omuna	6,7	z_{10}	z_{35}	
I	M	Ona	28	g,s,t	–	
I	D_1	Onarimon	1,9,12	b	1,2	
I	H	Onderstepoort	1,6,14,[25]	e,h	1,5	
I	E_1	Onireke	3,10	d	1,7	
I	D_2	Ontario	9,46	d	1,5	
I	C_1	Oranienburg	6,7	m,t	–	IP combined Theilallee (6,7,14:m,t:-) with Oranienburg to form Oranienburg 6,7,14:m,t:-. Oranienburg may possess H phase Rz_{57}.
I	T	Orbe	42	b	1,6	
I	52	Ord	52	a	e,n,z_{15}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	G	Ordonez	1,13,23	y	l,w	
I	I	Orientalis	16	k	e,n,z_{15}	
I	E_1	Orion	3,10	y	1,5	IP combined Binza (3,15:y:1,5) and Thomasville (3,15,34:y:1,5) with Orion to form Orion 3,10,[15],[15,34]:y:1,5.
I	C_1	Oritamerin	6,7	i	1,5	
I	K	Orlando	18	l,v	e,n,z_{15}	
I	U	Orleans	43	d	1,5	
ßßßI	D_1	Os	9,12	a	1,6	
I	M	Oskarshamn	28	y	1,2	
I	C_1	Oslo	6,7,14	a	e,n,x	
I	F	Osnabrueck	11	l,z_{13},z_{28}	e,n,x	
I	C_1	Othmarschen	6,7,14	g,m,[t]	–	
I	D_1	Ottawa	1,9,12	z_{41}	1,5	
II	R	Ottershaw	40	d	–	
I	D_2	Ouakam	9,46	z_{29}	–	
I	G	Oudwijk	13,22	b	1,6	
I	R	Overchurch	1,40	l,w	[1,2]	
I	51	Overschie	51	l,v	1,5	
I	N	Overvecht	30	a	1,2	
I	E_1	Oxford	3,10	a	1,7	IP combined Khartoum (3,15,34:a:1,7) with Oxford to form Oxford 3,10,[15],[15,34]:a:1,7.
I	C_1	Oyonnax	6,7	y	1,6	
II	C_1	Oysterbeds	6,7	z	z_{42}	
I	C_2	Pakistan	8	l,v	1,2	
I	V	Palamaner	1,44	d	z_{35}	
I	C_1	Palime	6,7	z_{35}	e,n,z_{15}	
I	D_1	Panama	1,9,12	l,v	1,5	Panama may possess H phase R1,11
I	E_2	Pankow	3,15	d	1,5	IP combined Pankow with Shangani (3,10:d:1,5) to form Shangani 3,10,15:d:1,5. Pankow is now called Shangani var. O 15+ by IP.
I	C_1	Papuana	6,7	r	e,n,z_{15}	
I	A	Paratyphi A	1,2,12	a	[1,5]	
I	B	Paratyphi B	1,4,[5],12	[b]	[1,2]	Paratyphi B is tartrate neg.; Paratyphi B var. Java (CDC calls this *S.* ser. Java) is often monophasic (1,4,5,12:b:-) and is tartrate pos. Paratyphi B and Java may possess H phase Rz_{33}.
I	C_1	Paratyphi C	6,7,[Vi]	c	1,5	
IV	C_2	Parera	11	z_4,z_{23}	–	
I	C_2	Paris	8,20	z_{10}	1,5	
I	E_4	Parkroyal	1,3,19	l,v	1,7	
II	E_1	Parow	3,10,[15]	g,m,s,t	–	
I	B	Pasing	4,12	z_{35}	1,5	
I	M	Patience	28	d	e,n,z_{15}	
I	D_1	Penarth	9,12	z_{35}	z_6	
I	M	Penilla	28	l,z_{13},z_{28}	e,n,z_{15}	
I	F	Pennsylvania	11	d	e,n,z_{15}	
I	D_1	Pensacola	1,9,12	m,t	[1,2]	
II	W	Perinet	45	g,m,t	e,n,x,z_{15}	
I	P	Perth	38	y	e,n,x	
I	E_4	Petahtikva	1,3,19	f,g,t	1,7	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	C_2	Phaliron	8	z	e,n,z_{15}	
I	F	Pharr	11	b	e,n,z_{15}	
II	X	Phoenix	47	b	1,5	
I	E_1	Pietersburg	3,10,[15,34]	z_{69}	1,7	
I	C_2	Pikine	8,20	r	z_6	Pikine was combined with Altona (8,20:r,[i]:z_6). The name Pikine has been dropped.
I	I	Pisa	16	i	l,w	
I	C_1	Planckendael	6,7	z_4,z_{23}	1,6	
I	V	Ploufragan	1,44	z_4,z_{23}	e,n,z_{15}	
I	D_2	Plymouth	9,46	d	z_6	
I	H	Poano	1,6,14,25	z	l,z_{13},z_{28}	
I	54	Poeseldorf	8,20,54	i	z_6	
I	C_1	Poitiers	6,7	z	1,5	
I	M	Pomona	28	y	1,7	Pomona may possess H phases Rz_{60}, Rz_{70} or Rz_{80}.
I	K	Pontypridd	18	g,m	–	
I	G	Poona	1,13,22	z	1,6	Poona may possess H phase Rz59.
I	C_2	Portanigra	8,20	d	1,7	
II	T	Portbech	42	l,v	e,n,x,z_{15}	
I	D_1	Portland	9,12	z_{10}	1,5	
I	E_2	Portsmouth	3,15	l,v	1,6	IP combined Portsmouth with London (3,10:l,v:1,6) to form London 3,10,[15]:l,v:1,6. Portsmouth is now called London var. O 15+ by IP.
I	K	Potengi	18	z	–	
I	H	Potosi	6,14	z_{36}	1,5	
I	C_1	Potsdam	6,7,14	l,v	e,n,z_{15}	
I	D_2	Potto	9,46	i	z_6	
I	D_1	Powell	9,12	y	1,7	
I	C_2	Praha	6,8	y	e,n,z_{15}	
I	E_1	Pramiso	3,10	c	1,7	
I	C_2	Presov	6,8	b	e,n,z_{15}	
I	B	Preston	1,4,12	z	l,w	
I	F	Pretoria	11	k	1,2	
I	D_1	Pullorum	1,9,12	–	–	IP combined Pullorum with Gallinarum (1,9,12:-:-). They must be identified biochemically.
I	G	Putten	13,23	d	l,w	
I	V	Quebec	44	c	e,n,z_{15}	
I	D_2	Quentin	9,46	d	1,6	
II	X	Quimbamba	47	d	z_{39}	
I	X	Quinhon	47	z_{44}	–	
I	C_2	Quiniela	6,8	c	e,n,z_{15}	
I	N	Ramatgan	30	k	1,5	
I	M	Ramsey	28	l,w	1,6	
II	T	Rand	42	z	e,n,x,z_{15}	
I	G	Raus	13,22	f,g	e,n,x	
I	K	Rawash	6,14,18	c	e,n,x	
I	B	Reading	1,4,[5],12	e,h	[1,5]	
I	C_2	Rechovot	8,20	e,h	z_6	
I	C_1	Redba	6,7	z_{10}	z_6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	F	Redhill	11	e,h	l,z_{13},z_{28}	
I	I	Redlands	16	z_{10}	e,n,z_{15}	
I	E_1	Regent	3,10	f,g,[s]	[1,6]	
I	B	Reinickendorf	4,12	l,z_{28}	e,n,x	
I	F	Remete	11	z_4,z_{23}	1,6	
I	C_2	Remiremont	8,20	z_{10}	l,w	
I	B	Remo	1,4,12,27	r	1,7	
I	C_2	Reubeuss	8,20	g,m,t	–	
II	D_1	Rhodesiense	9,12	d	e,n,x	
I	L	Rhone	21	c	e,n,x	
I	I	Rhydyfelin	16	e,h	e,n,x	
I	C_1	Richmond	6,7	y	1,2	
I	E_4	Rideau	1,3,19	f,g	–	
I	D_1	Ridge	9,12	c	z_6	
I	G	Ried	1,13,22	z_4,z_{23}	[e,n,z_{15}]	
I	C_1	Riggil	6,7	g,t	–	
I	R	Riogrande	40	b	1,5	
I	C_1	Rissen	6,7	f,g	–	IP combined Ardwick (6,7,14:f,g:-) with Rissen for form Rissen 6,7,14:f,g:-.
I	P	Rittersbach	38	b	e,n,z_{15}	
I	W	Riverside	45	b	1,5	
I	P	Roan	38	l,v	e,n,x	
I	Z	Rochdale	50	b	e,n,x	
II	51	Roggeveld	51	–	1,7	
I	M	Rogy	28	z_{10}	1,2	
I	G	Romanby	1,13,23	z_4,z_{24}	–	
I	G	Roodepoort	1,13,22	z_{10}	1,5	
II	H	Rooikrantz	1,6,14	m,t	1,5	
I	E_2	Rosenthal	3,15	b	1,5	IP combined Rosenthal and unnamed 3,15,34:b:1,5 with Butantan (3,10:b:1,5) to form Butantan 3,10,[15],[15,34]:b:1,5. Rosenthal is now called Butantan var. O 15+ by IP.
I	54	Rossleben	54	e,h	1,6	
I	D_1	Rostock	1,9,12	g,p,u	–	
IV	C_1	Roterberg	6,7	z_4,z_{23}	–	
I	P	Rothenburgsort	38	m,t	–	
II	G	Rotterdam	1,13,22	g,t	1,5	
I	G	Rottnest	1,13,22	b	1,7	
I	I	Rovaniemi	16	r,[i]	1,5	
II	I	Rowburton	16	m,t	[z_{42}]	
I	H	Royan	1,6,14,25	z	e,n,z_{15}	
I	D_1	Ruanda	9,12	z_{10}	e,n,z_{15}	
I	F	Rubislaw	11	r	[e,n,x]	
I	L	Ruiru	21	y	e,n,x	
I	B	Ruki	4,5,12	y	e,n,x	IP combined Ruki with Ball (1,4,12,27:y:e,n,x) and Dalat (4,5,27:y:e,n,x) to form Ball 1,4,[5],12,27:y:e,n,x. The name Ruki has been dropped.
I	C_1	Rumford	6,7	z_{38}	1,2	
I	H	Runby	1,6,14,25	c	e,n,x	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_1	Rutgers	3,10	Rl,z_{40}	1,7	Rutgers has been dropped from the scheme and the H phase Rl,z_{40} is now considered an R phase of Give.
I	E_1	Ruzizi	3,10	l,v	e,n,z_{15}	
I	D_1	Saarbruecken	1,9,12	a	1,7	
I	I	Saboya	16	e,h	1,5	
IV	R	Sachsenwald	1,40	z_4,z_{23}	–	
I	N	Sada	30	z_{10}	1,2	
I	52	Saintemarie	52	g,t	–	
I	B	Saintpaul	1,4,[5],12	e,h	1,2	
I	X	Saka	47	b	–	IP combined Saka with Sya (47:b:z_6) and called it Sya.
II	Y	Sakaraha	48	[k]	z_{39}	
I	I	Salford	16	l,v	e,n,x	
I	B	Salinatis	4,12	d,e,h	d,e,n,z_{15}	IP states that Salinatis was combined with Duisburg (1,4,12,27:d:e,n,z_{15}). This is incorrect; IP should have stated that it was combined with Sandiego (4,[5],12:e,h:e,n,z_{15}), because Salinatis loses the d and becomes Sandiego.
I	I	Saloniki	16	z_{29}	–	
I	S	Samaru	41	i	1,5	
I	E_4	Sambre	1,3,19	z_4,z_{24}	–	
I	B	Sandiego	4,[5],12	[e,h]	e,n,z_{15}	
I	C_2	Sandow	6,8	f,g	e,n,z_{15}	
I	C_2	Sanga	8	b	1,7	
I	D_2	Sangalkam	9,46	m,t	–	
I	I	Sangera	16	b	e,n,z_{15}	
I	C_1	Sanjuan	6,7	a	1,5	
I	M	Sanktgeorg	28	r,[i]	e,n,z_{15}	
I	E_4	Sanktmarx	1,3,19	e,h	1,7	
I	M	Santander	28	z_{35}	e,n,z_{15}	
I	R	Santhiaba	40	l,z_{28}	1,6	
I	C_2	Santiago	8,20	c	e,n,x	
I	E_4	Sao	1,3,19	e,h	e,n,z_{15}	
I	I	Saphra	16	y	1,5	
I	H	Sara	1,6,14,25	z_{38}	[e,n,x]	
I	B	Sarajane	1,4,[5],12,27	d	e,n,x	
II	I	Sarepta	16	l,z_{28}	z_{42}	
I	R	Saugus	40	b	1,7	
I	H	Schalkwijk	6,14,[24]	i	e,n,..	
I	B	Schleissheim	4,12,27	b	–	
I	E_4	Schoeneberg	1,3,19	z	e,n,z_{15}	
I	C_1	Schwabach	6,7	c	1,7	
I	B	Schwarzengrund	1,4,12,27	d	1,7	
I	C_2	Schwerin	6,8	k	e,n,x	
I	I	Sculcoates	16	d	1,5	
II	Z	Seaforth	50	k	z_6	
I	M	Seattle	28	a	e,n,x	
I	V	Sedgwick	44	b	e,n,z_{15}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_1	Seegefeld	3,10	r,[i]	1,2	
I	E_1	Sekondi	3,10	e,h	z_6	
I	E_2	Selandia	3,15	e,h	1,7	IP combined Selandia with Nyborg (3,10:e,h:1,7) to form Nyborg 3,10,[15]:e,h:1,7. Selandia is now called Nyborg var. O 15+ by IP.
I	M	Selby	28	y	z_6	
IV	R	Seminole	1,40	g,z_{51}	–	
I	D_1	Sendai	1,9,12	a	1,5	Sendai must be differentiated from Miami with biochemical tests. Sendai is neg. for H_2S, citrate, and tartrate; Miami is pos.
I	F	Senegal	11	r	1,5	
I	E_4	Senftenberg	1,3,19	g,[s],t		Senftenberg may possess H phase Rz_{37} or Rz_{43} or Rz_{45} or Rz_{46}. Simsbury (1,3,19:Rz_{27}:-) is now considered an H phase Rz_{27} of Senftenberg.
I	N	Senneville	30	z_{10}	1,5	
I	D_1	Seremban	9,12	i	1,5	
I	E_1	Serrekunda	3,10	k	1,7	
II	60	Setubal	60	g,m,t	z_6	
I	I	Shamba	16	c	e,n,x	
I	E_1	Shangani	3,10	d	1,5	IP combined Pankow (3,15:d:1,5) with Shangani to form Shangani 3,10,[15]:d:1,5.
ßßI	I	Shanghai	16	l,v	1,6	
I	E_1	Shannon	3,10	z_{35}	l,w	
I	F	Sharon	11	k	1,6	
I	P	Sheffield	38	c	1,5	
I	I	Sherbrooke	16	d	1,6	
I	R	Shikmonah	40	a	1,5	
I	C_2	Shipley	8,20	b	e,n,z_{15}	
I	M	Shomolu	28	y	l,w	
IIIa	K	Shomron	18	z_4,z_{32}	–	Shomron was formerly in Subspecies II, but is now combined with *Arizona* 7a,7b:1,7,8:-. The name Shomron has been dropped.
I	D_2	Shoreditch	9,46	r	e,n,z_{15}	
I	B	Shubra	4,[5],12	z	1,2	
I	S	Sica	41	b	e,n,z_{15}	
I	K	Siegburg	6,14,18	z_4,z_{23}	[1,5]	IP combined Siegburg with Cerro (18:z_4,z_{23}:[1,5]) to form Cerro 6,14,18:z_4,z_{23}:[1,5]. Siegburg is now called Cerro var. O 14+. The name Siegburg has been dropped.
I	E_1	Simi	3,10	r	e,n,z_{15}	
II	H	Simonstown	1,6,14	z10	1,5	
I	E_4	Simsbury	1,3,19	Rz_{27}	–	IP combined Simsbury with Senftenberg 1,3,19:g,[s],t:-. Simsbury is now considered an R phase of Senftenberg. The name Simsbury has been dropped.
I	E_1	Sinchew	3,10	l,v	z_{35}	
I	C_2	Sindelfingen	8,20	y	l,w	
I	C_1	Singapore	6,7	k	e,n,x	
I	E_1	Sinstorf	3,10	l,v	1,5	
I	K	Sinthia	18	z_{38}	–	
I	T	Sipane	1,42	r	e,n,z_{15}	
I	C_2	Skansen	6,8	b	1,2	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_4	Slade	1,3,19	y	e,n,z_{15}	
I	B	Sladun	1,4,12,27	b	e,n,x	IP combined Sladun with Abony (1,4,5,12:b:e,n,x) to form Abony 1,4,[5],12,27:b:e,n,x. Sladun is now called Abony var. O 27+. The name Sladun has been
II	H	Slangkop	1,6,14	z_{10}	z_6:z_{42}	
II	N	Slatograd	30	g,t	–	
I	X	Sljeme	1,47	f,g	–	
I	B	Sloterdijk	1,4,12,27	z_{35}	z_6	
I	H	Soahanina	6,14,24	z	e,n,x	
I	N	Soerenga	30	i	l,w	
IV	L	Soesterberg	21	z_4,z_{23}	–	
II	B	Sofia	1,4,12,27	b	[e,n,x]	
I	D_2	Sokode	9,46	r	z_6	
I	M	Solna	28	a	1,5	
I	F	Solt	11	y	1,5	
I	C_1	Somone	6,7	z_4,z_{24}	–	
I	M	Soumbedioune	28	b	e,n,x	
I	B	Southampton	1,4,12,27	r	z_6	
I	E_1	Southbank	3,10,15,34	m,t	[1,6]	
II	F	Soutpan	11	z	z_{39}	
I	E_1	Souza	3,10	d	e,n,x	IP combined Eschersheim (3,15:d:e,n,x) with Souza to form Souza 3,10,[15]:d:e,n,x.
I	T	Spalentor	1,42	y	e,n,z_{15}	
I	L	Spartel	21	d	1,5	
I	V	Splott	44	g,s,t	–	
II	R	Springs	40	a	z_{39}	
VI	F	Srinagar	11	b	e,n,x	
I	P	Stachus	38	z	–	
I	B	Stanley	4,5,12	d	1,2	IP combined Cairo (1,4,12,27:d:1,2) with Stanley to form Stanley 1,4,[5],12,27:d:1,2
I	B	Stanleyville	1,4,[5],12	z_4,z_{23}	[1,2]	IP combined Jaja (4,12,27:z_4,z_{23}:-) with Stanleyville to form Stanleyville 1,4,[5],12,27:z_4,z_{23}:[1,2].
I	X	Staoueli	47	k	1,2	
I	N	Steinplatz	30	y	1,6	
I	54	Steinwerder	3,15,54	y	1,5	
II	D_1	Stellenbosch	1,9,12	z	1,7	
I	X	Stellingen	47	d	[e,n,x]	
I	F	Stendal	11	l,v	1,2	
I	N	Sternschanze	30	g,s,t	–	Sternschanze may possess H phase Rz_{59}.
I	C_2	Sterrenbos	6,8	d	e,n,x	
II	G	Stevenage	1,13,23	[z_{42}]	1,[5],7	
II	E_1	Stikland	3,10	m,t	e,n,x	
I	E_1	Stockholm	3,10	y	z_6	IP combined Tournai (3,15:y:z6) with Stockholm to form Stockholm 3,10,[15]:y:z_6.
I	N	Stoneferry	30	z_4,z_{23}	–	
I	E_1	Stormont	3,10	d	1,2	
I	C_2	Stourbridge	6,8	b	1,6	
I	F	Straengnaes	11	z_{10}	1,5	
I	D_2	Strasbourg	9,46	d	1,7	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	E_4	Stratford	1,3,19	i	1,2	
I	C_1	Strathcona	6,7	l,z_{13},z_{28}	1,7	
I	E_4	Stuivenberg	1,3,19	l,z_{13},z_{28}	1,5	
I	C_1	Stuttgart	6,7,14	i	z_6	
II	R	Suarez	1,40	c	e,n,x,z_{15}	
I	E_1	Suberu	3,10	g,m	–	
I	U	Sudan	43	l,z_{13}	–	
II	D_1	Suederelbe	1,9,12	b	z_{39}	
I	W	Suelldorf	45	f,g	–	
II	C_1	Sullivan	6,7	z_{42}	1,7	
I	H	Sundsvall	[1],6,14,[25]	z	e,n,x	
I	C_2	Sunnycove	8	y	e,n,x	
II	R	Sunnydale	1,40	k	e,n,x,z_{15}	
I	H	Surat	[1],6,14,[25]	[r],[i]	e,n,z_{15}	
I	E_4	Svedvi	1,3,19	l,v	e,n,z_{15}	
I	X	Sya	47	b	z_6	
IIIb	Y	Sydney	48	i	z	Sydney was formerly in subspecies II, but it is now combined with *Arizona* 5:33:31. The name Sydney has been dropped.
I	H	Sylvania	[1],6,14,[25]	g,p	–	
I	I	Szentes	16	k	1,2	
I	X	Tabligbo	47	z_4,z_{23}	[e,n,z_{15}]	
I	C_2	Tado	8,20	c	z_6	
II	E_1	Tafelbaai	3,10	z	z_{39}	
I	B	Tafo	1,4,12,27	z_{35}	1,7	
I	C_2	Takoradi	6,8	i	1,5	
I	E_4	Taksony	1,3,19	[i]	z_6	
I	C_2	Tallahassee	6,8	z_4,z_{32}	–	
I	C_2	Tamale	8,20	z_{29}	[e,n,z_{15}]	
I	E_4	Tambacounda	1,3,19	b	e,n,x	
I	X	Tamberma	47	z_4,z_{24}	–	
I	C_1	Tamilnadu	6,7	z_{41}	z_{35}	
I	C_1	Tampico	6,7	z_{36}	e,n,z_{15}	
I	C_2	Tananarive	6,8	y	1,5	
I	G	Tanger	1,13,22	y	1,6	
I	G	Tanzania	1,13,22	z	e,n,z_{15}	
I	D_1	Tarshyne	9,12	d	1,6	
I	T	Taset	1,42	z_{41}	–	
I	M	Taunton	28	k	e,n,x	
I	O	Tchad	35	b	–	
I	J	Tchamba	17	z	e,n,z_{15}	
I	M	Techimani	28	c	z_6	
I	B	Teddington	1,4,12,27	y	1,7	
I	I	Tees	16	f,g	–	
I	B	Tejas	4,12	z_{36}	–	
I	H	Teko	1,6,14,25	d	e,n,z_{15}	
I	M	Telaviv	28	y	e,n,z_{15}	
I	G	Telelkebir	13,23	d	e,n,z_{15}	
I	F	Telhashomer	11	z_{10}	e,n,x	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	M	Teltow	28	z_4,z_{23}	1,6	
I	T	Tema	1,42	z_{35}	z_6	
I	C_1	Tennessee	6,7,14	z_{29}	[1,2,7]	
I	B	Tennyson	4,5,12	g,z_{51}	e,n,z_{15}	
I	X	Teshie	1,47	l,z_{13},z_{28}	e,n,z_{15}	
I	B	Texas	4,[5],12	k	e,n,z_{15}	
I	B	Thayngen	1,4,12,27	z_{41}	1,(2),5	
I	U	Thetford	43	k	1,2	
I	P	Thiaroye	38	e,h	1,2	
I	C_1	Thielallee	6,7,14	m,t	–	IP combined Thielallee with Oranienburg (6,7:m,t:-) to form Oranienburg 6,7,14:m,t:-. Theilallee is now called Oranienburg var. O 14+ by IP.
I	E_4	Thies	1,3,19	y	1,7	
I	E_3	Thomasville	3,15,34	y	1,5	IP combined Thomasville and Binza (3,15:y:1,5) with Orion (3,10:y:1,5) to form Orion 3,10,[15],[15,34]:y:1,5. Thomasville is now called Orion var. O 15+ by IP.
I	C_1	Thompson	6,7,14	[k]	[1,5]	IP combined Cardiff that contains H phase R1,10 (6,7:k:R1,10) with Thompson.
I	C_1	Tienba	6,7	z_{35}	1,6	
I	V	Tiergarten	44	a	e,n,x	
I	R	Tiko	40	l,z_{13},z_{28}	1,2	
I	E_4	Tilburg	1,3,19	d	l,w	Tilburg may possess H phase Rz_{49}.
I	R	Tilene	1,40	e,h	1,2	
I	B	Tinda	1,4,5,27	a	e,n,z_{15}	
I	51	Tione	51	a	e,n,x	
I	I	Togba	16	a	e,n,x	
I	B	Togo	4,12	l,w	1,6	
II	57	Tokai	57	z_{42}	$1,6:z_{53}$	
I	B	Tokoin	4,12	z_{10}	e,n,z_{15}	
I	T	Tomegbe	1,42	b	e,n,z_{15}	
I	E_4	Tomelilla	1,3,19	l,z28	1,7	
I	54	Tonev	21,54	b	e,n,x	
I	F	Toowong	11	a	1,7	
I	N	Torhout	30	e,h	1,5	
I	T	Toricada	1,42	z_4,z_{24}	–	
I	W	Tornow	45	g,m,[s],[t]	–	
I	D_2	Toronto	9,46	l,v	$e,n,x;[z_{44}]$	
II	C_1	Tosamanga	6,7	z	1,5	
I	Y	Toucra	48	z	1,5	Toucra may possess H phase Rz_{58}.
I	K	Toulon	18	l,w	e,n,z_{15}	
I	C_2	Tounouma	8,20	b	z_6	
I	E_2	Tournai	3,15	y	z_6	IP combined Tournai with Stockholm (3,10:y:z_6) to form Stockholm 3,10,[15]:y:z_6. Tournai is now called Stockholm var. O 15+ by IP.
I	B	Trachau	4,12,27	y	1,5	
II	55	Tranoroa	55	k	z_{39}	
I	W	Transvaal	45	z_4,z_{24}	-	
I	B	Travis	4,[5],12	g,z_{51}	1,7	
I	51	Treforest	1,51	z	1,6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	D1	Treguier	1,9,12	z_{10}	z_6	
I	I	Trier	16	z_{35}	1,6	
I	D_2	Trimdon	9,46	z_{35}	z_6	
I	B	Tripoli	1,4,12,27	b	z_6	
I	R	Trotha	40	z_{10}	z_6	
I	E_1	Truro	3,10	i	1,7	
I	G	Tschangu	1,13,23	e,h	1,5	
I	B	Tsevie	4,12	i	e,n,z_{15}	
I	C_2	Tshiongwe	6,8	e,h	e,n,z_{15}	
I	H	Tucson	[1],6,14,[25]	b	[1,7]	
I	B	Tudu	4,12	z_{10}	1,6	
I	E_2	Tuebingen	3,15	y	1,2	IP combined Tuebingen with Amager (e,10:y:1,2) to form Amager 3,10,[15]:y:1,2. Tuebingen is now called Amager var. O 15+ by IP.
IV	U	Tuindorp	43	z_4,z_{32}	–	
II	C_2	Tulear	6,8	a	z_{52}	
I	B	Tumodi	1,4,12	i	z_6	
I	G	Tunis	1,13,23	y	z_6	
II	G	Tygerberg	1,13,23	a	z_{42}	
I	D_1	Typhi	9,12,[Vi]	d	–	Typhi may possess H phase Rj or Rz_{66}.
I	B	Typhimurium	1,4,5,12	i	1,2,[7]	
I	B	Typhimurium var. Copenhagen		1,4,12	i	1,2
I	C_1	Typhisuis	6,7	c	1,5	Typhisuis is a bioserotype found in pigs. It is like Choleraesuis except tartrate negative.
I	B	Tyresoe	4,12	l,[z_{13}],z_{28}	1,5	
I	54	Uccle	3,54	g,s,t	–	
I	E_1	Uganda	3,10,15	l,z_{13}	1,5	
I	E_1	Ughelli	3,10	r	1,5	
I	V	Uhlenhorst	44	z	l,w	
I	52	Uithof	52	a	1,5	
I	G	Ullevi	1,13,23	b	e,n,x	
I	M	Umbilo	28	z_{10}	e,n,x	
I	C_1	Umhlali	6,7	a	1,6	
I	O	Umhlatazana	35	a	e,n,z_{15}	
I	C_2	Uno	6,8	z_{29}	[e,n,z_{15}]	
II	T	Uphill	42	b	e,n,x,z_{15}	
I	B	Uppsala	4,12,27	b	1,7	
I	N	Urbana	30	b	e,n,x	
I	T	Ursenbach	1,42	z	1,6	
I	K	Usumbura	6,14,18	d	1,7	
I	C_2	Utah	6,8	c	1,5	
II	O	Utbremen	35	z_{29}	e,n,x	
I	52	Utrecht	52	d	1,5	
I	H	Uzaramo	1,6,14,25	z_4,z_{24}	–	
I	G	Vaertan	13,22	b	e,n,x	
I	C_2	Valdosta	6,8	a	1,2	
I	I	Vancouver	16	c	1,5	
I	M	Vanier	28	z	1,5	
I	S	Vaugirard	41	b	1,6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	U	Veddel	43	g,t	–	
I	I	Vegesack	16	b	l,w	
I	E_1	Vejle	3,10	e,h	1,2	IP combined Goerlitz (3,15:e,h:1,2) with Vejle to form Vejle 3,10,[15]:e,h:1,2.
I	B	Vellore	1,4,12,27	z_{10}	z_{35}	
I	F	Veneziana	11	i	e,n,x	
II	J	Verity	17	e,n,x,z_{15}	1,6	
I	S	Verona	41	i	1,6	
I	W	Verviers	45	k	1,5	
I	D_1	Victoria	1,9,12	l,w	1,5	
I	J	Victoriaborg	17	c	1,6	
I	S	Vietnam	41	b	z_6	
II	S	Vietnam var. subsp. II	41	b	–	
I	E_4	Vilvoorde	1,3,19	e,h	1,5	
I	M	Vinohrady	28	m,t	[e,n,z_{15}]	
I	C_1	Virchow	6,7	r	1,2	
I	C_2	Virginia	8	d	[1,2]	
I	E_4	Visby	1,3,19	b	1,6	
I	M	Vitkin	28	l,v	e,n,x	
I	V	Vleuten	44	f,g	–	
I	T	Vogan	1,42	z_{38}	z6	
IV	U	Volksdorf	43	z_{36},z_{38}	–	
I	M	Volksmarsdorf	28	i	1,6	
I	F	Volta	11	r	l,z_{13},z_{28}	
I	B	Vom	1,4,12,27	l,[z_{13}],[z_{28}]	e,n,z_{15}	
I	U	Voulte	43	i	e,n,x	
II	G	Vredelust	1,13,23	l,z_{28}	z_{42}	
I	G	Vridi	1,13,23	e,h	l,w	
VI	W	Vrindaban	45	a	e,n,x	
I	B	Vuadens	4,12,27	z_4,z_{23}	z_6	
I	I	Wa	16	b	1,5	
I	D_2	Waedenswil	9,46	e,h	1,5	
I	E_1	Wagadugu	3,10	z_4,z_{23}	z_6	
I	B	Wagenia	1,4,12,27	b	e,n,z_{15}	
II	L	Wandsbek	21	z_{10}	[z_6]	
I	Q	Wandsworth	39	b	1,2	
I	D_1	Wangata	1,9,12	z_4,z_{23}	[1,7]	
I	T	Waral	1,42	m,t	–	
I	J	Warengo	17	z	1,5	
I	W	Warmsen	45	d	e,n,z_{15}	
I	M	Warnemuende	28	i	e,n,x	
I	C_2	Warnow	6,8	i	1,6	
I	H	Warragul	[1],6,14,[25]	g,m	–	
I	J	Warri	17	k	1,7	
I	G	Washington	13,22	m,t	–	
IV	Z	Wassenaar	50	g,z_{51}	–	
I	S	Waycross	41	z_4,z_{23}	[e,n,z_{15}]	
IV	S	Waycross var. subsp.IV	41	z_4,z_{23}	–	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	N	Wayne	30	g,z_{51}	–	
I	M	Wedding	28	c	e,n,z_{15}	
I	I	Welikade	16	l,v	1,7	
I	E_1	Weltevreden	3,10	r	z_6	IP combined Lanka 3,15:r:z_6 with Weltevreden to form Weltevreden 3,10,[15]:r:z_6.
I	X	Wenatchee	47	b	1,2	
I	F	Wentworth	11	z_{10}	1,2	
I	D_2	Wernigerode	9,46	f,g	–	
I	T	Weslaco	42	z_{36}	–	
I	D_1	Westafrica	9,12	e,h	1,7	
I	I	Westeinde	16	l,w	1,6	
I	E_4	Westerstede	1,3,19	l,z_{13}	[1,2]	
I	E_1	Westhampton	3,10	g,s,t	–	IP combined Halmstad (3,15:g,s,t:-) and Canoga (3,15,34:g,s,t:-) with Westhampton to form Westhampton 3,10,[15],[15,34]:g,s,t:-. Westhampton may possess H phase Rz_{37} or Rz_{43} or Rz_{45}.
I	E_1	Westminster	3,10,[15]	b	z_{35}	CDC has no 3,10:b:z35.
I	I	Weston	16	e,h	z6	
II	E_1	Westpark	3,10	l,z_{28}	e,n,x	
I	O	Westphalia	35	z_4,z_{24}	–	
I	E_1	Weybridge	3,10	d	z_6	
I	G	Wichita	1,13,23	d	1,6	Wichita may possess H phase Rz_{37}.
I	O	Widemarsh	35	z_{29}	–	
I	B	Wien	1,4,12,27	b	l,w	
I	C_1	Wil	6,7	d	l,z_{13},z_{28}	
I	E_3	Wildwood	3,15,34	e,h	l,w	IP combined Wildwood and Cambridge (3,15:e,h:l,w) with Meleagridis (3,10:e,h:l,w) to form Meleagridis 3,10,[15],[15,34]:e,h:l,w. Wildwood is now called Meleagridis var. O 15+, 34+ by IP.
I	B	Wilhelmsburg	1,4,[5],12,27	z_{38}	[e,n,z_{15}]	
II	52	Wilhemstrasse	52	z_{44}	1,5	IP combined Wilhemstrasse with Lobatsi (52:z_{44}:1,5,7). The name Wilhemstrasse has been dropped.
I	G	Willemstad	1,13,22	e,h	1,6	
I	E_1	Wilmington	3,10	b	z_6	
I	E_1	Wimborne	3,10	k	1,2	
II	E_1	Winchester	3,10	z_{39}	1,[5],7	
I	Q	Windermere	39	y	1,5	
II	W	Windhoek	45	g,m,s,t	1,5	
I	C_2	Wingrove	6,8	c	1,2	
I	B	Winneba	4,12	r	1,6	
I	54	Winnipeg	54	e,h	1,5	
I	C_1	Winston	6,7	m,t	1,6	
I	E_4	Winterthur	1,3,19	l,z_{13}	1,6	
I	C_2	Wippra	6,8	z_{10}	z_6	
I	I	Wisbech	16	i	1,7	
II	J	Woerden	17	c	z_{39}	
I	F	Wohlen	11	b	1,6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
I	B	Womba	4,12,27	c	1,7	IP combined Womba with Altendorf (4,12:c:1,7) to form Altendorf 4,12,27:c:1,7. Womba is now called Altendorf var. O 27+. The name Womba has been dropped.
I	H	Woodhull	1,6,14,25	d	1,6	
I	F	Woodinville	11	c	e,n,x	
II	I	Woodstock	16	z_{42}	1,[5],7	
I	D_2	Worb	9,46	b	e,n,x	
II	G	Worcester	1,13,23	m,t	e,n,x	
I	G	Worthington	1,13,23	z	l,w	Worthington may possess H phase Rz_{45}.
I	N	Wuiti	30	z_{35}	e,n,z_{15}	
I	D_2	Wuppertal	9,46	z_{41}	–	
I	G	Wyldegreen	1,13,23	a	l,w	
II	D_1	Wynberg	1,9,12	z_{39}	1,7	
I	E_1	Yaba	3,10,[15]	b	e,n,z_{15}	
I	E_4	Yalding	1,3,19	r	e,n,z_{15}	
I	B	Yaounde	1,4,12,27	z_{35}	e,n,z_{15}	
I	M	Yardley	28	g,m	1,6	
I	C_2	Yarm	6,8	z_{35}	1,2	
I	G	Yarrabah	13,23	y	1,7	
I	E_1	Yeerongpilly	3,10	i	z_6	
I	F	Yehuda	11	z_4,z_{24}	–	
I	R	Yekepa	1,40	z_{35}	e,n,z_{15}	
I	54	Yerba	54	z_4,z_{23}	–	
I	P	Yoff	38	z_4,z_{23}	1,2	
I	C_2	Yokoe	8,20	m,t	–	
I	O	Yolo	35	c	[e,n,z_{15}]	
I	W	Yopougon	45	z	e,n,z_{15}	
I	I	Yoruba	16	c	l,w	
I	C_2	Yovokome	8,20	d	1,5	
I	E_1	Yundum	3,10	k	e,n,x	
I	D_2	Zadar	9,46	b	1,6	
I	D_1	Zaiman	9,12	l,v	e,n,x	
I	N	Zaire	30	c	1,7	
I	E_1	Zanzibar	3,10,[15]	k	1,5	
I	J	Zaria	17	k	e,n,z15	
I	D_1	Zega	9,12	d	z6	
I	N	Zehlendorf	30	a	1,5	
II	K	Zeist	18	z_{10}	z_6	
I	C_2	Zerifin	6,8	z_{10}	1,2	
I	I	Zigong	16	l,w	1,5	
I	V	Zinder	44	z_{29}	–	
I	E_1	Zongo	3,10	z_{35}	1,7	
II	D_3	Zuerich	1,9,12,46,27	c	z_{39}	
I	E_4	Zuilen	1,3,19	i	l,w	
I	I	Zwickau	16	r,i	e,n,z_{15}	
II	B		1,4,[5],12,27	a	e,n,x	
II	B		1,4,12,27	a	z_{39}	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	B		4,12	b	1,5	
II	B		4,12	e,n,x	1,2,7	
II	B		4,[5],12	f,g,t	z_6,z_{42}	
II	B		4,12	(f),g	–	Not in IP book
II	B		4,12	g,m,t	z_{39}	
II	B		4,12	g,m,t	–	IP calls this monophasic var. of Bechuana.
II	B		4,12	g,z_{62}	–	
II	B		4,12,27	i	z_{35}	
II	B		1,4,12,27	k	1,6	
II	B		1,4,12,27	l,v	e,n,x	
II	B		1,4,12,27	l,v	z_{39}	
II	B		4,12	l,z_{28}	–	
II	B		1,4,12,27	z	1,5	
II	B		4,12	z	1,7	
II	B		4,12	z	z_{39}	
II	B		4,12	–	1,6	
II	C_1		6,7,14	a	1,5	
II	C_1		6,7	a	z_6	
II	C_1		6,7	b	z_{39}	
II	C_1		6,7	d	z_{42}	
II	C_1		6,7	g,m,[s],t	e,n,x	
II	C_1		6,7	(g),m,[s],t	[1,5]	
II	C_1		6,7	g,[m],s,t	[z_{42}]	
II	C_1		6,7	g,t	e,n,x:z_{42}	
IV	C_1		6,7	g,z_{51}	–	
II	C_1		6,7	k	[z6]	
IIIa	C_1		6,7	(k)	z:[z_{55}]	(Ar. 27:22:31:[37])
IIIb	C_1		6,7	l,v	z_{53}	(Ar. 27:23:25)
II	C_1		6,7	l,w	1,5,7	
II	C_1		6,7	l,w	z_{42}	
II	C_1		6,7	l,z_{28}	e,n,x	
II	C_1		6,7	l,z_{28}	z_6	
II	C_1		6,7	m,t	–	
II	C_1		6,7	z	z_6	
II	C_1		6,7	z	z_{39}	
II	C_1		6,7	z_4,z_{24}	z_{42}	
II	C_1		6,7	z_{10}	z_{35}	
II	C_1		6,7	z_{29}	–	
VI	C_1		6,7	z_{41}	1,7	
II	C_1		6,7	z_{42}	e,n,x:1,6	
II	C_1		6,7	–	1,6	
II	C_2		6,8	a	e,n,x	
II	C_2		6,8	a	z_{39}	
II	C_2		6,8	b	1,5	
II	C_2		6,8	d	z_6:z_{42}	
II	C_2		6,8	f,g,m,t	[e,n,x]	
II	C_2		6,8	g,m,t	1,7	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	C_2		6,8	l,v	e,n,x	
II	C_2		6,8	l,w	z_6:z_{42}	
II	C_2		6,8	l,z_{28}	e,n,x	
II	C_2		6,8	y	1,6:z_{42}	
II	C_2		6,8	z	1,5	
II	C_2		6,8	z_{29}	1,5	
II	C_2		8	z_{29}	e,n,x:z_{42}	
II	C_2		6,8	z_{29}	e,n,x	
II	C_2		6,8	–	1,5,7	
II	D_1		9,12	a	1,5	
II	D_1		1,9,12	a	e,n,x	
II	D_1		9,12	a	z_{39}	
II	D_1		1,9,12	a	z_{42}	
II	D_1		9,12	d	z_{39}	
II	D_1		9,12	e,n,x	1,6	
II	D_1		1,9,12	g,m,[s],t	[1,5,7]:[z_{42}]	
II	D_1		1,9,12	g,z_{62}	–	
II	D_1		9,12	l,v	e,n,x	
II	D_1		9,12	l,v	z_{39}	
II	D_1		9,12	l,z_{28}	1,5:[z_{42}]	
II	D_1		9,12	l,z_{28}	e,n,x	
II	D_1		1,9,12	m,t	1,5	
II	D_1		1,9,12	m,t	z_{39}	
II	D_1		9,12	m,t	–	
II	D_1		1,9,12	z_{29}	e,n,x	
II	D_1		1,9,12	z_{42}	1,[5],7	
II	D_2		9,46	e,n,x	1,5,7	
II	D_2		9,46	g,z_{62}	–	
II	D_2		9,46	m,t	e,n,x	
II	D_2		9,46	z	1,5	
II	D_2		9,46	z_{10}	z_{39}	
II	D_2		9,46	z_{10}	z_6	
II	D_2		9,46	z_{39}	1,7	
II	D_3		9,12,46,27	g,t	e,n,x	
II	D_3		1,9,12,46,27	l,z_{13},z_{28}	z_{39}	
II	D_3		1,9,12,46,27	y	z_{39}	
II	D_3		1,9,12,46,27	z_{10}	1,5	
II	D_3		1,9,12,46,27	z_{10}	e,n,x	
II	D_3		1,9,12,46,27	z_{10}	z_{39}	
II	D_3		1,9,12,46,27	z_4,z_{24}	[1,5]	
II	E_1		3,10	a	z_{39}	
II	E_1		3,10	b	e,n,x	
II	E_1		3,10	b	z_{39}	
II	E_1		3,10	d	e,n,x	
II	E_1		3,10	l,v	e,n,x	
II	E_1		3,10	l,z_{28}	1,5	
II	E_1		3,10	l,z_{28}	z_{39}	
II	E_1		3,10	m,t	1,5	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	E_1		3,10	z_4,z_{24}	–	
II	E_1		3,10	z_{29}	e,n,x	
II	E_1		3,10	z_{29}	–	
VI	F		11	a	1,5	
VI	F		11	b	1,7	
II	F		11	c	e,n,z_{15}	
IIIb	F		11	k	z53	(Ar. 17:29:25)
IIIb	F		11	l,v	z	(Ar. 17:23:31). May possess H phase Rz_{56} (Ar. 38).
IIIb	F		11	l,v	z_{53}	(Ar. 17:23:25)
II	F		11	z	e,n,x	
IIIa	F		11	z_4,z_{23}	–	(Ar. 17:1,2,5:-)
IV	F		11	z_4,z_{32}	–	
II	F		11	–	1,5	
II	G		1,13,23	a	1,5	
II	G		13,22	a	e,n,x	
II	G		1,13,22	b	z_{42}	
II	G		13,23	d	e,n,x	
II	G		1,13,23	d	e,n,z_{15}	
II	G		1,13,23	g,m,s,t	1,5	
II	G		1,13,23	g,m,s,t	z_{42}	
II	G		1,13,23	g,[s],t	z_{42}	
IIIa	G		1,13,23	g,51	–	(Ar. 18:13,14:-)
V	G		1,13,22	i	–	
II	G		13,22	k	1,5:z_{42}	
II	G		13,23	k	z_{41}	
IIIb	G		13,22	l,v	1,5,7	(Ar. 18:23:30)
II	G		13,23	l,w	e,n,x	
II	G		13,22	l,z_{28}	1,5	
II	G		13,23	l,z_{28}	1,5	
II	G		13,23	l,z_{28}	z_6	
II	G		13,22	m,t	z_{42}:z_{39}	
V	G		13,22	r	–	
II	G		13,22	z	–	
II	G		1,13,23	z	z_{42}	
IIIa	G		13,22	z_4,z_{23}	–	(Ar. 18:1,2,5:-)
IIIa	G		13,23	z_4,z_{23},z_{32}	–	(Ar. 18:1,6,7:-). CDC would call this 1,6,7,9.
IIIa	G		1,13,23	z_4,z_{24}	–	(Ar. 18:1,3,11:-)
II	G		1,13,22	z_{10}	z_6	
II	G		1,13,23	z_{29}	1,5	
II	G		1,13,23	z_{29}	e,n,x	
II	G		1,13,23	z_{39}	1,5,7	
II	G		13,22	z_{39}	1,7	
II	G		13,23	–	1,6	
VI	H		[1],6,14	a	1,5	
IIIb	H		(6),14	b	e,n,x,z_{15}	(Ar. 7a,7c:43:28)
II	H		6,14,[24]	k	1,6	
II	H		6,14	k	[e,n,x]	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIb	H		(6),14	k	z	(Ar. 7a,7c:29:31)
II	H		1,6,14	k	z_6:z_{42}	
IIIb	H		(6),14	k	z_{53}	(Ar. 7a,7c:29:25)
IIIb	H		(6),14	l,v	z	(Ar. 7a,7c:23:31)
IIIb	H		(6),14	l,v	z_{35}	(Ar. 7a,7c:23:21)
IIIb	H		(6),14	l,v	z_{53}	(Ar. 7a,7c:23:25)
IIIb	H		(6),14	r	z	(Ar. 7a,7c:24:31)
IV	H		6,14	z_4,z_{23}	-	
IIIb	H		(6),14	z_{10}	e,n,x,z_{15}	(Ar. 7a,7c:27:28)
IIIb	H		(6),14	z_{10}	z:[z_{53}]	(Ar. 7a,7c:27:31:[25])
IIIb	H		(6),14	z_{10}	z_{53}	(Ar. 7a,7c:27:25)
II	H		1,6,14	z_{42}	1,6	
IIIb	H		(6),14	z_{52}	e,n,x,z_{15}	(Ar. 7a,7c:26:28)
IIIb	H		(6),14	z_{52}	z_{35}	(Ar. 7a,7c:26:21)
II	I		16	b	e,n,x	
II	I		16	b	z_{39}	
II	I		16	b	z_{42}	
II	I		16	d	1,5	
II	I		16	g,[m],[s],t	z_{42}	
IIIb	I		16	i	z_{35}	(Ar. 25:33:21)
IIIb	I		16	k	z	(Ar. 25:29:31)
IIIb	I		16	k	z_{53}	(Ar. 25:29:25)
IIIb	I		16	(k)	z_{35}	(Ar. 25:22:21)
IIIb	I		16	l,v	1,5,7	(Ar. 25:23:30)
IIIb	I		16	l,v	z:[z_{61}]	(Ar. 25:23:31:[41])
IIIb	I		16	l,v	z_{35}	(Ar. 25:23:21)
IIIb	I		16	l,v	z_{53}	(Ar. 25:23:25)
II	I		16	m,t	e,n,x	
II	I		16	z	z_{42}	
II	I		16	z_4,z_{24}	–	
II	I		16	z_6	1,6	
IIIb	I		16	z_{10}	1,5,7	(Ar. 25:27:30)
IIIb	I		16	z_{10}	e,n,x,z_{15}	(Ar. 25:27:28)
II	I		16	z_{29}	1,5	
IV	I		16	z_{36}	–	
IIIb	I		16	z_{52}	z_{35}	(Ar. 25:26:21)
II	J		17	b	z_6	
II	J		17	e,n,x,z_{15}	1,[5],7	
II	J		17	g,m,s,t	–	
II	J		17	g,t	z_{39}	
IIIb	J		17	i	z_{35}	(Ar. 12:33:21)
IIIb	J		17	k	z	(Ar. 12:29:32)
II	J		17	k	–	
IIIb	J		17	l,v	e,n,x,z_{15}	(Ar. 12:23:28)
IIIb	J		17	l,v	z_{35}	(Ar. 12:23:21)
II	J		17	m,t	–	
IIIb	J		17	r	z	(Ar. 12:24:31)
II	J		17	y	–	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIa	J		17	z_4,z_{23}	–	(Ar. 12:1,2,5:- and 12:1,2,6:-)
IIIa	J		17	z_4,z_{23},z_{32}	–	(Ar. 12:1,6,7,9:-)
IIIa	J		17	z_4,z_{24}	–	(Ar. 12:1,3,11:-)
IIIa	J		17	z_4,z_{32}	–	(Ar. 12:1,6,7:- and 12:1,7,8:-)
IIIb	J		17	z_{10}	e,n,x,z_{15}	(Ar.12:27:28). May possess H phase Rz_{56} (Ar. 38).
IIIb	J		17	z_{10}	z	(Ar. 12:27:31)
IIIa	J		17	z_{29}	–	(Ar. 12:16,17,18:-)
IV	J		17	z_{29}	–	
IIIa	J		17	z_{36}	–	(Ar. 12:17,20:-)
IV	J		17	z_{36}	–	
IIIa	K		18	g,z_{51}	–	(Ar. 7a,7b:13,14:-)
IIIb	K		18	(k)	z_{53}	(Ar. 7a,7b:22:25)
IIIb	K		18	(k)	z_{54}	(Ar. 7a,7b:22:34)
IIIb	K		18	l,v	e,n,x,z_{15}	(Ar. 7a,7b:23:28)
IIIb	K		18	l,v	z	(Ar. 7a,7b:23:31)
IIIb	K		18	l,v	z_{53}	(Ar. 7a,7b:23:25)
II	K		18	m,t	1,5	
IIIb	K		18	r	z	(Ar. 7a,7b:24,31)
II	K		18	y	e,n,x,z_{15}	
II	K		18	z_4,z_{23}	–	
IIIa	K		18	z_4,z_{23}	–	(Ar. 7a,7b:1,2,5:- and 7a,7b:1,2,6:-)
II	K		18	z_4,z_{24}	–	
IIIa	K		18	z_4,z_{32}	–	(Ar. 7a,7b:1,6,7:- and 7a,7b:1,7,8:-)
IIIb	K		18	z_{10}	e,n,x,z_{15}	(Ar. 7a,7b:27:28)
IV	K		18	z36,z38	–	
II	L		21	b	1,5	
II	L		21	c	e,n,x	
IIIb	L		21	c	e,n,x,z_{15}	(Ar. 22:32:28)
II	L		21	g,[m],[s],t	–	
IIIa	L		21	g,z_{51}	–	(Ar. 22:13,14:-)
IV	L		21	g,z_{51}	–	
IIIb	L		21	i	1,5,7	(Ar. 22:33:30)
IIIb	L		21	i	e,n,x,z_{15}	(Ar. 22:33:28)
IIIb	L		21	k	e,n,x,z_{15}	(Ar. 22:29:28)
IIIb	L		21	k	z	(Ar. 22:29:31)
IIIb	L		21	l,v	z	(Ar. 22:23:31)
IIIb	L		21	l,v	z_{57}	(Ar. 22:23:40)
II	L		21	m,t	–	
IIIb	L		21	r	z	(Ar. 22:24:31)
II	L		21	z	–	CDC does not have this.
IIIa	L		21	z_4,z_{23}	–	(Ar. 22:1,2,5:- and 22:1,2,6:-)
IIIa	L		21	z_4,z_{24}	–	(Ar. 22:1,3,11:-)
IV	L		21	z_4,z_{32}	–	
IIIb	L		21	z_{10}	e,n,x,z_{15}	(Ar. 22:27:28)
IIIb	L		21	z_{10}	z	(Ar. 22:27:31)
IIIb	L		21	z_{10}	z_{53}	(Ar. 22:27:25)
IIIa	L		21	z_{29}	–	(Ar. 22:16,17,18:-)
IV	L		21	z_{36}	–	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIb	L		21	z_{65}	e,n,x,z_{15}	(Ar. 22:32:28)
II	M		28	a	e,n,x	
II	M		28	b	e,n,x	
II	M		28	e,n,x	1,7	
II	M		28	g,m,t	e,n,x	
II	M		28	g,m,t	z_{39}	
II	M		28	g,s,t	e,n,x	
II	M		28	l,z_{28}	1,5	
II	M		28	m,t	[e,n,x]	
II	M		28	z	1,5	
IIIb	M		28	z_{10}	z	(Ar. 35:27:31)
IIIb	M		28	z_{10}	z:[z_{57}]	(Ar. 35:27:31:[40])
II	M		28	z_{29}	1,5	
II	M		28	z_{29}	e,n,x	
II	N		30	b	z_6	
II	N		30	c	z_{39}	
II	N		30	g,m,s	e,n,x	
II	N		30	k	e,n,x,z_{15}	
II	N		30	l,z_{28}	z_6	
II	N		30	m,t	–	
II	N		30	z_6	1,6	
II	N		30	z_{39}	1,7	
II	O		35	d	1,5	
II	O		35	g,m,s,t	–	
II	O		35	g,t	1,5	
II	O		35	g,t	z_{42}	
IIIa	O		35	g,z_{51}	–	(Ar. 20:13,14:-)
IIIb	O		35	i	e,n,x,z_{15}	(Ar. 20:33:28)
IIIb	O		35	i	z	(Ar. 20:33:31)
IIIb	O		35	i	z_{35}	(Ar. 20:33:21)
IIIb	O		35	i	z_{53}	(Ar. 20:33:25)
IIIb	O		35	k	e,n,x,z_{15}	(Ar. 20:29:28)
IIIb	O		35	k	z	(Ar. 20:29:31)
IIIb	O		35	k	z_{53}	(Ar. 20:29:25). May possess H phase Rz_{50} (Ar.42).
IIIb	O		35	(k)	z	(Ar. 20:22:31)
IIIb	O		35	(k)	z_{35}	(Ar. 20:22:21)
IIIb	O		35	l,v	1,5,7	(Ar. 20:23:30)
IIIb	O		35	l,v	e,n,x,z_{15}	(Ar. 20:23:28)
IIIb	O		35	l,v	z_{35}	(Ar. 20:23:21)
II	O		35	l,z_{28}	–	
II	O		35	m,t	–	
IIIb	O		35	r	e,n,x,z_{15}	(Ar. 20:24:28)
IIIb	O		35	r	z	(Ar. 20:24:31)
IIIb	O		35	r	z_{35}	(Ar. 20:24:21)
IIIb	O		35	r	z_{61}	(Ar. 20:24:41)
IIIa	O		35	z_4,z_{23}	–	(Ar. 20:1,2,6:-)
IIIa	O		35	z_4,z_{32}	–	(Ar. 20:1,7,8:-)
IIIb	O		35	z_{10}	z_{35}	(Ar. 20:27:21)

