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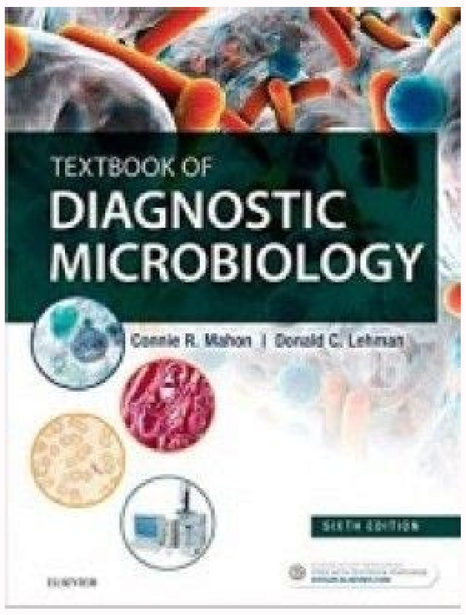

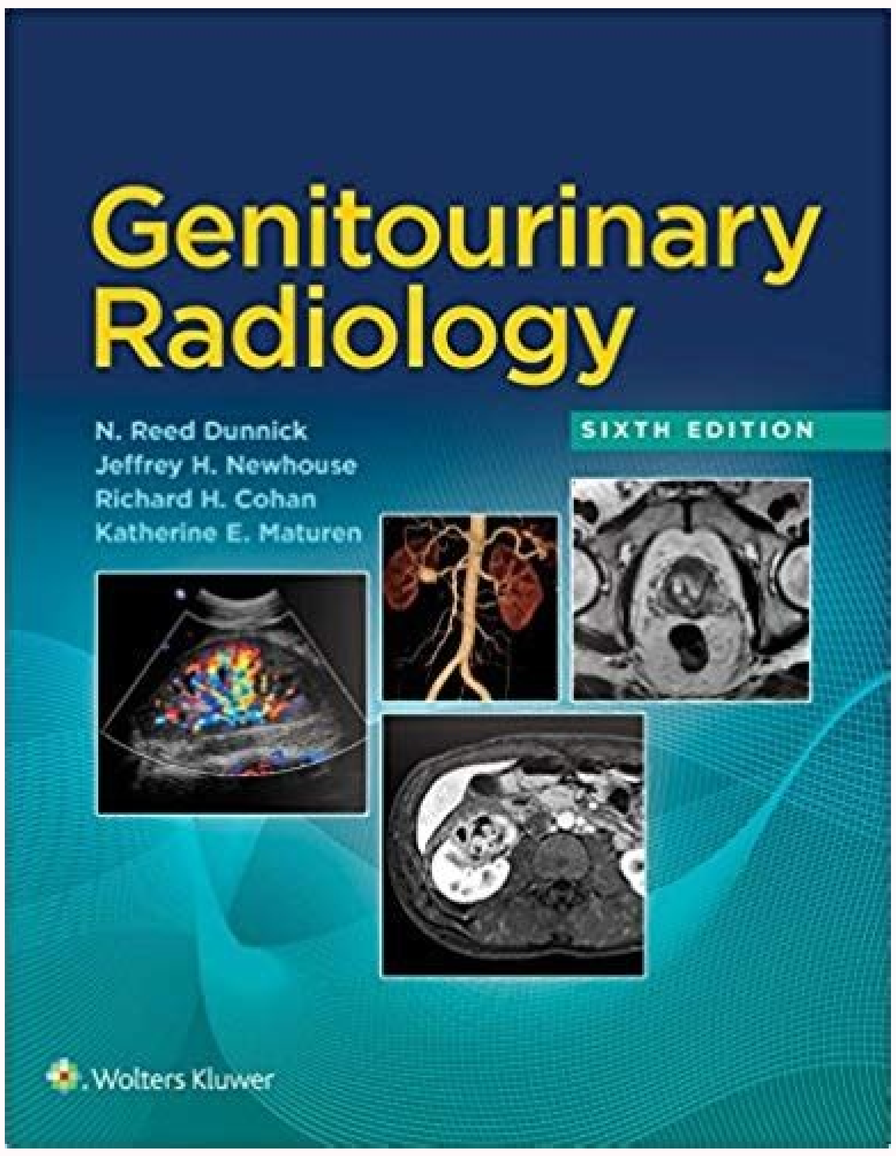

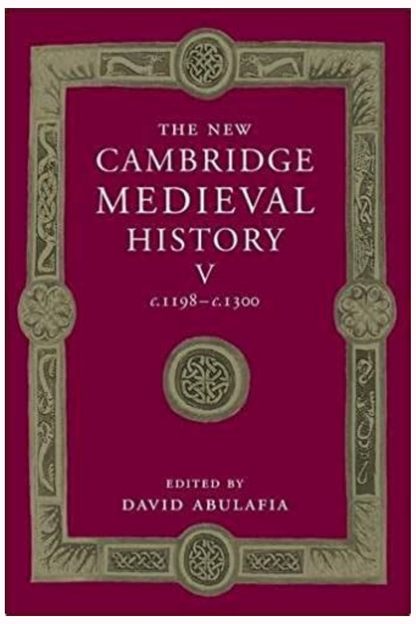

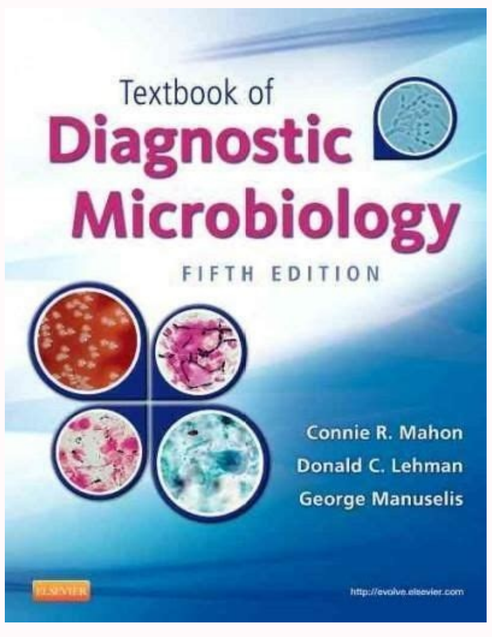

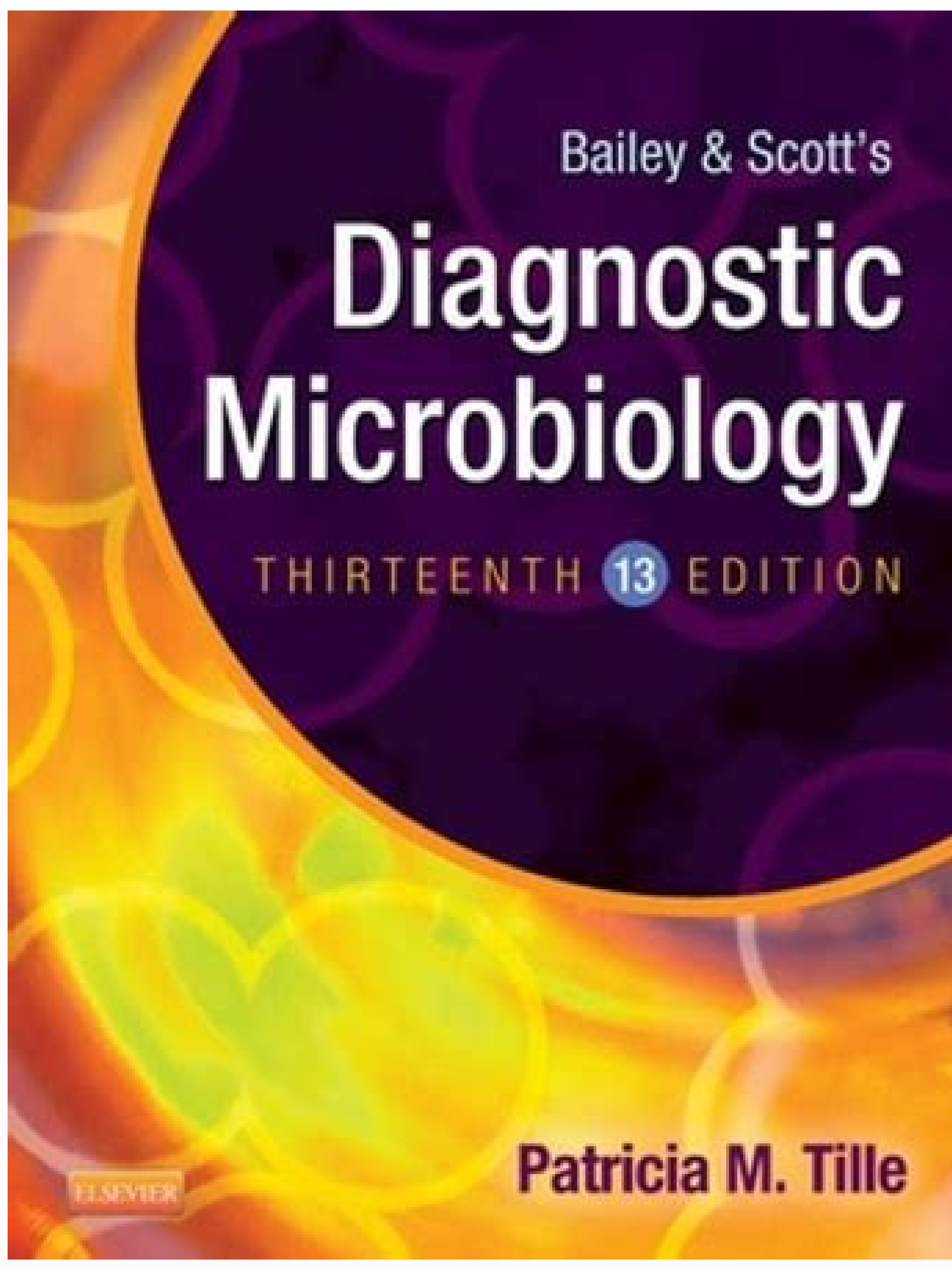

Types of microbiology test. Who is the father of bacteriology and protozoology. Who is the father of medical microbiology. Types of microbial analysis. Types of microbiology pdf.