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIa	O		35	z_{29}	–	(Ar. 20:16,17,18:-)
IIIa	O		35	z_{36}	–	(Ar. 20:17,20:-)
IIIb	O		35	z_{52}	1,5,7	(Ar. 20:26:30)
IIIb	O		35	z_{52}	e,n,x,z_{15}	(Ar. 20:26:28)
IIIb	O		35	z_{52}	z	(Ar. 20:26:31)
IIIb	O		35	z_{52}	z_{35}	(Ar. 20:26:21)
II	P		38	b	1,2	
IIIa	P		38	g,z_{51}	–	(Ar. 16:13,14:-)
IV	P		38	g,z_{51}	–	
IIIb	P		38	i	z	(Ar. 16:33:31)
IIIb	P		38	i	z_{53}	(Ar. 16:33:25)
IIIb	P		38	k	e,n,x,z_{15}	(Ar. 16:29:28)
IIIb	P		38	k	z	(Ar. 16:29:31)
IIIb	P		38	k	z_{53}	(Ar. 16:29:25)
IIIb	P		38	(k)	1,5,7	(Ar. 16:22:30)
IIIb	P		38	(k)	z	(Ar. 16:22:31)
IIIb	P		38	(k)	z_{35}	(Ar. 16:22:21). May possess H phase Rz_{56} (Ar. 38).
IIIb	P		38	(k)	z_{54}	(Ar. 16:22:34)
IIIb	P		38	(k)	z_{55}	(Ar. 16:22:37)
IIIb	P		38	l,v	z	(Ar. 16:23:31)
IIIb	P		38	l,v	z_{35}	(Ar. 16:23:21)
IIIb	P		38	l,v	z_{53}:[z_{54}]	(Ar. 16:23:25:[34])
IIIb	P		38	r	1,5,7	(Ar. 16:24:30)
IIIb	P		38	r	e,n,x,z_{15}	(Ar. 16:24:28)
IIIb	P		38	r	z:[z_{57}]	(Ar. 16:24:31:[40])
IIIb	P		38	r	z_{35}	(Ar. 16:24:21)
IIIa	P		38	z_4,z_{23}	–	(Ar. 16:1,2,6:-)
IV	P		38	z_4,z_{23}	–	
IIIb	P		38	z_{10}	z	(Ar. 16:27:31)
IIIb	P		38	z_{10}	z_{53}	(Ar. 16:27:25)
IIIb	P		38	z_{47}	z_{53}	(Ar. 16:39:25)
IIIb	P		38	z_{52}	z_{35}	(Ar. 16:26:21)
IIIb	P		38	z_{52}	z_{53}	(Ar. 16:26:25)
IIIb	P		38	z_{53}	–	(Ar. 16:25:-). May possess H phase Rz_{50} (Ar. 42) or Rz_{76} (Ar. Rz_{76}). CDC does not have monophasic.
IIIa	P		38	z_{61}	–	(Ar. 16:41:-)
IIIb	P		38	z_{61}	z_{53}	(Ar. 16:41:25)
II	Q		39	a	z_{39}	
II	Q		39	c	e,n,x	
II	Q		39	e,n,x	1,7	
II	Q		39	g,m,t	–	
II	Q		39	l,v	1,5	
II	Q		39	l,z_{28}	z_{39}	
II	Q		39	m,t	e,n,x	
II	Q		39	–	1,7	
II	R		1,40	a	1,5	
II	R		1,40	a	z_6	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	R		40	b	–	
II	R		1,40	c	z_{39}	
II	R		1,40	e,n,x	1,[5],7	
II	R		1,40	e,n,x,z_{15}	1,6	
II	R		1,40	g,t	1,5	
II	R		1,40	g,t	[e,n,x]	
II	R		1,40	g,t	e,n,x,z_{15}	
II	R		40	g,t	z_{39}	
II	R		1,40	g,[m],[s],t	z_{42}	
IIIb	R		40	g,z_{51}	[e,n,x,z_{15}]	(Ar. 10a,10b:13,14:[28])
IIIb	R		40	i	1,5,7	(Ar. 10a,10b:33:30)
IIIb	R		40	k	z:z_{57}	(Ar. 10a,10b:29:31:40)
II	R		40	k	z_6	
IIIb	R		40	k	z_{53}	(Ar. 10a,10b:29:25)
IIIb	R		40	l,v	z	(Ar. 10a,10b,(10c):23:31)
IIIb	R		40	l,v	z_{53}	(Ar. 10a,10b:23:25)
II	R		1,40	l,z_{28}	1,5:z_{42}	
II	R		1,40	l,z_{28}	z_{39}	
II	R		40	m,t	z_{39}	
II	R		1,40	m,t	z_{42}	
IV	R		40	m,t	-	
II	R		1,40	z	z_6	
II	R		1,40	z	z_{39}	
II	R		40	z	z_{42}	
IIIa	R		40	z_4,z_{23}	–	(Ar. 10a,10b:1,2,5:-; 10a,10b:1,2,5,6:-; and 10a,10b:1,2,6:-)
IIIa	R		40	z_4,z_{24}	–	(Ar. 10a,10b:1,3,11:-)
IV	R		40	z_4,z_{24}	–	Also called Degania var. subsp. IV.
IIIa	R		40	z_4,z_{32}	–	(Ar. 10a,10b:1,2,10:-; 10a,10c:1,2,10:-; and 10a,10b:1,7,8:-)
IIIa	R		40	z_4,z_{32}	–	
II	R		1,40	z_6	1,5	
IIIb	R		40	z_{10}	z_{35}	(Ar. 10a,10b:27:21)
IIIa	R		40	z_{29}	–	(Ar. 10a,10b:16,18:-)
V	R		1,40	z_{35}	–	
IIIa	R		40	z_{35}	–	(Ar. 10a,10b:17,20:-)
II	R		40	z_{39}	1,5:z_{42}	
II	R		40	z_{39}	1,7	
II	R		1,40	z_{42}	1,6	
II	R		1,40	[z_{42}]	1,(5),7	
V	R		40	z_{81}	–	H z_{81} was formerly H a in *S. bongori*.
II	S		41	b	[1,5]	
VI	S		41	b	1,7	
IIIb	S		41	c	e,n,x,z_{15}	(Ar. 13:32:28)
II	S		41	c	[z6]	
II	S		41	g,m,s,t	z_6	
IIIa	S		41	g,z_{51}	–	(Ar. 13:13,14:-)
II	S		41	k	1,6	
II	S		41	k	[z_6]	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIb	S		41	(k)	[z_{35}]	(Ar. 13:22:[21])
II	S		41	l,z_{13},z_{28}	e,n,x,z_{15}	
IIIa	S		41	z_4,z_{23}	–	(Ar. 13:1,2,5:- and 13:1,2,6:-)
IV	S		41	z_4,z_{23}	–	Also called Waycross var. subsp. IV.
IIIa	S		41	z_4,z_{23},z_{32}	–	(Ar. 13:1,6,7,9:-)
IIIa	S		41	z_4,z_{24}	–	(Ar. 13:1,3,11:-)
IIIa	S		41	z_4,z_{32}	–	(Ar. 13:1,6,7:- and 13:1,7,8:-)
IIIa	S		41	z_{29}	–	(Ar. 13:16,17,18:-)
IV	S		41	z_{29}	–	
IIIa	S		41	z_{36}	–	(Ar. 13:17,20:-)
IV	S		41	z_{52}	–	
II	S		41	–	1,6	
II	T		42	b	z_6	
II	T		42	d	z_6	
II	T		42	[e,n,x]	1,6	
IIIa	T		42	g,z_{51}	–	(Ar. 15:13,14:-)
IV	T		1,42	g,z_{51}	–	
IIIb	T		42	k	–	(Ar. 15:29:-)
IIIb	T		42	k	e,n,x,z_{15}	(Ar. 15:29:28)
IIIb	T		42	k	z	(Ar. 15:29:31)
IIIb	T		42	k	z_{35}	(Ar. 15:29:21)
IIIb	T		42	(k)	z_{35}	(Ar. 15:22:21)
IIIb	T		42	l,v	1,5,7	(Ar. 15:23:30)
IIIb	T		42	l,v	e,n,x,z_{15}	(Ar. 15:23:28)
IIIb	T		42	l,v	z	(Ar. 15:23:31)
IIIb	T		42	l,v	z_{53}	(Ar. 15:23:25)
II	T		1,42	l,w	e,n,x	
II	T		42	l,[z_{13}],z_{28}	[z_6]	
II	T		42	m,t	[e,n,x,z_{15}]	
IIIb	T		42	r	–	(Ar. 15:24:-). May possess H phase Rz_{50} (Ar. 42).
IIIb	T		42	r	z	(Ar. 15:24:31)
IIIb	T		42	r	z_{53}	(Ar. 15:24:25)
IIIa	T		42	z_4,z_{23}	–	(Ar. 15:1,2,5:- and 15:1,2,6:-)
IIIa	T		42	z_4,z_{24}	–	(Ar. 15:1,3,11:-)
IV	T		1,42	z_4,z_{24}	–	
II	T		42	z_6	1,6	
IIIb	T		42	z_{10}	–	(Ar. 15:27:-). May possess H phase Rz_{56} (Ar. 38) and Rz_{50} (Ar. 42).
II	T		42	z_{10}	1,2	
II	T		42	z_{10}	e,n,x,z_{15}	
IIIb	T		42	z_{10}	e,n,x,z_{15}	(Ar. 15:27:28)
IIIb	T		42	z_{10}	z	(Ar. 15:27:31)
II	T		42	z_{10}	z_6	
IIIb	T		42	z_{10}	z_{35}	(Ar. 15:27:21)
IIIb	T		42	z_{10}	z_{67}	(Ar. 15:27:46)
IV	T		42	z_{36}	–	
IIIb	T		42	z_{52}	z	(Ar. 15:26:31)
II	U		43	a	1,5	

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	U		43	a	z_6	
II	U		43	d	e,n,x,z_{15}	
II	U		43	d	z_{39}	
II	U		43	d	z_{42}	
II	U		43	e,n,x,z_{15}	1,(5),7	
II	U		43	e,n,x,z_{15}	1,6	
II	U		43	g,t	1,5	
IIIa	U		43	g,z_{51}	–	(Ar. 21:13,14:-)
IV	U		43	g,z_{51}	–	
II	U		43	g,z_{62}	e,n,x	
IIIb	U		43	k	z	(Ar. 21:29:31)
IIIb	U		43	l,v	z_{53}:[Rz_{56}]	(Ar. 21:23:25:[38])
II	U		43	l,z_{13},z_{28}	1,5	
IIIb	U		43	r	e,n,x,z_{15}	(Ar. 21:24:28)
IIIb	U		43	r	z	(Ar. 21:24:31)
IIIb	U		43	r	z_{53}	(Ar. 21:24:25)
II	U		43	z	1,5	
IV	U		43	z_4,z_{23}	–	
IIIa	U		43	z_4,z_{23}	–	(Ar. 21:1,2,5:- and 21:1,2,6:-)
IIIa	U		43	z_4,z_{24}	–	(Ar. 21:1,3,11:-)
IV	U		43	z_4,z_{24}	–	
IV	U		43	z_{29}	–	
II	U		43	z_{29}	e,n,x	
II	U		43	z_{29}	z_{42}	
IIIa	U		43	z_{36}	–	(Ar. 21:17,20:-)
IIIb	U		43	z_{52}	z_{53}	(Ar. 21:26:25)
II	V		1,44	e,n,x	1,6	
II	V		44	g,t	z_{42}	
IV	V		44	g,z_{51}	–	
II	V		44	z_4,z_{23}	–	
IIIa	V		44	z_4,z_{23}	–	(Ar. 1,3:1,2,5:- and 1,3:1,2,6:-)
IV	V		44	z_4,z_{23}	–	
IIIa	V		44	z_4,z_{23},z_{32}	–	(Ar. 1,3:1,6,7,9:-)
IIIa	V		44	z_4,z_{24}	–	(Ar. 1,3:1,3,11:-)
IV	V		44	z_4,z_{24}	–	
IIIa	V		44	z_4,z_{32}	–	(Ar. 1,3:1,2,10:- and 1,3:1,7,8:-). IP calls z_4,z_{23},z_{32}, Ar. 1,2,10.
IV	V		44	z_{29}	–	
II	V		44	z_{29}	e,n,x:z_{42}	
IV	V		44	z_{36},[z_{38}]	–	
V	V		44	z_{39}	–	
IIIa	W		45	g,z_{51}	–	(Ar. 11:13,14:-)
IV	W		45	g,z_{51}	–	
II	W		45	m,t	1,5	
II	W		45	z	1,5	
IIIa	W		45	z_4,z_{23}	–	(Ar. 11:1,2,5:-)
IV	W		45	z_4,z_{23}	–	
IIIa	W		45	z_4,z_{24}	–	(Ar. 11:1,3,11:-)

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIa	W		45	z_4,z_{32}	–	(Ar. 11:1,7,8:-)
IIIa	W		45	z_{29}	–	(Ar. 11:16,18:-)
II	W		45	z_{29}	1,5	
II	W		45	z_{29}	e,n,x	
II	W		45	z_{29}	z_{42}	
IV	W		45	z_{36},z_{38}	–	
II	X		47	a	e,n,x,z_{15}	
II	X		47	b	z_6	
IIIb	X		47	c	1,5,7	(Ar. 28:32:30)
IIIb	X		47	c	e,n,x,z_{15}:[z_{57}]	(Ar. 23:32:28 and 28:32:28:[40])
IIIb	X		47	c	z	(Ar. 28:32:31)
IIIb	X		47	c	z_{35}	(Ar. 28:32:21)
II	X		47	d	e,n,x,z_{15}	
II	X		47	e,n,x,z_{15}	1,6	
II	X		47	g,t	e,n,x	
IIIa	X		47	g,z_{51}	–	(Ar. 28:13,14)
IIIb	X		47	i	e,n,x,z_{15}	(Ar. 23:33:28). May possess H phase Rz_{50} (Ar. 42).
IIIb	X		47	i	z	(Ar. 28:33:31)
IIIb	X		47	i	z_{35}	(Ar. 23:33:21 and 28:33:21)
IIIb	X		47	i	z_{53}:[z_{57}]	(Ar. 23:33:25 and 28:33:25:[40])
IIIb	X		47	k	1,5,7	(Ar. 28:29:30)
IIIb	X		47	k	e,n,x,z_{15}	(Ar. 28:29:28)
IIIb	X		47	k	z	(Ar. 28:29:31)
IIIb	X		47	k	z_{35}	(Ar. 23:29:21)
IIIb	X		47	k	z_{53}	(Ar. 23:29:25)
IV	X		47	l,v	–	
IIIb	X		47	l,v	1,5,(7)	(Ar. 23:23:30). May possess H phase Rz_{50} (Ar. 42).
IIIa	X		47	l,v	e,n,x,z_{15}	(Ar. 28:23:28)
IIIb	X		47	l,v	z	(Ar. 23:23:31)
IIIb	X		47	l,v	z_{35}	(Ar. 28:23:21)
IIIb	X		47	l,v	z_{53}	(Ar. 28:23:25)
IIIb	X		47	l,v	z_{57}	(Ar. 28:23:40)
IIIa	X		47	r	–	(Ar. 23:24:-). CDC does not have this.
IIIb	X		47	r	1,5,7	(Ar. 23:24:30)
IIIb	X		47	r	z	(Ar. 23:24:31)
IIIb	X		47	r	z_{35}	(Ar. 23:24:21 and 28:24:21)
IIIb	X		47	r	z_{53}	(Ar. 23:24:25). May possess H phase Rz_{74} (Ar. Rz74)
IIIb	X		47	r	z_{53}:[z_{60}]	(Ar. 23:24:25:[44]). May possess H phase Rz_{70} and Rz_{72} (Ar. Rz_{70} or Rz_{72}).
IIIb	X		47	r	z_{53}:Rz_{50}:z_{60}	(Ar. 28:24:25:42:44). Not in IP book.
II	X		47	z	e,n,x,z_{15}	
IIIa	X		47	z_4,z_{23}	–	(Ar. 28:1,2,5:-)
II	X		47	z_6	1,6	
IIIb	X		47	z_{10}	1,5,7	(Ar. 28:27:30)
IIIb	X		47	z_{10}	z	(Ar. 28:27:31)
IIIb	X		47	z_{10}	z_{35}	(Ar. 28:27:21)

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIa	X		47	z_{29}	–	(Ar. 28:16,18:-)
II	X		47	z_{29}	e,n,x,z_{15}	
IV	X		47	z_{36}	–	
IIIb	X		47	z_{52}	1,5,7	(Ar. 28:26:30)
IIIb	X		47	z_{52}	e,n,x,z_{15}	(Ar. 28:26:28)
IIIb	X		47	z_{52}	z	(Ar. 28:26:31)
IIIb	X		47	z_{52}	z_{35}	(Ar. 28:26:21)
II	Y		48	a	z_6	
IIIb	Y		48	a	z_{35}	(Ar. 5:35:21). Not in 1992 IP, but is in Bergey.
II	Y		48	a	z_{39}	
V	Y		48	b	–	
II	Y		48	b	[z_6]	
IIIb	Y		48	c	z	(Ar. 29:32:31)
II	Y		48	d	1,2	
IIIa	Y		48	g,z_{51}	–	(Ar. 5:13,14:-)
IIIb	Y		48	i	z:[z_{72}]	(Ar. 5,29:33:31:[z_{72}]). CDC does not have z_{72} strain.
IIIb	Y		48	i	z_{35}:[z_{57}]	(Ar. 29:33:21:[40])
IIIb	Y		48	i	z_{53}	(Ar. 5:33:25)
IIIb	Y		48	i	z_{61}	(Ar. 5,29:33:41)
IIIb	Y		48	k	1,5,(7)	(Ar. 5:29:30)
II	Y		48	k	e,n,x,z_{15}	
IIIb	Y		48	k	e,n,x,z_{15}	(Ar. 5:29:28)
IIIb	Y		48	k	z	(Ar. 5,29:29:31)
IIIb	Y		48	k	z_{35}:[Rz_{75}]	(Ar. [5:29:21:Rz_{75}]). CDC does not have Rz_{75}.
IIIb	Y		48	k	z_{53}	(Ar. 5,29:29:25)
IIIb	Y		48	(k)	z_{53}	(Ar. 5:22:25 and Ar. 5,29:22:25). Called 5:22:25 by IP.
IIIb	Y		48	l,v	1,5,(7)	(Ar. 5:23:30). May possess H phase Rz_{47} or Rz_{50} (Ar. 39 or 42).
IIIb	Y		48	l,v	z	(Ar. 5,29:23:31)
IIIb	Y		48	r	e,n,x,z_{15}	(Ar. 5:24:28)
IIIb	Y		48	r	z	(Ar. 5,29:24:31)
II	Y		48	z	1,5	
IIIa	Y		48	z_4,z_{23}	–	(Ar. 5:1,2,5:-; 5:1,2,5,6:-; and 5:1,6:-)
IV	Y		48	z_4,z_{23}	–	
IIIa	Y		48	z_4,z_{23},z_{32}	–	(Ar. 5:1,6,7,9:-). IP calls this 5:1,6,7:-.
IIIa	Y		48	z_4,z_{24}	–	(Ar. 5:1,3,11:-)
IIIa	Y		48	z_4,z_{32}	–	(Ar. 5:1,6,7:-; 5:1,7,8:-; and Ar. 5:1,2,10:-). IP calls z_4,z_{32}, Ar. 1,7,8; and would call z_4,z_{23},z_{32}, Ar. 1,2,10.
IV	Y		48	z_4,z_{32}	–	
VI	Y		48	z_{10}	1,5	
IIIb	Y		48	z_{10}	e,n,x,z_{15}	(Ar. 5:27:28)
IIIb	Y		48	z_{10}	z	(Ar. 5,29:27:31)
II	Y		48	z_{29}	–	
IIIa	Y		48	z_{29}	–	(Ar. 5:16,18). This is not in IP book, but is on Rohde's list.
IV	Y		48	z_{29}	–	
IIIb	Y		48	z_{35}	z_{52}	(Ar. 5:21:26)

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIa	Y		48	z_{36}	–	(Ar. 5,29:17,20:-)
IV	Y		48	z_{36},[z_{38}]	–	
V	Y		48	z_{39}	-	
IIIb	Y		48	z_{52}	e,n,x,z15	(Ar. 29:26:28)
IIIb	Y		48	z_{52}	z	(Ar. 5:26:31)
V	Y		48	z_{65}	-	
V	Y		48	z_{81}	-	
IV	Z		50	a	-	
IV	Z		50	b	-	
II	Z		50	b	z_6	
IV	Z		50	d	-	
II	Z		50	g,z_{62}	e,n,x	
IIIb	Z		50	i	1,5,7	(Ar. 9a,9c:33:30)
IIIb	Z		50	i	e,n,x,z_{15}	(Ar. 9a,9c:33:28)
IIIb	Z		50	i	z	(Ar. 9a,9c:33:31)
IIIb	Z		50	k	1,5,7	(Ar. 9a,9c:29:30)
IIIb	Z		50	k	e,n,x,z_{15}	(Ar. 9a,9c:29:28)
II	Z		50	k	e,n,x:z_{42}	
IIIb	Z		50	k	z	(Ar. 9a,9b:29:31 and 9a,9c:29:31). Ar. 9a,9b may possess H phase Rz_{50} (Ar. 42).
IIIb	Z		50	k	z_{35}	(Ar. 9a,9b:29:21)
IIIb	Z		50	k	z_{53}	(Ar. 9a,9b:29:25 and 9a,9c:29:25). IP and Rohde only list the 9a,9c.
IIIb	Z		50	(k)	z	(Ar. 9a,9b:22:31)
IIIb	Z		50	(k)	z_{35}	(Ar. 9a,9b:22:21)
IIIb	Z		50	l,v	e,n,x,z_{15}	(Ar. 9a,9b:23:28)
IIIb	Z		50	l,v	z	(Ar. 9a,9b:23:31 and 9a,9c:23:31). IP only lists 9a,9c.
IIIb	Z		50	l,v	z_{35}	(Ar. 9a,9c:23:21)
II	Z		50	l,w	e,n,x,z_{15}:z_{42}	
II	Z		50	l,z_{28}	z_{42}	
IIIb	Z		50	r	1,5,(7)	(Ar. 9a,9b:24:30)
IIIb	Z		50	r	e,n,x,z_{15}	(Ar. 9a,9c:24:28)
IIIb	Z		50	r	z	(Ar. 9a,9b:24:31 and 9a,9c:24:31).
IIIb	Z		50	r	z_{35}	(Ar. 9a,9b:24:21). May possess H phase Rz_{58} (Ar. Rz58). This is not in IP book, but is on Rohde's list.
IIIb	Z		50	r	z_{53}	(Ar. 9a,9b:24:25). May possess H phase Rz_{50} (Ar. 42). This is not in IP book, but is on Rohde's list.
IIIa	Z		50	z_4,z_{23}	–	(Ar. 9a,9b:1,2,5:- and 9a,9b:1,2,6:-)
IIIa	Z		50	z_4,z_{23},z_{32}	–	(Ar. 9a,9b:1,2,10:-). Called 9a,9b:1,6,7:- by IP and Rohde.
IIIa	Z		50	z_4,z_{24}	–	(Ar. 9a,9b:1,3,11:-)
IV	Z		50	z_4,z_{24}	–	
IIIa	Z		50	z_4,z_{32}	–	(Ar. 9a,9b:1,2,10; 9a,9b:1,6,7:-; and 9a,9b:1,7,8:-). 9a,9b:1,2,10:- and 9a,9b:1,7,8:- used by IP and Rohde.
IIIb	Z		50	z_{10}	z	(Ar. 9a,9c:27:31). May possess H phase Rz_{56} (Ar. 38).
IIIb	Z		50	z_{10}	z_{53}	(Ar. 9a,9c:27:25)

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIa	Z		50	z_{29}	–	(Ar. 9a,9b:16,18:-)
IIIa	Z		50	z_{36}	–	(Ar. 9a,9b:17,20:-)
IIIb	Z		50	z_{52}	1,5,7	(Ar. 9a,9b:26:30 and 9a,9c:26:30)
IIIb	Z		50	z_{52}	z	(Ar. 9a,9b:26:31 and 9a,9c:26:31)
IIIb	Z		50	z_{52}	z_{35}	(Ar. 9a,9b:26:21 and 9a,9c:26:21)
IIIb	Z		50	z_{52}	z_{53}	(Ar. 9a,9b:26:25 and 9a,9c:26:25)
IV	51		51	b	–	
II	51		51	c	–	
II	51		51	g,s,t	e,n,x	
IIIa	51		51	g,z_{51}	–	(Ar. 1,2:13,14:-)
IIIb	51		51	k	z_{35}	(Ar. 1,2:29:21)
IIIb	51		51	l,v	z	(Ar. 1,2:23:31)
IIIa	51		51	z_4,z_{23}	–	(Ar. 1,2:1,2,5:- and 1,2:1,2,6:-)
IIIa	51		51	z_4,z_{24}	–	(Ar. 1,2:1,3,11:-)
IIIa	51		51	z_4,z_{32}	–	(Ar. 1,2:1,7,8:-)
II	51		51	z_{29}	e,n,x,z_{15}	
II	52		52	c	k	
IIIb	52		52	c	k	(Ar. 31:32:29). This is not in IP book, but is on Rohde's list.
II	52		52	d	e,n,x,z_{15}	
II	52		52	d	z_{39}	CDC does not have this.
II	52		52	g,t	–	
IIIb	52		52	k	z_{35}	(Ar. 31:29:21)
IIIb	52		52	k	z_{53}	(Ar. 31:29:25)
IIIb	52		52	(k)	z_{35}	(Ar. 31:22:21)
IIIb	52		52	l,v	z_{53}	(Ar. 31:23:25)
II	52		52	z	z_{39}	
IIIb	52		52	z_{52}	z	(Ar. 31:26:31)
II	53		53	c	1,5	
II	53		53	d	1,5	
II	53		1,53	d	z39	
II	53		53	d	z42	
IIIa	53		53	g,z_{51}	–	(Ar. 1,4:13,14:-)
IV	53		1,53	g,z_{51}	–	
IIIb	53		53	i	z	(Ar. 1,4:33:31)
IIIb	53		53	k	e,n,x,z_{15}	(Ar. 1,4:29:28)
IIIb	53		53	k	z	(Ar. 1,4:29:31)
IIIb	53		53	(k)	z	(Ar. 1,4:22:31)
IIIb	53		53	(k)	z_{35}	(Ar. 1,4:22:21)
IIIb	53		53	l,v	e,n,x,z_{15}	(Ar. 1,4:23:28)
IIIb	53		53	l,v	z_{35}	(Ar. 1,4:23:21)
II	53		53	l,z_{28}	e,n,x	
II	53		53	l,z_{28}	z_6	
IIIb	53		53	r	z	(Ar. 1,4:24:31)
IIIb	53		53	r	z_{35}	(Ar. 1,4:24:21)
IIIb	53		53	r	z_{68}	(Ar. 1,4:24:47). This was formerly called z_{56} (Ar. 38), but was changed to z_{68} (Ar. 47).
II	53		53	z	1,5	
IIIb	53		53	z	1,5,(7)	(Ar. 1,4:30:31)

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	53		53	z	z_6	
IIIa	53		53	z_4,z_{23}	–	(Ar. 1,4:1,2,5:- and 1,4:1,2,6:-)
IV	53		53	z_4,z_{23}	–	
IIIa	53		53	z_4,z_{23},z_{32}	–	(Ar. 1,4:1,6,7,9:-)
IIIa	53		53	z_4,z_{24}	–	(Ar. 1,4:1,3,11:-)
IIIa	53		53	z_4,z_{32}	–	(Ar. 1,4:1,6,7:-). IP combined this with 53:z_4,z_{23},z_{32}:- (Ar. 1,4:1,6,7,9:-).
IIIb	53		53	z_{10}	z_{35}	(Ar. 1,4:27:21)
IIIa	53		53	z_{29}	–	(Ar. 1,4:16,18:-)
IIIb	53		53	z_{52}	z_{35}	(Ar. 1,4:26:21)
IIIb	53		53	z_{52}	z_{53}	(Ar. 1,4:26:25)
II	56		56	d	–	
II	56		56	e,n,x	1,7	
II	56		56	l,v	z_{39}	
II	56		56	l,z_{28}	–	
II	56		56	z	z_6	
IIIa	56		56	z_4,z_{23}	–	(Ar. 14:1,2,5:- and 14:1,2,6:-)
IIIa	56		56	z_4,z_{23},z_{32}	–	(Ar. 14:1,6,7,9:-)
II	56		56	z_{10}	e,n,x	
IIIa	56		56	z_{29}	–	(Ar. 14:16,18:-)
II	57		57	a	z_{42}	
IIIb	57		57	c	z:[z_{60}]	(Ar. 34:32:31:[44])
II	57		57	d	1,5	
II	57		57	g,[m],s,t	z_{42}	
II	57		57	g,t	–	
IIIb	57		57	i	e,n,x,z_{15}	(Ar. 34:33:28)
IIIb	57		57	i	z	(Ar. 34:33:31)
IIIb	57		57	k	e,n,x,z_{15}	(Ar. 34:29:28). CDC does not have this and not on Rohde's list.
IV	57		57	z_4,z_{23}	–	
IIIb	57		57	z_{10}	z	(Ar. 34:27:31)
II	58		58	a	z6	
II	58		58	b	1,5	
II	58		58	c	z_6	
II	58		58	d	z_6	
IIIb	58		58	i	e,n,x,z_{15}	(Ar. 1,33:33:28)
IIIb	58		58	k	z	(Ar. 1,33:29:31)
IIIb	58		58	l,v	e,n,x,z_{15}	(Ar. 1,33:23:28)
IIIb	58		58	l,v	z_{35}	(Ar. 1,33:23:21)
II	58		58	l,z_{13},z_{28}	z_6	
IIIb	58		58	r	e,n,x,z_{15}	(Ar. 1,33:24:28)
IIIb	58		58	r	z	(Ar. 1,33:24:31)
IIIb	58		58	r	z_{53}	(Ar. 1,33:24:25). May possess H phase Rz_{47} (Ar. 39) or Rz_{57} (Ar. 40) or Rz_{70} (Ar. Rz_{70}).
II	58		58	z_6	1,6	
II	58		58	z_{10}	1,6	
IIIb	58		58	z_{10}	e,n,x,z_{15}	(Ar. 1,33:27:28)
II	58		58	z_{10}	z_6	
IIIb	58		58	z_{10}	z_{53}	(Ar. 1,33:27:25). May possess H phase Rz_{50} (Ar. 42).

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
II	58		58	z_{39}	e,n,x,z_{15}	
IIIb	58		58	z_{52}	z	(Ar. 1,33:26:31)
IIIb	58		58	z_{52}	z_{35}	(Ar. 1,33:26:21)
IIIb	59		59	c	e,n,x,z_{15}	(Ar. 19:32:28)
IIIb	59		59	i	e,n,x,z_{15}	(Ar. 19:33:28)
IIIb	59		59	i	z	(Ar. 19:33:31)
IIIb	59		59	i	z_{35}	(Ar. 19:33:21)
IIIb	59		59	k	z_{53}	(Ar. 19:29:25)
IIIb	59		59	(k)	e,n,x,z_{15}	(Ar. 19:22:28)
IIIb	59		59	(k)	z	(Ar. 19:22:31)
IIIb	59		59	(k)	z_{35}	(Ar. 19:22:21)
IIIb	59		59	l,v	z	(Ar. 19:23:31)
IIIb	59		59	l,v	z_{53}	(Ar. 19:23:25)
IIIb	59		59	r	z_{35}	(Ar. 19:24:21)
II	59		1,59	z	z_6	
IIIa	59		59	z_4,z_{23}	–	(Ar. 19:1,2,5:- and 19:1,2,6:-)
IIIb	59		59	z_{10}	z_{53}	(Ar. 19:27:25)
IIIb	59		59	z_{10}	z_{57}	(Ar. 19:27:40)
IIIa	59		59	z_{29}	–	(Ar. 19:16,18:-)
IIIa	59		59	z_{36}	–	(Ar. 19:17,20:-)
IIIb	59		59	z_{52}	[z_{53}]	(Ar. 19:26:[25])
II	60		60	b	[1,16]	
IIIb	60		60	i	–	(Ar. 24:33:-). May possess H phase Rz_{50} (Ar. 42).
IIIb	60		60	i	e,n,x,z_{15}	(Ar. 24:33:28)
IIIb	60		60	i	z_{35}	(Ar. 24:33:21)
IIIb	60		60	k	z	(Ar. 24:29:31)
IIIb	60		60	k	z_{35}	(Ar. 24:29:21)
IIIb	60		60	(k)	z_{53}	(Ar. 24:22:25)
IIIb	60		60	l,v	z	(Ar. 24:23:31)
IIIb	60		60	r	e,n,x,z_{15}	(Ar. 24:24:28)
IIIb	60		60	r	z	(Ar. 24:24:31)
IIIb	60		60	r	z_{35}	(Ar. 24:24:21)
IIIb	60		60	r	z_{53}	(Ar. 24:24:25)
IIIb	60		60	z_{10}	z	(Ar. 24:27:31)
IIIb	60		60	z_{10}	z_{35}	(Ar. 24:27:21)
IIIb	60		60	z_{10}	z_{53}	(Ar. 24:27:25)
II	60		60	z_{29}	e,n,x	
V	60		60	z_{41}	–	
IIIb	60		60	z_{52}	1,5,[7]	(Ar. 24:26:30)
IIIb	60		60	z_{52}	z	(Ar. 24:26:31)
IIIb	60		60	z_{52}	z_{35}	(Ar. 24:26:21)
IIIb	60		60	z_{52}	z_{53}	(Ar. 24:26:25)
IIIb	61		61	c	1,5,(7)	(Ar. 26:32:30)
IIIb	61		61	c	z_{35}	(Ar. 26:32:21)
IIIb	61		61	i	e,n,x,z_{15}	(Ar. 26:33:28)
IIIb	61		61	i	z	(Ar. 26:33:31)
IIIb	61		61	i	z_{35}	(Ar. 26:33:21)
IIIb	61		61	i	z_{53}	(Ar. 26:33:25)

SUBSPECIES	O ANTIGEN GROUP	SEROTYPE	O ANTIGENS	PHASE 1	PHASE 2	NOTE
IIIb	61		61	k	1,5,(7)	(Ar. 26:29:30)
IIIb	61		61	k	z_{35}	(Ar. 26:29:21). CDC does not have this.
IIIb	61		61	(k)	z_{53}	(Ar. 26:22:25)
IIIb	61		61	l,v	1,5,7:[z_{57}]	(Ar. 26:23:30:[40])
IIIb	61		61	l,v	z	(Ar. 26:23:31)
IIIb	61		61	l,v	z_{35}	(Ar. 26:23:21)
IIIb	61		61	r	1,5,7	(Ar. 26:24:30)
IIIb	61		61	r	z	(Ar. 26:24:31)
IIIb	61		61	r	z_{35}	(Ar. 26:24:21)
IIIb	61		61	r	z_{53}	(Ar. 26:24:25). May possess H phase Rz_{47} (Ar. 39).
IIIb	61		61	z_{10}	z_{35}	(Ar. 26:27:21)
V	61		61	z_{35}	-	
IIIb	61		61	z_{52}	1,5,7	(Ar. 26:26:30)
IIIb	61		61	z_{52}	z	(Ar. 26:26:31)
IIIb	61		61	z_{52}	z_{35}	(Ar. 26:26:21)
IIIb	61		61	z_{52}	z_{53}	(Ar. 26:26:25)
IIIa	62		62	g,z_{51}	–	(Ar. 6:13,14:-)
IIIa	62		62	z_4,z_{23}	–	(Ar. 6:1,2,5:-)
IIIa	62		62	z_4,z_{32}	–	(Ar. 6:1,7,8:-)
IIIa	62		62	z_{29}	–	(Ar. 6:17,18:-)
IIIa	62		62	z_{36}	–	(Ar. 6:17,20:-)
IIIa	63		63	g,z_{51}	–	(Ar. 8:13,14:-)
IIIa	63		63	z_4,z_{23}	–	(Ar. 8:1,2,5:-)
IIIa	63		63	z_4,z_{32}	–	(Ar. 8:1,7,8:-)
IIIa	63		63	z_{36}	–	(Ar. 8:17,20:-)
IIIb	65		65	c	1,5,7	(Ar. 30:32:30)
IIIb	65		65	c	z	(Ar. 30:32:31)
IIIb	65		65	c	z_{53}	(Ar. 30:32:25)
II	65		65	g,t	–	
IIIb	65		65	i	e,n,x,z_{15}	(Ar. 30:33:28)
IIIb	65		65	(k)	z	(Ar. 30:22:31)
IIIb	65		65	(k)	z_{35}	(Ar. 30:22:21)
IIIb	65		65	(k)	z_{53}	(Ar. 30:22:25)
IIIb	65		65	l,v	e,n,x,z_{15}	(Ar. 30:23:28)
IIIb	65		65	l,v	z	(Ar. 30:23:31)
IIIb	65		65	l,v	z_{35}	(Ar. 30:23:21)
IIIb	65		65	l,v	z_{53}	(Ar. 30:23:25)
IIIb	65		65	r	z_{35}	(Ar. 30:24:21)
IIIb	65		65	z_{10}	e,n,x,z_{15}	(Ar. 30:27:28)
IIIb	65		65	z_{10}	z	(Ar. 30:27:31)
IIIb	65		65	z_{52}	e,n,x,z_{15}	(Ar. 30:26:28)
IIIb	65		65	z_{52}	z	(Ar. 30:26:31)
IIIb	65		65	z_{52}	z_{35}	(Ar. 30:26:21)
IIIb	65		65	z_{52}	z_{53}	(Ar. 30:26:25)
II	65		65	–	1,6	
V	66		66	z_{39}	–	
V	66		66	z_{81}	–	

Bacto® Shigella Antisera

Shigella Antiserum Poly Group A · Shigella Antiserum Poly Group A_1 · Shigella Antiserum Poly Group B · Shigella Antiserum Poly Group C · Shigella Antiserum Poly Group C_1 Shigella Antiserum Poly Group C_2 · Shigella Antiserum Poly Group D · Alkalescens-Dispar Antiserum Poly

Intended Use

Bacto Shigella Antisera are used for identifying *Shigella* species by the slide agglutination test. Bacto Alkalescens-Dispar Antiserum Poly is used for identifying the Alkalescens-Dispar Group of microorganisms by the slide agglutination test.

Summary and Explanation

Shigella species cause the human diarrheal disease shigellosis (classic bacillary dysentery). The range of illness is from mild diarrhea to severe dysentery characterized by abdominal cramps and frequent passage of bloody, mucoid stools. While the disease is usually self-limiting, it can be life threatening to the young, the elderly and malnourished persons. *Shigella* species are carried primarily in humans and are not generally distributed in nature. While transmission is usually direct person-to-person and through contaminated water supplies, foodborne outbreaks do occur.

The genus *Shigella* belongs to the family Enterobacteriaceae. *Shigella* species are facultatively anaerobic, gram-negative bacilli that typically are oxidase negative, lactose negative, H_2S negative and non-gas producing. *Shigella* and *Escherichia* are genetically related. Certain strains of *E. coli* may resemble *Shigella* biochemically because both can be lactose-negative, nonmotile or non-gas-producing. These anaerogenic, nonmotile types have historically been called the Alkalescens-Dispar group and are presently classified as *E. coli*.

Serological testing with polyvalent and group specific antisera should be used to confirm the identification of isolates that are morphologically and biochemically identified as *Shigella* species. *Shigella* species are nonmotile, so serological identification is based on somatic ("O") antigens. However, some strains have envelope antigens that prevent agglutination in somatic antisera. Heating the suspension at 100°C for 15-60 minutes destroys these interfering antigens. The four named species or serotypes of *Shigella* are *S. dysenteriae* (10 serovars), *S. flexneri* (six serovars), *S. boydii* (15 serovars) and *S. sonnei*. For a complete and current explanation of the classification of *Shigella*, consult appropriate references.[1]

The Alkalescens-Dispar group of microorganisms is currently recognized as anaerogenic, nonmotile biotypes of *E. coli*. Consult appropriate references for biochemical tests specific for differentiating these strains from *Shigella*.[1-6]

User Quality Control

Identity Specifications

Shigella Poly Antisera Group A-D

Lyophilized appearance: Light gold to amber, button to powdered cake.