Erythromycin is important for eradication of the organism and prevention of secondary cases but has clinical efficacy only if treatment is started during the catarrhal phase of disease. Except for B. Some species have been recovered only from environmental sources. Patients may have nausea and vomiting, urticaria, and dyspnea before the rupture of the worm's uterus. Most isolates that are not capable of sexual reproduction or whose sexual state has not yet been discovered are correctly termed yeastlike fungi. Winn W Jr, et al, editors: Koneman's color atlas and textbook of diag nostic microbiology, ed 6, Philadelphia, 2006, Lippincott Williams & Wilkins. Laboratory Diagnosis. The yeastlike cells of P. If a chamber is not available, the grinding must be accomplished as quickly as possible at the workbench. and Aggregatibacter segnis comb. The egg, however, is usually detected. Lysozyme is found in serum, tissue fluids, tears, breast milk, saliva, and sweat. In Brenner DJ, et al, editors: Bergey's manual of systematic bacteriology, ed 2, vol 2: the proteobacteria, part C, New York, 2005, Springer, p. Some yeast isolates, referred to as phaeoid yeasts, are darkly pigmented because of melanin in their cell walls. Permanent stains such as trichrome and Giemsa may also demonstrate the trophozoite in clinical specimens. Patients in the intensive care unit (ICU) who are mechanically ventilated may quickly become colonized with P. The major renal lesion is an interstitial nephritis with associated glomerular swelling and hyperplasia that does not affect the glomeruli. This virus derives its name from the fact that under electron microscopy, the virion is surrounded by a corona, or halo (×100,000). However, some species appear gram-variable and some, such as C. Biochemical-Based Multitest Systems A commercially available alternative to conventional tubed media is the biochemical-based identification systems manufactured by Analytab Products (API; bioMérieux, Durham, NC). The decision to administer a second dose of vaccine to school-age children has drastically reduced the incidence of measles in the United States. Roundworms Human roundworms include those that infect the intestinal tract and blood and tissue. sonnei is the predominant isolate, followed by S. Young children in daycare centers, particularly infants younger than 1 year of age, are the most susceptible. The pathogenic borreliae commonly are arthropod-borne (by a tick or louse) and cause relapsing fever and Lyme disease. hepatica–Fasciolopsis buski egg usually not seen in a stool sample unless it is a purged specimen. As the host cell ruptures, the morulae break into many individual EBs, which continue the infective cycle. Compare the confirmatory tests commonly used to identify these isolates. Morganella is motile but does not swarm. The stool contains no leukocytes, which distinguishes it from dysentery caused by Shigella spp. The organisms gain entrance into the lymphatic system and are sustained in the mesenteric lymph nodes. Within the macrophage, it converts to the amastigote stage and multiplies within the cell. Peptostreptococcus spp. In urinary schistosomiasis, microscopic bleeding into the urine is present during the acute phase. Epidemiology Pertussis is a human disease; no animal reservoir or vector has been found. In tropical areas, agents such as Phialophora spp. gondii) and the organ donor is seropositive. This test is also useful for other molds and yeast. A few of the released amastigotes will transform into trypomastigotes in the bloodstream, which can be taken in by the insect to continue the life cycle. Lower g force necessitates longer centrifuge time. CNS complications may develop. Identification Procedure. For suspected cases of blood parasites, a thick film and a thin film should be made. Early-onset congenital syphilis, onset at less than 2 years of age, is characterized by mucocutaneous lesions, osteochondritis, anemia, hepatosplenomegaly, and central nervous system involvement and occurs when mothers have early syphilis during pregnancy. Blastomyces dermatitidis b. The physician initially ordered a complete blood count. It is widely used by many practitioners for all types of infection, including vaginitis and thrush. At one time, measles (rubeola) was the most common viral disease in children in the United States. The El Tor biogroup differs from the classic biogroup in that El Tor is Voges-Proskauer-positive, hemolyzes erythrocytes, is inhibited by polymyxin B (50 µg), and is able to agglutinate chicken red blood cells. Table 1-1 compares the major characteristics of eukaryotic and prokaryotic cells. Although treatment data are too sparse to be definitive, penicillin is considered beneficial and alters the course of the disease if treatment is initiated before the fourth day of illness. If the technologist forgets to add the Gram's iodine to the staining procedure, what will most likely occur? ■ Case in Point A 29-year-old man arrived at a local medical clinic in Los Angeles complaining of diarrhea, fever, chills, muscle aches, and headaches. Some tests are Clinical Laboratory Improvement Act (CLIA)–waived, which brings viral identification services into physicians' offices and clinics. Although most clinicians rely on wet mount preparations, the method has relatively low sensitivity (30% to 70%). See Chapters 7, 8, 9, 12, and 13 for more detailed information. mutans, S. Which method is preferred for the detection of Bordetella in nasopharyngeal smears? Staphylococcus epidermidis and Propionibacterium spp. influenzae and S. The nonpathogenic Neisseria spp. Treatment. P. On arrival in the laboratory, the swab should be placed into a tube containing about 0.5 mL of sterile thioglycollate broth. A Gram stain of the conjunctival exudates (product of acute inflammation with white blood cells and fluid) showed gram-positive intracellular and extracellular, faint-staining, coccobacillary organisms. These variable regions create two active sites for antigen binding on each IgG molecule (see Figure 2-6). EIA methods can also be used but are less sensitive than fluorescent antibody methods. Although some laboratories prepare the stain in house, manufacturers provide prepared stains and reagents for this procedure. It acts in conjunction with other compounds to exert a bactericidal effect. In addition, biogroup 1 may be indole-positive (60%). Some viruses also have a phospholipid labile envelope surrounding the virion. Once the mature oocyst has been ingested, the presence of bile and trypsin in the small intestine helps trigger release of sporozoites, which invade the intestinal stain, ×1000). Background staining in the Weber stain is pale green, whereas in the Ryan stain it is blue. Regional lymphadenopathy is common with minor constitutional symptoms. Eumycotic mycetoma is caused by fungi and bacteria and results in draining sinus tracts and tissue destruction. Although antifungal therapy was initiated, the patient died on day 22. In the case of intestinal infection, spores are passed in the feces; in infection of the urinary tract, they can be detected in urine. PIV-4 generally causes mild upper respiratory tract infections. All these must be balanced to provide the most cost-effective and complete diagnostics that will meet the needs of the clinical staff. Colony Morphology. The typical colony morphology of C. Flood the slides with carbolfuchsin stain for 5 minutes. The material is spun for 10 minutes. It is based on similarities and differences in genotype (genetic makeup of an organism, or combinations of forms of one or a few genes under scrutiny in an organism's genome) and phenotype (readily observable physical and functional features of an organism expressed by its genotype). The most common serotypes are 1 to 8, 11, 21, 35, 37, and 40. LGV is uncommon in the United States and is usually seen in immigrants from and returning travelers to countries in which the disease is endemic, typically the tropics and subtropics. The API 20NE performs well for most commonly isolated nonfermenters and is used in many clinical laboratories. She also complained of her eyelids sticking together because of exudative discharge. Yeasts such as Cryptococcus grow well. Gu W, Levin RE: Quantitative detection of Plesiomonas shigelloides in clam and oyster tissue by PCR, Int J Food Microbiol 111:81, 2006. infect humans—Taenia saginata, the beef tapeworm, which is found primarily in beef-eating countries of the world, and Taenia solium, the pork tapeworm, which is found in areas of the world with a high consumption of pork, such as Latin America. Albendazole is another drug that may be used. This area may also contain regulatory tahir99 VRG 20 PART I Introduction to Clinical Microbiology regions to which molecules may attach and cause either a decrease or an increase in transcription. Clostridium. can cause bacillary dysentery. Other variations can occur. tahir99 VRG CHAPTER 26 Mycobacterium tuberculosis and Nontuberculous Mycobacteria Acid-fast bacillus Growth rate 3 to 7 days on LJ at 37° C 3-Day arylsulfatase test + – Nitrate reduction + – Mycobacterium fortuitum Mycobacterium chelonae Pigment Buff Rough Mycobacterium smegmatis Colony Yellow to orange Smooth Rough Other rapidly growing Mycobacterium species Mycobacterium phlei Colony Smooth Mycobacterium vaccae FIGURE 26-9 Schematic diagram for the identification of rapidly growing Mycobacterium species. MIF also can distinguish an IgM from an IgG response. The mental and physical development of a child may be affected by chronic heavy infections because of the complications of anemia and malnutrition. Rapid and Semiautomated Identification Systems. Although rapid and semiautomated identification systems contain databases of the more commonly encountered clinical vibrios, they generally are inadequate for accurate identification of these species, particularly those that are less commonly encountered. schenckii infection is lymphocutaneous sporotrichosis. The condition is characterized by chronic nonhealing lesions, especially on the extremities and face. The eggs are embryonated when passed, and the miracidium is released when the egg reaches water. Four genera with human clinical significance belong to the family Picornaviridae—Enterovirus, Rhinovirus, Hepatovirus, and Parechovirus. T. The protein is dissipated into a filter pad, leaving the background clearer for viewing gram-negative morphotypes. It is often difficult to determine whether the organisms recovered in culture from these specimens are pathogens or just colonizers. In Versalovic J, et al, editors: Manual of clini cal microbiology, ed 10, Washington, DC, 2011, ASM Press. Discuss the conditions that must be present or events that must occur for a microorganism to cause disease. Influenza A (H1N1), the Spanish flu, appeared in 1918 to 1919. Why would final species identification be made from the thin smear? Resistance is expressed as the lowest concentration of drug that inhibits all or almost all of the growth—that is, the MIC. There has been significant reorganization in the order Rickettsiales in recent years. This H3N2v virus was first detected in humans in January 2011, and it had genes from avian, swine and human viruses and the 2009 H1N1 pandemic virus M gene. are implicated as pathogens. The B moiety, or binding portion, confers the specificity. In some patients, the chronic form may manifest as megacolon, megastomach, or megaesophagus, which results in difficulty in swallowing and disturbances to intestinal motility. Liquid Media Mycobacterium spp. Risk factors in patients with AIDS include sexual transmission, a CD4 count of less than 100 cells/mm3, exposure to water by swimming, and contact with animals. Isolates are usually motile, catalase positive, and indole negative (nonpigmented strains may be indole positive). Metronidazole (Flagyl), which has been the drug of choice for treating giardiasis, may be prescribed. Serologic tests for antibody to malaria are not useful in an endemic area but may be useful for diagnosis in those who have traveled to an endemic area and have clinical symptoms of malaria. The RPR test is more commonly used; it uses carbon particles and is read macroscopically. The presence of multiple distinct morphologic forms suggests that a polymicrobic infectious process is present. Neuroinvasive disease typically manifests as meningitis or encephalitis. gladioli is difficult without the use of molecular tools for confirmation because isolates can be misidentified in commercial phenotypic systems as a member of the B. Renal complications such as nephrotic syndrome or even renal failure can occur with P. Isolation of patients in mobile isolation chamber units is often enforced when a CRAB is isolated. Kalayoglu MV: Chlamydia pneumoniae in cardiovascular disease: update on Chsp60 and other emerging virulence determinants, Med Chem Rev 1:475, 2004. Despite treatment, systemic infections have a poor tahir99 VRG CHAPTER 28 Diagnostic Parasitology prognosis, and many patients with keratitis lose their sight in the affected eye. For example, the potential pathogen list for a healthy 20-year-old college student is much shorter than for a healthy 90-year-old person, a transplant recipient, or a 20-year-old college student with AIDS. Recurrent herpes infections involve the same sites as primary infections, but the urethra is less commonly involved. Based on virulence factors, clinical manifestation, epidemiology, and different O and H serotypes, there are five major categories of diarrheogenic E. As a result of this reorganization, Coxiella has been removed from the family Rickettsiaceae. This enables differentiation between aerobic and capnophilic organisms. Holt JG, et al: Bergey's manual of determinative bacteriology, ed 9, Baltimore, 1993, Williams & Wilkins. The Quantitative Buffy Coat (QBC; Becton-Dickinson, Sparks, MD) procedure uses the fluorescent dye acridine orange to stain nuclear material for detecting malarial organisms in blood. Extraintestinal Infections Aeromonads are also responsible for extraintestinal infections; septicemia, meningitis, and wound infections are the most common. Common stains used to visualize microorganisms microscopically also are presented. Viral transport medium, saline, or trypticase soy broth can be added to sterile containers to keep tissues from drying. Organisms that are pH-susceptible and survive are generally protected by being enmeshed in food, and they move to the small intestine. The cilia-lined cytostome is located at the slightly pointed anterior end. Infants fed with cow's milk do not have the high colonization by Bifidobacterium spp. difficile to multiply with less competition and produce two toxins: toxin A, an enterotoxin, and toxin B, a cytotoxin. Virus isolation is preferable, although physicians rarely have trouble recognizing mumps clinically. The viruses acquire the envelope from the host membrane as they bud from host cells. Human beings are 99.9% identical. Similarly, fimbriae are essential for gonococci to infect the epithelial cells of the genitourinary tract. pertussis appears to be maintained in the human population by adults who become transiently colonized. The organism also grows on routine isolation media, such as SBA and MAC agar. The scolex is released in the intestine, where it develops into an adult worm. When a probe is used on a contaminated specimen, the resulting hybridization percentage may incorrectly fall below accepted cutoff hybridization levels, leading to a false-negative result. Other stains, including DiffQuik (Baxter Scientific, Kansas City, MO) and Giemsa, can be used to facilitate observation of the organisms. Fluorescent staining of exudates using acridine orange will demonstrate a typical fluorescent morphology, with a yellow-green nucleus. Because they are susceptible to the acid pH of stool, feces suspected of containing Shigella organisms should be plated immediately onto laboratory media to increase recovery of the organism. In gas gangrene exotoxins, such as α-toxin produced by C. They are 28 to 30 µm long and have a vase or flask shape. fetus subsp. Bacterial Biochemistry and Metabolism Metabolism Microbial metabolism consists of the biochemical reactions bacteria use to break down organic compounds and the reactions tahir99 VRG CHAPTER 1 Bacterial Cell Structure, Physiology, Metabolism, and Genetics they use to synthesize new bacterial parts from the resulting carbon skeletons. rubidaea, and S. The tissue or fluid should be collected aseptically and placed in a