Rehydrated appearance: Light gold to amber, clear liquid.

Alkalescens-Dispar Antiserum Poly

Lyophilized appearance: Light gold to amber, button to powdered cake.

Rehydrated appearance: Light gold to amber, clear liquid.

Culture Response

Rehydrate Shigella Antiserum Poly Groups A-D and Alkalescens-Dispar Antiserum Poly per label directions. Perform the slide agglutination test using appropriate QC Antigens Shigella Group A-D or Alkalescens-Dispar.

SHIGELLA ANTISERUM	QC ANTIGEN	REACTION
Poly Group A	Shigella Group A	3+
Poly Group A_1	Shigella Group A_1	3+
Poly Group B	Shigella Group B	3+
Poly Group C	Shigella Group C	3+
Poly Group C_1	Shigella Group C_1	3+
Poly Group C_2	Shigella Group C_2	3+
Poly Group D	Shigella Group D	3+
Alkalescens-Dispar Antiserum Poly	Alkalescens-Dispar Group 1	3+

Principles of the Procedure

Identification of *Shigella* species includes the isolation of the microorganism, biochemical identification and serological confirmation. Serological confirmation involves the reaction in which the microorganism (antigen) reacts with its corresponding antibody. This *in vitro* reaction produces macroscopic clumping called agglutination. The desired homologous reaction is rapid, does not dissociate (high avidity) and binds strongly (high affinity).

Because a microorganism (antigen) may agglutinate with antibodies produced in response to other species, heterologous reactions are possible. These are characterized as weak in strength or slow in formation.

Such unexpected and, perhaps, unpredictable reactions may lead to some confusion in serological identification. Therefore, a positive homologous agglutination reaction should support the morphological and biochemical identification of the microorganism. Homologous reactions occur rapidly and are strong. Heterologous reactions form slowly and are weak.

Reagents

Shigella Antisera Poly and Alkalescens-Dispar Antiserum Poly are lyophilized, polyclonal rabbit antisera containing approximately 0.04% Thimerosal as a preservative.

Shigella Antisera Poly are absorbed when necessary to render each lot of serum as specific as practical. Antisera are absorbed to a certain point without reducing homologous reactions to an unsatisfactory level. They have been absorbed inter- and intra-specifically except that *Shigella* antisera are not prepared from or tested for:

S. dysenteriae provisional serotypes,

S. flexneri X and Y variants, or

Alkalescens-Dispar Groups other than types 1-4.

ANTISERUM	REACTS WITH
Shigella Antiserum Poly Group A	*S. dysenteriae* types 1-7
Shigella Antiserum Poly Group A_1	*S. dysenteriae* types 8ab, 8ac, 9, 10
Shigella Antiserum Poly Group B	*S. flexneri* types 1-6
Shigella Antiserum Poly Group C	*S. boydii* types 1-7
Shigella Antiserum Poly Group C_1	*S. boydii* types 8-11
Shigella Antiserum Poly Group C_2	*S. boydii* types 12-15
Shigella Antiserum Poly Group D	*S. sonnei* I and II
Alkalescens-Dispar Antiserum Poly	Alkalescens-Dispar Groups 1,2,3 and 4

When rehydrated and used as described, each vial of Shigella Antisera Poly and Alkalescens-Dispar Antiserum Poly contains sufficient reagent for 60 slide tests.

Precautions

1. For In Vitro Diagnostic Use.
2. **Shigella Antiserum Poly Group A**
   Shigella Antiserum Poly Group A_1
   Shigella Antiserum Poly Group B
   Shigella Antiserum Poly Group C
   Shigella Antiserum Poly Group C_1
   Shigella Antiserum Poly Group C_2
   Shigella Antiserum Poly Group D
   Alkalescens-Dispar Antiserum Poly

   The Packaging of This Product Contains Dry Natural Rubber.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated Shigella Antisera Poly and Alkalescens-Dispar Antiserum Poly at 2-8°C. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Lyophilized Shigella Antisera Poly and Alkalescens-Dispar Poly are stable through the expiration date on the label when stored as described.

Rehydrated Shigella Antisera Poly and Alkalescens-Dispar Antiserum Poly that are cloudy or have a precipitate at any time during the period of use should be discarded.

Procedure

Materials Provided

Shigella Antisera Poly Group A
Shigella Antisera Poly Group A_1
Shigella Antisera Poly Group B
Shigella Antisera Poly Group C
Shigella Antisera Poly Group C_1
Shigella Antisera Poly Group C_2
Shigella Antisera Poly Group D
Alkalescens-Dispar Antiserum Poly

Materials Required But Not Provided

Agglutination slides with 1 inch squares
Applicator sticks
Waterbath, boiling
Sterile 0.85% NaCl solution
QC Antigen Shigella Groups
QC Antigen Alkalescens-Dispar Group 1

Reagent Preparation

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergents.

Shigella Antisera Poly and Alkalescens-Dispar Antiserum Poly: To rehydrate, add 3 ml of sterile 0.85% NaCl solution and rotate gently to completely dissolve the contents. The rehydrated antiserum is considered a 1:2 working dilution. Subsequent dilutions are based on this as a starting dilution.

Specimen Collection and Preparation

From clinical specimens, *Shigella* can be recovered on selective differential media such as Hektoen Enteric Agar or XLD Agar. For specific recommendations, consult appropriate references.[2,3,4] Determine that a pure culture of the microorganism has been obtained and that biochemical test reactions are consistent with the identification of the organism as a *Shigella* species. After these criteria are met, serological identification can be performed.

Shigella can be recovered from various types of foods when samples are processed to recover injured microorganisms and to prevent overgrowth of competing microorganisms. Consult appropriate references for recommended procedures when testing food samples.[5,6] After following an established protocol, determine that a pure culture of the microorganism has been obtained. Biochemical test reactions

should be consistent with the identification of the organism as a *Shigella* species. After these criteria are met, serological identification can be performed.

Test Procedure

Use this procedure to test the isolate with each selected Shigella Antisera Poly or Alkalescens-Dispar Antiserum Poly.

1. **Shigella Antiserum**: Dispense 1 drop (35 µl) of the antiserum to be tested on an agglutination slide.
2. **Negative control**: Dispense 1 drop of sterile 0.85% NaCl solution on an agglutination slide.
3. **Test isolate**: Transfer a loopful of growth of the test organism to the drops of antisera and NaCl solution and mix thoroughly.
4. **Positive control**: Dispense 1 drop of the Shigella Antiserum to be tested on an agglutination slide. Add 1 drop of the appropriate QC Antigen.
5. Mix each reaction area with a separate applicator stick and rock for 1 minute. Read for agglutination.

Results

1. Read and record results as follows:
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. **Positive control**: Should show 3+ or greater agglutination.
3. **Negative control**: Should show no agglutination. If autoagglutination occurs, tests results cannot be reported. To test for autoagglutination, transfer the isolate to selective medium.
4. **Test isolates**: 3+ or greater agglutination within 1-2 minutes is a positive result.
5. If no agglutination occurs or agglutination is weak, follow this procedure to remove blocking envelope antigens:
   - Prepare a dense suspension of the isolate from an agar medium in 3-5 ml of sterile 0.85% NaCl solution.
   - Heat in a boiling waterbath for 30-60 minutes and cool. The suspension should not show precipitation after heating. If this occurs, select another colony for testing.
   - Centrifuge at 1,000 rpm for 10-15 minutes.
   - Aspirate and discard the supernatant.
   - Resuspend the sediment in 0.5 ml sterile 0.85% NaCl solution.
   - Use a drop of the suspension and perform the slide agglutination test as outlined above.
6. A partial (less than 3+) or delayed agglutination reaction should be considered negative.
7. If test results for either the positive control or negative control are not as described, the test is invalid and results cannot be reported.

Limitations of the Procedure

1. Correct interpretation of serological reactions depends on culture purity, morphological characteristics and biochemical reactions that are consistent with identification of the microorganism as a *Shigella* species.
2. Serological methods alone cannot identify the isolate as a *Shigella* species.
3. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent making a smooth suspension of the microorganism or cause evaporation or precipitation of the test mixture. False-positive reactions may occur.
4. Rough culture isolates do occur and will agglutinate spontaneously, causing agglutination of the negative control (autoagglutination). Smooth colonies must be selected and tested in serological procedures.
5. Shigella Antisera Poly and Alkalescens-Dispar Poly have been tested using cultures taken directly from agar media. These antisera have not been tested using antigen suspensions in NaCl solution or other diluents. If the user applies variations in the recommended steps, each lot of antiserum must be tested with known control cultures to verify expected reactions under the modified procedure.

References

1. **Ewing, W.H. (ed.).** 1986. Edwards and Ewing's identification of Enterobacteriaceae, 4th ed. Elsevier Science Publishing Co., Inc., New York, NY.
2. **Gray, L. D.** 1995. *Escherichia, Salmonella, Shigella and Yersinia*, p. 450-456. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
3. **Baron, E. J., L. R. Peterson, and S. M. Finegold.** 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc., St. Louis, MO.
4. **Pezzlo, M. (ed.).** 1992. Aerobic bacteriology, p. 1.0.1-1.20.47. *In* H. D. Isenberg (ed.), Clinical microbiology procedures handbook, vol. 1. American Society for Microbiology, Washington, D.C.
5. **Andrews, W. H., G. A. June, and P. S. Sherrod.** 1995. Shigella, p. 6.01-6.06. *In* FDA Bacteriological Analytical Manual, 8th ed. AOAC International, Gaithersburg, MD.
6. **Vanderzant, C., and D. F. Splittstoesser (eds.).** 1992. Compendium of methods for the microbiological examination of foods, 3rd edition. American Public Health Association, Washington, D.C.

Packaging

Shigella Antiserum Poly Group A	3 ml	2834-47
Shigella Antiserum Poly Group A_1	3 ml	2776-47
Shigella Antiserum Poly Group B	3 ml	2835-47
Shigella Antiserum Poly Group C	3 ml	2836-47
Shigella Antiserum Poly Group C_1	3 ml	2777-47
Shigella Antiserum Poly Group C_2	3 ml	2778-47
Shigella Antiserum Poly Group D	3 ml	2837-47
Alkalescens-Dispar Antiserum Poly	3 ml	2838-47

Bacto® Streptococcus Antigens and Antisera

Streptococcus Antiserum Group A · Streptococcus Antiserum Group B · Streptococcus Antigen Group A · Streptococcus Antigen Group B

Intended Use

Bacto Streptococcus Antisera are used in the serological grouping of Group A and Group B streptococci by the capillary tube precipitin technique.

Bacto Streptococcus Antigens are used in the quality control testing of Bacto Streptococcus Antisera Groups A and B.

Summary and Explanation

Streptococci are gram-positive cocci that are facultative anaerobes. They are catalase negative and may be alpha-, beta- or non-hemolytic.

Streptococcus pyogenes (Group A) is the most common cause of bacterial pharyngitis in children. Symptoms include fever, pharyngeal erythema and edema, tonsillar exudate and enlarged cervical lymph nodes. Physical findings alone cannot distinguish between Group A streptococcal pharyngitis and pharyngitis caused by other agents such as viruses or mycoplasma. Other infections caused by Group A streptococci include scarlet fever, impetigo and skin infections that range from mild to severe with toxic shock symptoms and tissue necrosis.

User Quality Control

Identity Specifications

Streptococcus Antisera Groups A and B

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated Appearance: Light gold to amber, clear liquid.

Streptococcus Antigens Groups A and B

Solution Appearance: Colorless to light yellow, clear liquid.

Quality Control Results

Rehydrate Streptococcus Antisera per label directions. Perform the capillary tube precipitin technique using appropriate Streptococcus Antigens.

ANTISERUM	ANTIGEN	REACTION
Streptococcus Antiserum Group A	Streptococcus Antigen Group A	Positive
Streptococcus Antiserum Group A	Streptococcus Antigen Group B	Negative
Streptococcus Antiserum Group B	Streptococcus Antigen Group B	Positive
Streptococcus Antiserum Group B	Streptococcus Antigen Group A	Negative

Streptococcus agalactiae (Group B streptococci) causes neonatal sepsis and meningitis. Other infections in children and adults include bacteremia, endocarditis and pneumonia.

Identification of Group A and Group B streptococci includes isolation of the microorganism and biochemical and serological identification. Serological identification involves the reaction in which the microorganism (antigen) reacts with its corresponding antibody. This *in vitro* reaction produces fine particles called precipitation.

Principles of the Procedure

Beta-hemolytic streptococci Group A and Group B have carbohydrate group-specific antigens that can be extracted. Streptococcus Antisera and Streptococcus Antigens are used together in the capillary precipitin test to serologically identify the microorganisms.

Reagents

Streptococcus Antisera Groups A and B are lyophilized, polyclonal rabbit antisera containing approximately 0.02% Thimerosal as a preservative. When rehydrated and used as described, each vial of Streptococcus Antisera contains sufficient reagent for 50 precipitin tests.

Streptococcus Antigens Groups A and B are ready-to-use cellular extracts of *S. pyogenes* and *S. agalactiae*, respectively, containing Thimerosal as a preservative. When used as described, each vial of Streptococcus Antigens contains sufficient reagent for 50 precipitin tests.

Precautions

1. For In Vitro Diagnostic Use.
2. **Streptococcus Antiserum Group A**
   Streptococcus Antiserum Group B
   Streptococcus Antigen Group A
   Streptococcus Antigen Group B
   The Packaging of This Product Contains Dry Natural Rubber.
3. Follow proper established laboratory procedures in handling and disposing of infectious materials.
4. Streptococcus Antigens are not intended for use in the immunization of humans or animals.

Storage

Store lyophilized and rehydrated Streptococcus Antisera at 2-8°C.

Store Streptococcus Antigens at 2-8°C.

Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications Rehydrated Streptococcus Antiserum that is cloudy or has a precipitate anytime during use should be discarded.

Antigens must be smooth uniform suspensions. Examine antigen vials for precipitation before use. Suspensions with precipitation are not usable and should be discarded.

Procedure

Materials Provided

Streptococcus Antiserum Group A
Streptococcus Antiserum Group B
Streptococcus Antigen Group A
Streptococcus Antigen Group B

Materials Required but not Provided

Capillary tubes
Sterile distilled or deionized water
Plasticine block

Reagent Preparation

Equilibrate all materials to room temperature before performing the tests. Ensure that glassware and pipettes are clean and free of residues such as detergent.

Streptococcus Antisera: To rehydrate, add 1 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

Streptococcus Antigens are ready to use.

Specimen Collection and Preparation

Group A and Group B streptococci can be recovered on routine culture media such as sheep blood agar. For specific recommendations on isolation and presumptive identification, consult appropriate references.[1,2] Determine that a pure culture of the microorganism has been obtained and that biochemical test reactions are consistent with the identification of the organism as a Group A or Group B *Streptococcus*. After these criteria are met, serological identification can be performed.

Test antigen extract: To prepare, extract the carbohydrate, group-specific antigen from a pure culture of the microorganism by the Lancefield hot HCl, autoclave, enzyme or other such method. For specific information on these methods, consult appropriate references.[1,2]

Test Procedure

Add the antiserum to the capillary tube first so that it will be layered above the extract.

1. **Streptococcus Antiserum**: Dip a capillary tube into the antiserum and allow a column of 2-3 cm to rise into the tube.
2. Holding the forefinger on the top end of the capillary tube, remove the tube from the antiserum vial. Clean the tip with a lint-free tissue to remove excess antiserum. Do not allow air into the tube. If this occurs, discard the tube and begin again.
3. **Test antigen extract**: Dip the capillary tube into the prepared extract until the antiserum and the antigen come in contact with each other. If an air bubble separates them, discard the tube and repeat steps 1-3.
4. Remove the tube from the extract and invert slightly to allow the column to move to the center of the tube.
5. Wipe excess fluid from the tube and insert in a plasticine block, antiserum end upward. Wipe the capillary tube so that it is free of fingerprints or any material that might interfere with a clear reading.
6. **Positive control**: Repeat steps 1-5, using (in step 3) a Streptococcus Antigen (Group A or B) that is *homologous* to the antiserum used in step 1.
7. **Negative control**: Repeat steps 1-5, using (in step 3) a Streptococcus Antigen (Group A or B) that is *not homologous* to the antiserum used in step 1.
8. Incubate all capillary tubes at 22 ± 2°C for 5 minutes. Examine for the formation of a white precipitate at the interface of the antiserum and the antigen. Observe at 5 minute intervals for up to 30 minutes.

Results

1. A strongly positive reaction develops within 5 minutes, a weaker reaction develops within 30 minutes.
2. Disregard any precipitate that appears after 30 minutes.
3. Precipitation in a tube indicates that the test antigen extract is homologous to the Streptococcus Antiserum Group A or Group B used.
4. Observe at 5 minute intervals within the 30 minute period because the precipitate may dissolve (prozone phenomenon).

Limitations of the Procedure

1. Correct interpretation of serological reactions depends on culture purity as well as on morphological characteristics and biochemical reactions that are consistent with identification of the microorganism as *S. pyogenes* or *S. agalactiae*.
2. Serological methods alone cannot identify the isolate as *S. pyogenes* or *S. agalactiae*.

References

1. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
2. **Isenberg, H. D. (ed.).** 1992. Clinical microbiology procedures handbook, vol. 2. American Society for Microbiology, Washington, D.C.

Packaging

Streptococcus Antiserum Group A	1 ml	2672-50
Streptococcus Antiserum Group B	1 ml	2741-50
Streptococcus Antigen Group A	1 ml	2978-50
Streptococcus Antigen Group B	1 ml	2979-50

Bacto® USR Antigen
Bacto USR Test Control Serum Set

Intended Use

Bacto USR Antigen is nontreponemal antigen used in the Unheated Serum Reagin (USR) Test.[1]

Bacto USR Test Control Serum Set is standardized human sera used for controlling the USR Test.

Summary and Explanation

Treponema pallidum is the causative agent of syphilis. Syphilis is a chronic infection with clinical manifestations that occur in distinct stages. Specific laboratory tests are recommended for the detection of each stage of the disease.

During the primary stage, treponemes present in the characteristic lesion, a chancre, are detectable by dark-field microscopy[2] or by the Direct Fluorescent Antibody Test for *T. pallidum* (DFA-TP). During the secondary stage, most serological tests for syphilis are reactive and treponemes may be found in the lesions by using dark-field microscopy. The latent period, which is asymptomatic, may last for years. Serological tests are usually reactive in the early latent period, but the reactivity of non-treponemal tests decreases during the late latent period. Symptoms of the tertiary or late stage of syphilis may occur 10-20 years after initial infection. Approximately 71% of patients in the tertiary stage of syphilis have reactive non-treponemal tests.[3,4] In the tertiary stage, treponemal tests will usually be reactive and are the only basis for diagnosis. The lesions in tertiary syphilis will have few treponemes. Neurosyphilis and late cardiovascular syphilis are complications of tertiary syphilis.

Since the clinical manifestations of syphilis can be confused with other infectious or noninfectious conditions, proper diagnosis must include microscopic examination of lesion material and serological results.[3]

The USR Antigen is a nontreponemal antigen composed of cardiolipin, cholesterol and lecithin. The antigen is a modification of the VDRL Antigen emulsion in a USR suspending solution. The antigen suspension contains choline chloride, which enhances the reactivity of reagin in unheated serum.[5] The USR Test is suitable for qualitative as well as quantitative determinations.

Nontreponemal tests measure reagin, an antibody-like substance that can be detected in syphilitic serum. Reagin is also occasionally found in the serum of persons with other acute or chronic diseases. Reactive nontreponemal tests aid in the diagnosis of latent subclinical syphilis and are effective tools for detecting cases in epidemiological investigations. Nontreponemal tests are superior to treponemal tests for following the response to therapy.[3]

Nontreponemal antigen tests are not entirely specific for syphilis, nor do they have satisfactory sensitivity in all stages of syphilis. Whenever the results of a nontreponemal antigen test disagree with the clinical impression, a treponemal antigen test such as the Fluorescent Treponemal Antibody-Absorption (FTA-ABS)[2,3] should be performed. Nontreponemal tests such as the USR, RPR and VDRL tests are used to screen patient serum. Treponemal tests such as the FTA-ABS are used for confirmation.

The likelihood of obtaining a reactive USR Test result in various stages of untreated syphilis has been reported as follows:[3]

STAGES OF UNTREATED SYPHILIS	% REACTIVE
Primary	80
Secondary	100
Latent	95

User Quality Control

Identity Specifications

USR Antigen

Appearance: Milky white, opaque suspension after gentle mixing.

Nontreponemal Antigen Reactive Serum

Lyophilized Appearance: White to cream colored, button to powdered cake.

Rehydrated Appearance: Light gold to light amber, clear to slightly opalescent.

USR Weakly Reactive Serum

Lyophilized Appearance: White to cream colored, button to powdered cake.

Rehydrated Appearance: Light gold to light amber, clear to slightly opalescent.

Nontreponemal Antigen Nonreactive Serum

Lyophilized Appearance: White to cream colored, button to powdered cake.

Rehydrated Appearance: Light gold to light amber, clear to slightly opalescent.

Performance Response

Rehydrate the sera contained in the USR Test Control Serum Set per label directions. Perform the USR Test according to the Test Procedure. Each serum in the USR Test Control Serum Set should yield appropriate reactions when tested with the USR Antigen.

Use the USR Antigen suspension only if it produces the expected reactivity with the control sera.

Principles of the Procedure

In the USR Test procedure, the patient's unheated serum is mixed with a buffered saline suspension of USR Antigen containing cardiolipin, lecithin and cholesterol. The combination of reagin and USR Antigen forms microscopic clumping called flocculation.

Reagents

USR Antigen is 0.03% cardiolipin and 0.9% cholesterol dissolved in absolute alcohol with sufficient lecithin (approximately 0.2%) to produce standard reactivity. The antigen is suspended in a solution

containing EDTA, choline chloride and phosphate with 0.2% Thimerosal as a preservative.[7,8]

USR Test Control Serum Set contains 3 ml, each, of the following lyophilized human sera: Nontreponemal Antigen Reactive Serum, USR Weakly Reactive Serum and Nontreponemal Antigen Nonreactive Serum. These reagents are standardized to provide reactive, weakly reactive and nonreactive readings, respectively, when tested according to the USR Test procedure.

Precautions

1. For In Vitro Diagnostic Use.
2. WARNING! POTENTIAL BIOHAZARDOUS REAGENTS. Each donor unit used in preparation of USR Antigen and USR Test Control Serum Set was tested by an FDA approved method for the presence of the antibody to human immunodeficiency virus (HIV) and for hepatitis B surface antigen and found negative (were not repeatedly reactive).

   Because no test method can offer complete assurance that HIV, hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen.[9]
3. **USR Test Control Serum Set**

   The Packaging of This Product Contains Dry Natural Rubber.
4. Observe universal blood and body fluid precautions in handling and disposing of specimens.[10,11]
5. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store USR Antigen at 2-8°C. If the original 3 ml quantity exceeds what is needed for one testing period, transfer the remainder from the first day's use to one or more aliquot vials and store at 2-8°C.

Store the lyophilized sera in the USR Test Control Serum Set at 2-8°C. Store the rehydrated control sera at 2-8°C or divide into aliquots sufficient for one day of testing and store at -20°C. Do not thaw and refreeze.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

USR Antigen
USR Test Control Serum Set

Materials Required But Not Provided

0.9% saline
Nondisposable glass syringe, 1-2 cc
Nondisposable calibrated 18-gauge needles without bevel
Micropipettor, 50 µl
Pipettes, serological, graduated to tip:
1.0 ml, graduated in 1/100 ml
5.0 ml, graduated in 1/10 ml
10.0 ml, graduated in 1/10 ml
Slides, 2 x 3 inches with paraffin or ceramic rings approximately 14 mm in diameter and high enough to prevent spillage during rotation.
Slide holder for 2 x 3 inch slides
Mechanical rotator, adjustable to 180 ± 2 rpm circumscribing a circle 19 mm in diameter on a horizontal plane.
Light microscope with 10X ocular and 10X objective
Sterile distilled or deionized water
Absolute alcohol
Acetone
Timer

Reagent Preparation

USR Antigen is ready to use.

Equilibrate all materials to room temperature (23-29°C) before performing the tests. Ensure that all glassware and pipettes are clean and free of detergent residues.

USR Test Control Serum Set: To rehydrate the control sera, add 3 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

Specimen Collection and Preparation

Collect a blood specimen by aseptic venipuncture into a clean, dry tube without anticoagulant. After the specimen has clotted, centrifuge the specimen at 1,500-2,000 rpm for five minutes to obtain test serum. Store serum specimens at room temperature for no longer than 4 hours; for prolonged storage, keep at 2-8°C for up to 5 days or maintain below -20°C. Serum specimens must be clear, free of hemolysis and show no visible evidence of bacterial contamination, such as turbidity or particulate matter. Refer to appropriate references for more information on collection of specimens.[1,3,12]

Test Procedure

Preparation of Specific Glassware

Syringes with needles:

1. Prerinse with tap water.
2. Soak and hand wash thoroughly in a glassware detergent solution.
3. Rinse with tap water 6-8 times.
4. Rinse with unused distilled or deionized water.
5. Rinse with absolute alcohol.
6. Rinse with acetone.
7. Air dry until the acetone odor is completely eliminated.
8. Remove needles from syringes for storage.

Ceramic-ringed slides:

1. Prerinse with tap water.
2. Wash with a glassware detergent solution. Avoid prolonged soaking of ceramic-ringed slides in detergent solution because the ceramic rings will become brittle and flake off.
3. Rinse with tap water 3-4 times.
4. Rinse with unused distilled or deionized water.
5. Wipe dry with a clean lint-free cloth. If cleaned slides do not allow serum to spread evenly within the inner surface of the circle, proceed as follows.
6. Scrub the slides with a nonscratching cleanser.
7. Rinse, dry and polish with a clean, lint-free cloth.

Testing the Accuracy of the Antigen Suspension Needle

1. The accuracy of the test depends on the amount of antigen suspension used. Check the calibration of the needle periodically to ensure delivery of the correct volume of USR Antigen suspension.
2. For the qualitative and quantitative tests on serum, dispense the antigen suspension from a syringe fitted with an 18-gauge needle without bevel that will deliver 45 ± 1 drops (22 ml) of antigen suspension per ml when held vertically.
3. Place the needle on a 1-2 ml syringe. Fill the syringe with 1 ml of USR Antigen suspension. Holding the syringe in a vertical position, count the number of drops delivered in 1.0 ml. The needle is correctly calibrated if 45 ± 1 drops are delivered in 1.0 ml.
4. Adjust or replace the needle if it does not meet this specification. Repeat calibration on the new or adjusted needle.

Testing and Storing the USR Antigen Suspension

1. Store the antigen suspension at 2-8°C.
2. For daily use, withdraw a sufficient amount of suspension for 1 day's testing and store the remainder at 2-8°C. Antigen suspensions must be at room temperature (23-29°C) before use.
2. Test antigen suspension reactivity with the Reactive, Weakly Reactive and Nonreactive control sera. Use the antigen suspension only if it produces the expected reactivity with the control sera.
3. After each day of use, clean the dispensing needle, bottle and syringe by rinsing with water, alcohol and acetone, in that order. Remove the needle from the syringe after cleaning.

USR Qualitative Slide Test on Serum[1,13]

For reliable and reproducible test results, the USR Antigen suspension, controls and test specimens must be at 23-29°C when tests are performed.

1. Pipette 50 µl of unheated serum into one ring of a paraffin- or ceramic-ringed slide using a safety pipetting device. Do not use a glass slide with concavities, wells or glass rings. Spread the serum with a circular motion of the pipette tip so that the serum covers the entire inner surface of the paraffin or ceramic ring. Include control sera when performing the test.
2. Gently resuspend the USR Antigen and withdraw the desired quantity with a syringe and needle.
3. Hold the syringe and needle containing the USR Antigen suspension in a vertical position. Dispense several drops to clear the needle of air. Add exactly 1 free-falling drop (22 µl) of antigen suspension to each circle containing serum. Do not allow the needle to touch the serum.
4. Place the slide on the mechanical rotator. Rotate the slide for 4 minutes at 180 ± 2 rpm. If the environment is dry, cover the slides with a moist, humidifying cover during rotation to prevent excessive evaporation.
5. Immediately after rotating the slide, remove the slide from the rotator and read the test results microscopically, using a 10X ocular and a 10X objective.

Results – Qualitative Test

1. Read and record results as follows:
   Medium to large clumps - Reactive (R)
   Small clumps - Weakly reactive (WR)
   No clumping or very slight roughness - Nonreactive (N)
2. Verify that the control sera results are as expected. If reactions are not as expected, the test is invalid and results cannot be reported.
3. Perform a quantitative test on all serum specimens that produce Reactive, Weakly Reactive or "rough" Nonreactive results, since prozone reactions are occasionally encountered.

USR Quantitative Test[1,13] on Serum

1. To quantitate serum samples to an endpoint titer, prepare serum dilutions on the slide at 1:1, 1:2, 1:4 and 1:8, as follows.
2. Dispense 50 µl of 0.9% saline in circles 2-4. Do not spread the saline.
3. Dispense 50 µl of serum in circles 1 and 2.
4. Mix the saline and the serum in circle 2 by drawing the mixture up and down in the pipette 8 times. Mix gently to prevent bubbles.
5. Transfer 50 µl from circle 2 (1:2) to circle 3 and mix.
6. Transfer 50 µl from circle 3 (1:4) to circle 4 (1:8), mix, and then discard 50 µl from circle 4.
7. Holding the syringe and needle containing the USR Antigen suspension in a vertical position, dispense several drops to clear the needle of air. Then, add exactly 1 free-falling drop (22 µl) of antigen suspension to each circle containing serum. Do not allow the needle to touch the serum.
8. Place the slide on the mechanical rotator. Rotate the slide for 4 minutes at 180 ± 2 rpm. If the environment is dry, cover the slides with a moist, humidifying cover during rotation to prevent excessive evaporation.
9. Immediately after rotating the slide, remove the slide from the rotator and read the test results microscopically using a 10X ocular and a 10X objective.
10. If the highest dilution tested (1:8) is reactive, prepare a 1:8 dilution of the test specimen by adding 0.1 ml of serum to 0.7 ml of 0.9% saline. Mix thoroughly. Retest as in steps 1-9, above.

Results – Quantitative Test

Report the titer as the highest dilution that produces a Reactive result.

Table 1. Sample quantitative USR Test results.

Undiluted (1:1)	1:2	1:4	1:8	1:16	1:32	
R	W	N	N	N	N	Reactive, undiluted
R	R	W	N	N	N	Reactive, 1:2 dilution
R	R	R	W	N	N	Reactive, 1:4 dilution
W	W	R	R	W	N	Reactive, 1:8 dilution
N (rough)	W	R	R	R	N	Reactive, 1:16 dilution
W	N	N	N	N	N	Weakly reactive, undiluted

If reactive results are obtained through dilution 1:32, prepare further twofold serial dilutions in 0.9% saline (1:64, 1:128 and 1:256) and retest using the quantitative test procedure.

Interpretation

1. The results of the serum USR Test must be confirmed by a treponemal test.
2. The diagnosis of syphilis depends on the results of the USR Test, treponemal confirmatory test, clinical signs and symptoms, and risk factors.
3. A Reactive USR Test may indicate past or present infection with a pathogenic treponeme. However, it may be a false-positive reaction. A false positive is determined if the confirmatory treponemal test is negative.
4. A Nonreactive USR Test with clinical evidence of syphilis may indicate early, primary syphilis, a prozone reaction in secondary syphilis, or late syphilis.
5. A Nonreactive USR Test with no clinical evidence of syphilis indicates no current infection or an effectively treated infection.
6. A quantitative USR Test detects changes in reagin titer. Therefore, a serum specimen showing a fourfold increase in titer on a repeat specimen may indicate an infection, a reinfection or a treatment failure. Likewise, a fourfold decrease during treatment indicates adequate syphilis therapy.

Limitations of the Procedure

1. A prozone reaction may occur in which reactivity with true positive undiluted serum is inhibited. The prozone phenomenon often gives Weakly Reactive or "rough" Nonreactive results in the qualitative test. Specimens with such nonreactive results must be quantitatively tested.
2. Biological false-positive reactions can occur with nontreponemal tests in persons who abuse drugs, have diseases such as lupus erythematosus, mononucleosis, malaria, leprosy or viral pneumonia, or who have recently been immunized.[1]
3. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
4. If the temperature of the testing area, specimens, or reagents is less than 23°C, test, reactivity is decreased. If the temperature is greater than 29°C, test reactivity is increased.[1]
5. Test results are unpredictable when testing hemolyzed, contaminated or extremely turbid serum specimens.
6. Test results may be erroneous if the speed and time of rotation are not correct.
7. Positive results obtained by using USR Antigen should not be considered as conclusive evidence that the patient is syphilitic. Conversely, a nonreactive USR Test, by itself, does not rule out the diagnosis of syphilis.

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Packaging

USR Antigen	6 x 3 ml	2405-46
USR Test Control Serum Set	1 set	3516-32
Contains:		
Nontreponemal Antigen Reactive Serum	3 ml	
USR Weakly Reactive Serum	3 ml	
Nontreponemal Antigen Nonreactive Serum	3 ml	
Aliquant Vials	3 vials	

Bacto® VDRL Antigen
Bacto VDRL Test Control Serum Set

Intended Use

Bacto VDRL Antigen with Bacto VDRL Buffered Saline is used in the Venereal Disease Research Laboratory (VDRL)[1] Test for detecting reagin, an antibody-like substance, by the qualitative and quantitative slide procedures.

Bacto VDRL Test Control Serum Set is used for controlling the VDRL Test.

Summary and Explanation

Treponema pallidum is the causative agent of syphilis. Syphilis is a chronic infection with many clinical manifestations which occur in distinct stages. Specific laboratory tests are recommended for the detection of each stage of the disease.[2-4]

The VDRL Antigen is a nontreponemal antigen composed of cardiolipin, cholesterol, and lecithin. The nontreponemal tests measure antilipid antibodies (IgG and IgM, which are formed by the host in response to lipoidal material released from damaged host cells early in infection with *T. pallidum*) and lipid like material from the treponemal cell surface.[5] During infection with syphilis, an antibody-like substance called reagin can be detected in the patient's serum. In syphilis infection of the central nervous system, reagin can be detected in the cerebrospinal fluid (CSF).

Reactive nontreponemal tests confirm the diagnosis in the presence of early or late lesion syphilis. They offer a clue in latent subclinical syphilis, and are effective tools for detecting cases in epidemiological investigations. Nontreponemal tests are superior to the treponemal test for following the response to therapy.[3]

Nontreponemal antigen tests are not entirely specific for syphilis, nor do they have satisfactory sensitivity in all stages of syphilis. Whenever the results of a nontreponemal antigen test disagree with the clinical impression, a treponemal antigen test such as the FTA-ABS[2,3] should be performed. Nontreponemal tests such as the VDRL are used to screen patient serum, while treponemal tests such as the FTA-ABS are used for confirmation. The likelihood of obtaining a reactive VDRL test result in various stages of untreated syphilis has been reported as follows[3]:

STAGES OF UNTREATED SYPHILIS	% REACTIVE VDRL TEST
Primary	78
Secondary	100
Latent	96
Late	71

User Quality Control

Identity Specifications

VDRL Antigen
Appearance: Clear, colorless solution.

VDRL Buffered Saline
Appearance: Clear, colorless solution.

Nontreponemal Antigen Reactive Serum
Lyophilized Appearance: White to cream colored, button to powdered cake.
Rehydrated Appearance: Light gold to light amber, clear to slightly opalescent.

VDRL Weakly Reactive Serum
Lyophilized Appearance: White to cream colored, button to powdered cake.
Rehydrated Appearance: Light gold to light amber, clear to slightly opalescent.

Nontreponemal Antigen Nonreactive Serum
Lyophilized Appearance: White to cream colored, button to powdered cake.
Rehydrated Appearance: Light gold to light amber, clear to slightly opalescent.

Performance Response

Rehydrate the sera contained in the VDRL Test Control Serum Set per label directions. Perform the VDRL Slide Test according to the Test Procedure. Each serum in the VDRL Test Control Serum Set should yield appropriate reactions when tested with the VDRL Antigen.

Use an antigen suspension only if it produces the expected reactivity with the control sera.

Principles of the Procedure

In the VDRL Test procedure, the patient's serum is heat-inactivated and then mixed with a buffered saline suspension of VDRL Antigen containing cardiolipin, lecithin and cholesterol. The combination of reagin and VDRL Antigen forms microscopic clumping called flocculation. With certain modification, the serum test procedure can be used for testing CSF.

Reagents

VDRL Antigen is 0.03% cardiolipin and 0.9% cholesterol dissolved in absolute alcohol with sufficient lecithin (approximately 0.18-0.2%) to produce standard reactivity.

It is prepared according to the modifications of Harris, Rosenberg and Riedel.[6] Cardiolipin and lecithin are prepared according to the directions of Pangborn.[7,8,9]

VDRL Buffered Saline is a 1% NaCl solution at pH 6.0 ± 0.1. It is packaged with VDRL Antigen and used to prepare the VDRL Antigen suspension.

Nontreponemal Antigen Reactive Serum is a lyophilized human serum standardized to provide a reactive reading when tested according to the USR or VDRL test procedure.

VDRL Weakly Reactive Serum is a lyophilized human serum standardized to provide a weakly reactive reading when tested according to the VDRL test procedure.

Nontreponemal Antigen Nonreactive Serum is a lyophilized human serum standardized to provide a nonreactive reading when tested according to the USR or VDRL test procedure.

Precautions

1. For In Vitro Diagnostic Use.
2. **WARNING!** POTENTIALLY BIOHAZARDOUS REAGENTS. Each donor unit used in preparation of VDRL Antigen and VDRL Test Control Serum Set was tested by an FDA approved method for the presence of the antibody to human immunodeficiency virus (HIV) and for hepatitis B surface antigen and found negative (were not repeatedly reactive).

   Because no test method can offer complete assurance that HIV, hepatitis B virus or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen.[10]
3. Observe universal blood and body fluid precautions in handling and disposing of specimens.[11,12]
4. **VDRL Antigen**

   HIGHLY FLAMMABLE. IRRITATING TO EYES, RESPIRATORY SYSTEM AND SKIN.[us] POSSIBLE RISK OF IRREVERSIBLE EFFECTS.[us] POSSIBLE RISK OF HARM TO THE UNBORN CHILD.[us] Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. Keep away from sources of ignition. No smoking. Target Organs: Blood, Intestines, Liver, Muscles, Nerves.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
5. **VDRL Test Control Serum Set**

   The Packaging of This Product Contains Dry Natural Rubber.
6. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store VDRL Antigen at 15-30°C in the dark.

Store VDRL Buffered Saline at 15-30°C. After the bottle is opened, store at 2-8°C.

Store the lyophilized control sera in the VDRL Test Control Serum Set at 2-8°C. Store the rehydrated control sera at 2-8°C or divide into aliquots sufficient for one day of testing and store at -20°C. Do not thaw and refreeze.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

VDRL Antigen with VDRL Buffered Saline
VDRL Test Control Serum Set

Materials Required But Not Provided

0.9% saline
Nondisposable syringe, 1 cc
Nondisposable calibrated needles without bevel:
  Serum test: 18 gauge
  CSF test: 21 or 22 gauge
Bottles, 30 ml, round, narrow-mouthed, 35 mm in diameter with glass stoppers and a flat inner-bottom surface
Micropipettor, 50 µl
Pipettes, serological, graduated to tip:
  1.0 ml, graduated in 1/100 ml
  5.0 ml, graduated in 1/10 ml
  10.0 ml, graduated in 1/10 ml
Slides:
  Serum test: 2 x 3 inches with paraffin or ceramic rings approximately 14 mm in diameter and high enough to prevent spillage during rotation
  CSF test: Kline concavity slides, 3 x 2 3 inches x 3 mm thick, 12 concavities measuring 16 mm in diameter and 1.75 mm in depth
Slide holder for 2 x 4 inch slides
Mechanical rotator adjustable to 180 ± 2 rpm, circumscribing a circle 19 mm in diameter on a horizontal plane
Waterbath, 56°C
Light microscope with 10X ocular and 10X objective
Sterile distilled or deionized water
Absolute alcohol
Acetone
Timer

Reagent Preparation

VDRL Antigen and VDRL Buffered Saline are ready to use in preparing VDRL Antigen suspension.

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of detergent residues.

VDRL Test Control Serum Set: To rehydrate the control sera, add 3 ml sterile distilled or deionized water each and rotate gently to completely dissolve the contents.

Specimen Collection and Preparation

Collect a blood specimen by aseptic venipuncture. After the specimen has clotted, centrifuge to obtain serum. Store serum specimens at room temperature for no longer than 4 hours; for prolonged storage, keep at 2-8°C for up to 5 days or maintain below -20°C. Serum specimens must be clear, free of hemolysis and show no visible evidence of bacterial contamination such as turbidity, hemolysis or particulate matter. Consult appropriate references for more information on collection of specimens.[1,3,13]

Before testing, heat the test sera at 56°C for 30 minutes. Specimens that are not tested within four hours must be reheated for 10 minutes at 56°C.

Preparation of Specific Glassware

Syringes with needles and emulsion bottles:

1. Pre-rinse with tap water.
2. Soak and hand wash thoroughly in a glassware detergent solution.
3. Rinse with tap water 6-8 times.
4. Rinse with unused distilled or deionized water.
5. Rinse with absolute alcohol.
6. Rinse with acetone.

7. Air dry until the acetone odor is completely eliminated.
8. Remove needles from syringes for storage.

Ceramic-ringed slides:

1. Pre-rinse with tap water.
2. Wash with a glassware detergent solution. Avoid prolonged soaking in detergent solution because the ceramic rings will become brittle and flake off.
3. Rinse with tap water 3-4 times.
4. Rinse with unused distilled or deionized water.
5. Wipe dry with a clean lint-free cloth. If, after cleaning, the slides do not allow serum to spread evenly within the inner surface of the circle, proceed as follows.
6. Scrub the slides with a nonscratching cleanser.
7. Rinse, dry and polish with a clean lint-free cloth.

Prepare the Antigen Suspension

Check the pH of VDRL Buffered Saline before preparing the VDRL Antigen emulsion. VDRL Buffered Saline outside the range of pH 6.0 ± 0.1 should be discarded.

Allow VDRL Antigen and VDR Buffered Saline to reach 23-29°C before preparing the VDRL Antigen emulsion.

Use only emulsion bottles with flat inner-bottom surfaces that allow the initial VDR Buffered Saline to evenly cover the inner-bottom surface of the bottle. If the VDRL Buffered Saline beads or does not spread evenly to cover the bottom of the bottle, rewash the bottle.

For reproducible results, the VDRL Antigen emulsion must be checked daily for proper reactivity by testing with VDRL Test Control Serum Set. Only those VDRL emulsions producing the established reactivity pattern of the control serum should be used.

1. Prepare a fresh VDRL Antigen suspension each testing day. The temperature of the VDRL Buffered Saline, VDRL Antigen and equipment should be between 23-29°C at the time the antigen suspension is prepared.
2. Pipette 0.4 ml of VDRL Buffered Saline to the bottom of a round, 30 ml glass-stoppered bottle with a flat inner-bottom surface. Gently tilt the bottle so that the VDR Buffered Saline will cover the entire inner-bottom surface of the bottle.
3. Add 0.5 ml of VDRL Antigen (from the lower half of a 1.0 ml pipette graduated to the tip) directly into the saline while continuously but gently rotating the bottle on a flat surface. Add antigen drop by drop at a rate allowing approximately 6 seconds for each 0.5 ml of antigen. Keep the pipette tip in the upper third of the bottle. Do not splash saline onto the pipette. The proper speed of rotation is obtained when the center of the bottle circumscribes a 2-inch diameter circle approximately three times per second.
4. Expel the last drop of antigen from the pipette without touching the pipette to the saline and continue rotating the bottle for 10 seconds.
5. Add 4.1 ml of buffered saline from a 5 ml pipette. Do not drop the saline directly onto the antigen; allow it to flow down the side of the bottle.
6. Cap the bottle and shake it from bottom to top and back approximately 30 times in 10 seconds. Let the VDRL Antigen emulsion stand without further disturbance for 10 minutes. The antigen suspension is ready for use and may be used during 1 day (8 hours).
7. Mix the VDRL Antigen suspension by gently swirling it each time it is used. Do not mix the suspension by forcing it back and forth through the syringe and needle, since this may cause breakdown of particles and loss of reactivity.

Testing the Accuracy of the Antigen Suspension Needle

1. The accuracy of the test depends on the amount of antigen suspension used. Check the calibration of the needle periodically to ensure delivery of the correct volume of VDRL Antigen suspension.
2. For the qualitative and quantitative tests on serum, dispense the antigen suspension from a syringe fitted with an 18-gauge needle without bevel that will deliver 60 ± 2 drops of antigen suspension per ml when held vertically.
3. Place the needle on a 1 ml syringe. Fill the syringe with VDRL Antigen suspension. Holding the syringe in a vertical position, count the number of drops delivered in 0.5 ml. The needle is correctly calibrated if 30 ± 1 drops are delivered in 0.5 ml.
4. Adjust or replace the needle if it does not meet this specification. Repeat calibration of the new needle.

Testing and Storing the VDRL Antigen Suspension

1. Prepare a fresh antigen suspension each testing day. Once prepared, it should be used within 8 hours.
2. Store the antigen suspension at 23-29°C.
3. Test the reactivity of the antigen suspension with the Reactive, Weakly Reactive and Nonreactive control sera. Test the serum dilutions within 1 hour after inactivation.
4. Use the antigen suspension only if it produces the expected reactivity with the control sera (Reactive, Weakly Reactive and Nonreactive).
5. After each day of use, clean the dispensing needle, bottle and syringe by rinsing with water, alcohol and acetone, as described above. Remove the needle from the syringe after cleaning.

VDRL Qualitative Slide Test on Serum

1. Slide flocculation tests for syphilis are affected by the temperature of the room. For reliable and reproducible test results, the VDRL Antigen suspension, controls and test specimens must be at 23-29°C when tests are performed.
2. Pipette 50 µl of serum into one ring of a paraffin or ceramic-ringed slide using a safety pipetting device. Do not use a glass slide with concavities, wells or glass rings. Spread the serum with a circular motion of the pipette tip so that the serum covers the entire inner surface of the paraffin or ceramic ring. Use only clean slides that allow the serum to evenly cover the entire surface within the ceramic or paraffin ring.
3. Gently Resuspend the VDRL Antigen suspension.
4. Holding the VDRL Antigen suspension dispensing needle and syringe in a vertical position, dispense several drops to clear the needle of air. Then add exactly 1 free-falling drop (17 µl) of antigen suspension to each circle containing serum. Do not allow the needle to touch the serum.
5. Place the slide on the mechanical rotator. Rotate the slide for 4 minutes at 180 ± 2 rpm. When performing the test in a dry climate, cover the slides with a moist, humidifying cover during rotation to prevent excessive evaporation.
6. Immediately after rotating the slide, remove it from the rotator and read the test results microscopically using a 10X ocular and a 10X objective.

Results - Qualitative Slide Test

1. Read and record results as follows:
   Reactive (R) - Medium to large clumps
   Weakly reactive (WR) - Small clumps
   Nonreactive (N) - No clumping or very slight roughness
2. Verify that the control sera results are as expected. If reactions are not as expected, the test is invalid and results cannot be reported.
3. Perform a quantitative test on all serum specimens that produce Reactive, Weakly Reactive or "rough" Nonreactive results, since prozone reactions are encountered occasionally.

VDRL Quantitative Test on Serum

1. To quantitate serum samples to an endpoint titer, prepare serum dilutions on the slide at 1:1, 1:2, 1:4 and 1:8, as follows.
2. Dispense 50 µl of 0.9% saline in circles 2-4. Do not spread the saline.
3. Dispense 50 µl of serum in circles 1 and 2.
4. Mix the saline and the serum in circle 2 by drawing the mixture up and down in the pipette 8 times. Avoid forming bubbles.
5. Transfer 50 µl from circle 2 (1:2) to circle 3 (1:4) and mix.
6. Transfer 50 µl from circle 3 (1:4) to circle 4 (1:8), mix, and then discard 50 µl from circle 4.
7. Gently resuspend the antigen suspension.
8. Holding the antigen suspension dispensing needle and syringe in a vertical position, dispense several drops to clear the needle of air. Then add exactly 1 free-falling drop (17 µl) of antigen suspension to each circle.
9. Place the slide on the mechanical rotator. Rotate the slide for 4 minutes at 180 ±2 rpm. When performing the test in a dry climate, place the slides under a moist, humidifying cover during rotation to prevent excessive evaporation.
10. Immediately after rotation, read the test results microscopically using a 10X ocular and a 10X objective.
11. If the highest dilution tested (1:8) is reactive, prepare a 1:8 dilution of the test specimen by adding 0.1 ml of serum to 0.7 ml of 0.9% saline. Mix thoroughly. Retest as in steps 1-10, above.

Results - Quantitative Test

Report the titer as the highest dilution that produces a Reactive (not Weakly Reactive) result.

Table 1. Sample quantitative VDRL Test results.

	SERUM DILUTIONS					REPORT
Undiluted (1:1)	1:2	1:4	1:8	1:16	1:32	
R	W	N	N	N	N	Reactive, undiluted
R	R	W	N	N	N	Reactive, 1:2 dilution
R	R	R	W	N	N	Reactive, 1:4 dilution
W	W	R	R	W	N	Reactive, 1:8 dilution
N (rough)	W	R	R	R	N	Reactive, 1:16 dilution
W	N	N	N	N	N	Weakly reactive, undiluted

If reactive results are obtained through dilution 1:32, prepare further twofold serial dilutions in 0.9% saline (1:64, 1:128 and 1:256) and retest using the quantitative test procedure.

Interpretation

1. The results of the serum VDRL Test must be confirmed by a treponemal test.
2. The diagnosis of syphilis depends on the results of the VDRL test, the treponemal confirmatory test, clinical signs and symptoms, and risk factors.
3. A reactive VDRL Test may indicate past or present infection with a pathogenic treponeme. However, it may be a false-positive reaction. A false positive is determined if the confirmatory treponemal test is negative.
4. A nonreactive VDRL Test with clinical evidence of syphilis may indicate early, primary syphilis, a prozone reaction in secondary syphilis, or late syphilis.
5. A nonreactive VDRL Test with no clinical evidence of syphilis indicates no current infection or an effectively treated infection.
6. A quantitative VDRL Test detects changes in reagin titer. Therefore a serum specimen showing a fourfold increase in titer on a repeat specimen may indicate an infection, a reinfection or a treatment failure. Likewise, a fourfold decrease during treatment indicates adequate syphilis therapy.

VDRL Test on Spinal Fluid

Consult an appropriate reference for the procedure to use when testing spinal fluids by the VDRL Test.[1]

Limitations of the Procedure

1. A prozone reaction may occur in which reactivity with undiluted serum is inhibited. The prozone phenomenon often gives Weakly Reactive or "rough" Nonreactive results in the qualitative test. Specimens with such results must be quantitatively tested.
2. Biological false-positive reactions can occur with nontreponemal tests in persons who abuse drugs, have diseases such as lupus erythematosus, mononucleosis, malaria, leprosy or viral pneumonia, or who have recently been immunized.[1]
3. During manufacturing, VDRL Antigen with VDRL Buffered Saline is tested only with serum. To modify the serum test products and procedures for testing CSF, consult the appropriate reference.[1] The user is responsible for modifying the products and procedures and for the required quality control standards according to this manual.
4. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
5. VDRL Buffered Saline showing turbidity or mold growth should be discarded.
6. If the temperature of the testing area, specimens or reagents is less than 23°C, test reactivity is decreased. If the temperature is greater than 29°C, test reactivity is increased.[1]
7. Test results are unpredictable when testing hemolyzed, contaminated or extremely turbid serum specimens.
8. For correct test results, adhere strictly to the correct speed and length of time for rotating the specimens and antigen.

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Packaging

VDRL Antigen w/Buffered Saline	5 ml	0388-56
	10 x 0.5 ml	0388-49
VDRL Test Control Serum Set	1 set	3520-32
Contains:		
Nontreponemal Antigen Reactive Serum	3 ml	
VDRL Weakly Reactive Serum	3 ml	
Nontreponemal Antigen Nonreactive Serum	3 ml	
Aliquant Vials	3 vials	

Bacto® Vibrio Cholerae Antisera

Vibrio Cholerae Antiserum Inaba · Vibrio Cholerae Antiserum Ogawa · Vibrio Cholerae Antiserum Poly

User Quality Control

Identity Specifications

Vibrio Cholerae Antiserum Inaba
Vibrio Cholerae Antiserum Ogawa
Vibrio Cholerae Antiserum Poly

Lyophilized appearance: Light gold to amber button to powdered cake

Rehydrated appearance: Light gold to amber, clear liquid

Performance Response

Rehydrate Vibrio Cholerae Antisera per label directions. Perform the slide agglutination test using appropriate known cultures of *Vibrio cholerae* as positive and negative controls.

Intended Use

Bacto Vibrio Cholerae Antisera are used for serotyping *Vibrio cholerae* in slide agglutination tests.

Summary and Explanation

V. cholerae are facultative anaerobes that in microscopic morphology are gram-negative, curved or straight bacilli. The microorganisms either require sodium chloride or grow best in its presence. Various media designed to select and cultivate the growth of this microorganism are used in the clinical laboratory, the food industry and environmental testing.

V. cholerae is the causative agent of a secretory diarrhea known as cholera. Two biotypes, El Tor and Classical, are associated with human disease.[1] These two biotypes cannot be differentiated serologically. The spread of the disease is primarily through contaminated

water and by the fecal-oral route. Infections with *V. cholerae* may be asymptomatic, mild or severe. If not treated, patients with severe cholera may die within five hours from massive fluid and electrolyte loss.[1] Because *Vibrio* species are natural inhabitants of aquatic environments, food products such as uncooked or incorrectly handled seafood can spread infection.

Gastrointestinal symptoms of cholera include "rice water stools" caused by a potent enterotoxin. The primary human specimen is feces. Seafood products are frequently tested as vehicles of human infection.

In 1935, Gardner and Ventkatraman[2] published a classification scheme for *Vibrio cholerae*. *V. cholerae* isolated from cholera patients was classified as O1. All other strains were designated non-O1. Non-O1 *V. cholerae* causes both gastroenteritis and systemic infections. Some strains produce cholera enterotoxin. Non-O1 *V. cholerae* has been isolated from blood, wounds, ears, sputum, cerebro-spinal fluid and urine.[3,4]

V. cholerae of the O1 serogroup are divided into the serotypes Ogawa, Inaba and Hikojima.[5,6] The antigenic factors are:

SEROTYPE	O ANTIGEN FACTORS
Ogawa	AB
Inaba	AC
Hikojima	ABC

Cholera can be diagnosed retrospectively. A fourfold rise in titer between acute-phase serum and that collected 10-14 days later is considered diagnostic.[7]

Principles of the Procedure

Identification of *V. cholerae* includes the isolation of the microorganism as well as biochemical identification and serological confirmation.

Serological confirmation requires that the microorganism (antigen) react with its corresponding antibody. This *in vitro* reaction produces macroscopic clumping called agglutination. The desired homologous reaction is rapid, does not dissociate (high avidity) and binds (high affinity).

Because a microorganism may agglutinate with an antibody produced in response to another species, heterologous reactions are possible. These are characterized as weak in strength or slow in formation. Such unexpected and unpredictable reactions may lead to some confusion in serological identification. A positive homologous agglutination reaction should support the morphological and biochemical identification of the microorganism.

Homologous reactions occur rapidly and are strong. Heterologous reactions form slowly and are weak.

Reagents

Vibrio Cholerae Antisera are lyophilized, polyclonal rabbit *Vibrio cholerae* O1 antisera containing approximately 0.04%Thimerosal as a preservative. Vibrio Cholerae Antisera Inaba and Ogawa are monospecific absorbed antisera.

Vibrio Cholerae Antisera possess the following antibodies:

ANTISERUM	O ANTIBODIES
Vibrio Cholerae Antiserum Inaba	C
Vibrio Cholerae Antiserum Ogawa	B
Vibrio Cholerae Antiserum Poly	ABC

Each vial of Vibrio Cholerae Antiserum contains sufficient reagent for 20 slide tests.

Precautions

1. For In Vitro Diagnostic Use.
2. **Vibrio Cholerae Antiserum Inaba**
   Vibrio Cholerae Antiserum Ogawa
   Vibrio Cholerae Antiserum Poly
   The Packaging of This Product Contains Dry Natural Rubber.
3. Follow proper established laboratory procedure in handling and disposing of infectious materials.

Storage

Store lyophilized and rehydrated Vibrio Cholerae Antisera at 2-8°C.

Expiration Date

The expiration date applies to product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Vibrio Cholerae Antiserum Inaba
Vibrio Cholerae Antiserum Ogawa
Vibrio Cholerae Antiserum Poly

Materials Required But Not Provided

Agglutination slides
Applicator sticks
Sterile 0.85% NaCl solution

Reagent Preparation

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergent.

Vibrio Cholerae Antisera: To rehydrate, add 3 ml sterile 0.85% NaCl solution and rotate gently to completely dissolve the contents. The rehydrated antiserum is considered a 1:2 working dilution.

Specimen Collection and Preparation

V. cholerae can be recovered from clinical specimens on selective media such as thiosulfate-citrate-bile salts-sucrose (TCBS) agar. For specific recommendations, consult appropriate references.[1,8,9]

V. cholerae can be recovered from various types of foods, particularly seafood. Samples are processed to prevent overgrowth of competing microorganisms and selective media are used to enhance the growth of the microorganism. Some isolation media have been specifically developed for the food industry. Consult appropriate references for recommended procedures for isolating *V. cholerae* from food.[10,11]

The isolate for serological testing should be subcultured from selective media to a nonselective agar such as Nutrient Agar. Consult standard protocols in appropriate references for the type of specimen and the appropriate medium.[1, 8-11]

Having followed an established protocol, determine that a pure culture of the microorganism has been obtained and that biochemical test reactions are consistent with the identification of the organism as *V. cholerae*. After these criteria are met, serological identification can be performed.

Testing the isolate for autoagglutination:

1. From the test culture, transfer a loopful of growth to a drop (35 µl) of sterile 0.85% NaCl solution on a clean slide and emulsify the organism.
2. Rotate the slide for one minute, then observe for agglutination.
3. If autoagglutination occurs, the culture is rough and cannot be tested. Subculture to a nonselective medium, incubate and test the organism again as described in steps 1 and 2.

   If no agglutination occurs, proceed with testing the organism as described below.

Test Procedure

Use Vibrio Cholerae Antiserum Poly to screen possible *V. cholerae* isolates. Continue testing with Vibrio Cholerae Antisera Inaba and Ogawa. Include known positive and negative control cultures.

1. **Vibrio Cholerae Antiserum**: Dispense a drop of the antiserum to be tested on an agglutination slide.
2. **Test organism**: Transfer a loopful of growth to the drop of antiserum and mix thoroughly.
3. Rotate the slide for one minute and read for agglutination.

Results

1. Read and record results as follows:

   4+ 100% agglutination; background is clear to slightly hazy.
   3+ 75% agglutination; background is slightly cloudy.
   2+ 50% agglutination; background is moderately cloudy.
   1+ 25% agglutination; background is cloudy.
   – No agglutination.

1. **Positive control**: Should produce 3+ or greater agglutination.
2. **Negative control**: Should show no agglutination.
3. Test isolates: 3+ or greater agglutination within one minute is a positive result.

Interpretation

Agglutination of the monospecific antiserum, when used, provides preliminary presumptive identification of the serotype.

0.85% NACL SOLUTION CONTROL	POLY	OGAWA	INABA	INTERPRETATION
–	–			Not *V. cholerae*
–	+			Presumptively *V. cholerae*
–	+	+	–	*V. cholerae* serotype Ogawa
–	+	–	+	*V. cholerae* serotype Inaba
–	+	+	+	*V. cholerae* serotype Hikojima
+	Any	Any	Any	Autoagglutination. Unsuitable test culture.

+ agglutination
– no agglutination

1. Positive agglutination using Vibrio Cholerae Antiserum Poly with typical biochemical test results gives presumptive identification of *V. cholerae* O1.
2. Cultures with positive agglutination in Vibrio Cholerae Antiserum Poly may be serotyped using the Vibrio Cholerae Antiserum Ogawa and Vibrio Cholerae Antiserum Inaba. Positive agglutination in both antisera is rare and, when it occurs, is usually interpreted as identifying *V. cholerae* serotype Hikojima.[6] *V. cholerae* serotype Hikojima is a rare serotype and should be sent to a reference laboratory for further study.
3. Positive agglutination will be immediate and strong. The strongest and most rapid reaction should be used to identify the serotype. *V. cholerae* O1 strains frequently cross-react slowly or weakly in monospecific antiserum for the other serotype.
4. Isolates that weakly or slowly agglutinate with Vibrio Cholerae Antiserum Poly and not with Vibrio Cholerae Antiserum Inaba or Vibrio Cholerae Antiserum Ogawa are usually considered *V. cholerae* non-O1. The isolate may be sent to a reference laboratory for further study.

Limitations of the Procedure

1. Correct interpretation of serological reactions depends on culture purity as well as morphological characteristics and biochemical reactions that are consistent with identification of the microorganism as *V. cholerae*.
2. Serological methods alone cannot identify the isolate as *V. cholerae*.
3. Excessive heat from external sources (hot bacteriological loop, burner flame, light source, etc.) may prevent a smooth suspension of the microorganism or cause evaporation or precipitation of the test mixture. False-positive reactions may occur.
4. Rough culture isolates do occur and will agglutinate spontaneously, causing agglutination of the negative control reaction (autoagglutination). Smooth colonies must be selected and tested in serological procedures.
5. Vibrio Cholerae Antisera have been tested using undiluted cultures taken from agar media. These antisera have not been tested using antigen suspensions in NaCl solution or other diluents. If the user applies variations to the recommended procedure, each lot of antiserum must be tested with known control cultures to verify expected reactions under the modified procedure.
6. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
7. Rehydrated Vibrio Cholerae Antiserum that is cloudy or has a precipitate at any time during its use should be discarded.

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Packaging

Vibrio Cholera Antiserum Inaba	3 ml	2430-47
Vibrio Cholera Antiserum Ogawa	3 ml	2431-47
Vibrio Cholera Antiserum Poly	3 ml	2432-47

Bacto® Widal Antigen Set

CONTAINS: Salmonella O Antigen · Salmonella H Antigens Salmonella Vi Antigen · Febrile Positive Control Polyvalent Febrile Negative Control

ALSO AVAILABLE: Salmonella O Antigens · Salmonella H Antigen

Intended Use

Bacto Widal Antigen Set, which contains four *Salmonella* antigens, is used in detecting *Salmonella* antibodies by slide and tube agglutination tests.

Bacto Salmonella H, O and Vi Antigens, available individually, are used in detecting *Salmonella* antibodies by slide and tube agglutination tests.

Summary and Explanation

Salmonellae represent many species of pathogenic microorganisms that, upon invasion, produce a fever in their host. Consequently, they are often called "Febrile Antigens". *Salmonella* species cause a variety of human diseases called salmonelloses. The range of disease is from mild self-limiting gastroenteritis to a more severe form, possibly with bacteremia or typhoid fever, which can be severe and life-threatening. Severe disease and bacteremia are associated primarily with *S.* Choleraesuis, *S.* Paratyphoid A and *S.* Typhi, while most of the other 2,300 or more strains are associated with gastroenteritis. The severity of the diarrheal disease depends on the virulence of the strain and the condition of the human host.