sterile container. Issues to Consider Case in Point A 48-year-old woman had a complete hysterectomy. Facultative anaerobes, such as Escherichia coli and Staphylococcus aureus, preferentially use oxygen as an electron acceptor if it is available but can grow well in the absence of oxygen. All data except those for oxidase production and nitrate reduction are for reactions that occur within 2 days at 35° to 37° C; oxidase production and nitrate reduction reactions are done only at day 1. Myonecrosis. Myonecrosis, or gas gangrene, usually occurs when organisms contaminate wounds, through trauma or surgery. It was thought that perhaps two strains of the organism existed, one pathogenic and one nonpathogenic. Colony Morphology On charcoal–horse blood and Regan-Lowe media, young colonies are smooth, glistening, and silver, resembling mercury droplets (Figure 18-31). Treatment with mebendazole may need to be repeated in several weeks to eliminate organisms that have matured as a result of ingestion of eggs remaining in the environment. With the methenamine silver stain, the cyst wall stains black. Activation of factor G by glucan produces coagulation. The bacteria are transmitted transovarially in chiggers. When Dr. Adams arrived at the clinic, many children were waiting for her, so she immediately began seeing her patients. HHV-8 DNA or antigens are rarely detected in immunocompetent individuals, even if they are seropositive. To be successful in establishing infection, infectious agents must be able to avoid or overcome these local factors. aeruginosa, Acinetobacter spp., Burkholderia spp., or S. Lactophenol Cotton Blue Lactophenol cotton blue is used to stain the cell walls of medically important fungi grown in slide culture (see Figure 1-6, G). fetus, C. (Giemsa stain, ×1000.) tahir99 VRG CHAPTER 28 Diagnostic Parasitology Although they are characteristic for babesiosis, the tetrad forms are not always seen. meningitidis, all potential pathogens, are also found in the nasopharynx of healthy individuals. Escherichia vulneris may also produce a yellow-pigmented colony, but the yellow is more prevalent in E. When treatment is necessary, a combination of antimicrobials such as trimethoprim-sulfamethoxazole is used for the tachyzoite stage. Some species are also nitratepositive. Classification Previously, the genus Salmonella comprised three biochemically discrete species: S. Patients with DHF develop the symptoms of classic DF, along with thrombocytopenia, hemorrhage and shock, and sometimes death. The pathology of the disease and clinical symptoms are the same as those seen with W. The envelope masks the shape of the virion, so most enveloped viruses are variably shaped or pleomorphic. Once the tahir99 VRG CHAPTER 28 Diagnostic Parasitology organism has reached the mature trophozoite stage, the chromatin begins to divide (developing schizont). Although environmental cultures are generally to be avoided, as in the introductory case, there are times when they become an important and often required element of an infection control program that affects the microbiology laboratory. Place the smear on a staining rack, and overlay the surface of the material to be stained with the stains in sequence as shown in the figures. It usually develops in patients with repeated infections and those undergoing quinine therapy. are important human pathogens responsible for a number of diseases including Rocky Mountain spotted fever, rickettsialpox, and typhus. In a study of 55 documented infections with S. Table 22-2 shows endogenous anaerobes commonly encountered in human infections. Virtually no other members of the Enterobacteriaceae synthesize the required enzyme, phenylalanine deaminase. As with bacterial testing, Mueller-Hinton agar is used, alleviating the need for multiple media to perform antimicrobial susceptibility testing. Although the rotavirus is present in large numbers in stools, it can be isolated only with special procedures. B, Selective BCYE agar plate inoculated with the same sputum specimen, which has been acid-washed before inoculation. It contains an invaginated scolex containing four suckers and a circle of hooklets on the rostellum. The various forms of ringworm continue to be described in these terms, as shown in Table 27-1. coli O157:H7, which ordinarily would not be distinguishable from other E. Coccidioides immitis c. tahir99 VRG CHAPTER 3 The Laboratory Role in Infection Control • How long does the environment need to be in contact with the agent to kill the virus? Treatment of severe P. sputorum, C. Christian Whelen CHAPTER OUTLINE Borrelia recurrentis and Similar Borreliae Borrelia burgdorferi ■ TREPONEMES General Characteristics Clinically Significant Species Treponema pallidum subsp. The most common and practical choices for anaerobic incubation systems for clinical laboratories are anaerobic chambers, anaerobic jars, and anaerobic bags or pouches. fowleri is relatively simple, consisting of three stages—free-living amebic trophozoite, transient flagellate form that appears when there is a scarcity of nutrients, and environmentally resistant cyst. The next step in viral replication is penetration. Toh HS, et al: Risk factor associated with Sphingomonas paucimobilis infection, J Microbiol Immunol Infect 44:289, 2011. Suspected colonies should be inoculated with a short streak onto a chocolate tahir99 VRG 512 PART II Laboratory Identification of Significant Isolates agar plate for incubation in a CO_2 incubator and an anaerobic blood agar plate incubated anaerobically. tahir99 VRG 596 PART II Laboratory Identification of Significant Isolates FIGURE 27-13 Microscopic structures of Hortaea werneckii, showing characteristic budding annelloconidia. IgG titers greater than 1 : 1024 are generally considered indicative of recent or active infection. TABLE FIGURE 22-19 Appearance of Bacteroides fragilis on a kanamycin–vancomycin–laked blood agar (left) and Bacteroides bile esculin (BBE) agar biplate (right). Instead, the cellophane tape preparation is routinely used for detection of suspected pinworm infections. FIGURE 27-25 Conversion of the mold phase of Blastomyces TABLE 27-5 Mold to Yeast Conversion of Thermally Dimorphic Fungi* Fungus Culture Media and Temperature Blastomyces dermatitidis Blood agar, 37° C Histoplasma capsulatum Pines medium, glucose-cysteine blood, or BHI-blood, 37° C BHI-blood, 37° C Paracoccidioides brasiliensis Yeast Form Large yeast (8-12 µm) Blastoconidia attached by broad base Small, oval yeast (2-5 µm) Multiple blastoconidia budding from single, large yeast (15-30 µm) BHI, Brain-heart infusion. by its ability to grow in nutrient broth with 0% NaCl and its inability to grow in nutrient broth with 6% NaCl (see Figure 20-8). Pyruvate can be further processed either fermentatively or oxidatively. For example, although inpatients frequently acquire nosocomial bacterial infections, it is highly unlikely that an inpatient would acquire a nosocomial parasitic infection. The Old World complex contains lymphocytic choriomeningitis (LCM) virus and the Lassa viruses, including the Lassa-like viruses Mopeia, Mobala, and Ippy. The four Vibrio spp. More recently, gastroenteritis has also been linked to H. ETEC infections must be differentiated from other diarrheal illnesses that may appear similar. • What might spread through a facility if proper barriers are not used to control demolition dust and debris? Some have been recognized as classic pathogens, whereas others are recognized only as environmental saprobes, living on nonliving material. ovale Enlarged, oval Fringed edge Schüffner's dots Normal size Multiple infections P. The term rickettsiae can specifically refer to the genus Rickettsia or it can refer to a group of organisms included in the order Rickettsiales. Compare the methods of identification currently used to diagnose infections caused by Bordetella spp. Aureobasidium. Infections by Aureobasidium spp. This process occurs rapidly after attachment. Genital herpes infections can as much as double the risk of sexual transmission of HIV. Botulinum toxin type A (botox) is also used medically to treat strabismus (wandering eye) and as a beauty enhancer by temporarily improving frown lines. In 2011, a review of the literature found 28 cases of C. If the deposit of cells is too heavy, a portion of the cellular deposition can be smeared (see Figure 7-5). Laboratory Diagnosis. The small size (4 to 6 µm), refractile appearance, and round shape of the oocyst make detection difficult with routine concentration procedures because the organism may resemble a yeast or RBC. Accessed March 21, 2013. In these patients, the infection usually occurs within the first 3 months after transplantation and may disseminate to multiple organs. The notable example is tinea imbricata, caused by Trichophyton concentricum. The specimen is sealed and incubated at 35° C. (Giemsa stain, ×1000.) cerca volvulus. The Lactobacillus acidophilus complex constitutes most of the tahir99 VRG CHAPTER 22 Anaerobes of Clinical Importance 503 lactobacilli of the healthy vagina, but L. Hybridization and Nucleic Acid Amplification Tests for Mycobacterium tuberculosis The use of nucleic acid hybridization techniques allows for the rapid identification of several common mycobacterial species. coli or a mixture of enteric bacteria other than anaerobes. Carbon represents 50% of the dry weight of a bacterium. The World Health Organization (WHO), after decades of aggressive vaccination, education, and eradication, officially declared the world free of smallpox in May 1980. Normally, abundant macroconidia with fewer microconidia are produced on vegetative hyphae (Figure 27-40). The printed phone books grew in popularity during the decades and centuries. The spirochetes are slender, flexuous, helically shaped, unicellular bacteria ranging from 0.1 to 0.5 µm wide and from 5 to 20 µm long, with one or more complete turns in the helix. Depending on the virus, the metabolism of the host cell may be completely stopped (as with polioviruses) or may continue on a restricted scale (as with influenza viruses). RT-PCR assays have demonstrated greater sensitivity and specificity and much shorter TATs. Other nucleic acid–based tests include isothermal amplification methods and loop-mediated amplification assays. Erythrocytic Phase. Merozoites have structures and proteins (e.g., erythrocyte-binding antigen) that selectively adhere to receptors on the RBC membrane. In the Enterotest, the patient swallows a gelatin capsule containing a weighted string. cholerae O1 strains occur in two biogroups, classic and El Tor. tahir99 VRG CHAPTER 28 Diagnostic Parasitology Commercial systems that incorporate collection container and preservatives are also available. CMV infection can also be transmitted sexually via semen and cervical and vaginal secretions and through blood and blood products. albicans produces chlamydoconidia along with hyphae, as shown in Figure 27-61. A wet mount of sputum demonstrates the egg in some patients. (Courtesy Jean Barnishan.) FIGURE 19-3 Mucoid appearance of Klebsiella pneumoniae on MacConkey (MAC) agar. Move slides to staining rack on acid alcohol collection container. The best tests to distinguish the aeromonads from Vibrio spp. Lake Victoria marburgvirus hemorrhagic fever was named after the location of one of the first outbreaks, Marburg, Germany. Note the wrinkled appearance of the colonies. trachomatis and C. There are also species-specific PCR assays available for identification. Influenza viruses have a worldwide distribution and originate as zoonotic infections, carried by several different species of birds and mammals. Endemic Syphilis Endemic syphilis (bejel) is caused by T. Like other pathogenic bacteria, anaerobes can produce a variety of virulence factors. FIGURE 27-40 Fusarium sp. Note the circular arrangement of intracystic bodies within a faint outline of the cyst wall in the center of the field (A, B, ×1000). Aeromonas 2. B, Legionella pneumophila (arrow) in specimen smear stained by DFA technique (×1000). • They do not produce H_2S. With most Exophiala spp., conidia are borne from annellides, with conidia aggregating in masses at the tips of the conidiophore, as seen in Figure 27-22. vermicularis and trophozoites of Trichomonas vaginalis can be detected in the sediment of a urine specimen. Virus isolates can be identified using serum neutralization, EIA, or IF tests. Symptoms that occur during the larval phase are the result of an intense inflammatory response by the host. mimicus. are grouped together in the tribe Escherichiae. Bergeyella (Weeksella) zoohelicum has been isolated from cases of cellulitis, tenosynovitis, septicemia, pneumonia, and meningitis, in particular in association with dog and cat bite wounds, because it is part of their normal oral biota. Disseminated disease appears in different forms. It is nonetheless important to be aware of the other species because they also cause significant infectious diseases. An enteric organism that is acquired by eating improperly prepared and cooked or preserved contaminated food and produces dysentery is: a. They have been isolated from respiratory specimens, contaminated blood products, urine, cosmetics, hospital equipment, and fluids. Initial positive or equivocal EIA results are confirmed with Western blot. Chapter 29 describes viral pathogens, including severe acute respiratory syndrome and the highly pathogenic avian influenza virus. The viruses can be identified by hemadsorption, IF, or EIA techniques. In the hospital environment, they have been associated with ventilators, humidifiers, catheters, and other devices. lari, and sometimes C. histolytica infection, the patient may shed pieces of intestinal mucosa. Knowledge of the biochemistry and metabolism of bacteria is important in the clinical laboratory. Parasites Certain eukaryotic parasites exist as unicellular organisms of microscopic size, whereas others are multicellular organisms. This is induced by antigenic response to the egg and is characterized by increased circulating immune complexes and eosinophils. With a Bunsen burner, heat the smears gently until steaming occurs. All of the above 6. Microscopes vary both in their ability to resolve small structures and in their modifications. −(55) −(94) −(70) +(92) + +(87) +(99) +(99) +(85) +(98) +(97) +(99) Tween Hydrolysis +10 Day − −(84) +(68) − +(75) +(99) +/−(93/47) +−(50) − +(66) + (99) −(75) +(99) −(83) +(98) V +(96) +(99) +(91) +(93) Urease‡ Descriptive term † Continued Modified from Kent PT, Kubica GP: Public health mycobacteriology: a guide for the level III laboratory, Atlanta, 1985, Centers for Disease Control and Prevention. Strains with dissimilar patterns would be determined to be unrelated. Song Y, Finegold SM: Peptostreptococcus, Finegoldia, Anaerococcus, Peptoniphilus, Veillonella, and other anaerobic cocci. On Gramstained smears, they can appear as coccobacilli or as straight rods. However, in virtually all other cases, the bacterium, virus, or fungus requires adherence to the host cell before infection and disease can progress. The case had a fatal outcome. Infection with N. Acinetobacter The genus Acinetobacter, now a member of the family Moraxellaceae, consists of 25 DNA homology groups or genomospecies. Both the isolate identification and serologic relatedness may be important determinants in an outbreak investigation. serotype Typhi − V + V + + + + − + − + − − + + + − − − + + − − + Other* + + + + + + + + Data from Farmer JJ, et al: Enterobacteriaceae: introduction and identification. This is especially pertinent for those who are immunocompromised or have serious underlying liver disease. A patient is complaining of a painful cervical lymph node following a case of pharyngitis. maltophilia; in addition, an Etest or agar dilution can be performed. Kiderlen AF, et al: Cytopathogenicity of Balamuthia mandrillaris, an opportunistic causative agent of granulomatous amebic encephalitis, J Eukaryot Microbiol 53:456, 2006. Sheoran A, et al: Infection with Cryptosporidium hominis provides incomplete protection of the host against Cryptosporidium parvum, J Infect Dis 205:1019, 2012. nov., a novel member of the Alcaligenaceae, isolated from various clinical specimens, Int J Syst Evol Microbiol 55:251, 2005. Many of these tests can be performed in a few minutes. Table 20-3 outlines eight key differential tests to divide the 10 clinically significant Vibrio spp. Define the following terms: sterilization, disinfection, and antiseptic. disiens, Actinomyces israelii (IUD associated) Bacteroides