Salmonella is found in nature and occurs in the intestinal tract of many animals, both wild and domestic. The microorganism can spread to man from contact with the environment or from eating contaminated meat or vegetable food products.

The genus *Salmonella* is in the family *Enterobacteriaceae.* Salmonellae are facultatively anaerobic, gram-negative bacilli that typically are oxidase negative, non-lactose fermenting, H_2S positive and produce gas. Serotypes of *Salmonella* are defined based on antigenic structure, both somatic or cell wall (O) antigens and flagellar (H) antigens. The antigenic formula lists the O antigen(s) followed by the H antigen(s).

In 1896, Widal introduced techniques for testing patient serum for antibodies in cases of typhoid fever.[1] The Widal test was used diagnostically in two ways. First, it was considered diagnostic when a single high antibody titer occurred during the first week of illness. Further, it was diagnostic if a greater than fourfold rise in titer existed in serum samples taken 1 to 2 weeks apart.[2,3,4] The Widal test was developed to include *Salmonella* Typhi and other species of *Salmonella* detected by a variety of O and H antigens. *S.* Typhi and *S.* Paratyphi A and B are the major pathogens in this group that can produce clinically distinct systemic illness. In areas such as developing countries where typhoid is highly endemic, the diagnostic value of the Widal test has been well documented.[5,6,7] The Widal test for antibodies to the O antigens of *Salmonella* serotypes most likely to cause typhoid fever (usually *S.* Typhi and *S.* Paratyphi A and B) can be useful in helping diagnose typhoid fever when other methods have failed.[8]

Diagnosis of the cause of febrile disease cannot be based solely on the analysis of serum samples for antibody response. Many factors may affect measurable antibody levels. For example, the patient's immune response can be affected by age, immune status, general state of health and previous immunizations. Patients with known typhoid fever have developed diagnostic titers of antibodies that are low.[9,10] Also, patients treated with antibiotics early in their disease may not develop a significant titer rise.[2]

The various species of *Salmonella* contain multiple antigens that are cross-reactive. This prevents using increased antibody levels, alone, to identify infecting organisms by species or serotype. Other non-typhoidal febrile illness or unrelated immunological disorders may produce significant elevations of antibody titers.[2]

Certain organisms may share cross-reacting antigens leading to the production of heterologous antibodies. These heterologous antibodies may react with one or more antigens in an antibody test procedure, resulting in low-level antibody titers that may not, singly, suggest disease.

The rapid slide procedure is a screening test designed to detect agglutinins. The macroscopic tube test[11] is a confirmatory procedure designed to quantitate agglutinin compositions. Any positive results obtained in the screening (slide) test of specimens must be confirmed by a tube test.

The rapid slide test is the most widely used procedure employing the Widal antigens because of the simplicity with which the results may be reported. Negative slide test reactions can usually be reported as such if all five serum dilutions have been used. Although the slide test is not quantitative, running the series of dilutions is necessary to detect agglutinins that might be overlooked with a "prozone phenomenon". This often occurs in serum containing a high titer of typhoid agglutinins where higher concentrations of the serum may yield negative results but a dilution of the serum is positive.

Principles of The Procedure

Agglutination tests involving the use of *Salmonella* antigens determine the presence of antibodies that react with the test antigen. The serological procedure involves serially diluting the patient serum and then adding a standard volume of antigen. The end point of the test is the last dilution of the serum that shows a specific amount of agglutination. The end point, reported as a dilution of the serum, is called the patient's antibody "titer."

User Quality Control

Identity Specifications

Salmonella O Antigens
Salmonella H Antigens
Salmonella Vi Antigen

Appearance: Turquiose-blue-violet suspension.

Febrile Positive Control Polyvalent

Lyophilized Appearance: Light gold to amber, button to powdered cake.

Rehydrated Appearance: Light gold to amber, clear liquid.

Febrile Negative Control

Lyophilized Appearance: Colorless to light gold, button to powdered cake.

Rehydrated Appearance: Colorless to light gold, clear liquid.

Performance Response

Rehydrate Febrile Positive Control Polyvalent and Febrile Negative Control per label directions. Perform the slide or tube agglutination test using Salmonella O, H or Vi Antigens and positive and negative controls diluted in the same proportion as a patient serum.

A Salmonella Antigen is considered satisfactory if it does not agglutinate with the negative control and gives a 2+ or greater reaction at a 1:80 dilution with the positive control.

Reagents

Antigens

1. Salmonella Antigens are ready-to-use suspensions of the *Salmonella* organisms listed below. Salmonella O Antigens contain 20% glycerin.

   Widal Antigen Set contents:
   Salmonella O Antigen Group D - *Salmonella* Typhi O901, factors 9,12 (selected strain)
   Salmonella H Antigen a - *Salmonella* Paratyphi A.
   Salmonella H Antigen b - *Salmonella* Paratyphi B.
   Salmonella H Antigen d - *Salmonella* Typhi H901.

   Salmonella O and H Antigens, available individually, are prepared from selected strains containing the following group-specific antigens:
   Salmonella H Antigen c - flagellar antigen c
   Salmonella O Antigen Group A - factors 1, 2, 12
   Salmonella O Antigen Group B - factors 1, 4, 5, 12
   Salmonella O Antigen Group C - factors 6, 7, (8), 20
   Salmonella Vi Antigen

   When used as described, each vial contains sufficient reagent for 20 slide tests or 25 tube tests.
2. **Concentration of Antigen**: Salmonella O, H and Vi Antigens are used undiluted for the slide test and diluted 1:20 for the tube test.
3. **Antigen Density**: Salmonella O, H and Vi Antigens are adjusted to a density approximating 20 times a McFarland Barium Sulfate Standard No. 3 (1.8×10^{10} organisms per ml).

   Because antigen density may vary, it is adjusted for optimum performance when standardized with hyperimmune sera obtained from laboratory animals.

   Variation in color intensity of the antigen is normal and will not affect the outcome of the test.
4. Salmonella Antigens contain the following preservatives:

   Salmonella O Antigens: 0.5% phenol, and approximately 0.002% crystal violet and 0.005% brilliant green.

   Salmonella H Antigens: 0.5% formaldehyde, and approximately 0.002% crystal violet and 0.005% brilliant green.

   Salmonella Vi Antigen: 0.5% phenol, and approximately 0.002% crystal violet and 0.005% brilliant green.

Antisera:

1. **Febrile Positive Control Polyvalent** is lyophilized, polyclonal, polyvalent goat antisera containing approximately 0.04% Thimerosal as a preservative. This reagent contains antibodies at a titer of 1:80 or greater for the Salmonella O and H Antigens in the Widal Antigen Set.

   Each vial of Febrile Positive Control Polyvalent contains sufficient reagent for 32 slide tests or 50 tube tests using the four antigens contained in the Widal Antigen Set. When using the Salmonella O, H and Vi Antigens separately, there is sufficient reagent for 20 slide or 25 tube tests.

2. **Febrile Negative Control** is a lyophilized, standard protein solution containing approximately 0.02% Thimerosal as a preservative.

   Each vial of Febrile Negative Control contains sufficient reagent for 32 slide tests when using the four antigens contained in the Widal Antigen Set. When using the Salmonella O, H and Vi Antigens separately, there is sufficient reagent for 20 slide tests.

Precautions

1. For In Vitro Diagnostic Use.
2. Observe universal blood and body fluid precautions in the handling and disposing of specimens.[12,13]
3. **Salmonella H Antigen a**
   Salmonella H Antigen b
   Salmonella H Antigen c
   Salmonella H Antigen d

   POSSIBLE RISK OF IRREVERSIBLE EFFECTS. (US) Avoid contact with skin and eyes. Do not breathe mist. Wear suitable protective clothing. Keep container tightly closed. TARGET ORGAN(S): Eyes, Kidneys, Lungs, Skin.

   FIRST AID: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Seek medical advice. If swallowed seek medical advice immediately and show this container or label.
4. Follow proper established laboratory procedure in handling and disposing of infectious materials.
5. Salmonella O, H and Vi Antigens are not intended for use in the immunization of humans or animals.

Storage

Store Salmonella O, H and Vi Antigens at 2-8°C.

Store lyophilized and rehydrated Febrile Positive Control Polyvalent at 2-8°C.

Store lyophilized and rehydrated Febrile Negative Control at 2-8°C.

Expiration Date

The expiration date applies to the product in its intact container when stored as directed. Do not use a product if it fails to meet specifications for identity and performance.

Procedure

Materials Provided

Widal Antigen Set:
Salmonella O Antigen Group D
Salmonella H Antigen Group a
Salmonella H Antigen Group b
Salmonella H Antigen Group d
Febrile Positive Control Polyvalent
Febrile Negative Control

Available separately:
Salmonella O Antigens
Salmonella H Antigens
Salmonella Vi Antigen
Febrile Positive Control Polyvalent
Febrile Negative Control
Droppers, 0.03 ml per drop (supplied)

Materials Required but not Provided

Slide test

Agglutination slides, 5 squares, 1” each
Applicator sticks
Sterile distilled or deionized water
Serological pipettes, 0.2 ml

Tube Test

Culture tubes 12 x 75 mm and rack
Waterbath, 50 ± 2°C
Serological pipettes, 1 ml and 5 ml
Sterile 0.85% NaCl solution

Reagent Preparation

Salmonella O, H and Vi Antigens are ready to use in the slide test. Salmonella O and H antigens must be diluted 1:20 for the tube tests. (See Test Procedure for preparation instructions).

Equilibrate all materials to room temperature before performing the tests. Ensure that all glassware and pipettes are clean and free of residues such as detergent.

Febrile Positive Control Polyvalent: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

Febrile Negative Control: To rehydrate, add 5 ml sterile distilled or deionized water and rotate gently to completely dissolve the contents.

Specimen Collection and Preparation

Collect a blood specimen by aseptic venipuncture. Serum is required for the test. Store serum specimens at room temperature for no longer than 4 hours; for prolonged storage, keep at 2-8°C for up to 5 days or maintain at or below -20°C. Serum specimens must be clear, free of hemolysis and show no visible evidence of bacterial contamination (turbidity, hemolysis or particulate matter). Refer to appropriate references for more information on collection of specimens.[14,15] Serum specimens must not be heated. Heat may inactivate or destroy certain antibodies.

Because changes in titer over a period of time are the best indicators of active infection, and because the accuracy and precision of the tests can be affected not only by test conditions but also by the subjectivity of the person reading the endpoint, the following protocol is recommended.

A preliminary test using either the rapid slide test and/or the macroscopic tube test may be performed on the initial serum specimen and reported to the physician at that time. An aliquot of the serum should be transferred to a sterile test tube, sealed tightly, and kept in the freezer. When the second serum is obtained, it should be run in parallel with the original specimen. In this manner, the original serum will serve as a control. Any difference in titer will be more credible, since the bias associated with the performance of the test and determining the endpoint will be reduced.

Test Procedure

Rapid Slide Test

Use the slide test only as a screening test. Confirm positive results with the tube test.

1. **Test serum**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of each test serum into a row of squares on an agglutination slide.
2. **Positive control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Febrile Positive Control Polyvalent into a row of squares on the agglutination slide.
3. **Negative control**: Using a 0.2 ml serological pipette, dispense 0.08, 0.04, 0.02, 0.01 and 0.005 ml of Febrile Negative Control into a row of squares on the agglutination slide.
4. **Salmonella Antigen**: Shake the vial of antigen well to ensure a smooth, uniform suspension. Place one drop (35 µl) of antigen suspension in each drop of test serum, positive control and negative control.
5. Mix each row of test sera and control sera, using a separate applicator stick for each row. Start with the most dilute mixture (0.005 ml) and work to the most concentrated (0.08 ml).
6. Rotate the slide for 1 minute and read for agglutination.
7. The final dilutions in squares 1-5 correspond with tube dilutions of 1:20, 1:40, 1:80, 1:160, 1:320, respectively.

Results

1. Read and record results as follows:
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
2. **Positive control**: Should show 2+ or greater agglutination at the 1:80 dilution.
3. **Negative control**: Should show no agglutination.
4. If results for either the positive or negative control are not as described, the test is invalid and results cannot be reported.
5. **Test specimens**: The serum titer is that dilution that shows a 2+ or greater agglutination. See Table 1.
6. The slide test is a screening test, only, and results must be confirmed with the tube test.

Table 1. Sample Rapid Slide Test reactions.

ml SERUM	CORRELATED DILUTION	REACTIONS		
		SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
0.08	1:20	3+	4+	4+
0.04	1:40	2+	3+	3+
0.02	1:80	1+	3+	2+
0.01	1:160	–	2+	+
0.005	1:320	–	1+	–
Serum titer		**1:40**	**1:160**	**1:80**

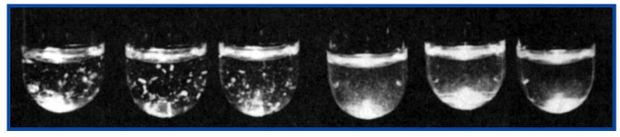
Macro Tube Test

Prepare a 1:20 dilution of the Salmonella O and H Antigens by adding 1 part of the antigen to 19 parts of sterile 0.85% NaCl solution.

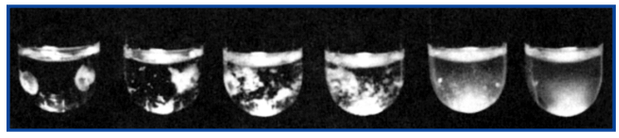
1. Prepare a row of eight culture tubes (12 x 75 ml) for each test serum, including a row for the Febrile Positive Control Polyvalent.
2. **Sterile 0.85% NaCl solution**: Dispense 0.9 ml in the first tube of each row and 0.5 ml in the remaining tubes.
3. **Test serum**: Using a 1 ml serological pipette, dispense 0.1 ml of test serum in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
4. **Positive control**: Using a 1 ml serological pipette, dispense 0.1 ml of Febrile Positive Control Polyvalent in the first tube in the row and mix thoroughly. Transfer 0.5 ml from tube 1 to tube 2 and mix thoroughly. Similarly, continue transferring 0.5 ml through tube 7, discarding 0.5 ml from tube 7 after mixing. Tube 8 is the antigen control tube and contains only sterile 0.85% NaCl solution.
5. **Salmonella Antigen**: Add 0.5 ml of the diluted antigen to all 8 tubes in each row and shake the rack to mix the suspensions.
6. The final dilutions in tubes 1-7 are 1:20, 1:40, 1:80, 1:160, 1:320, 1:640 and 1:1280, respectively.
7. **H antigen tests**: Incubate in a waterbath at 50 ± 2°C for 1 hour.
   O antigen tests: Incubate in a waterbath at 50 ± 2°C for 17 ± 1 hours.
8. Remove from the waterbath. Avoid excessive shaking before reading the reactions, either when the tubes are in the waterbath or when removing them from the waterbath.
9. Read and record the results.

Results

1. Tube agglutination reactions detect antibodies to either somatic (O) antigens or flagellar (H) antigens and these antibodies give two different reactions. An O antigen and the corresponding antibody give a coarse, compact agglutination which may be difficult to disperse. An H antigen and its corresponding antibody give a loose, flocculent agglutination. Do not vigorously shake tubes containing H antigens. Characteristic O and H agglutination is shown in the following diagrams.



Somatic "O" Agglutination



Flagellar "H" Agglutination

2. Read and record results as follows:
   - 4+ 100% agglutination; background is clear to slightly hazy.
   - 3+ 75% agglutination; background is slightly cloudy.
   - 2+ 50% agglutination; background is moderately cloudy.
   - 1+ 25% agglutination; background is cloudy.
   - – No agglutination.
3. **Positive control**: Should show 2+ or greater agglutination at the 1:80 dilution.

4. **Antigen control**: Tube #8 of each row should show no agglutination.
5. If results of the positive control and antigen control are not as described, the test is invalid and results cannot be reported.
6. **Test serum**: The serum titer is that dilution which shows 2+ or greater agglutination. See Table 2.

Table 2. Sample Macroscopic Tube Test reactions.

REACTIONS			
SERUM DILUTION	SPECIMEN 1	SPECIMEN 2	SPECIMEN 3
1:20	4+	3+	4+
1:40	4+	2+	4+
1:80	3+	1+	4+
1:160	2+	–	4+
1:320	1+	–	3+
1:640	–	–	2+
1:1280	–	–	1+
Serum Titer	**1:160**	**1:40**	**1:640**

Interpretation

For a single serum specimen, a titer of 1:80 suggests infection.

A pair of serum specimens (acute and convalescent) showing a two-dilution increase in titer is significant and suggests infection. A one dilution difference is within the limits of laboratory error.

Table 3 presents data that will be helpful in interpreting serological tests with the *Salmonella* antigens. The values tabulated will vary in certain cases.

Table 3. Disease states and associated Salmonella Antigens.

SALMONELLA ANTIGEN	SUGGESTED PATHOLOGY	TIME TO MAXIMUM TITER	SIGNIGICANT TITER
Salmonella H Antigen d (Typhoid H)	Typhoid Fever	4-5 weeks	1:80
Salmonella O Antigen Group D (Typhoid O)	Typhoid Fever	3-5 weeks	1:80
Salmonella H Antigen a (Paratyphoid A)	Paratyphoid Fever	3-5 weeks	1:80
Salmonella H Antigen b (Paratyphoid B)	Paratyphoid Fever	3-5 weeks	1:80

Limitations of the Procedure

1. The slide test is intended for screening only and should be confirmed by the tube test. Slide test dilutions are made to detect a prozone reaction and do not represent true quantitation of the antibody. A serum specimen with a prozone reaction shows no agglutination because of excessively high antibody concentrations. To avoid this occurrence, all five serum dilutions of the slide test should be run.
2. Detection of antibodies in serum specimens may complete the clinical picture of a patient having infection. However, isolation of the causative agent from patient specimens may be required. A definitive diagnosis must be made by a physician and must be based on patient history, physical examination and data from all laboratory tests.
3. In some cases of typhoid fever, sera may show a prozone reaction, the inability of an antigen to react in higher serum antibody concentrations. It is advised that all five serum dilutions be run in the rapid slide test, rather than just one dilution, to eliminate the possibility of missing positive reactions due to the prozone phenomenon.
4. Cross-reacting heterologous antibodies are responsible for many low titer reactions. Infections with other organisms, vaccinations and a history of disease may result in a low level of antibody titer. Antimicrobial therapy may suppress antibody production.
5. Previous immunization with typhoid vaccine or previous infection with *Salmonella* species sharing common antigens with *S.* Typhi can cause elevated antibody titers for prolonged periods. Other non-typhoid febrile illnesses may cause elevation of cross-reacting antibodies.
6. While a single serum specimen showing a titer of 1:80 suggests infection, it is not diagnostic.
7. Nonspecific agglutination has been noted with *Salmonella* O Group D antigen in the sera of patients with influenza.
8. Sera from narcotic addicts appear to contain broad nonspecific activity to the Widal antigen.[16]
9. Sera from patients with chronic active liver disease may show high agglutinin titers.[17]
10. To test for a significant rise in antibody titer, at least two specimens are necessary: an acute specimen (obtained at the time of initial symptoms) and a convalescent specimen (obtained 7 to 14 days later). A two-dilution difference in the titer is a significant increase in antibody level and suggests infection.
11. Antibody titers may be clinically useful in detecting chronic carriers of *S.* Typhi and in diagnosing typhoid fever in endemic areas. Detection of antibodies to heat-labile *Salmonella* envelope antigen (Vi) may be useful for the detection of a chronic carrier state for *S.* Typhi. The presence of capsular Vi antigen may mask somatic (O) antigen activity.
12. In *Salmonella* infections, the stage of the patient's disease is important and may affect test results. The optimum time for detecting peak titers may be missed if symptoms do not correlate with increased antibody levels. Antibodies to O antigens appear earlier and disappear earlier than antibodies to H antigens.
12. Prolonged exposure of reagents to temperatures other than those specified is detrimental to the products.
13. Exposure to temperatures below 2°C can cause autoagglutination. Antigens must be smooth uniform suspensions; before use, examine antigen vials for agglutination. Suspensions with agglutination are not usable and should be discarded.
14. Discard rehydrated Febrile Positive Control Polyvalent or Febrile Negative Control that is cloudy or has a precipitate anytime during its period of use.

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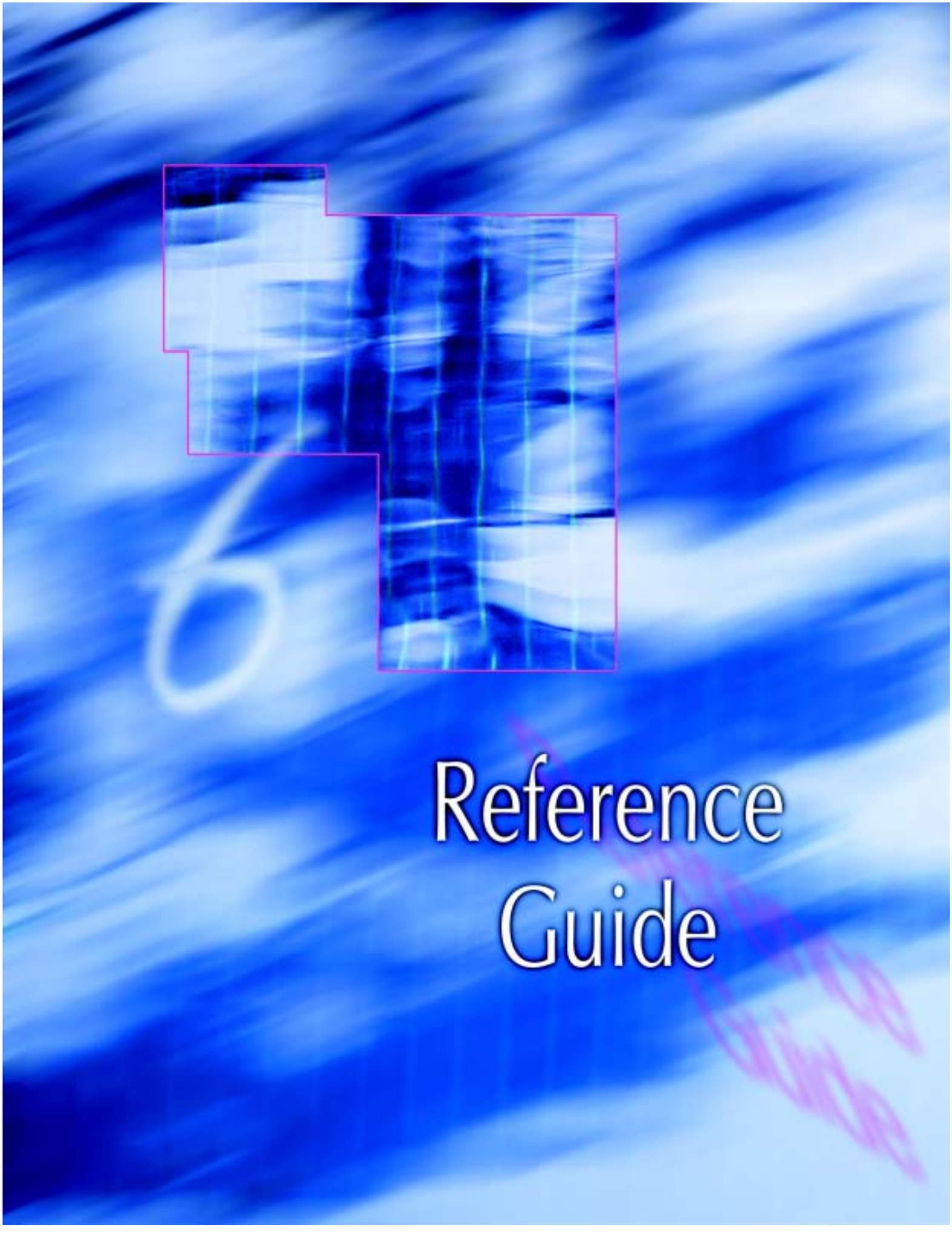
Packaging

Widal Antigen Set	6 x 5 ml	2642-32

Contains:
- Salmonella O Antigen Group D
- Salmonella H Antigen a
- Salmonella H Antigen b
- Salmonella H Antigen d
- Febrile Positive Control Polyvalent
- Febrile Negative Control

AVAILABLE SEPARATELY:

Salmonella H Antigen a	5 ml	2844-56
Salmonella H Antigen b	5 ml	2845-56
Salmonella H Antigen c	5 ml	2846-56
Salmonella H Antigen d	5 ml	2847-56
Salmonella O Antigen Group A	5 ml	2839-56
Salmonella O Antigen Group B	5 ml	2840-56
Salmonella O Antigen Group C	5 ml	2841-56
Salmonella O Antigen Group D	5 ml	2842-56
Salmonella Vi Antigen	5 ml	2953-56


Reference
Guide

Agar Selection Guide

APPLICATIONS	BACTERIOLOGICAL USES OF AGARS		
	Bacto® Agar	Agar, Granulated	Agar, Technical
Auxotrophic studies	++	+	
Bacteriology, research	++	+	
Bacteriology, general purpose	++	+	
Bacteriophage studies	++		
Biotechnology production	+	++	+/–
General microbial production	+	++	+/–
Growth of fastidious organisms	++		
Identification of pathogenic organisms	++	+	+/–
Microaerophilic studies	++		
Molecular genetics	++	+	
Prepared plate manufacture	+	++	+
Quality control, production	++	+	+/–
Quality control, environmental	+	++	+/–
Transformation of bacteria	++		
Transformation of yeast	++		

APPLICATIONS	NON-BACTERIOLOGICAL USES OF AGARS		
	Bacto® Agar	Agar, Granulated	Agar, Technical
Immunodiffusion		++	
Electrophoresis		++	
Tissue Culture, mammalian		++	
Tissue Culture, plant	++	+	
Histology, tissue embedding	+	++	
Histology, bone marrow embedding	++	+	
Insect growth substrate			++

Key

++ Recommended
+ Suitable
+/– Marginal

Anaerobes - General

ANAEROBES - GENERAL
Anaerobe Broth MIC
Anaerobic Agar
Blood Culture Bottles Columbia Broth w/CO_2
Blood Culture Bottles Columbia Broth w/SPS and CO_2
Blood Culture Bottles Fluid Thioglycollate Medium w/SPS and CO_2
Blood Culture Bottles Thioglycollate w/CO_2
Blood Culture Bottles Thioglycollate w/SPS and CO_2
Brain Heart Infusion Agar
Brewer Anaerobic Agar
Brewer Thioglycollate Medium
CHO Medium Base
Clostridium Difficile Antimicrobic Supplement CC
Cooked Meat Medium
ESP Anaerobic Broth
Differential Reinforced Clostridial Agar
Fluid Thioglycollate Medium
Liver Veal Agar
McClung Toabe Agar Base
NIH Thioglycollate Broth
Reinforced Clostridial Medium
Schaedler Agar
Schaedler Broth
SFP Agar Base
Sterility Bottles with Septum Fluid Thioglycollate Medium
Sterility Bottles with Screw Cap Fluid Thioglycollate Medium
SPS Agar
Sulfite Agar
Thioglycollate Medium USP Alternative
Thioglycollate Medium w/o Dextrose
Thioglycollate Medium w/o Dextrose or Indicator
Thioglycollate Medium w/o Indicator
Wilkens-Chalgren Agar

Antimicrobic Selective Agents For Culture Media

AGENT	CONCENTRATION PER VIAL	PRODUCT NAME
Cefsulodin-novobiocin	4 mg/2.5 mg	Yersinia Antimicrobic Supplement CN
Ceftozidime	40 mg	Palcam Antimicrobic Supplement
Chloramphenicol	0.05 g	Rose Bengal Antimicrobic Supplement C
Chlortetracycline (Aureomycin®)	25 mg	Antimicrobic Vial A
Colistin Sulfate - Nystatin - Vancomycin	7,500 mcg/12,500 units/ 3,000 mcg	Antimicrobic Vial CNV
Colistin Sulfate - Nystatin - Vancomycin Trimethoprim	7,500 mcg/12,500 units/ 3,000 mcg/5,000 mcg (10 ml vial)	Antimicrobic Vial CNVT
Cyclohexamide - Colistin Sulfate - Acriflavine - Cefotetan - Fosfomycin	400 mg/20 mg/5 mg/2 mg/ 10 mg	Oxford Antimicrobic Supplement
Cycloserine - Cefoxitin	125 mg/ 5 mg	Clostridium Difficile Antimicrobic Supplement CC
Kanamycin	25,000 mcg	Antimicrobic Vial K
Moxalactam	20 mg	Moxalactam Antimicrobic Supplement
Moxalactam - Colistin Sulfate	20 mg/10 mg	Modified Oxford Antimicrobic Supplement
Novobiocin	20 mg	Novobiocin Antimicrobic Supplement
Oxytetracycline	100 mg	Antimicrobic Vial Oxytetracycline
Polymyxin B	30,000 units	Antimicrobic Vial P
Potassium Tellurite Solution 1%	1%	Chapman Tellurite Solution
Potassium Tellurite Solution 3.5%	3.5%	Potassium Tellurite Solution 3.5%
Sodium 7- ethyl - 2 - methyl - 4 - undecyl sulfate		XLT4 Supplement

Cosmetic Testing

PRODUCTS	APPLICATIONS: Environmental (See also Environmental Sampling and Disinfectant Testing)	Gram-Negative Screening	Pseudomonas Isolation	Staphylococcus	Sterility Testing	Yeast & Mold Isolation
AC Broth					✔	
AC Broth Medium w/o Dextrose†					✔	
Cetrimide Agar Base/**PSEUDOSEL** Agar†			✔			
RODAC™ Plates	✔					
D/E Neutralizing Agar†	✔					
D/E Neutralizing Broth†	✔					
EMB Agar/Eosin Methylene Blue Agar Modified†		✔				
Fluid Thioglycollate Medium†					✔	
HC Agar Base†						✔
HYcheck	✔					
Letheen Agar†	✔					
Letheen Broth/Letheen Broth AOAC†	✔					
m Staphylococcus Broth				✔		
MacConkey Agar†		✔				
Malt Agar†						✔
Malt Extract Agar						✔
Malt Extract Broth†						✔
Mannitol Salt Agar†				✔		
Microbial Content Test Agar/ **TRYPTICASE**™ Soy w/Lec. poly.†	✔					
Modified Letheen Agar					✔	
Modified Letheen Broth					✔	
Mycological Agar/**MYCOPHIL**™ Agar†						✔
Mycobiotic Agar/**MYCOPHIL** Agar†						✔
Neutralizing Buffer†	✔					
Phenylethanol Agar/Phenylethyl Alcohol Agar†				✔		
Potato Dextrose Agar†						✔
Pseudomonas Agar F/Flo Agar†			✔			
Pseudomonas Agar P/Tech Agar†			✔			
Pseudomonas Isolation Agar/Pseudomonas ISO †			✔			
Sabouraud Dextrose Agar†						✔
Staph Latex Test Kit/**STAPHYLOSLIDE**™ Test Kit†				✔		
Staphylococcus Medium 110/Staphylococcus Agar #10†				✔		
Sterility Test Bottles, Prepared †					✔	
TAT Broth Base†					✔	
Tryptic Soy Broth/ **TRYPTICASE**™ Soy Broth†					✔	
VJ Agar/Vogel & Johnson Agar†				✔		

† Available from Difco & Becton Dickinson Microbiology Systems.