fragilis group, other Bacteroides spp., Fusobacterium spp., C. The slide is removed. coli and the Vi antigen of Salmonella enterica subsp. Available at: mm5539a3.htm. Separate clusters of sporangiophores are joined by stolons, arching filaments that terminate at the rhizoids. The areas involved include the anterior portion of the legs; some patients have reported nodules on their arms. Trichoderma. Trichoderma spp. Adherence to this preventive medicine likely contributed to disease avoidance in these individuals. Many of these systems use enzyme and assimilation reactions to aid in yeast identification. Surface Polymers Various pathogenic bacteria produce a discrete organized covering termed a capsule. It has been found frequently in hospital acquired infections of the urinary or respiratory tract and in bacteremic outbreaks in nurseries and cardiac surgery and burn units. Gastric ulcers d. Patients present with severe abdominal pain and fever; the abdominal pain is concentrated in the right lower quadrant. Another bacterial structure that protects organisms from phagocytosis is protein A. If you're looking on a site with a map function, you may also see a map with the location pinned and an option to get turn-by-turn directions to the place you're calling.Reverse Phone Number LookupA reverse phone number lookup is done when you have only a phone number and want to know who it belongs to before you call. This isolate grows rapidly and produces white to dark gray colonies on potato dextrose agar at 22° C and 35° C. Except for C. They are often first seen on fungal media, such as Sabouraud dextrose agar, and do not grow as well on SBA, or CHOC, modified Thayer-Martin, or buffered charcoal yeast extract agars. Patients infected with B. 17. Plague is a disease primarily of rodents. The Gram stain of the stock Staphylococcus (gram-positive) and Escherichia coli (gram-negative) showed gram-positive reactions for both organisms on review of the stained quality control organisms. For other species, broth microdilution, not disk diffusion, is recommended if antimicrobial susceptibility testing is done at all. In some isolates, the distal end of the macroconidium might bear a thin, filamentous tail. After growth of the isolate appears on an egg-based medium slant, rusty brown colonies appear in a positive reaction on the addition of 20% aqueous solution of ferric ammonium citrate; color formation is the result of iron uptake (Figure 26-14). This organism is part of the GI biota in about 5% of individuals, although the colonization rate in patients associated with long-term care facilities, such as nursing homes and rehabilitation facilities, can reach 20% of the population. Serologic results can be confusing because of cross-reactions, but patients with rising antibody levels to HHV-7 but not to HHV-6 may have an active HHV-7 infection. Once the patient enters the chronic stage of the disease, there are periods of intermittent parasitemia of trypomastigotes, during which time the organism may be transmitted. tularensis samples. Methods such as flow cytometry are being investigated as a means to detect low number of oocysts in a stool specimen. Patients with leukopenia also may have few inflammatory cells within their inflammatory debris. Direct smear examination of stool samples is not particularly helpful in identifying enteric pathogens but may reveal the presence of inflammatory cells. Overview of the Microbial World The study of microorganisms by the Dutch biologist and lens maker Anton van Leeuwenhoek has evolved immensely from its early historical beginnings. Although the amino acid sequence of some regions of the polypeptides is nearly identical among all IgG molecules (conserved regions), one of the ends of each polypeptide is highly variable. In 1978, Levine and colleagues attempted to settle the dispute concerning the pathogenic role of EPEC by challenging volunteers with EPEC strains that lacked the toxins of ETEC and the invasiveness of EIEC. Rhinoviruses are the major cause of the common cold. Tests that detect E. These microorganisms are eliminated either by the host inherent immune defenses or by competition with the resident flora. (Courtesy J. interrogans sensu lato have been reported. gypseum is a rapidly growing geophilic species found in soils worldwide. Bloody diarrhea that progresses to dysentery may appear within a few hours to a few days. Clinical manifestations of myonecrosis include pain and swelling in the affected area. For that reason, health care–associated RSV is a problem in many medical treatment facilities. The identification can be made within minutes with a high level of accuracy and reproducibility as compared with a gold standard of molecular sequencing. Because Colorado tick fever is not reportable, actual numbers of cases are unknown. Chemical or physical methods may be used to accomplish this form of microbial destruction. Even under the most stringent and optimal conditions, isolation of chlamydiae is only approximately 80% sensitive. These viruses occur in Asia and Europe, with the exception of the Seoul virus, which is found worldwide. The life cycle of C. Accessed November 13, 2013. Document the investigation and communicate with all involved parties. This section briefly reviews some of the basic terminology and concepts of bacterial genetics. urethralis, formerly Moraxella urethralis, and O. (Data from Mangels JI: Anaerobic bacteriology. Part II highlights methods for the identification of clinically significant isolates. georgiana. The LGV serovars have also been linked to Parinaud oculoglandular conjunctivitis. The physician suspects malaria and orders a blood smear for examination. hermannii. This characteristic swarming is a result of a tightly regulated cycle of differentiation from standard vegetative cells (swimmers) to hyperflagellated, elongated, polyploid cells (swarmers) capable of coordinated surface movement. cholerae, have been documented since 1817. The source of the infection has been attributed primarily to poultry, milk, eggs, and egg products as well as to handling pets. What is the most likely identification of the organism? Flocked swabs (Copan Diagnostics, Corona, CA) are reported to collect significantly more epithelial cells than rayon swabs from the nasopharynx. The disease was characterized by high fever, pneumonia, and in some patients, acute respiratory distress syndrome. The system comes with patient instructions and a self-sealing plastic bag for transport. Decontamination and Digestion Agents Each laboratory should maintain a proper balance between the rate of recovery of mycobacteria and suppression of contaminating growth. mic dadei, L. The monoclonal antibodies eliminate falsepositive and false-negative results. The organism can be an occupational hazard for people in constant contact with seawater, such as fishermen or sailors. Arenaviruses are commonly divided into two groups, Old World and New World viruses. These fungi form a rapidly growing phaeoid colony that is cottony and dirty gray to black. Available at: 11a3.htm. Polymicrobial presentations in smears require more interpretation and must take into account smear background, the morphology of the organisms, and the anatomic location of the suspected infection as well as accompanying clinical symptoms. The patient recovered once the diabetes was stabilized. Isolate, identify, and perform susceptibility testing for all species of Mycobacterium tahir99 VRG 582 PART II Laboratory Identification of Significant Isolates collaboration with the Association of State and Territorial Public Health Laboratory Directors, surveyed mycobacterial laboratories for their practices in isolation, identification, and susceptibility testing of MTB. The virus grows on PMK cells, causing the formation of distinctive spindle-shaped or multinucleated cells. FIGURE 27-58 Schematic diagram showing how the germ tube test can be used to identify yeasts presumptively. Members of the first group, the autotrophs (lithotrophs), are able to grow simply, using carbon dioxide as the sole source of carbon, with only water and inorganic salts required in addition. With age, phaeoid hyphae develop and break up into arthroconidia, which do not bear hyaline conidia. Available at: 13/2/06-0527_article.htm. Rhizopus. Rhizopus spp. Rotaviruses are spread by the fecal-oral route and have an incubation period of 1 to 4 days. In an iodine wet mount, the nuclei appear as yellowish refractile bodies within the cyst; chromatoidal bars do not take up stain and appear as colorless areas. However, this assay is technically demanding and can take 2 to 3 days for results to be obtained. Serologic grouping is also important in the identification of enterovirulent E. influenzae are associated with highly invasive infections and are known to be more virulent than nonencapsulated strains. These tests can involve the detection of viral antigens by a number of methods, such as immunofluorescence (IF) or enzyme immunoassay (EIA). and the macroconidia resemble Sepedonium spp., neither saprobe produce two types of conidia in a single culture, nor are they dimorphic. The importance of natural immune mechanisms rests in their rapid response to invading organisms. During the febrile period, diagnosis of relapsing fever is readily made by Giemsa or Wright staining of blood smears. Deeper scrapings are necessary to prepare a KOH preparation and inoculate media. Rickettsia Rickettsiae are short, nonmotile, gram-negative bacilli about 0.8 to 2.0 µm × 0.3 to 0.5 µm in size. It can be differentiated from Y. This requires the use of an oxygen reduction indicator, which can be methylene blue or resazurin. If specimens cannot be processed immediately after collection, they should be stored at 4° C. meningoseptica can cause pneumonia, endocarditis, bacteremia, and meningitis, especially in critically ill patients. Most outbreaks occur in the winter months in the temperate zones and year-round in subtropical and tropical regions. Wash slides with running tap water. cincinnatiensis Group 3 − + + + − −/NG −/NG −/NG G. The application of matrixassisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry in microbial identification has been added to Chapter 11. This method provides limited information and has certain inherent problems. Unlike the case with RMSF, the rash does not involve the palms of the hands and soles of the feet, and the face is also not involved. gastri, M. capsulatum. Actinomyces israelii Bacteroides spp. Antibiotic resistance 8. A quadrivalent vaccine, Gardasil (Merck), against HPV types 6, 11, 16, and 18 to prevent cervical cancer was approved by the FDA in 2006 for females ages 9 to 26 years; the vaccine was approved for use in males in 2009. The principles of the TSIA reactions are explained in Chapter 9. Tracheal cytotoxin contributes to pathogenesis by causing ciliostasis, inhibiting DNA synthesis, and promoting cell death. However, when none, or only one, of the first three sputum smears is positive, additional specimens are needed for culture confirmation. Compare the pathogenesis of B. An interrelationship exists between the mechanisms of the innate and the adaptive. In vivo, host cells are primarily the nonciliated, columnar, or transitional epithelial cells that line the conjunctiva, respiratory tract, urogenital tract, and rectum. The use of a solid-based medium, such as LJ medium, in combination with a liquid-based medium is recommended for routine culturing of specimens for the recovery of AFB. Initial FIGURE 27-27 Spherules of Coccidioides immitis in tissue (×300). Overwhelming septicemia can occur. morganii Providencia Salmonella* M. There are at least 19 Cryptosporidium species recognized as animal parasites that were initially identified as causing zoonotic infections in veterinarians and other animal handlers. Campylobacter jejuni is most noted for causing: a. However, an oxidase test performed on SBA colonies will easily separate the oxidase-positive aeromonads from the oxidase-negative yersinias. With what types of infections are Chlamydophila pneumoniae associated? Schriefer ME: Borrelia. aeruginosa, A. The VDRL test uses a cardiolipin antigen that is mixed with the patient's serum or CSF. However, mortality associated with disseminated neonatal disease is about 60% in treated neonates but exceeds 70% in untreated neonates. Winn W, et al, editors: Koneman's color atlas and textbook of diagnostic microbiology, ed 6, Philadelphia, 2005, Lippincott Williams & Wilkins. Two other echinocandins are anidulafungin and micafungin. The diarrhea is cholera-like, with bits of mucus and little fecal material. Two nuclei are visible (trichrome stain, ×1000). Mortality in children who have malaria is also significantly associated with infection by P. However, resistance to tetracycline and doxycycline has been reported. Unbound probe is chemically degraded. Carbohydrate Assimilation. Sugar fermentation tests, although valuable, are time- and labor-intensive, making them impractical for the routine microbiology laboratory. A biletolerant, gram-negative anaerobic bacillus indicates that the isolate is likely a member of the B. • Set up an aerotolerance test. Other pathogens that grow in culture but not in routine bacterial culture can be recognized on smear and redirected for appropriate culture. pestis and Y. However, improperly preserved and handled foods, including fish and seafood, milk, ice cream, and unpreserved meat, have been responsible for outbreaks. They are found in the environment worldwide. botulinum spores. Entamoeba hartmanni. Entamoeba hartmanni, once known as small race E. Gentamicin and chloramphenicol inhibit bacterial growth, whereas cycloheximide inhibits bacteria and many of the tahir99 VRG 618 TABLE PART II Laboratory Identification of Significant Isolates 27-9 Staining Characteristics of Fungi Stain Periodic acid–Schiff Gomori methenamine silver Giemsa India ink KOH KOH-calcofluor white Masson-Fontana Color of Fungal Element Background Color Magenta Black Pink or green Green Purple-blue yeast with clear halo (capsule) Yeast with clear halo (capsule) Refractile Fluorescent Brown Pink-purple Black Clear Dark Pink-purple KOH, Potassium hydroxide. The infection is often more serious in immunocompromised patients, including those with AIDS and those with conditions such as Hodgkin disease, lymphoproliferative disorders, or lymphoblastic leukemia. Neisseria gonorrhoeae c. maltophilia, Acinetobacter spp., and Burkholderia cepacia, the Clinical and Laboratory Standards Institute (CLSI) recommends that a broth microdilution or Kirby-Bauer disk diffusion assay be performed. tuberculosis. tahir99 VRG CHAPTER 22 Anaerobes of Clinical Importance TABLE 22-11 Fluorescence Under Long-Wave Ultraviolet Light Organism Prevotella (pigmented) P. Symptoms of late onset congenital syphilis occur after 2 years of age but generally are not apparent until the second decade of life. Many Salmonella serotypes are typically found in cold-blooded animals as well as in rodents and birds, which serve as their natural hosts. This can be inoculated, incubated overnight at 35° C, and subsequently subcultured to appropriate plate media. The incidence is underestimated because the disease manifests as a nonspecific fever, headache, nausea, vomiting, and lethargy. Along with E. After an 8- to 30-hour incubation period, the patient experiences diarrhea and cramping abdominal pain for about 24 hours. rubrum isolates are negative or require more than 5 days to give a positive reaction. Approximately 50% of the isolates of M. Laboratory Diagnosis Specimen Collection and Handling. The most common and productive specimen collected for the laboratory diagnosis of B. The first section of the chapter describes the origin of the indigenous microbial flora and the composition at different body sites. The infection is typically a meningitis or septicemia in a newborn, especially in conjunction with prematurity. FIGURE 19-15 Flow chart for the presumptive identification of commonly encountered Enterobacteriaceae on triple sugar iron (TSI) agar. Yeast fungi can be classified into one of two groups, yeasts and yeastlike fungi. hortae grows slowly on Sabouraud dextrose agar at room temperature. These reports must be tempered by public health needs and individual confidentiality restrictions. tarda is an opportunist, causing bacteremia and wound infections. 595 Laboratory Diagnosis. When infected hairs are removed and placed in 10% to 20% KOH, the nodules may be crushed open to reveal the asci. Other species associated with diarrhea include A. The fourth step is to gather as much information as possible about the cases with respect to person, place, and time. aeruginosa, as well as other gramnegative bacilli, are available to increase the recovery of B. In very severe infections, exchange transfusion may be used. Clinical symptoms vary according to the phase of the life cycle and worm burden. The crystal violet stain is not tied into the organism until the iodine is added. Other important species are L. Unlike infections with Acanthamoeba spp., which occur primarily in immunocompromised hosts, infection with B. Figure 19-6 illustrates the pigmentation of S. Khan NA: Acanthamoeba and the blood-brain barrier: the breakthrough, J Med Microbiol 57:1051, 2008. Compare the metabolic pathways used by nonfermentative and fermentative organisms. trachomatis, unless proven otherwise. Specific restriction enzymes are produced that cut incoming, foreign DNA at specific DNA sequences. These strains are known as highfrequency (Hfr) strains. The steps of the technique are as follows: 1. Note overgrowth of respiratory biota. Digital oxygen indicators are available that offer faster response times to changes in atmospheric concentrations. The book details the emergence of Lassa fever, which in retrospect is eerily similar to the emergence of other hemorrhagic fevers that would be discovered later in Africa, including the Ebola virus. Other complications of shigellosis include seizures, which may occur during any Shigella sp. Their general susceptibility to the vibriostatic agent O/129 (150 µg) and positive string test distinguishes them from Aeromonas. Fungal organisms, which are unresponsive to antibacterial antibiotics Direct Examination Summary Once this stepwise evaluation of the smear has been completed, the information yielded, along with the clinical setting, allows for reasonable management of infected patients until the subsequent steps of culture isolation, susceptibility testing, or antibody or antigen detection can occur. Table 2-3 lists many bacterial exotoxins that are important in disease production as examples. The patterns of the fragments are compared among strains of microbes recovered in a possible outbreak. The bacteria are primarily found in monocytes. Oxalic Acid. Oxalic acid, 5%, is used to decontaminate specimens contaminated with Pseudomonas aeruginosa, such as sputum specimens from patients with CF. An emergent phenotype, the sorbitol-fermenting, nonmotile E. When specimens are inoculated to Middlebrook 7H10 and 7H11 media and incubated in an atmosphere of 10% CO_2 and 90% air, 99% of the positive cultures are detected in 3 to 4 weeks, earlier than for those detected on egg-based media. acidovorans are usually aminoglycoside-resistant. Which nonfermenters are most commonly isolated from patients with CF? An inner membrane with inconspicuous polar thickenings but no polar filaments surrounds the oncosphere (Figure 28-54). ■ There are notable exceptions to the Enterobacteriaceae family "gold standard" definition of glucose fermenters that are able to reduce nitrate to nitrite and are oxidase-negative. Blood Blood must be cultured as to recover any bacteria or yeasts that might be present. From 2000 to 2003, about 250 cases of mumps were reported in the United States annually. Infections caused by many yeasts are extremely aggressive and difficult to treat. C, Schistosoma japonicum egg (unstained wet mounts, ×400). The sporangia are typically fragile and are not easily retained when making slide culture preparations, resulting in an umbrella-shaped structure at the end of the conidiophores. fonticola. The infant was admitted to the intensive care unit (ICU) for antimicrobial therapy and monitoring of feeding and breathing. Subcutaneous fungal infections may be grouped together by the disease processes they cause or by the causative agents involved. Rift Valley fever virus targets the brain and liver to cause encephalitis and hepatitis. Latex agglutination assays are also available for detecting C. However, they are more expensive than tuberculin skin testing. Cystoisospora belli. Cystoisospora belli (formerly Isospora belli) is an opportunistic organism seen less frequently than C. This infection manifests as an initial lesion that may increase in size, invading and destroying the mucosal surfaces of the nose and mouth. Points to Remember ■ ■ ■ ■ ■ ■ ■ 9. Some studies linked the presence of B. The organism can also secrete chemoattractants for neutrophils. Other key tests for the identification of mycobacteria include rate of growth, nitrate reduction, niacin production, presence of heatstable catalase, and

sensitivity to T2H. Adults of F. Bacilli Bacteroides spp. in culture? Enterohemorrhagic Escherichia coli. In 1982, the O157: H7 strain of E. pneumoniae on MAC agar. intercalatum and S. vesicularis is also oxidase positive and oxidizes glucose and maltose. Conidia have both transverse and longitudinal cross walls and are often echinulate. If the isolate fails to agglutinate in the sera, it can be sent to a state or reference laboratory for additional serogrouping and serotyping. 1169. pneumoniae subsp. Notice dark staining, highly vacuolated cytoplasm (trichrome stain). All types of meat, including lamb, pork, beef, and game animals such as deer and bear, have been implicated in this mechanism. Studies indicate that the monoclonal antibody test demonstrates greater sensitivity and specificity than the modified acid-fast stain. septicum, C. Sterility should be strictly maintained in collection and transport. Shigellae are fragile organisms. The diagnostic method used is the skin snip, in which a small slice of skin is obtained and placed on a saline mount. Processed meats such as undercooked hamburgers served at fast-food restaurants, unpasteurized dairy products and apple cider, bean sprouts, and spinach all have been implicated in the spread of infection. Longer rods and filaments may be straight or wavy and branched. pneumophila, and 20% to 25% of cases are associated with travel. Pneumocystis sp. fowleri as the “braineating ameba.” In recent years, Balamuthia mandrillaris has been identified as another agent of CNS infection in humans. Cramps and bloody diarrhea often follow the initial signs. Personal protective equipment provides extra safety for staff working in the mycobacteriology laboratory. In chronic cases, the intestinal villi may show signs of atrophy caused by inflammation, and the brush border of the cells is disrupted because of invasion by the organism. Although they can be inoculated aerobically, they require anaerobic incubation. These antimicrobials include cephalosporins, penicillins, carbapenems, and aminoglycosides. The establishment of interventions to stop the outbreak probably occurs from the initial recognition and heightened awareness of a problem. Such an assessment is necessary in construction activities and must be done before any decision to culture the air can be made. are urease-positive. During this period, the patient may experience generalized symptoms such as fever, headache, and chills as well as localized swelling, redness, and lymphangitis, primarily at sites in the male and female genitalia and extremities. Differentiate among the mechanisms of transformation, transduction, and conjugation in the transfer of genetic material from one bacterium to another. A substance that plays a major role in resistance to infection is lysozyme, a low-molecular-weight (approximately 20,000 D) enzyme that hydrolyzes the peptidoglycan layer of bacterial cell walls. Likewise, serologic testing can identify more cases, but it is not generally used because it has not been standardized and is not widely available. A positive oxidase distinguishes aeromonads from the family Enterobacteriaceae (except for Plesiomonas shigelloides), and most clinically relevant aeromonads are indole-positive. Infected individuals are most infectious during the early days of a primary infection. Treponemes can cross the placenta and be transmitted from an infected mother to her fetus. A long asymptomatic stage is followed by death years later. Negative; can; positive d. cloacae, E. enterica (e.g., S. General Characteristics The characteristics of the fungi differ from those of plants or bacteria. and B. The organisms cannot be readily stained, but they can be impregnated with silver and visualized. Branda JA, et al: A rational approach to the stool ova and parasite examination, Clin Infect Dis 42:972, 2006. Meningitis was suspected, and the patient was admitted with a diagnosis of polyradiculopathy (neuropathy of the spinal nerve roots) secondary to acquired immunodeficiency syndrome. TABLE 615 Blood and Bone Marrow Blood from septicemic patients can harbor known pathogenic and opportunistic fungi. This swab method of preparation is adequate but may produce less desirable results than other methods. When viruses that normally circulate in animals infect humans, they are termed variant viruses. This results in a spastic type of paralysis, with continuous muscular spasms leading to trismus (lockjaw), risus sardonicus (distorted grin), and difficulty breathing. One of the major difficulties in a large outbreak (e.g., the state fair outbreak) is the reduced ability to collect and transport specimens from persons from out of state. Chloroplasts, found in plant cells, are the sites of photosynthesis. Parasitic protozoan nuclei are red. Hong DK, et al: Severe cryptosporidiosis in a seven-year-old renal transplant recipient—case report and review of the literature, Pediatr Transplant 11:94, 2007. Conversely, early antimicrobial intervention with macrolides may decrease antigen excretion in some patients. The family Anaplasmataceae includes the genera Ehrlichia, Anaplasma, Cowdria, Neorickettsia, and Wolbachia. They are often opportunistic pathogens in immunocompromised hosts. The incubation period is about 10 days, after which a papule forms at the site of inoculation. The immature schizont (multinucleate stage) is characterized by a splitting of the chromatin mass. Explain the importance of understanding microbial metabolism in clinical microbiology. cause intracellular infections in humans but also survive in an extracellular environment. Gastric Aspirates and Washings Gastric aspirates are used to recover mycobacteria that may have been swallowed during the night. are an emerging pathogen in the immunocompromised host that can cause a range of infections, including pulmonary and skin infections. A poisoned catalyst results from the gases, particularly H_2S, produced by anaerobes. Immunologic Assays Latex agglutination tests are available for the rapid identification of colonies of enteric campylobacters on primary isolation media. The scolex, proglottid, and egg are diagnostic structures that can be found in a fecal specimen. Some results may be free, while others may require a fee to unlock the information.Reverse Address LookupA reverse address lookup is another type of search you can do if you only have part of the information about the number you need to find. Most members of the family Bunyaviridae cause a febrile illness, hemorrhagic fever, or encephalitis. The most commonly recommended container is a sterile, wide-mouth cup with a tightly fitted lid. Bacteria process pyruvic acid further using various fermentation pathways. In this disease, organisms spread through the lymphatics and invade organs of the reticuloendothelial system, including the liver, spleen, lymph nodes, and bone marrow. infection, and HUS, a complication among the shigellae exclusively associated with S. Bacterial spores are formed as a result of harsh environments. A. Because children have difficulty producing sputum, gastric aspiration is recommended. Zephiran is bacteriostatic for tubercle bacilli, necessitating neutralization before plating or the use of egg-based media to exploit its inherent neutralizing capacity. Bacteria are swallowed into the gastrointestinal tract either as part of the mouth flora and upper respiratory tract or in liquids and food. None of the above 10. Direct microscopic examination of the granules collected from the draining lesion immediately differentiates eumycotic from actinomycotic mycetomas (Table 27-3). lincolnii, and M. Likewise, consider how a sterile environment might affect a newborn. ozaenae, K. mansoni, which is most commonly found in Africa, parts of South America, the West Indies, and Puerto Rico, lives in venules of the mesentery and large intestines. Table 28-2 lists the characteristics of the classes in which most medically important human parasites are found; they are described in the remainder of this chapter. are rare but can be traced to contaminated dialysis lines and other similar devices. 16. These insects live in the mud or thatch walls of a dwelling during the day and come out at night to take a blood meal from the human inhabitants. diphtheriae. As with other areas of clinical microbiology, the use of molecular methods rather than phenotypic characteristics to determine taxonomic placement of an organism has resulted in an explosion of taxonomic changes. In patients with a leptospiral bacteremia, immunoglobulin M (IgM) antibodies are detected tahir99 VRG CHAPTER 23 The Spirochetes within 1 week after onset of disease and may persist in high titers for many months. The polar heads of the phospholipids are hydrophilic (water loving) and lie on both the intracellular and the extracellular fluids; their nonpolar tails are hydrophobic (water hating) and avoid water by lining up in the center of the PM “tail to tail.” This type of hydrophobic makeup of the interior of the PM makes it potentially impermeable to water-soluble molecules. Enterobacter cloacae 10. malariae infections can lead to nephrotic syndrome, which arises from the deposition of circulating immune complexes of malarial antigen and antibody on the basement membrane of the glomeruli, causing an autoimmune reaction. Right, Mucoid colonies. coli. These three domains are the largest and most inclusive taxa. However, researchers had to discount a viral cause, because the agent was not filterable. (indole-negative). Certain individuals may be genetically more susceptible to SARS than others. malariae nor P. If an enrichment procedure is desired to enhance isolation of vibrios, alkaline peptone water with 1% NaCl (pH 8.5) can be inoculated (at least 20 mL) and incubated for 5 to 8 hours at 35° C before subculturing to TCBS. However, careful examination is critical of organisms recovered from, for example, the respiratory tract, urogenital tract, stool, and wounds in open sites that are inhabited by other endogenous microbiota. D, P. aureus PEA or CNA B. Although they may appear to be strict anaerobes, they have been grown in a microaerophilic environment. tahir99 VRG 479 CHAPTER 21 Nonfermenting and Miscellaneous Gram-Negative Bacilli Burkholderia cepacia Achromobacter xylosoxidans Stenotrophomonas maltophilia Burkholderia pseudomallei Pseudomonas stutzeri Sphingomonas paucimobilis Pseudomonas mendocina Ralstonia pickettii Delftia acidovorans Pseudomonas pseudoalcaligenes Alcaligenes faecalis Achromobacter denitricans Oligella ureolytica Bordetella bronchiseptica Pseudomonas alcaligenes Brevundimonas diminuta Brevundimonas vesicularis Comamonas testosteroni Shewanella putrefaciens / / Pseudomonas aeruginosa Oxidase Pyocyanin Fluorescein MOTILE, WEAK, OR NONSACCHAROLYTIC NONFERMENTERS Pseudomonas fluorescens/putida MOTILE, STRONGLY SACCHAROLYTIC NONFERMENTERS / / / // / / / / / / / Urea // DNase ONPG Indole / Glucose Xylose / Mannitol /// Lactose Maltose 42° C Esculin / / / ///// / / / / ///// // / / / Motility Flagella 1 >1 >1 P >1 >1 1 1 1 1 1 >1 1 P P P P 1 1 1 >1 1 H 2S / / / Pigment B,F,G F Y Y B,Y Y B,Y B // N2 gas Growth on MacConkey FIGURE 21-2 Biochemical and morphologic characteristics of selected nonfermentative gram-negative bacilli. B, 5′ and 3′ antiparallel polarity and “twisted ladder” configuration of DNA. Attachment One of the most effective defenses bacteria have against phagocytosis is the capsule. Expectorated sputum, which collects in the lower lung airways and is expelled through the mouth, is the most common contaminated specimen managed by the clinical laboratory. Laboratory Diagnosis. Feces serve as the usual diagnostic specimen, but shedding of the cysts is irregular, and multiple stool specimens are often required for diagnosis. Other less serious conditions associated with P. Isolates grow well at 5° C to 25° C but rarely at 