Dairy Product Testing - Products and Applications

PRODUCTS	APPLICATIONS								
	Beta-Lactams in Milk	*Clostridium*	Coliform Analysis	Environmental	Lipolytic Microorganisms	*Listeria* Analysis	Standard Plate Count	*Streptococcus* Detection	Yeast & Mold Analysis
Antibiotic Medium 1†	✔								
Antibiotic Medium 4†	✔								
Azide Blood Agar Base†								✔	
Brilliant Green Bile 2%/Brilliant Green Bile Broth†			✔						
Bryant and Burkey Medium		✔							
Concentration Disks 1/2" Penase	✔								
Concentration Disks 1/2" Penicillin 0.05 Unit/PM Discs, 0.05, 1/4" Taxo™†	✔								
Concentration Disks 1/2" Sterile Blanks, Antibiotic Detect Disc 1/2" Taxo†	✔								
Desoxycholate Lactose Agar†			✔						
D/E Neutralizing Agar†				✔					
D/E Neutralizing Broth†				✔					
EC Medium w/MUG/EC Broth w/ MUG†			✔						
m FC Agar/M-FC Agar†			✔						
Fraser Broth Base and/Fraser Broth Base Modified†						✔			
Fraser Broth Supplement/Fraser Broth Additive†						✔			
HYcheck™				✔					
LPM Agar Base†						✔			
Lactose Broth†			✔						
Lauryl Tryptose Broth w/MUG/ Lauryl Sulfate Broth w/ MUG†			✔						
Letheen Agar†				✔					
Letheen Broth/Letheen Broth AOAC†				✔					
Levine EMB Agar/Levine Eosin Methylene Blue Agar†			✔						
Listeria Antisera						✔			
M17 Broth†								✔	
Malt Extract Agar†									✔
Malt Extract Broth†									✔
McBride Listeria Agar†						✔			
Microbial Content Test Agar/ Trypticase™ Soy Agar w/Lec & Polysorbate†				✔					
Milk Agar IDF Formulation							✔		
Modified Listeria Enrichment Broth						✔			

† Available from Difco & Becton Dickinson Microbiology Systems.

Dairy Product Testing - Products and Applications

PRODUCTS	APPLICATIONS								
	Beta-Lactams in Milk	*Clostridium*	Coliform Analysis	Environmental	Lipolytic Microorganisms	*Listeria* Analysis	Standard Plate Count	*Streptococcus* Detection	Yeast & Mold Analysis
Modified Oxford Antimicrobic Supplement						✔			
Motility Test Medium†						✔			
Moxalactam Antimicrobic Supplement						✔			
Neutralizing Buffer†				✔					
Nutrient Agar†				✔					
Nutrient Broth†			✔						
Oxford Antimicrobic Supplement						✔			
Oxford Medium Base/ Oxford Agar Base Modified†						✔			
PALCAM Medium Base and PALCAM Antimicrobic Supplement						✔			
PM Indicator Agar	✔								
PM Negative Control	✔								
PM Positive Control	✔								
Penase Concentrate/Penicillinase Concentrate†	✔								
Phenylethanol Agar/Phenylethanol Alchol Agar†								✔	
Plate Count Agar/Standard Methods Agar†							✔		
Potato Dextrose Agar†									✔
Spirit Blue Agar w/Lipase Reagent					✔				
Strep Grouping Kit†								✔	
Subtilis Spore Suspension	✔								
Thermospore Suspension PM	✔								
Tryptic Soy Broth/**TRYPTICASE**™ Soy Broth†				✔					
Tryptone Glucose Extract Agar/ **TRYPTICASE** Glucose Extract Agar							✔		
UVM Modified Enrichment Broth/ UVM Mod. Listeria Enrichment Broth†						✔			
Universal Preenrichment Broth						✔			
Violet Red Bile Agar†			✔						
Violet Red Bile Agar w/MUG†			✔						
Yeast Extract Glucose Chloramphenicol Agar									✔

† Available from Difco & Becton Dickinson Microbiology Systems.

Environmental Sampling and Disinfectant Testing

CULTURE MEDIA	HYGIENE CONTACT SLIDES
Aseptic Commissioning Medium Bushnell-Haas Broth D/E Neutralizing Agar† D/E Neutralizing Broth† Disinfectant Test Broth AOAC Egg Meat Medium Letheen Agar† Letheen Broth/Letheen Broth AOAC† Microbial Content Test Agar /Trypticase™ Soy Agar w/ Lec. & Polysorbate† Neutralizing Buffer† Synthetic Broth AOAC	HYcheck™ D/E Neutralizing Agar HYcheck for Disinfection Control HYcheck for Enterobacteriaceae HYcheck for Plate Count Agar with TTC HYcheck for Total Count HYcheck for Yeasts and Molds HYcheck for Yeasts and Molds with TTC
CONTACT PLATES	**SETTLING PLATES (TRIPLE WRAPPED)**
DOUBLE WRAPPED (IRRADIATED) D/E Neutralizing Agar Sabouraud Dextrose Agar w/Lecithin and Polysorbate 80 Standard Methods Agar Tryptic Soy Agar w/Lecithin and Polysorbate 80	**STERILE 100mm OR 150mm** Sabouraud Dextrose Agar Tryptic Soy Agar Tryptic Soy Agar w/Lecithin and Polysorbate 80

Fermentation Products

FERMENTATION*		
Beef Extract, Desiccated†	Malt Extract†	Todd Hewitt Broth†
Brain Heart Infusion†	Neopeptone	Tryptic Soy Broth/**TRYPTICASE**™ Soy Broth
Brucella Broth†	Peptamin	Tryptic Soy Broth w/o Dextrose/ **TRYPTICASE** Soy Broth w/o Dextrose†
Casamino Acids/Select Casamino Acids†	Peptone, Bacto®	Tryptone/Select **TRYPTICASE** Peptone†
Casamino Acids, Technical†	Peptone Bacteriological, Technical	Tryptose/ **POLYPEPTONE**™ Peptone†
Casitone	Proteose Peptone/Meat Peptone†	Tryptose Phosphate Broth†
Eugon Broth/**EUGONBROTH**™	Proteose Peptone No. 2	Yeast Extract/Select Yeast Extract†
Gelatone/Gelysate Peptone†	Proteose Peptone No. 3	Yeast Extract, Technical†
Heart Infusion Broth†	Soytone/**PHYTONE**™ Peptone†	
M Broth†	TC Lactalbumin Hydrolysate	
	TC Yeastolate/Yeastolate, TC†	

*Custom formulations and packaging are also available.

† Available from Difco & Becton Dickinson Microbiology Systems.

Escherichia coli - Products and Applications

PRODUCTS	APPLICATIONS: Nonselective Media	Purity Plate	Selective-Differential Media	Serology	Specimen Collection and Shipment	Rapid Test
Brain Heart Infusion Agar†	✔					
BAGG Broth			✔			
Brilliant Green Bile Agar†			✔			
CULTURESWAB™ Transport System Amies Medium					✔	
CULTURESWAB Transport System Amies Medium w/o Charcoal/**CULTURETTE**™ Amies w/o Charcoal†					✔	
CULTURESWAB Transport System Cary-Blair Medium/ Anaerobic **CULTURETTE** Cary-Blair Single†					✔	
CULTURESWAB Transport System Stuart's Medium Modified/**CULTURETTE** Modified Stuart's Medium†					✔	
EC Medium w/MUG†			✔			
EMB Agar/Eosin Methylene Blue Agar Modified†			✔			
E. coli H Antiserum H7†				✔		
E. coli O Antiserum O157†				✔		
m Endo Agar LES/M-Enda Agar LES†			✔			
m Endo Broth MF®/M-Endo Broth			✔			
EZ Coli™ Rapid Detection System for *E. coli* 0157						✔
Lauryl Tryptose Broth w/MUG/Lauryl Sulfate Broth w/MUG†			✔			
MacConkey Agar†			✔			
MacConkey Sorbitol Agar/MacConkey II Agar w/Sorbitol†			✔			
Nutrient Agar†	✔					
Nutrient Agar w/MUG†	✔					
Transport Medium Amies					✔	
Transport Medium Stuart†					✔	
Transport Medium Amies w/o Charcoal					✔	
Tryptic Soy Agar/**TRYPTICASE**™ Soy Agar†	✔	✔				
TSA Blood Agar Base	✔	✔				
Veal Infusion Agar†	✔	✔				
Violet Red Bile Agar†			✔			
Violet Red Bile Agar w/MUG†			✔			

BIOCHEMICAL TESTS

Purple Agar/Broth Base		Ammonium Citrate	–	Tryptone Water	+
Adonitol	–	Gelatin	–	Urease	–
Dulcitol	V	H_2S	–	Voges-Proskauer	–
Glucose	+	Indole	+		
Inositol	–	KCN	–	**Key**	
Lactose	V	Methyl Red	+	– Negative	
Mannitol	+	Phenylalanine	–	+ Positive	
Salicin	V	Sodium Malonate	–	d Delayed	
Sucrose	V			V Variable +, d or –	

† Available from Difco & Becton Dickinson Microbiology Systems.

Food and Beverage Testing - Products and Applications

PRODUCTS	APPLICATIONS													
	Bacillus	Beverage Analysis	Campylobacter Analysis	Clostridium	Coliform Analysis	Environmental	Lactobacillus	Listeria Analysis	Salmonella/Shigella spp.	Staphylococcus	Total Plate Count	Vibrio	Yeast & Mold Analysis	Yersinia spp.
A-1 Medium/A-1 Broth†					✔									
APT Agar†							✔							
APT Broth†							✔							
Baird-Parker Agar Base w/EY Tellurite Enrichment†										✔				
Bismuth Sulfite Agar†									✔					
Brilliant Green Agar†									✔					
Brilliant Green Agar Modified (Edel-Kampelmacher)									✔					
Brilliant Green Bile 2%/Brilliant Green Bile Broth 2%†					✔									
Brucella Agar†			✔											
Brucella Broth†			✔											
Bryant and Burkey Medium				✔										
Buffered Peptone Water†									✔					
Campylobacter Agar Kit Blaser/Campylobacter Agar w/5 Antimicrobics and 10% Sheep Blood†			✔											
Campylobacter Agar Kit Skirrow			✔											
Campylobacter Agar Base			✔											
Coagulase Plasma (Rabbit)†										✔				
Coagulase Plasma EDTA (Rabbit)/ Coagulase Plasma, Rabbit w/ EDTA†										✔				
Cooke Rose Bengal Agar													✔	
Cooked Meat Medium†				✔										
D/E Neutralizing Agar†						✔								
D/E Neutralizing Broth†						✔								
DNAse Test Agar†										✔				
DNAse Test Agar w/Methyl Green/ DNAse Test Agar w/Toluidine Blue†										✔				
DRBC Agar													✔	
Demi-Fraser Broth Base								✔						
Desoxycholate Citrate Agar Hynes†									✔					
Differential Reinforced Clostridial Agar				✔										
EC Medium/EC Broth†					✔									
EC Medium with MUG/EC Broth w/MUG†					✔									
Elliker Broth†							✔							
m Endo Agar LES†					✔									
m Endo Broth MF®/ mEndo Broth, ALPHA†					✔									
EZ Coli™ Rapid Detection System					✔									
Fluid Thioglycollate Medium†				✔										
Fraser Broth Base/Fraser Broth Base Modified†								✔						
Fraser Broth Supplement/Fraser Broth Base Supplement†								✔						
Hektoen Enteric Agar†									✔					
HYcheck™ D/E Neutralizing Agar						✔								
HYcheck for Disinfection Control						✔								
HYcheck for Enterobacteriaceae						✔								
HYcheck for Total Count						✔								
HYcheck for Yeasts and Molds						✔								
HYcheck for Yeasts and Molds w/TTC						✔								
LPM Agar Base†								✔						

† Available from Difco & Becton Dickinson Microbiology Systems.

Food and Beverage Testing - Products and Applications

PRODUCTS	APPLICATIONS													
	Bacillus	Beverage Analysis	Campylobacter Analysis	Clostridium	Coliform Analysis	Environmental	Lactobacillus	Listeria Analysis	Salmonella/Shigella	Staphylococcus	Total Plate Count	Vibrio	Yeast & Mold Analysis	Yersinia sp.
Lactobacilli MRS Agar/MRS Agar†							✔							
Lactobacilli MRS Broth/MRS Broth†							✔							
Lactose Broth†									✔					
Lauryl Tryptose Broth/Lauryl Sulfate Broth†					✔									
Lauryl Tryptose Broth with MUG/Lauryl Sulfate Broth w/MUG†					✔									
Letheen Agar†						✔								
Letheen Broth/Letheen Broth AOAC†						✔								
Levine EMB Agar/Levine Eosin Methylene Blue Agar†					✔									
Listeria Enrichment Broth†								✔						
Listeria O Antisera								✔						
Liver Veal Agar†				✔										
Lysine Medium		✔											✔	
MYP Agar	✔													
M Broth†									✔					
MacConkey Agar†					✔				✔					
MacConkey Sorbitol Agar/MacConkey II Agar w/Sorbitol†					✔									
Malt Agar†													✔	
Malt Extract Agar†													✔	
Malt Extract Broth†													✔	
Mannitol Salt Agar†										✔				
McBride Listeria Agar†								✔						
McClung Toabe Agar Base†				✔										
Microbial Content Test Agar/ **TRYPTICASE**™ Soy Agar w/Lec. & Polysorbate 80†						✔								
Minerals Modified Glutamate Agar					✔									
Modified Listeria Enrichment Broth								✔						
Modified Oxford Antimicrobic Supplement								✔						
Modified EC Medium w/Indicator and Novobiocin Antimicrobic Supplement					✔									
Motility Test Medium†								✔						
Moxalactam Antimicrobic Supplement†								✔						
Muller Kauffmann Tetrathionate Broth Base									✔					
Mycological Agar/**MYCOPHIL**™ Agar†													✔	
Neutralizing Buffer†						✔								
OGYE Agar Base w/Antimicrobic Vial Oxytetracycline													✔	
Orange Serum Agar†		✔												
Orange Serum Broth Concentrate 10X		✔												
Oxford Antimicrobic Supplement								✔						
Oxford Medium Base/Oxford Agar Base Modified†								✔						
PALCAM Medium Base with PALCAM Antimicrobic Supplement								✔						
Plate Count Agar/Standard Methods Agar†											✔			
Potato Dextrose Agar†													✔	
Potato Dextrose Broth†													✔	
Rappaport-Vassiliadis (MSRV) Medium Semisolid Modification									✔					

† Available from Difco & Becton Dickinson Microbiology Systems.

Food and Beverage Testing - Products and Applications

PRODUCTS	APPLICATIONS													
	Bacillus	Beverage Analysis	Campylobacter Analysis	Clostridium	Coliform Analysis	Environmental	Lactobacillus	Listeria Analysis	Salmonella/Shigella sp.	Staphylococcus	Total Plate Count	Vibrio	Yeast & Mold Analysis	Yersinia sp.
Rappaport-Vassiliadis R10 Broth								✔						
Raka-Ray No. 3 Broth		✔					✔							
Raka-Ray No. 3 Medium		✔					✔							
Reinforced Clostridial Agar†				✔										
Rogosa SL Agar†							✔							
Rogosa SL Broth†							✔							
Rose Bengal Agar Base													✔	
Rose Bengal Antimicrobic Supplement C/ Chloramphenicol Selective†													✔	
SFP Agar Base/TSN Agar†				✔										
SS Agar/Salmonella Shigella Agar†								✔						
Sabouraud Dextrose Agar†													✔	
Sabouraud Dextrose Broth													✔	
Salmonella H Antiserum Poly a-z†								✔						
Salmonella O Antisera †								✔						
Selenite Broth/Selenite F Broth†								✔						
Selenite Cystine Broth†								✔						
Special Yeast and Mold Medium													✔	
m Staphylococcus Broth									✔					
Staphylococcus Medium 110/Staphylococcus Agar 110†									✔					
Staph Latex Test/**STAPHYLOSLIDE**™ Test†									✔					
Sulfite Agar				✔										
TCBS Agar†												✔		
TT Broth Base, Hajna/Tetrathionate H†								✔						
Tetrathionate Broth Base†								✔						
Tomato Juice Agar†						✔								
Tryptic Soy Broth/**TRYPTICASE**™ Soy Broth†					✔									
Tryptone Water					✔									
UBA Medium/Universal Beer Agar†		✔												
UVM Modified Listeria Enrichment Broth†							✔							
Universal Preenrichment Broth							✔	✔						
Vibrio Cholerae Antisera												✔		
Violet Red Bile Agar†					✔									
Violet Red Bile Agar w/MUG†					✔									
Violet Red Bile Glucose Agar					✔									
WL Differential Medium/WL Differential Agar†		✔												
WL Nutrient Broth		✔												
WL Nutrient Medium†		✔												
Wort Agar†		✔											✔	
XLD Agar †								✔						
XLT4 Agar Base and XLT4 Supplement								✔						
YM Agar													✔	
YM Broth													✔	
Yeast Extract Glucose Chloramphenicol Agar													✔	
Yersinia Selective Agar Base/CIN Agar Base†														✔
Yersinia Antimicrobic Supplement CN/CN Inhibitor†														✔

† Available from Difco & Becton Dickinson Microbiology Systems.

McFarland Standard Preparation

"MCFARLAND"	SULFURIC ACID, 1% AQUEOUS SOLUTION ML	BARIUM CHLORIDE, 1% AQUEOUS SOLUTION ML	CORRESPONDING DENSITY OF BACTERIA -10^6	INTERNATIONAL UNITS (IU) OF OPACITY
1	9.9	0.1	300	3
2	9.8	0.2	600	7
3	9.7	0.3	900	10
4	9.6	0.4	1200	12
5	9.5	0.5	1500	15
6	9.4	0.6	1800	–
7	9.3	0.7	2100	20
8	9.2	0.8	2400	–
9	9.1	0.9	2700	–
10	9.0	1.0	3000	30

1. Prepare the tubes by mixing 1% sulfuric acid with 1% barium chloride according to the table.
2. Make sure the tubes are uniform in size and made of chemically resistant glass.
3. Plug the tubes with rubber stoppers and carefully seal with paraffin. Store the tubes upright.
4. To estimate bacterial cell density, compare the bacterial suspension with the standards.
5. The above set is used to determine bacterial density in saline suspension. To estimate bacterial density in broth, make the set by dissolving the sulfuric acid and barium chloride in sterile broth.

REFERENCE IN: Gradwohl's Clinical Laboratory Methods and Diagnosis. In. A.C. Sonnenwirth and L. Jarett (ed.). C.V. Mosby Company, 1980 p. 1363.

Molecular Genetics - Media and Ingredients

LB MEDIA	NZ MEDIA AND INGREDIENTS	GENERAL MEDIA AND INGREDIENTS
LB Agar, Lennox/LB Agar (Lennox LAgar)†	Casein Digest/Casein Digest Peptone†	M9CA Medium
LB Agar, Miller (Luria-Bertani)/ Luria Agar†	NZCYM Broth†	M9 Minimal Salts, 5x/ M9 Minimal Salt†
LB Broth, Lennox/LB Broth†	NZM Broth	Minimal Agar Davis
LB Broth, Miller/Luria Broth†	NZYM Broth/NZY Broth†	Minimal Broth Davis w/o Dextrose
Luria Agar Base, Miller		SOB Medium
Luria Broth Base, Miller		Terrific Broth
		2xYT
		YPD Agar/YEPD Agar†
		YPD Broth/YEPD Broth†
		Yeast Nitrogen Base†
		Yeast Nitrogen Base w/o Amino Acids†
		Yeast Nitrogen Base w/o Amino Acids & Ammonium Sulfate

† Available from Difco & Becton Dickinson Microbiology Systems.

Mycobacteria

MYCOBACTERIA		
ATS Medium	ESP Myco GS	Middlebrook 7H9 Broth/Middlebrook 7H9 Broth Base†
Dubos Albumin Broth	ESP Myco PVNA	Middlebrook 7H10 Agar/Middlebrook & Cohn 7H10 Agar Base†
Dubos Broth Base	Lowenstein Medium Base/ Lowenstein Jensen Medium Base†	Middlebrook ADC Enrichment†
Dubos Medium Albumin	Lowenstein Medium Gruft	Middlebrook OADC Enrichment
Dubos Oleic Agar Base	Lowenstein Medium, Jensen	Middlebrook OADC Enrichment w/WR 1339
Dubos Oleic Albumin Complex/ Oleic Albumin Complex†	Lowenstein Medium, Jensen Deeps	Mycobacteria 7H11 Agar/Seven H11 Agar Base†
ESP Myco	Lowenstein Medium w/5% NaCl	Petragnani Medium

† Available from Difco & Becton Dickinson Microbiology Systems.

Mycology

CLINICAL REAGENTS FOR DETECTION OF *CANDIDA ALBICANS*

BiGGY Agar†
Candida Isolation Agar
Candida Albicans Antiserum

MEDIA FOR CLASSIFICATION OF YEASTS

Yeast Carbon Base
Yeast Morphology Agar
Yeast Nitrogen Base†
Yeast Nitrogen Base w/o Amino Acids†
Yeast Nitrogen Base w/o Amino Acids and Ammonium Sulfate

NON-INHIBITORY MEDIA FOR FUNGI

MEDIA	General Use	Vitamin Assay	*Candida* and/or Dermatophytes
Blood Agar Base†	✔		
Brain Heart Infusion†	✔		
Brain Heart Infusion Agar†	✔		
Brain Heart Infusion w/PAB†	✔		
Corn Meal Agar†			✔
Fluid Sabouraud Medium	✔		
Malt Agar†	✔		
Malt Extract Agar†	✔		
Malt Extract Broth†	✔		
Mycological Agar/**MYCOPHIL**™ Agar†	✔		
Mycological Agar w/Low pH/**MYCOPHIL**™ w/ Low pH†	✔		
Oatmeal Agar	✔		
Pagano Levin Base and TTC			✔
Potato Dextrose Agar†	✔		
Potato Dextrose Broth†	✔		
Rice Extract Agar†			✔
SABHI Agar Base/SABHI Agar†	✔		
Sabouraud Agar Modified/ Sabouraud Dextrose Agar Emmons†	✔		
Sabouraud Dextrose Agar†	✔		
Sabouraud Dextrose Broth	✔		
Sabouraud Maltose Agar†	✔		
Special Yeast and Mold Medium	✔		
YM Agar	✔		
YM Broth	✔		

† Available from Difco & Becton Dickinson Microbiology Systems.

Mycology

Reagents for Direct Microscopic Detection of Fungi

PRODUCT	USES
Gram Stain Sets and Reagents†	General screening
3-Step™ Gram Stain Sets and Reagents	General screening
SpotTest™ Calcofluor White Reagent/Calcofluor White Droppers† A nonspecific fluorochrome stain which allows the rapid examination of clinical specimens for the presence of fungi under an FA microscope	Screening of cultures for presence of fungi
SpotTest India Ink/India Ink Droppers† For use in the direct microscopic examination of clinical material for the presence of encapsulated yeast cells	Screening CSF for Cryptococcus neoformans Negative staining
SpotTest KOH 10%/Potassium Hydroxide 10% Droppers† A 10% solution of potassium hydroxide	Used in wet mounts of clinical specimens to examine for the presence of fungi
SpotTest Lactophenol Cotton Blue Stain/ Lactophenol Cotton Blue Droppers† For use in the direct mounting and staining of yeast and molds	Teased mount method Slide culture method

Selective and/or Differential Media for Fungi

GENERAL USE	*CANDIDA* AND/OR DERMATOPHYTES
Brain Heart CC Agar†	BiGGY Agar†
Cooke Rose Bengal Agar and Antimicrobic Vial A	Candida BCG Agar Base
DRBC Agar	Candida Isolation Agar
HC Agar Base†	DTM Agar
Mycobiotic Agar/Mycosel™ Agar†	Littman Oxgall Agar
Mycological Agar w/Low pH/Mycophil™ Agar w/Low pH†	Trichophyton Agars 1-7
Rose Bengal Agar Base and Rose Bengal Antimicrobic Supplement C	
Yeast Extract Glucose Chloramphenicol Agar	

† Available from Difco & Becton Dickinson Microbiology Systems.

Pasco® Panel Contents - Selection Guide

ANTIMICROBIAL AGENT (MCG/ML)		MIC GRAM-NEG.	MIC/ID GRAM-NEG.	MIC/ID GRAM-NEG. SPECIAL	BREAKPOINT/ID GRAM-NEG.	MIC ORAL	MIC SUPP.	MIC GRAM-POS.	MIC/ID GRAM-POS.
AK	Amikacin	32,24,16-1	32-4	32-4	32,24,16-4	———	———	———	———
A/C	Amoxicillin/ Clavulanic Acid	———	———	16-4/8-2	———	16-0.5/8-0.25	16-0.25/8-0.12	———	———
AM	Ampicillin	16-2	16-2	16-8	16-8	16-0.12	———	8-0.12	8-0.12
A/S	Ampicillin/ Sulbactam	16-2/8-1	16-8/8-4	16-8/8-4	16-8/8-4	———	———	16-2/8-1	16-2/8-1
AZT	Aztreonam	16-4	16-8	16-8	16-8	———	———	———	———
CB	Carbenicillin	———	———	———	———	256-128	256-128	———	———
CCL	Cefaclor	———	———	———	———	16-1	16-1	———	———
CFZ	Cefazolin	16-2	16-2	16-8	16-8	———	———	16-2	16-8
FIX	Cefixime	2-1	2-1	———	2-1	2-0.25	———	2-0.5	2-1
CPZ	Cefoperazone	32-4	32-16	32-16	32-16	———	———	———	———
CTX	Cefotaxime	32-8	———	32-8	32-8	———	16-0.12	32-4	32-8
CTN	Cefotetan	32-8	32-8	32-16	32-16	———	———	———	———
CX	Cefoxitin	16-2	16-4	16-8	16-8	———	———	———	———
TAZ	Ceftazidime	16-2	16-4	16-4	16-8	———	———	———	———
CTZ	Ceftizoxime	32-4	32-8	———	32-8	———	———	———	———
FRX	Ceftriaxone	32-4	32-8	32-8	32-8	———	8-0.25	32-4	32-8
CFX	Cefuroxime	16-2	16-2	16-8	16-8	16-0.5	4-0.25	16-4	16-8
CF	Cephalothin	———	———	16-8	16-8	16-1	16-8	———	———
C	Chloramphenicol	16-2	———	16-8	16-8	16-0.5	8-1	16-4	16-4
CIP	Ciprofloxacin	2-0.25	2-0.25	2-1	2-1	2-0.06	———	2-0.25	2-0.25
CLM	Clarithromycin	———	———	———	———	4-0.25	4-0.12	4-0.5	4-0.5
CD	Clindamycin	———	———	———	———	2-0.25	2-0.06	2-0.25	2-0.25
EN	Enoxacin	———	———	———	4-2	———	———	———	———
E	Erythromycin	———	———	———	———	4-0.25	4-0.25	4-0.5	4-0.5
GM	Gentamicin	8,6,4-0.25	8,6,4-0.5	8,6,4-0.5	8,6,4-1	———	———	500,8,6,4-0.25	500,8,6,4-1
IMI	Imipenem	———	———	8-4	———	———	8-0.06		
LOM	Lomefloxacin	4-2	4-2	———	4-2	4-2	———	4-2	4-2
MZ	Mezlocillin	64-8	64-8	64-16	———	———	———	———	———
NET	Netilmicin	16-8	———	———	———	———	———	———	———
FD	Nitrofurantoin	64-32	64-32	64-32	64-32	64-32	64-32	———	64-32
NOR	Norfloxacin	8-4	_____	8-4	8-4	8-4	8-2	———	———
OFX	Ofloxacin	4-0.5	4-0.5	4-2	4-2	4-0.12	8-1	4-0.25	4-1
OX	Oxacillin	———	———	———	———	6,4-0.5	———	6.4-0.5	6,4-1
P	Penicillin	———	———	———	———	8-0.03	2-0.03	8-0.03	8-0.03
PIP	Piperacillin	64-8	_____	64-16	64-8	———	———	———	———
P/T	Piperacillin/ Tazobactam	64-8/4	64-8/4	64-16/4	64-16/4	———	———	16-2/4	16-4/4
RI	Rifampin	———	———	———	———	2-1	4-0.5	2-1	2-1
STR	Streptomycin	———	———	———	———	———		1000	1000
SFX	Sulfisoxasole	———	———	———	———	256	———	———	———
TE	Tetracycline	———	———	8-4	8-4	8-0.5	8-1	8-2	———
TC	Ticarcillin	———	———	———	64-8	———	———	———	———
T/C	Ticarcillin/ Clavulanic Acid	———	———	64-16/2	———	———	64-16/2	———	———
TO	Tobramycin	8,6,4-0.25	8,6,4,-0.5	8,6,4,-0.5	8,6,4-1	———	———	8,6,4-0.25	8,6,4-1
T/S	Trimethoprim/ Sulfamethoxazole	2-1/38-19	2-1/38-19	2-38	2/38	2-0.5/38-9.5	2-0.5/38-9.5	2-0.5/38-9.5	2/38
VA	Vancomycin	———	———	———	———	16-4	4-0.5	16-1	16-2
Biochemical Substrates			30*	30*	30*				18**

* Gram-Negative Panels
** Gram-Positive Panels

Peptones & Hydrolysates Selection Guide

Typical Analyses

PRODUCT	AMINO ACIDS - %																	
	Alanine	Arginine	Aspartic Acid	Cystine	Glutamic Acid	Glycine	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Proline	Serine	Threonine	Tryptophan	Tyrosine	Valine
Beef Extract	2.54	1.39	1.67	0.18	6.01	4.14	4.94	0.53	1.00	1.45	0.30	<0.01	2.16	0.90	0.67	0.05	1.99	0.86
Beef Extract, Dessicated	8.96	5.66	4.30	0.17	12.55	16.25	2.50	1.45	3.63	3.27	1.08	2.00	9.58	2.10	1.42	0.32	1.03	2.62
Casamino Acids	3.26	2.20	4.76	0.16	15.30	1.31	1.66	3.34	5.47	5.71	1.28	2.11	6.17	2.19	2.41	<0.01	0.47	4.30
Casamino Acids, Technical	1.64	1.77	3.42	0.34	10.97	1.09	1.31	0.13	2.54	2.14	1.19	5.23	4.44	2.64	1.99	0.01	1.33	3.24
Casein Digest	2.92	3.02	6.75	0.17	23.10	1.93	2.52	4.66	8.29	7.70	2.66	4.27	11.04	5.55	4.33	1.16	2.54	6.51
Casitone	3.01	3.76	6.61	0.02	20.03	1.97	2.17	4.16	8.74	13.62	1.71	4.02	8.57	4.82	3.74	0.14	2.09	4.06
Neopeptone	4.03	4.14	6.19	0.26	13.22	7.02	<0.01	0.36	3.65	5.16	2.00	8.67	6.73	4.22	3.69	0.96	4.21	4.96
Bacto Peptone	8.67	6.76	5.60	0.20	10.21	15.59	0.58	1.45	3.01	3.42	1.19	1.81	8.80	2.87	1.81	0.36	0.64	2.35
Proteose Peptone	6.50	5.12	7.28	0.87	11.95	9.68	2.01	3.04	5.66	5.33	1.97	2.86	5.93	3.49	3.14	0.60	2.35	3.76
Proteose Peptone No. 2	6.08	5.47	7.45	0.40	10.57	10.84	<0.01	1.00	3.57	5.22	1.51	7.94	5.31	4.64	3.90	0.94	1.92	4.73
Proteose Peptone No. 3	5.99	5.49	6.92	1.12	12.38	9.26	1.74	2.65	5.70	5.02	1.86	2.72	4.94	3.65	3.32	0.59	1.96	3.62
Soytone	2.46	3.82	7.27	1.45	12.76	2.51	1.24	2.37	4.03	3.45	0.86	2.46	2.92	2.87	2.17	0.47	1.93	2.65
TC Lactalbumin Hydrolysate	3.70	2.67	7.13	0.55	16.30	2.02	1.83	1.18	5.43	4.11	2.11	10.81	6.62	4.62	4.76	1.75	1.22	5.82
TC Yeastolate	4.84	2.99	5.58	0.45	10.53	4.02	<0.01	0.56	3.23	3.82	0.96	5.59	2.59	2.81	2.80	0.79	1.21	3.80
Tryptone	2.86	3.03	6.11	0.42	17.05	1.75	2.02	4.40	7.11	6.70	2.57	3.71	7.45	4.29	3.58	0.71	1.42	5.00
Tryptose	4.45	4.65	6.34	0.44	13.92	2.84	<0.01	0.34	3.67	4.64	1.92	7.52	6.33	4.09	3.55	0.62	2.21	1.93
Yeast Extract	5.36	3.02	6.69	0.74	14.20	3.25	1.20	3.23	4.69	5.15	1.05	2.53	2.60	2.84	2.95	1.36	1.20	3.79