35° C. These continuous monitoring systems have similar performance and operational features. Biochemical Identification A panel of biochemical tests can identify most mycobacteria isolates, but because growth of Mycobacterium spp. Steere AC: Borrelia burgdorferi (Lyme disease, Lyme borreliosis). fragilis has apparently permanently lost its flagella. In contrast to Escherichia spp., Shigella spp. tahir99 VRG 408 PART II Laboratory Identification of Significant Isolates BIBLIOGRAPHY Al Dahouk S, et al: Laboratory-based diagnosis of brucellosis—a review of the literature, part II: serological tests for brucellosis, Clin Lab 49:577, 2003. Role of the Microbial Flora in the Host Defense Against Infectious Disease The microbial flora provides beneficial effects. tahir99 VRG CHAPTER 2 Host-Parasite Interaction 37 The importance of phagocytosis is seen in patients with defects in the numbers or function of phagocytic cells. Cows d. Adenine of one chain always pairs with thymine of the other chain, and cytosine of one chain pairs with guanine of the other chain. caviae is the species most frequently associated with gastrointestinal infections, especially in neonate and pediatric populations; it has been associated with inflammatory bowel disease. Clinical Infections In humans, salmonellosis may occur in several forms, as follows: • Acute gastroenteritis or food poisoning characterized by vomiting and diarrhea • Typhoid fever, the most severe form of enteric fever, caused by Salmonella serotype Typhi, and enteric fevers caused by other Salmonella serotypes (e.g., Salmonella Paratyphi and Choleraesuis) • Nontyphoidal bacteremia • Carrier state following Salmonella infection Humans acquire the infection by ingesting the organisms in food, water, and milk contaminated with human or animal excreta. Some patients develop an ameboma (amebic granuloma), a tumor-like lesion that forms in the submucosa of the intestine. are the most common Mucorales causing human disease. Mucor spp. PCR tests are especially useful in detecting infection in asymptomatic men. A comparison of the CNS infections caused by N. In addition, the high parasitemia that often accompanies P. The patient has severe headaches, may be confused, and ultimately lapses into a coma. Monoclonal antibodies specific for C. Available at: . In addition, no standardized method currently exists for performing these tests. are sometimes implicated in subcutaneous infections, usually following traumatic inoculation. (Nomarski optics, ×650.) FIGURE 27-42 Paecilomyces sp. There they develop into infective metacyclic trypomastigotes, which are transmitted to humans in saliva when the fly takes another blood meal. Hymenolepis. The dwarf tapeworm, Hymenolepis nana, is found worldwide and is a common tapeworm in children, whereas Hymenolepis diminuta, the rat tapeworm, is seen less frequently. Lesions associated with tinea versicolor typically appear as pale patches in individuals with darkly pigmented skin, but they can also be described as fawn-colored liver spots in individuals with fair complexions. Up to 50% of erythrocytes may be infected, and the primary complication is development of cerebral malaria. An improperly collected specimen may result in the growth of many different anaerobes, but determining which, if any, are responsible for the infection might be impossible. Enterobiasis is frequently found in families, kindergartens, daycare centers, or crowded conditions in which the eggs can be easily transmitted. Wet mount preparations of the material showed budding yeast cells and hyphal elements. On Sabouraud dextrose agar, they produce pink, mucoid, almost runny colonies (Figure 21-15); however, they do not appear black under long-wavelength ultraviolet light, as do the Methylobacterium spp. 687 Vannier E, Krause PJ: Human babesiosis, N Engl J Med 366:2397, 2012. A U.S. Food and Drug Administration–approved, visually read IgM enzyme-linked immunosorbent assay (ELISA; Pan Bio INDx, Baltimore, MD) is available and has been shown to have high sensitivity (98%) and specificity (90.6%) in acute leptospirosis cases. Its true significance is unknown, but initial evidence suggests that it is nonpathogenic. ■ The usual microbial flora present at each site in the body is dictated by nutritional and environmental factors. B, B. Other information that may be included if you’re looking up a business is the company profile and a link to the company website. When infections are diagnosed early, they usually can be treated successfully with a macrolide such as azithromycin or a fluoroquinolone. A rate of infections in 1000 device-related events b. Available at: .gov/vaccines/schedules/hcp/child-adolescent.html. IgM antibody does not cross the blood-brain barrier; therefore, the presence of IgM in CSF strongly suggests CNS infection. (Courtesy Fred A. Figure 27-54, B, shows the cyst stained with Giemsa stain. The use of these assays has led to a better understanding of viruses and helped develop better patient therapies. However, the genus Peptostreptococcus has been reclassified recently into at least five different genera—Peptostreptococcus, Anaerococcus, Finegoldia, Parvimonas and Peptoniphilus. tuberculosis resistant to PZA are often pyrazinamidase-negative. In general, the interior organization of eukaryotic cells is more complex than that of prokaryotic cells (see Figure 1-1). septicum C. For example, in an acute care hospital, infection prevention and control committees may expect reports with various types of microbiologic information. Adults are thin, tapered at both ends, and 1 to 2.5 cm long. Botulinum toxin is an extremely potent neurotoxin; it takes only a small amount to produce paralysis and death. The diagnosis can be made by radiologic examination, ultrasound, or other imaging techniques. Chapter 31 describes an increasingly complex entity—biofilms. On average, the mortality rate for smallpox was 30%. In Murray PR, et al, editors: Manual of clinical microbiology, ed 9, Washington, DC, 2007, ASM Press. Generally, two swabs are collected, one through each of the external nares; the swabs should be inserted as far back as possible into the nasopharynx, rotated, held a few seconds, and then gently withdrawn. Serotyping for both O and H antigens is often useful in identification of strains, particularly strains associated with serious enteric disease. The asexual spores (sporangiospores) are Hyphae Arthroconidia FIGURE 27-7 Arthroconidia, another form of asexual reproduction, are formed by fragmentation of fertile hyphae. The colonies are straw to creamcolored and yeastlike. luteolus and P. Urine is an important specimen to be collected for antigen detection, but it is most useful for L. Adenylate cyclase toxin inhibits host epithelial and immune effector cells by inducing supraphysiologic concentrations of cyclic adenosine monophosphate. In this procedure, an aliquot of the specimen is first diluted 1 : 10 with 0.2 N KCl-HCl and allowed to stand for 5 minutes. ONPG, O-nitrophenyl-β-D-galactopyranoside; −, ≤9% of strains positive; +, ≥90% of strains positive; V, 10% to 89% of strains positive. The incubation period may be prolonged, with reactivation occurring long after exposure. In addition, all rapid growers are able to reduce tellurite in 3 days. Antimicrobial Susceptibility Borreliae are susceptible to many antimicrobial agents; however, tetracyclines are the drugs of choice because they reduce the relapse rate and rid the central nervous system of spirochetes. Mixed or engineered cell cultures are lines of cells that contain a mixture of two different cell types or are made up of cells genetically modified to make identification of viral infection easier. focal areas of Latin America and Africa. The patient’s blood is reacted with monoclonal antibody labeled with dye or gold particles. With the B FIGURE 28-10 A, Endolimax nana cyst, identified by arrow (trichrome stain). The size, shape, and interior location of the spore, for example, at one end (terminal), subterminal, or central, can be used as identifying characteristics. Identification of clinically encountered clostridia using cultural and biochemical characteristics is summarized in Table 22-15. The rash involves the palms of the hands and soles of the feet, just as in RMSF. on Gram-stained smear (×1000). These tests are inexpensive to perform, demonstrate rising and falling reagin titers, and correlate with the clinical status of the patient. Heparinized bone marrow specimens should be plated directly onto media at bedside; blood culture bottles are not recommended. Other named species TABLE of Chlamydia exist, but they are rarely isolated from humans. FIGURE 27-31 Yeast phase (“mariner’s wheel”) of Paracoccidioides brasiliensis with multipolar budding (Nomarski optics, ×1250.) Serologic assays have shown to be important in early diagnosis; however, none are commercially available. Disease involves no inflammatory or other tissue reaction to the infecting fungus. Rectangular fragments of hyphae should be accompanied by blastoconidia for an isolate to be considered a yeast. perfringens, other Clostridium spp., peptostreptococci (frequently polymicrobial) Clostridium perfringens, C. Trichuris trichiura. Ascaris lumbricoides, Trichuris trichiura, and two genera of hookworms, Ancylostoma and Necator, are the most common soil-transmitted helminths. Hosts acquire infections directly by contact with the urine of carriers or indirectly by contact with bodies of water contaminated with the urine of carriers. As many as 45% of patients with a tracheostomy may be colonized. Because infection does not confer complete immunity, multiple infections can occur throughout life and can be severe in older adults, the immunocompromised, and those with cardiac and respiratory problems. Outbreaks may 55 Noroviruses Rotavirus Hepatitis A Hepatitis E Bacteria Parasites Salmonella spp. difficile is the most common but not the sole cause of antibiotic-associated diarrhea and pseudomembranous colitis. The most commonly used target is the gene coding for 16S rRNA. marneffei can be detected in Wright-stained smears from skin lesions or biopsy specimens. Actinomycosis Antibiotic-associated diarrhea; pseudomembranous colitis Bacteremia Brain abscess Infections of the female genitourinary tract Intraabdominal infections, liver abscess, peritonitis, perineal and perirectal infections Myonecrosis Oral, sinus, dental infections Aspiration pneumonia, pleuropulmonary infections Anaerobe Actinomyces israelii, other Actinomyces spp. Most clinical specimens, such as sputum, contain mucin or organic debris that surrounds the bacteria in the sample. Occasional isolates that are not β-hemolytic must be indole-positive, lactose-positive on MAC or EMB, and must exhibit a negative pyrrolidonyl arylamidase (PYR) test to be identified as E. Detection of diarrheal illness attributable to EPEC depends primarily on the suspicion of the physician. Figure 28-21 shows the four life cycle stages of the hemoflagellates. pneumophila groups 1 to 7, L. Infections are typically confined to the sinuses or following traumatic inoculation. Currently, several characteristics are still used to place an organism in the family Enterobacteriaceae (Box 19-1). Coy Laboratory Products (Grass Lake, MI) has recently developed a hydrogen sulfide removal column that circulates the air inside the chamber through layers of media, removing the toxic gas. Several of these are important in the killing and digestion of the engulfed bacterial cell. In developing countries in Africa, 20-6 Clinically Significant Campylobacter Species and Campylobacter-Like Organisms Species Clinical Significance Arcobacter butzleri Arcobacter cryaerophilus Arcobacter skirrowii Campylobacter jejuni Campylobacter coli Campylobacter fetus subsp. The upper respiratory tract is composed of the mouth, nasopharynx, oropharynx, and larynx; the lower respiratory tract is composed of the trachea, bronchi, and pulmonary parenchyma. (A, ×1000; B, original magnification ×1000.) A 637 B FIGURE 28-8 A, Entamoeba coli cyst (merthiolate-iodine-formalin) wet mount. bronchiseptica and B. It should be noted that there have been reports of a case of sepsis with an extendedspectrum β-lactamase (ESBL)–producing A. Erect sporangiophores, solitary or in groups (slightly branched), terminate in an apophysis surrounded by a sporangium. faecalis and A. polymerase) using one strand of the DNA as a template. Genes that code for antibiotic resistance (and sometimes toxins or other virulence factors) are often located on plasmids. § Data on M. Microaerophilic organisms require oxygen, but at a concentration less than that of room air; 5% is normally optimal. Deschaght P, et al: Psychrobacter isolates of human origin, other than P. Turbid or thick fluids can be more efficiently prepared by the previously described method. (Nomarski optics, ×450.) phialoconidia remain in long chains or are easily disturbed into individual phialoconidia. The urethra is commonly involved in both men and women. The groups of organisms discussed in this chapter include the Pseudomonas spp., Acinetobacter spp., Stenotrophomonas maltophilia and other lactosenegative oxidizers, and asaccharolytic species of gram-negative bacilli. Intestinal Flukes. Fasciolopsis buski, known as the giant intestinal fluke, is found in the Far East, including China, Vietnam, and India. Continuously drop 1% sulfuric acid on the smear until the washing becomes colorless. putida produce pyoverdin, but neither produces pyocyanin or grows at 42° C, the key characteristics of P. Nutritional Requirements for Growth Bacteria are classified into two basic groups according to how they meet their nutritional needs. vulgaris b. Figure 19-8 shows the antigenic structures used in serologic grouping and their locations. Some Salmonella infections are transmitted by human carriers. Generally, conidial chains of the saprophytic species break up easily, whereas those of pathogenic species remain connected. With heavy exposure, however, acute pulmonary disease can occur. Sparganosis. Human infection with the plerocercoid larva (sparganum) of a dog or cat tapeworm can result in sparganosis. albicans by its inability to grow at 42° C. Pectobacterium spp. In the diagnostic microbiology laboratory, the eukaryotic cell type occurs in medically important fungi and in parasites. Al-Hasan MN, et al: Invasive enteric infections in hospitalized patients with underlying strongyloidiasis, Am J Clin Pathol 128:622, 2007. More oocysts are seen in liquid stool than in formed stool. Most cases of HME are diagnosed retrospectively by serologic testing; IFA is the most widely used method. Patients then enter a phase of hypotensive shock and pulmonary edema. These include the anaerobic cocci, Fusobacterium, Prevotella, Bacteroides, and Lactobacillus. The different body sites may have the same or different flora, depending on local conditions. Bartlett’s method for scoring sputum and the Murray-Washington method for contamination assessment document the association of 10 to 20 squamous epithelial cells (SECs) per ×10 microscopic field with unacceptable specimens and 10 to 25 PMNs per ×10 field with significant specimens. Benedetti P, et al: Septic shock, pneumonia, and soft tissue infection due to Myroides odoratimimus: report of a case and review of Myroides infections, Infection 39:161, 2011. A major disadvantage is the potential contamination of the colony and temporary nature of this preparation. Although most isolates of E. The catalyst, usually tahir99 VRG CHAPTER 22 Anaerobes of Clinical Importance palladium-coated alumina pellets, removes residual oxygen from the atmosphere in the chamber. catarrhalis. Within about 1 week, reticulocytosis occurs and the patient recovers. coli remains one of the most common causes of septicemia and meningitis among neonates, accounting for about 40% of the cases of gram-negative meningitis. EHEC d. caviae. Further investigation reveals that the patient consumed a medium-cooked wild rabbit in a restaurant in Germany 2 months earlier. The rodents are infected for long periods and typically do not become ill from the viruses, which they shed in urine, feces, and saliva. This group of bacteria belongs within the class Mollicutes. Centers for Disease Control and Prevention: Investigation update: ongoing outbreak of human Salmonella Typhimurium infec tions associated with African dwarf frogs. gillenii and C. This general definition has been broadened over the years to mean a reaction to any foreign substance, including proteins and polysaccharides as well as invading microorganisms. 