PRODUCT	INORGANICS - %															CARBOHYDRATE
	Calcium	Chloride	Cobalt	Copper	Iron	Lead	Magnesium	Manganese	Phosphate	Potassium	Sodium	Sulfate	Sulfur	Tin	Zinc	Total (%)
Beef Extract	0.068	1.284	<0.001	<0.001	<0.001	<0.001	0.239	<0.001	5.458	5.477	2.315	0.629	0.707	<0.001	<0.001	0.2
Beef Extract, Dessicated	0.018	1.576	<0.001	0.001	0.011	<0.001	0.022	<0.001	0.345	1.994	2.774	0.829	0.661	<0.001	0.002	<0.1
Casamino Acids	<0.001	7.400	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	3.325	0.410	8.710	0.045	0.420	<0.001	<0.001	
Casamino Acids, Technical	0.019	21.212	<0.001	<0.001	<0.001	<0.001	0.008	<0.001	1.358	0.273	13.721	0.167	0.424	<0.001	0.002	3.4
Casein Digest	0.019	0.059	<0.001	<0.001	0.002	<0.001	0.018	<0.001	2.670	0.078	2.937	0.167	0.621	<0.001	0.002	4.2
Casitone	0.010	0.110	<0.001	<0.001	0.003	<0.001	0.019	<0.001	2.604	0.162	3.073	0.339	0.676	<0.001	0.004	0.2
Neopeptone	0.012	0.344	<0.001	<0.001	<0.001	<0.001	0.006	<0.001	2.209	0.149	2.057	0.340	0.657	<0.001	<0.001	0.8
Bacto Peptone	0.008	1.086	<0.001	<0.001	0.004	<0.001	0.007	<0.001	0.445	0.203	1.759	0.244	0.410	<0.001	0.001	6.9
Proteose Peptone	0.021	4.510	<0.001	<0.001	0.002	<0.001	0.027	<0.001	0.872	0.685	3.677	0.162	0.812	<0.001	0.002	<0.1
Proteose Peptone No. 2	0.024	3.644	<0.001	<0.001	<0.001	<0.001	0.024	<0.001	1.674	0.815	3.956	0.232	0.698	<0.001	0.003	1.3
Proteose Peptone No. 3	0.023	3.581	<0.001	<0.001	0.002	<0.001	0.027	<0.001	1.447	0.982	3.815	0.232	0.975	<0.001	0.007	1.4
Soytone	0.055	0.165	<0.001	<0.001	0.008	<0.001	0.161	<0.001	0.820	2.220	3.404	2.334	1.660	<0.001	0.001	24.0
TC Lactalbumin Hydrolysate	0.095	<0.010	<0.001	<0.001	0.002	<0.001	0.026	<0.001	1.309	0.932	1.357	0.379	0.750	<0.001	0.002	9.2
TC Yeastolate	0.002	0.299	<0.001	<0.001	<0.001	<0.001	0.025	<0.001	2.633	5.085	0.819	0.488	0.528	<0.001	0.007	10.3
Tryptone	0.013	0.186	<0.001	<0.001	<0.001	<0.001	0.017	<0.001	2.669	0.229	2.631	0.241	0.740	<0.001	0.003	7.7
Tryptose	0.001	1.886	<0.001	<0.001	0.002	<0.001	0.022	<0.001	2.144	0.679	3.410	0.308	0.737	<0.001	0.005	7.1
Yeast Extract	0.013	0.380	<0.001	<0.001	<0.001	<0.001	0.075	<0.001	3.270	3.195	1.490	0.091	0.634	<0.001	0.011	17.5

Peptones & Hydrolysates Selection Guide

Typical Analyses

PRODUCT	PHYSICAL CHARACTERISTICS						BIOLOGICAL TESTING - CFU/g						NITROGEN CONTENT		
	Ash (%)	Clarity 1% Soln (NTU)	Filterability (g/cm²)	Loss on Drying (%)	pH, 1% Soln		Coliform	Salmonella	Spore Count	Standard Plate Count	Thermophile Count		Total Nitrogen (%)	Amino Nitrogen	AN/TN (%)
Beef Extract	24.1	116.8	0.1	77.2*	5.4		neg	neg	299	117	33		11.2	3.8	33.8
Beef Extract, Dessicated	10.2	1.7	0.6	2.5	6.9		neg	neg	585	690	28		14.0	2.2	15.7
Casamino Acids	24.4	0.5	2.9	4.5	6.4		neg	neg	390	950	25		10.5	8.8	83.8
Casamino Acids, Technical	38.3	0.3	2.6	4.5	6.7		neg	neg	2375	2250	<50		8.1	6.4	79.0
Casein Digest	6.4	0.4	2.6	4.7	7.2		neg	neg	235	250	178		13.4	7.2	53.9
Casitone	7.0	0.6	1.7	3.7	7.2		neg	neg	300	1850	100		13.3	4.7	35.3
Neopeptone	7.0	1.2	0.3	3.2	7.4		neg	neg	175	400	75		13.7	3.3	23.8
Bacto Peptone	4.4	0.5	0.5	3.0	7.0		neg	neg	90	273	13		15.5	3.1	20.0
Proteose Peptone	11.1	1.4	0.9	3.1	7.2		neg	neg	393	443	73		14.0	2.9	20.7
Proteose Peptone No. 2	12.7	1.5	0.6	3.5	7.2		neg	neg	75	1450	<50		12.6	5.0	39.7
Proteose Peptone No. 3	11.4	2.2	0.5	4.0	7.2		neg	neg	890	915	25		13.2	3.5	26.5
Soytone	12.0	1.0	1.2	4.6	7.2		neg	neg	10	38	<3		9.4	3.1	33.0
TC Lactalbumin Hydrolysate	7.2	0.4	7.3	4.6	7.1		neg	neg	<50	300	<50		13.0	6.3	48.3
TC Yeastolate	13.0	1.4	4.5	3.6	6.9		neg	neg	175	175	<50		10.8	6.5	59.8
Tryptone	6.8	0.5	1.3	3.7	7.2		neg	neg	73	870	8		13.0	5.2	40.0
Tryptose	9.7	0.8	2.3	3.2	7.4		neg	neg	875	825	100		13.4	4.4	32.5
Yeast Extract	11.2	1.5	2.7	3.1	6.7		neg	neg	9	60	<5		10.9	6.0	55.0

PRODUCT	VITAMINS - µg/g											
	Biotin	Choline	Cyanocobalamin	Folic Acid	Inositol	Nicotinic Acid	PABA	Pantothenic Acid	Pyridoxine	Riboflavin	Thiamine	Thymidine
Beef Extract	0.1	1171.5	0.5	3.3	4113.2	774.7	20.0	91.0	7.3	0.4	<0.1	1093.4
Beef Extract, Dessicated	0.1	1300.0	<0.1	0.6	2100.0	138.1	40.5	8.7	2.8	<0.1	<0.1	111.3
Casamino Acids	<0.1	160.0	<0.1	<0.1	<100.0	<20.0	<5.0	<0.1	<0.1	1.8	1.2	<30.0
Casamino Acids, Technical	<0.1	<50.0	<0.1	<0.1	<38.0	<0.1	9.8	<0.6	<0.1	0.2	0.2	<14.0
Casein Digest	0.1	<40.0	<0.1	1.0	490.0	14.1	6.1	6.7	0.4	<0.1	1.5	8297.0
Casitone	0.2	550.0	<0.1	0.8	980.0	20.3	15.9	7.7	1.3	0.4	<0.1	342.9
Neopeptone	0.2	3100.0	<0.1	0.4	3600.0	52.2	2.9	16.0	2.3	1.3	<0.1	<14.0
Bacto Peptone	0.2	2000.0	<0.1	0.3	2400.0	21.9	<0.5	5.9	1.7	3.9	<0.1	413.0
Proteose Peptone	0.1	2300.0	<0.1	0.4	5000.0	79.9	4.2	20.0	1.1	<0.1	1.2	99.7
Proteose Peptone No. 2	0.3	4500.0	<0.1	0.5	4700.0	157.1	1.2	47.0	4.0	6.4	1.6	1319.0
Proteose Peptone No. 3	0.4	3700.0	<0.1	0.3	8900.0	124.2	<0.5	20.0	1.3	6.8	0.1	659.6
Soytone	0.2	2200.0	<0.1	3.0	2100.0	19.1	9.0	13.0	11.0	<0.1	1.2	113.2
TC Lactalbumin Hydrolysate	<0.1	280.0	<0.1	0.2	360.0	<0.1	11.3	4.3	1.0	8.5	0.5	<14.0
TC Yeastolate	6.7	3400.0	<0.1	25.2	1900.0	945.0	96.6	300.0	77.5	21.8	54.3	2975.0
Tryptone	0.1	350.0	<0.1	0.3	1400.0	97.8	3.7	5.3	0.6	<0.1	0.4	93.4
Tryptose	0.2	2700.0	<0.1	0.4	5400.0	47.4	11.4	16.0	1.4	4.3	0.1	769.0
Yeast Extract	3.3	300.0	<0.1	1.5	1400.0	597.9	763.0	273.7	43.2	116.5	529.9	217.5

*Represents Total Solids value rather than loss on drying.

Pharmaceutical Testing - Products and Applications

PRODUCTS	APPLICATIONS: Sterility Testing	Antibiotic Assay	Vitamin/Amino Acid Assay
Agar Medium No. F	✔		
Antibiotic Medium 1†		✔	
Antibiotic Medium 2/Base Agar, Penicillin Assay†		✔	
Antibiotic Medium 3†		✔	
Antibiotic Medium 4/Yeast Beef Agar†		✔	
Antibiotic Medium 5/ Streptomycin Assay Agar w/YE†		✔	
Antibiotic Medium 8/Base Agr w/Low pH†		✔	
Antibiotic Medium 9/Polymixin Base Agar†		✔	
Antibiotic Medium 10/Polymixin Seed Agar†		✔	
Antibiotic Medium 11/Neomycin Assay Agar†		✔	
Antibiotic Medium 12		✔	
Antibiotic Medium 19/Nystatin Assay Agar†		✔	
APT Agar†			✔
APT Broth†			✔
Aseptic Commissioning Medium	✔		
B12 Assay Medium USP			✔
B12 Culture Agar USP			✔
B12 Inoculum Broth USP			✔
Biotin Assay Medium			✔
Cystine Assay Medium			✔
Fluid Thioglycollate Medium†	✔		
Fluid Thioglycollate Medium w/Beef Extract†	✔		
Fluid Thioglycollate Medium w/K Agar	✔		
Folic Acid Assay Medium			✔
Folic Acid Casei Medium			✔
Folic AOAC Medium			✔
Folic Buffer A, Dried			✔
Inositol Assay Medium			✔
Lactobacilli Agar AOAC			✔
Lactobacilli Broth AOAC			✔
Lysine Assay Medium			✔
Methionine Assay Medium			✔

† Available from Difco & Becton Dickinson Microbiology Systems.

Pharmaceutical Testing - Products and Applications

PRODUCTS	APPLICATIONS: Sterility Testing	Antibiotic Assay	Vitamin/Amino Acid Assay
Micro Assay Culture Agar			✔
Micro Inoculum Broth			✔
Neurospora Culture Agar			✔
Niacin Assay Medium			✔
NIH Thioglycollate Medium†	✔		
Panthenol Assay Medium			✔
Pantothenate Assay Medium			✔
Pantothenate Medium AOAC USP			✔
Pyridoxine Y Medium			✔
Riboflavin Assay Medium			✔
Sterility Bottles w/ Screw Cap Fluid Thioglycollate Medium/ Fluid Thioglycollate Medium†	✔		
Sterility Bottles w/ Screw Cap Tryptic Soy Broth/Trypticase™ Soy Broth†	✔		
Sterility Bottles w/ Septum Fluid A/ Peptone Water (0.1%)†	✔		
Sterility Bottles w/ Septum Fluid D/ Peptone Water (0.1%) w/Polysorbate†	✔		
Sterility Bottles w/ Septum Fluid Thioglycollate Medium†	✔		
Sterility Bottles w/ Septum Tryptic Soy Broth/ Septum Fluid Thioglycollate Medium†	✔		
Thiamine Assay Medium LV			✔
Tryptic Soy Broth/Trypticase™ Soy Broth†	✔		
Vitamin B12 Assay Medium			✔

† Available from Difco & Becton Dickinson Microbiology Systems.

Salmonella - Products and Applications

PRODUCTS	APPLICATIONS: Biochemical Tests	Differential Agar	Enrichment	Highly Selective Media	Primary Differential Media	Serological Identification	Streak lactose negative colonies on
Adonitol/Adonitol CP†	–						
Alginate	–						
Arginine	+ or (+)						
Bismuth Sulfite Agar†				✔			
BG Sulfa Agar				✔			
Brilliant Green Agar†				✔			
Brilliant Green Agar Modified (Edel-Kampelmacher)				✔			
Desoxycholate Agar†		✔					
Desoxycholate Citrate Agar†				✔			
Dulcitol	(+) d						
EMB Agar†		✔					✔
Erythritol	–						
Esculin	–						
Gelatin	–						
Glucose	+						
GN Broth Hajna†			✔				
Hektoen Enteric Agar†		✔					
H_2S	+(–)						
Individual O and H Antisera						✔	
Indole	–						
Inositol	d						
KCN	–						
Lactose	–						
Lysine	+						
Lysine Iron Agar†					✔		
MacConkey Agar†		✔					✔
MacConkey Agar CS		✔					
Methyl Red	+						

Key

– Negative
+ Positive
d Delayed
(+) Variable
(–) Variable

† Available from Difco & Becton Dickinson Microbiology Systems.

Salmonella - Products and Applications

PRODUCTS	APPLICATIONS						
	Biochemical Tests	Differential Agar	Enrichment	Highly Selective Media	Primary Differential Media	Serological Identification	Streak lactose negative colonies on
MIO Medium					✔		
Muller Kauffmann Tetrathionate Broth Base				✔			
Nitrate Reduction	+						
Ornithine	+ or (+)						
Oxidase	–						
Phenylalanine	–						
Polyvalent Antisera						✔	
Raffinose	–						
Salicin	–						
SBG Enrichment			✔				
SBG Sulfa Enrichment			✔				
Selenite Broth			✔				
Selenite Cystine Broth†			✔				
SIM Medium†					✔		
Simmons Citrate†	(+) d						
Sodium Malonate	–						
SS Agar†				✔			
Sucrose	–						
m Tetrathionate Broth Base			✔				
Tetrathionate Broth Base†			✔				
Triple Sugar Iron Agar†					✔		
Universal Preenrichment Broth			✔				
Urea Agar Base†					✔		
Urease	–						
VP	–						
XLD Agar†		✔					
XLT4 Agar Base and XLT4 Supplement		✔					

Key

– Negative
+ Positive
d Delayed
(+) Variable
(–) Variable

† Available from Difco & Becton Dickinson Microbiology Systems.

Shigella and *Alkalescens-Dispar* Group - Products and Applications

PRODUCTS	APPLICATIONS: Biochemical Tests: Shigella	Biochemical Tests: A-D Group	Enrichment	Primary Differential Media	Primary Plating Media	Streak lactose negative colonies on
Acetate Differential Agar				✔		
Adonitol	–	–				
Arabinose	d	+				
Arginine Dihydrolase	d	d				
Christensen Citrate	–	d				
Desoxycholate Agar					✔	
Desoxycholate Citrate Agar					✔	
Dulcitol	d	d				
EMB Agar†					✔	✔
Gas from Glucose	–(1)	–				
Gelatin (22°C)	–	–				
GN Broth Hajna†			✔			
Hektoen Enteric Agar†					✔	
H_2S	–	–				
Indole	– or+	+				
Inositol	–	–				
KCN	–	–				
Lactose	–(1)	d				
Lysine Decarboxylase	–	d				
Lysine Iron Agar†				✔		
MacConkey Agar†					✔	✔
MacConkey Agar CS					✔	
Malonate	–	–				
Maltose	d	+				
Mannitol	+ or–	+				
Methyl Red	+	+				
Motility	–	–				

Key

– Negative

\+ Positive

d Different reactions

(1) Certain biotypes of *S. flexneri* produce gas; cultures of *S. sonnei* ferment lactose and sucrose slowly and decarboxylate ornithine.

(–) Variable

† Available from Difco & Becton Dickinson Microbiology Systems.

Shigella and *Alkalescens-Dispar* Group - Products and Applications

PRODUCTS	APPLICATIONS: Biochemical Tests: *Shigella*	Biochemical Tests: A-D Group	Enrichment	Primary Differential Media	Primary Plating Media	Streak lactose negative colonies on
Mucate	–	d				
Ornithine Decarboxylase	d	d				
Phenylalanine	–	–				
Raffinose	d	d				
Rhamnose	d	d				
Salicin	–	–				
Simmons Citrate Agar†	–	–				
Sodium Acetate	–	+(+)				
Sucrose	–(1)	d				
Triple Sugar Iron Agar†				✔		
Urease	–	–				
Voges-Proskauer	–	–				
XLD Agar†					✔	
Xylose	d	+				

Key

– Negative
+ Positive
d Different reactions
(1) Certain biotypes of *S. flexneri* produce gas; cultures of *S. sonnei* ferment lactose and sucrose slowly and decarboxylate ornithine.
(–) Variable

† Available from Difco & Becton Dickinson Microbiology Systems.

Sterility Testing

STERILITY TESTING, MANUAL

AC Broth
AC Medium
Agar Medium No. F
Brewer Thioglycollate Medium
Fluid Thioglycollate Medium†
Fluid Thioglycollate Medium w/Beef Extract†
Fluid Thioglycollate Medium w/K Agar
NIH Thioglycollate Medium
Sterility Bottles w/Screw Cap Fluid Thioglycollate Medium
Sterility Bottles w/Screw Cap Tryptic Soy Broth
Sterility Bottles w/Septum Fluid A
Sterility Bottles w/Septum Fluid D
Sterility Bottles w/Septum Fluid Thioglycollate Medium
Sterility Bottles w/Septum Tryptic Soy Broth
Thioglycollate Medium w/o Dextrose
Thioglycollate Medium w/o Indicator
Thioglycollate Medium w/o Dextrose or Indicator
Tryptic Soy Broth

See also: Environmental Sampling Section of the Reference Guide

STERILITY TESTING, AUTOMATED

See ESP, Industrial Applications

† Available from Difco & Becton Dickinson Microbiology Systems.

Veterinary Testing - Products and Applications

PRODUCTS	APPLICATIONS								
	Collection/Transport Bacterial	Collection/Transport, Viral	General Purpose Bacterial Culture Media	*Brucella*	*Clostridium*	*Francisella*	Gram Negative Enteric Bacteria (General)	*Leptospira*	*Pseudomonas*
Brain Heart Infusion†			✔						
Brain Heart Infusion Agar†			✔						
Brain Heart Infusion Agar and Clostridium Difficile Antimicrobic Supplement CC†					✔				
Brucella Agar†				✔					
Brucella Broth†				✔					
Brucella Antisera				✔					
Cell*matics*™ Viral Transport Pack/ **CULTURETTE**™ Viral Single Swab†		✔							
Cetrimide Agar Base/**PSEUDOSEL**™ Agar†									✔
Cooked Meat Medium†					✔				
CULTURESWAB™ Amies Medium	✔								
CULTURESWAB Amies Mediumw/o Charcoal/ **CULTURETTE** Amies w/o Charcoal†	✔								
CULTURESWAB Cary-Blair Medium/Anaeno **CULTURETTE** Cary-Blair Single†	✔								
CULTURESWAB Perinasal Swab w/Amies Medium	✔								
CULTURESWAB Urethral Swab w/Amies Medium	✔								
CULTURESWAB Stuart's Medium Modified/ **CULTURETTE** Modified Stuart's Medium†	✔								
Cystine Heart Agar						✔			
EMB Agar/Eosin Methylene Blue Agar Modified†							✔		
Eugon Agar/**EUGONAGAR**™†				✔					
Eugon Broth/**EUGONBROTH**™†				✔					
Fletcher Medium Base								✔	
Fluid Thioglycollate Medium†			✔						
Francisella Tularensis Antigen						✔			
Francisella Tularensis Antiserum†						✔			
Leptospira Enrichment EMJH								✔	
Leptospira Medium Base EMJH								✔	
Levine EMB Agar/Levine Eosin Methylene Blue Agar†							✔		
MacConkey Agar							✔		
McClung Toabe Agar Base†					✔				
Pseudomonas Agar F/Flo Agar†									✔
Pseudomonas Agar P/Tech Agar†									✔
Pseudomonas Isolation Agar/Pseudomonas ISO†									✔
Reinforced Clostridium Medium/ Reinforced Clostridum Agar†					✔				

† Available from Difco & Becton Dickinson Microbiology Systems.

Veterinary Testing - Products and Applications

PRODUCTS	APPLICATIONS								
	Collection/Transport Bacterial	Collection/Transport, Viral	General Purpose Bacterial Culture Media	*Brucella*	*Clostridium*	*Francisella*	Gram Negative Enteric Bacteria (General)	*Leptospira*	*Pseudomonas*
SFP Agar Base/TSN Agar†					✔				
Simmons Citrate Agar†							✔		
SPS Agar†					✔				
Sterile Swab/Aerobic Collection & Trans. System w/o Agar†	✔								
Triple Sugar Iron Agar†							✔		
Tryptic Soy Agar/**TRYPTICASE**™ Soy Agar†			✔						
Tryptic Soy Broth/**TRYPTICASE**™ Soy Broth†			✔						
Tryptose Phosphate Broth†			✔						

PRODUCTS	APPLICATIONS							
	Staphylococcus	Oxidation-Fermentation (O-F) Test	Mycological Media	Blood Culture	*Salmonella/Shigella*	*Streptococcus*	*Vibrio*	Susceptibility Testing
Azide Blood Agar Base†						✔		
Baird-Parker Agar Base w/ EY Tellurite Enrichment†	✔							
Bismuth Sulfite Agar†					✔			
Blood Culture Bottles Brain Heart Infusion w/ PAB, SPS + CO_2					✔			
Blood Culture Bottles Columbia Broth w/CO_2					✔			
Blood Culture Bottles Columbia Broth w/SPS + CO_2					✔			
Blood Culture Bottles Fluid Thioglycollate Medium w/SPS + CO_2					✔			
Blood Culture BottlesThiol Broth w/CO_2					✔			
Blood Culture BottlesThiol Broth w/SPS + CO_2					✔			
Blood Culture Bottles Tryptic Soy Broth w/CO_2					✔			
Blood Culture Bottles Tryptic Soy Broth w/SPS + CO_2/ **SEPTIC-CHEK**™ TSB w/ SPS + CO_2†					✔			
Brain Heart Infusion Agar†				✔				
Brilliant Green Agar†						✔		
Coagulase Plasma (Rabbit)/ Coagulase Plasma, Rabbit†	✔							

† Available from Difco & Becton Dickinson Microbiology Systems.

Veterinary Testing - Products and Applications

PRODUCTS	APPLICATIONS: Staphylococcus	Oxidation-Fermentation (O-F) Test	Mycological Media	Blood Culture	Salmonella/Shigella	Streptococcus	Vibrio	Susceptibility Testing
Coagulase Plasma EDTA (Rabbit)/ Coagulase Plasma, Rabbit w/EDTA†	✔							
Columbia CNA Agar†						✔		
Corn Meal Agar†			✔					
Decarboxylase Medium Base		✔						
Dispens-O-Disc Susceptilbility Disks: Apramycin 15 mcg								✔
Ceftiofur 30 mcg								✔
Enrofloxacin 5 mcg								✔
Tilmicosin 15 mcg								✔
DNase Test Agar†	✔							
DNase Test Agar w/ Methyl Green/DNase Test Agar w/Toluidine Blue†	✔							
DTM Agar			✔					
Lysine Iron Agar†					✔			
Mannitol Salt Agar†	✔							
MIO Medium					✔			
MR-VP Medium/MR-VP Broth†					✔			
Mueller Hinton Medium/Mueller Hinton II Agar†								✔
Mycological Agar/**MYCOPHIl**™ Agar†			✔					
OF Basal Medium†		✔						
Phenol Red Broth Base†		✔						
Phenylethanol Agar/Phenylethyl Alcohol Agar†	✔					✔		
Purple Agar Base		✔						
Purple Broth Base†		✔						
Sabouraud Dextrose Agar†			✔					
Sabouraud Dextrose Broth			✔					
Selenite Broth/Selenite F Broth					✔			
Selenite Cystine Broth†					✔			
SS Agar/Salmonella Shigella Agar†					✔			
Staph Latex Test/**STAPHYLOSLIDE**™ Test Kit†	✔							
Strep Grouping Kit†						✔		
Tetrathionate Broth Base†					✔			
TCBS Agar†							✔	
Urea Agar Base†			✔					
Vibrio Cholerae Antisera							✔	
VJ Agar (Vogel-Johnson Medium)†	✔							
XLD Agar†					✔			
XLT4 Agar Base and XLT4 Supplement					✔			

† Available from Difco & Becton Dickinson Microbiology Systems.

Water/Wastewater Testing - Products and Applications

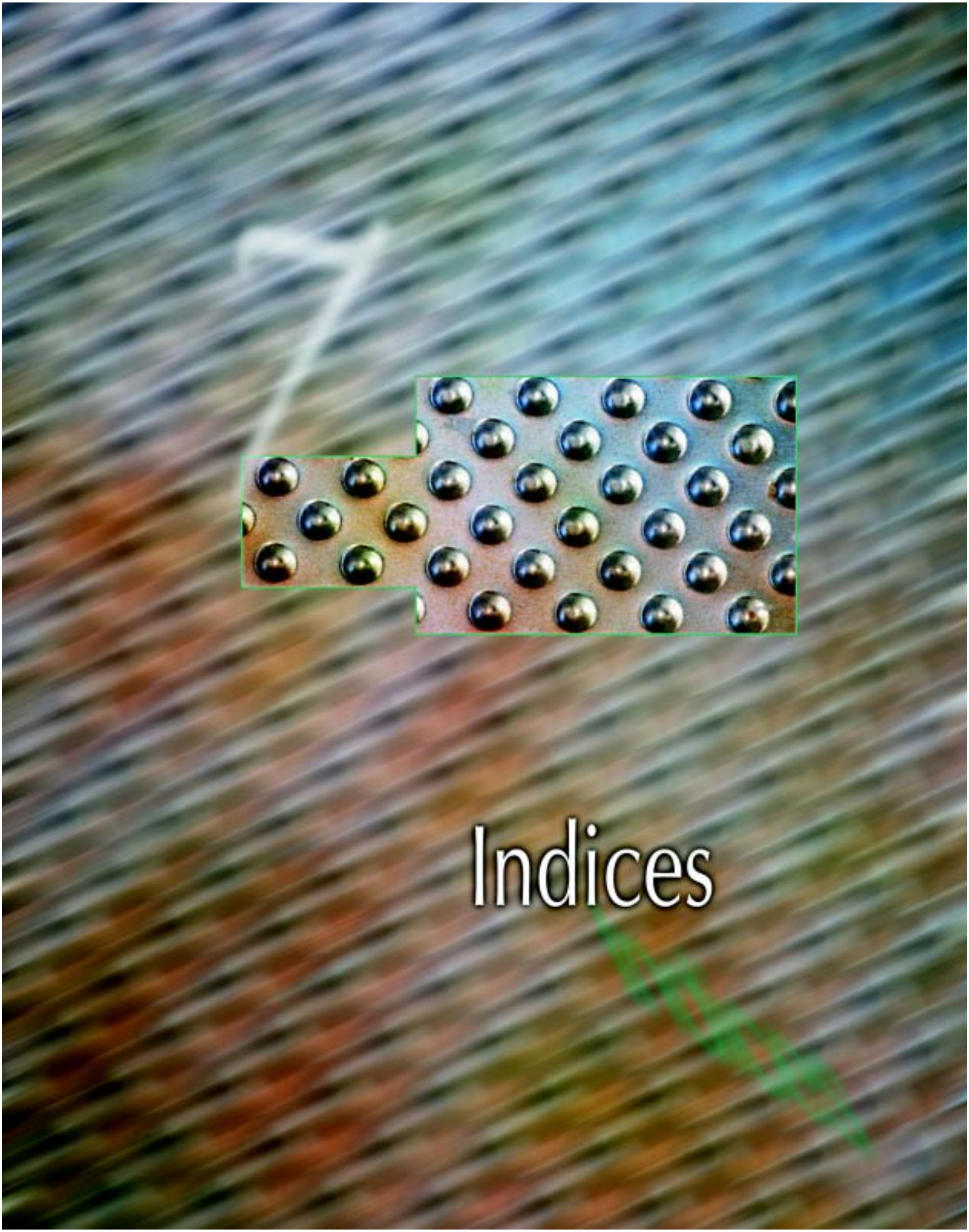
PRODUCTS	APPLICATIONS						
	Standard Plate Count	Total Coliforms	Fecal Coliforms / *E. coli*	Fecal Streptococcus	*Salmonella*	Stressed Organisms	*Staphylococcus*
A-1 Medium/A-1 Broth†			✔				
Azide Dextrose Broth†				✔			
BAGG Broth			✔				
Baird-Parker Agar Base w/ EY Tellurite Enrichment/Egg Yolk Tellurite Solution†							✔
Bile Esculin Agar†				✔			
Bile Esculin Azide Agar/**ENTEROCOCCOSEL**™ Agar†				✔			
Bismuth Sulfite Agar†					✔		
Brilliant Green Agar†					✔		
Brilliant Green Agar Modified (Edel-Kempelmacher)					✔		
Brilliant Green Bile 2%/Brilliant Green Bile Broth 2%†		✔					
m Brilliant Green Broth					✔		
Desoxycholate Lactose Agar†		✔					
m E Agar/M-E Agar Base†				✔			
EC Medium/EC Broth†			✔				
EC Medium with MUG/EC Broth w/ MUG†			✔				
m Endo Agar LES/m Endo Agar LES†		✔					
m Endo Broth MF®/m Endo Broth†		✔					
m Enterococcus Agar/m Enterococcus Agar†				✔			
Esculin Iron Agar				✔			
EVA Broth/Ethyl Violet Azide Broth†				✔			
m FC Agar/ m FC Agar†			✔				
m FC Basal Medium			✔				
m FC Broth Base/m FC Broth†			✔				
m HPC Agar/m HPC Agar Base†	✔						
Lactose Peptone Broth		✔					
Lauryl Tryptose Broth/Lauryl Sulfate Broth†		✔					
Lauryl Tryptose Broth with MUG/ Lauryl Sulfate Broth w/ MUG†		✔					
Levine EMB Agar/Levine Eosin Methylene Blue Agar†		✔					
Minerals Modified Glutamate Broth		✔					
Muller Kauffmann Tetrathionate Broth Base					✔		

† Available from Difco & Becton Dickinson Microbiology Systems.

Water/Wastewater Testing - Products and Applications

PRODUCTS	APPLICATIONS: Standard Plate Count	Total Coliforms	Fecal Coliforms / *E. coli*	Fecal Streptococcus	*Salmonella*	Stressed Organisms	*Staphylococcus*
Nutrient Agar w/ MUG†			✔				
Plate Count Agar/Standard Methods Agar†	✔						
Presence-Absence Broth†		✔					
R2A Agar†						✔	
Rosolic Acid			✔				
Selenite Broth/Selenite F Broth†					✔		
Selenite Cystine Broth†					✔		
m Staphylococcus Broth							✔
m T7 Agar						✔	
m TEC Agar						✔	
m Tetrathionate Broth Base					✔		
Tetrathionate Broth Base†					✔		
Tryptone Water			✔				
XLD Agar†					✔		

† Available from Difco & Becton Dickinson Microbiology Systems.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