1943. A, Gram-stained appearance of terminal spores of Clostridium tetani (×1000). Some assays are able to distinguish between immunoglobulin M (IgM) and immunoglobulin G (IgG); the presence of IgM indicates an acute (recent) infection. Ewing, Jr., Centers for Disease Control and Prevention, Atlanta, GA.) As with HSV, serology is not as helpful as a culture in diagnosing the infection. Onchocerca volvulus. Infection with O. Consistency may help determine the type of preservative to be used, indicate the forms of parasites expected to be present, or dictate the immediacy of examination. If stain is discarded into sink, flush with large volumes of water to prevent azide buildup, which may react with lead and copper plumbing to form highly explosive metal azides. Once disseminated, the organisms cause vasculitis in the blood vessels of the lungs, brain, and heart, leading to pneumonitis, central nervous system manifestations, and myocarditis. Although complete conversion is seldom noted, a combination of both mycelial and yeast forms is sufficient for identification. In colonies of M. Antifungal Susceptibility Antifungal Agents In the last 25 years, fungal infections have steadily increased in incidence. About 24 hours after infection, the dividing organisms begin reorganizing into infective EBs. At about 30 hours, multiplication ceases, and by 35 to 40 hours, the disrupted host cell dies, releasing new EBs (Figure 24-2) that can infect other host cells, continuing the cycle. In the postantimicrobial era, the case-fatality rates for F. Patients with liver dysfunction and syndromes that result in increased serum levels of iron (e.g., hemochromatosis, cirrhosis, thalassemia major, hepatitis) are particularly predisposed to this scenario. The isolate is then heavily inoculated into the water and incubated at 37° C. Isolation and Identification Isolation Methods The most important test for legionnaires’ disease is culture of the organism and should always be attempted even when employing a rapid test such as urine antigen detection. Contact time should be at least 30 minutes. In tissue, these fungi may form yeastlike cells that are solitary or in short chains or hyphae that are septate, tahir99 VRG CHAPTER 27 Medically Significant Fungi branched, or unbranched and often swollen to toruloid (irregular or beaded). Most transport media consist of a buffered isotonic solution with a protein such as albumin, gelatin, or serum to protect less stable viruses. The appearance of the spirochete among the red cells is characteristic (Figure 23-2). vulnificus − + + + − − + V. If the catalyst performs properly, water vapor will be present on the inside of the jar, and the indicator strip will be white. Despite these problems, AMB remains the drug of choice for most life-threatening fungal disease. The counterstain does not penetrate the mycobacteria to affect the color of the primary stain. bongori, should be designated. The spirochetes exhibit various types of motion in liquid media. Erythromycin d. VZV can be cultured on human embryonic lung or Vero cells. The specimen should be inoculated into the pouch within 30 minutes of collection to ensure optimal organism viability. Legionella pneumophila, the agent of this outbreak, became the first named member of the family Legionellaceae. The diagnosis of HHV-6 infection is usually made clinically. In BV, a shift in the vaginal biota occurs, resulting in the overgrowth of other endogenous anaerobes of the vagina such as Mobiluncus spp., Bacteroides spp., Prevotella spp., anaerobic gram-positive cocci, and the aerobic bacterium Gardnerella vaginalis. mesnili, which measures 6 to 10 µm, is lemon-shaped, with an anterior nipple. Rhizoids (see Figure 27-3, B), rootlike structures, might be seen in some of the Zygomycetes, and their presence and placement can assist with identification. Nitrate Disk. The nitrate reduction disk test is a miniaturized version of the conventional nitrate reduction test. Currently, 30,000 to 50,000 cases of Japanese encephalitis are reported annually. ferment lactose slowly and are positive for the o-nitrophenyl-β-D-galactopyranoside (ONPG) test. Extrachromosomal Elements In addition to the genetic information encoded in the bacterial chromosome, many bacteria contain extra information on small circular pieces of extrachromosomal, dsDNA called plasmids. Studies in Bangladesh, however, indicate a rapidly occurring reemergence of the classic biogroup. cholerae isolates. gilardi, C. Semiquantitation of catalase production is based on the addition of Tween 80 (a detergent) and hydrogen peroxide to a 2-week-old culture grown in an agar deep. To establish itself and cause disease, a pathogen must be able to replicate after attachment to host cells. (weak fermenters) Sphingobacterium spp. A shell vial is a small, round, flat-bottomed tube, generally with a screw cap. Superficial wound or abscess specimens aspirated by needle and syringe are much better specimens for anaerobic bacteriology than material collected by swabs; the latter often are contaminated with anaerobes of the skin microbiota. Cook, MD Professor of Infectious Disease Co-Director, Infectious Disease and Immunology Institute Loyola University Maywood, Illinois Director, Division of Infectious Diseases Department of Medicine Loyola University Medical Center Maywood, Illinois Chief, Infectious Diseases Edward Hines Jr. VA Hospital Chicago, Illinois Robert C. Hot water systems, cooling towers, and evaporative condensers are major reservoirs. The one exception is the rapidly growing mycobacteria. falciparum infection as a result of tubular necrosis brought about by tissue anoxia. Some laboratories also routinely include a CNA blood agar plate to select for gram-positive bacteria. APPLICATION MATERIALS A 1% (wt/vol) aqueous solution of calcofluor white is prepared by dissolving the powder in distilled water with gentle heating. Colonies are wooly and grow rapidly, completely covering the culture medium. morganii subsp. gondii tachyzoites in lung tissue. Infected patients develop fever, photophobia, myalgia, arthralgia, and chills. In more severe cases, the sole of the foot may develop extensive scaling, with fissuring and erythema. The febrile episode becomes more evident during this release of the organisms into the circulatory system. Background debris and yeasts stain green. 603. cdc.gov/ecoli/2011/ecolio104. However, for medical purposes, and because of the useful association of the genus epithet with the distinct disease shigellosis or bacillary dysentery, they remain as separate genera. fluvialis, V. tuberculosis infections. This stain is selective for the cell wall of acid-fast bacteria. Rapid membranebound colorimetric tests are also available. Most nonfermentative, gram-negative bacilli are oxidasepositive, a feature that differentiates them from the Enterobacteriaceae (Plesiomonas sp. • Is necrotic (amorphous) debris in the background? albicans grows at 45° C, whereas C. (Courtesy Bionique Testing Laboratories, Saranac Lake, NY.) 25-1 Divergent Ecosystems Inhabited by Genera of the Class Mollicutes Ecosystem Soil and grasses Crops and plants Mown hay Water Deciduous trees Humans Cattle Mycoplasma Ureaplasma Acholeplasma Spiroplasma Thermoplasma Anaeroplasma − − − − − + + − − − − − − + + − − − − + − − − − − + − − + − − − − − + − − − − − − − − − − − + + +, Present in ecosystem; −, rarely associated with ecosystem. You can search these sites by name, keywords or location and, sometimes, you can enter a phone number to do a reverse search.Telkom Cell Phone ContractsAs Africa’s biggest integrated telecommunications company, Telkom mobile contracts are also part of the company’s offerings. For each drug tested, a standardized inoculum is added into a drug-free and drug-containing vial. In bone marrow transplant patients, mortality from infections caused by the fusaria approaches 100%. Within the cell, the amastigote multiplies and causes the cell to rupture. While kayaking the Segama River, his kayak capsized and he had inadvertently swallowed several mouthfuls of river water. Fever is relatively uncommon, and radiographs show isolated pneumonitis. The life cycle of D. Left to right, Negative, weak positive, positive. This migration is called chemotaxis (a chemical “taxi” or chemically caused movement). Although the organism is occasionally seen in a Gram-stained smear, wet mounts of material are often unrewarding because of the small numbers of organisms present. Within the snail, a complex development of germinal tissue occurs, resulting first in sporocysts, which contain undifferentiated germinal structures, and then in rediae, which contain partially differentiated germinal material. baumannii demonstrates variable susceptibility to the aminoglycosides and β-lactam plus β-lactamase inhibitor combinations (e.g., ampicillinsulbactam, piperacillin-tazobactam). These two antifungals tend to have lower minimum inhibitory concentrations (MICs) than CAS and may be options when MICs to CAS are above desired limits. Some species harbor plasmids that can provide antimicrobial resistance genes. There is no specific treatment for WNV but in severe cases requiring hospitalization, supportive care, including IV fluids, respiratory support, and prevention of secondary infection, may be warranted. Issues to Consider After reading the patient’s case history, consider: ■ The various organisms that can be recovered from exudative material from newborns ■ The clinical infections and disease spectrum associated with these organisms ■ How these organisms are transmitted and the risk factors associated with the diseases produced ■ The appropriate methods of laboratory diagnosis tahir99 VRG 537 538 PART II Laboratory Identification of Significant Isolates Key Terms Brill-Zinsser disease Bubo Elementary body (EB) Human granulocytic anaplasmosis (HGA) Human monocytic ehrlichiosis (HME) Lymphogranuloma venereum (LGV) Morulae Pelvic inflammatory disease (PID) Reiter syndrome Reticulate body (RB) Trachoma T he genus Chlamydia is in the family Chlamydiaceae; members of the family share selected characteristics (Table 24-1) and have a unique life cycle. Organisms that are isolated must be tested for toxin production because a small percentage of the human population can carry C. Therefore, the risk of infection from all Vibrio spp. have been recovered from nails and skin lesions. The stool typically contains large amounts of mucus, but apparent blood is not present. All have been isolated from rodents of the family Muridae. Titball RW, et al: Will the enigma of Francisella tularensis virulence soon be solved? The dominant strains of influenza A since 1977 have been influenza A (H1N1) and influenza A (H3N2). For example, large quantities of gas in the specimen might be caused by gas-producing organisms such as E. 9. Levine discovered that DNA contained phosphates, five-carbon sugars (cyclic pentose), and nitrogen-containing bases. sonnei growing on MAC agar after 48 hours of incubation. histolytica trophozoite. nov., Cronobacter genomospecies 1, and of three subspecies, Cronobacter dublinensis subsp. These organisms are motile by means of one or two polar flagella and are catalase and urease positive. In Versalovic J, et al, editors: Manual of Clinical Microbiology, ed 10, Washington, DC, 2011, ASM Press, p. Characteristics of Viruses Structure At a minimum, viruses contain a viral genome of RNA or DNA and a protein coat—the capsid. In the mold phase, conidia are borne on short lateral branches that are ovoid to dumbbell-shaped and vary in diameter from 2 to 10 µm. A pink to red color is indicative of a positive reaction. Adults are approximately 3 cm long and have a prominent cephalic cone. The most commonly infected sites are striated muscle, the eye, and the brain. Issues to Consider After reading the patient’s case history, consider: ■ The role of microscopic morphology in presumptive identification ■ Significance of observable cellular structures ■ Importance of quality control in assessing and interpreting direct smear results ■ Unique characteristics of organisms, such as cellular structure and metabolic and physiologic pathways, in initiating infection and disease in hosts Key Terms Acid-fast Aerotolerant anaerobes Anticodon Archaea Archaeobacteria Autotrophs Bacteria Bacteriophage Capnophilic Capsule Codon Competent Conjugation Differential media Dimorphic Eukarya Eukaryotes Facultative anaerobes Family Fermentation Fimbriae Flagella Fusiform Genotype Genus Gram-negative Gram-positive Heterotrophs Hyphae Lysogeny Mesophiles Microaerophilic Minimal medium Mycelia Nutrient media Obligate aerobes Obligate anaerobes Pathogenic bacteria Phenotype Phyla Pili Plasmids Pleomorphic Prokaryotes Protein expression Psychrophiles Respiration Restriction enzymes Selective media Species Spores Strains Taxa Taxonomy Temperate Thermophiles Transduction Transformation Transport medium 3 I n this chapter, the basic concepts of prokaryotic and eukaryotic cells and viral and bacterial cell structure physiology, metabolism, and genetics are reviewed. For example, an increasing number of E. fortuitum. Aerosolized ribavirin can be used to treat infection. Gram-negative anaerobes frequently stain a pale pink when safranin is used as the counterstain and thus may be overlooked in Gram-stained smears of clinical specimens and blood cultures. It is a frequent colonizer in the CF patient population. Adenovirus infections occur throughout the year and affect every age group. Similar to F. Lactobacillus, anaerobic bacilli Clostridium, Bacillus spp. The patient was immediately scheduled for surgical débridement and was placed on a broad-spectrum antimicrobial agent. Serologic tests are useful if invasive methods cannot be used for identification. sordellii are spot indole– positive. Those who have traveled to endemic areas are at risk for infection with B. Once ETEC strains are established, they can release one or both of two toxins into the small intestine. If an isolate is suspected of being one of these three serotypes, Salmonella Typhi, Salmonella Paratyphi, or Salmonella Choleraesuis, because of the medical implications it must be biochemically identified and serologically confirmed. Two U.S. Food and Drug Administration (FDA)–approved allylamines are terbinafine and naftifine. Reports of isolating E. The second theory suggests that the disease was endemic in Africa and transported to Europe via the migration of armies and civilians. celatum, M. burgdorferi sensu lato infection is serum for serology. Ideally, laboratories should process specimens for mycobacteria daily because delays in processing may lead to false-negative cultures and increased bacterial contamination. Patients may experience fever and chills and, rarely, nausea and vomiting. Given an organism’s characteristic growth on nonselective and selective differential media, presumptively identify the isolate to the genus level. Taxa (plural of taxon), for example, the levels of classification, are the categories or subsets in taxonomy. Progressive pulmonary or invasive disease may follow, resulting in ulcerative lesions of the skin and bone. chaffeensis infections; however, this method is not very Anaplasma Anaplasma phagocytophilum, formerly known as Ehrlichia phagocytophilum, causes a disease referred to as human granulocytic anaplasmosis (HGA). Describe the mode of transmission of Bordetella spp. This virus is discussed in detail in the hepatitis viruses section of this chapter. *Results of TSI and LIA reactions in this category indicate a potential pathogen; additional tests must be performed. cdc.gov/eid/content/15/5/pdfs/v15-n5.pdf. The typhus group contains only two species, R. Although treatment is not always necessary, albendazole has been reported to be effective, especially in undernourished children. The trophozoite ranges in size from 10 to 25 µm and moves by explosively extend ing large, broad pseudopods (lobopodia). Develop a new isolation precaution d. The risk of metastatic complications could be more severe than the bacteremia itself, even in individuals who do not have underlying diseases. If more than 5 mL is submitted, the CSF may be filtered through a membrane filter and portions of the filter placed on media. (A, ×200; B, ×200.) tahir99 VRG 642 PART II Laboratory Identification of Significant Isolates Human ingestion of infective cyst in contaminated food or water Asexual reproduction Formed stool Cyst—infective Liquid or soft stool Trophozoite—not infective FIGURE 28-16 Generalized life cycle for intestinal flagellates. Giardia lamblia. G. ✓ Case Check 22-2 The patient’s infection in the Case in Point is a typical scenario of gas gangrene (myonecrosis) caused by C. Because the symptoms resemble those of bacterial meningitis, specific treatment may be delayed, but the possibility of cure depends on early diagnosis. Lehman George Manuselis Acknowledgments W e are grateful to all contributing authors, students, and instructors and many other individuals who have made significant suggestions and invaluable comments on ways to improve this edition. Name risk factors associated with acquiring infections by these organisms. tahir99 VRG 502 PART II Laboratory Identification of Significant Isolates Clostridium difficile–Associated Diseases. C. vaginalis are usually asymptomatic and serve as carriers, although they can develop nonspecific urethritis with a milky discharge that lasts up to 4 weeks. Formerly, serology provided only limited information to aid in the diagnosis of HSV infections. Vibrios can be easily confused with other genera, including Aeromonas, Plesiomonas, Enterobacteriaceae, and even Pseudomonas. Four workers in the animal facility developed antibodies to EBO-R but did not develop disease. Typing genital lesion isolates can be prognostic in that HSV-2 reactivation occurs more readily than HSV-1. Capnophilic bacteria require extra carbon dioxide (5% to 10%) for growth; an example is H. Virus can also be isolated from urine and feces, suggesting other potential routes of transmission. braziliensis complex is the causative agent of mucocutaneous leishmaniasis or espundia. Table 25-2 compares features of three genera known to be pathogenic for humans. There are two pyrimidines in the molecule: thymine (T) and cytosine (C). Clinical and Laboratory Standards Institute: Abbreviated identification of bacteria and yeast: approved guideline—second edition, CLSI Document M35-MA2, Wayne, PA, 2008, CLSI. Tests that yield negative results may need to be repeated in several weeks. Ectopic infections have also caused endometritis, urethritis, and salpingitis. What are the typical susceptibility patterns of the most commonly isolated nonfermenters? Mucorales generally produce profuse, gray to white, aerial mycelium characterized by the presence of hyaline, sparsely septate hyphae. Each contains malachite green to suppress the growth of gram-positive bacteria. Diagnosis of filarial worm infection is made by observing the microfilariae in blood or tissue specimens. At this point, the patient is infectious. A novel parvovirus was described in 2005. Does the specimen fluoresce brick red when exposed to long-wave (366-nm) ultraviolet light? Two years previously, the patient had been diagnosed with human immunodeficiency virus infection. Humans acquire the infection by ingesting a copepod (Cyclops) that contains an infective larva. Preliminary Identification of Mycobacteria Once an isolate has been recovered in the mycobacteriology laboratory, certain characteristics can be used to classify the isolate before performing biochemical tests. illustrates the Gram stain of a bronchial specimen containing P. Paramyxoviridae Parainfluenza Viruses Several genera belong to the family Paramyxoviridae, including Morbillivirus, Paramyxovirus, Pneumovirus, and Rubulavirus. FIGURE 2-7 Immunoglobulin M (IgM). Paired sera can be tested for mumps antibody by EIA, IF, and hemagglutination inhibition tests. Clinical Infections. Clinical infections range from single, self-healing skin ulcers to visceral disease affecting multiple organs. The nature of the adaptive immune response varies according to the type of organism and is designed to eliminate it efficiently. Microconidia are abundantly formed by most isolates, and these may be the only conidia maintained in cultures that have been serially transferred. The nitrate reduction test differentiates M. The vectors include the human louse (Pediculus humanus; Figure 24-10), squirrel flea (Orchopeas howardii), and squirrel louse (Neohaematopinus sciuriopteri). Flaviviridae The family Flaviviridae contains a number of important human pathogens, many of which are zoonotic arboviruses, including Japanese encephalitis virus, dengue virus, yellow fever virus, St. Louis encephalitis (SLE) virus, and WNV. Discuss the appropriate use of the following skin antiseptics in health care settings by health care personnel: handwash, surgical hand scrub, and patient preoperative skin preparation. The borreliae are typically cultivated in the clinical laboratory using Kelly medium. Pertussis toxin (PT) is a protein exotoxin that produces a wide variety of responses in vivo. List the four divisions of fungi. A second vaccine that protects against HPV types 16 and 18, Ceravix (GlaxoSmithKline, Middlesex, United Kingdom), was approved for use in women in 2009. kansasii M. Nonpigmented strains have been confused with Aeromonas spp. The cell structure and biochemical pathways of an organism determine its susceptibility to various antibiotics. At this point, the infection generally remains dormant for the life of the host unless the immune system is compromised. This is the only stand-alone test for C. Biopsy Specimens Biopsy specimens are usually needed to diagnose infections with Leishmania spp. When large sections of tissue are submitted, suspicious areas, such as purulent or discolored sections, are selected for mincing and grinding prior to subsequent culture. Misra, MS, SM(NRM), SM(ASCP) Manager, Microbiology, Immunology, & Virology University of Delaware Christiana Care Health Services Newark, Delaware Paula C. Burkholderia Burkholderia cepacia Burkholderia cepacia is a complex of at least 17 distinct genomic species (genomovars) that was formerly called P. Carranza PG, Lujan HD: New insights regarding the biology of Giardia lamblia, Microbes Infect 12:71, 2010. In this figure, the appliqué form is seen in the lower left and upper right of the photograph. The FDA has cleared an EIA for detecting NoV during outbreaks. 435 Enteric Fevers. The clinical features of enteric fevers include: • Prolonged fever • Bacteremia • Involvement of the reticuloendothelial system, particularly the liver, spleen, intestines, and mesentery • Dissemination to multiple organs Enteric fever caused by Salmonella Typhi is known as typhoid fever, a febrile disease that results from the ingestion of food contaminated with the organisms originating from infected individuals or carriers. The patient was now clammy with a weak pulse, marked swelling, and bullous formation with gangrenous changes apparent on the left hand. Hence, it is vital to have an accurate Gram stain to arrive at an accurate identification. The laboratory is frequently asked for additional input. furnissii, which differs from V. Diagnostic laboratory media for bacteria are usually adjusted to a final pH between 7.0 and 7.5. Temperature influences the rate of growth of a bacterial culture. Fungal disease is frequently categorized based on the site of the infection —superficial, cutaneous, subcutaneous, and systemic mycoses. Only 11 species have been officially named; the two species most commonly seen in clinical specimens are A. tahir99 VRG CHAPTER 27 Medically Significant Fungi Urogenital and Fecal Specimens Laboratory scientists often receive specimens such as urine, feces, and vaginal secretions for bacteriologic culture; on occasion, these specimens grow yeast that requires identification. Today, many of these reference laboratories probably use a nucleic acid sequencing or mass spectrophotometric method for the identification and not rely on biochemical or phenotypic identification for obtaining a definitive answer. As the inclusions mature, they develop morulae (mulberry-like bodies; Figure 24-11). Conidia are formed from annellides, which increase in length as conidia are formed. They contain their own DNA and the electron transport system that produces energy for cell functions. Microscopic examination of agar for microcolonies allows earlier detection of growth. Yeast such as C. In addition, whether inpatient or outpatient, the presence of symptoms should dictate whether three specimens are needed. coli, such as cytolysins and aerobactins. Cases are likely unrecognized nationwide and also go unreported in Hawaii. Black dot ringworm consists of endothrix hair involvement. Results are most consistent when the test is performed on egg-based media. Species identification among the genomovars of B. possess O antigens, and certain strains can possess K antigens. Samples sent for focused screening cultures usually are not stained. Microscopes FIGURE 7-4 Smears from thin fluids can be prepared by placing a single drop of fluid or resuspended fluid sediment on a well-marked area of the slide. The presence of skin bacteria inhibits the growth of more pathogenic bacterial species, providing benefits to the host. These include length of hospital stay, weakened immune response of elderly patients, and underlying serious illnesses. Reemerging Pathogens The reemergence or potential reemergence of pathogens once thought to be eliminated demands collaboration with the infection control program. enterocolitica infection, usually following a GI episode or an appendicitis-like syndrome. The assays are based on (1-3)-β-D-glucan activating the coagulation cascade in Limulus amebocyte lysate, material found in the North American horseshoe crab. FIGURE 18-26 Gram stain of specimen demonstrating intracellular and extracellular Legionella pneumophila (×1000). Vaccine development using hookworm antigens is being targeted as a way to help control infections. Middlebrook 7H11 medium also contains 0.1% casein hydrolysate, which improves the recovery of isoniazid-resistant strains of M. Describe the key characteristics associated with the identification of the clinically significant fungi. In the United States and other industrialized countries, shigellosis is probably underreported because most patients are not hospitalized and usually recover from the infection without culture to identify the etiologic agent. Oxalic acid–treated specimens can be used with the broth-based culture systems. Both b and c 8. One distinguishing characteristic of C. Each component of this relationship is in constant interaction with the other. † Some cultures of Proteus mirabilis may yield positive Voges-Proskauer tests. Once merozoites have attached, endocytic invagination of the red cell membrane allows the organism to enter the red cell within a vacuole. Routine bacterial cultures were negative; however, a rapid nucleic acid amplification test was diagnostic. tahir99 VRG 34 PART I Introduction to Clinical Microbiology Endotoxins Endotoxins are composed of the LPS portion of the outer membrane on the cell wall of gram-negative bacteria. tahir99 VRG CHAPTER 7 Microscopic Examination of Materials from Infected Sites TABLE 7-3 Observing Microbial Pathogens Tools Magnification (×) Application Eyes Magnifying glass Dissecting microscope 0 5 2.5-30 Gross examination Gross examination Gross detailed examination and manipulation Compound light microscope Bright-field Dark-field 10-2000 10-400 Phase-contrast Fluorescence 10-400 10-400 Cells stained Cells not readily stained for bright-field microscopy Living or unstained cells Preparations using fluorochrome stains, which can directly stain cells or be connected to antibodies that attach to cells Electron microscopes Transmission electron 150-10 million Scanning electron 20-10,000 Determine ultrastructure of cell organelles Determine surface shapes and structures observations are reported everyone is able to understand the implications of the descriptions. When the abovementioned two subunits 50S and 30S bind together, there is a loss of surface area, and the two subunits add up to only 70S in size. Personnel Safety The administration of the microbiology laboratory must ensure that each employee is: (1) provided with adequate safety equipment, (2) trained in safe laboratory procedures, (3) informed of the hazards associated with the procedures, (4) prepared for action following an unexpected accident, and (5) monitored regularly by medical personnel. aeruginosa can colonize mucosal surfaces, such as the oropharynx. piechaudii were repeatedly isolated. The rickettsiae also survive in nature, to a lesser extent, by transovarial transmission. diphtheriae for observation of metachromatic granules (see Figure 1-6, F). The laboratory technologist should be instrumental in the interpretation of the results. usually produce ornithine decarboxylase; lysine decarboxylase is produced by most species but not by E. were isolated from nonsterile sites such as urine and many different types of respiratory specimens, they were usually considered insignificant colonizers or contaminants. Otitis media is a less frequent infection. The isolate was catalase-, indole-, and oxidase-negative. A few coronaviruses are responsible for a small percentage of pediatric diarrhea cases. In 2011, an outbreak in Europe caused by STEC (O104:H4) that also had virulence related to EAEC resulted in more than 3400 total infections and about 850 cases of HUS with 32 HUS-related deaths. Also, despite influenza surveillance programs, reliable vaccines, and dependable antiviral medications, more than 30,000 U.S. citizens die each year of influenza. Bacteria that grow best at cold temperatures are called psychrophiles (optimal growth at 10° to 20° C). Aeromonads are responsible for a diverse spectrum of disease syndromes in warm- and cold-blooded animals, including fish, reptiles, amphibians, mammals, and humans. Each of the media has different nutritional ingredients; after inoculation and incubation, growth is scored on a scale of 1 to 4. Is antibiotic susceptibility testing necessary, or is identification of the suspected pathogen sufficient? These tachyzoites, as they are now called, are responsible for tissue destruction. immitis from C. C, Appearance of K. Diseases with which they have been associated (in particular A. venerealis. Describe the typical biochemical reactions and characteristic features of the most commonly encountered nonfermentative organisms. Measles is highly contagious and spreads by aerosol. (Courtesy Anaerobe Systems, Morgan Hill, CA.) tahir99 VRG CHAPTER 22 Anaerobes of Clinical Importance TABLE 507 22-8 Primary Setup Media Recommended for Recovery of Anaerobes Medium Anaerobic blood agar (CDC) Bacteroides bile esculin (BBE) agar Brucella blood agar (BRU/BA) Kanamycin–vancomycin–laked blood (KVLB) agar Phenylethyl alcohol (PEA) agar Colistin nalidixic acid (CNA) blood agar plate Anaerobic broth (e.g., thioglycollate [THIO] and chopped or cooked meat) Organisms Comments Supports growth of almost all obligate and facultative anaerobes, best for anaerobic gram-positive cocci Supports growth of bile-tolerant Bacteroides spp., some strains of Fusobacterium mortiferum, Klebsiella pneumoniae, enterococci, and yeast may grow to a limited extent An enriched medium containing sheep blood for enrichment and detection of hemolysis, vitamin K (required by some Porphyromonas spp.), and yeast extract A selective medium containing gentamicin (which inhibits most aerobic organisms), 20% bile (which inhibits most anaerobes), and esculin; used primarily for rapid isolation and presumptive identification of members of the B. (unstained, ×200). Such procedures are useful in screening large numbers of specimens during epidemio logic studies. It is likely that the 2-month-old child described in the Case in Point had incomplete immunity. vivax, as well as P. loss, abdominal pain, nausea and vomiting, and chronic diarrhea. Even these organisms probably Part of organism Plague Systemic disease following local lesion at inoculation site Warm, moist skin is more susceptible Bite from infected rat flea Bite from infected mosquito Infection restricted to skin, nails, and hair From Mims CA: The pathogenesis of infectious disease, ed 5, San Diego, 2001, Academic Press. In 2011, an outbreak of about 24 cases of GBS affected Yuma County, Arizona. The characteristic coiled structure of a Curschmann’s spiral is more easily recognized on low power. When the composition of the indigenous flora is altered (e.g., by antibiotic therapy with broad-spectrum antibiotics), other organisms capable of causing disease may fill the void. One potential pitfall with these systems is that the codebook compendium is divided by Gram-staining characteristics of the organism. During approximately the first year of an infant’s life, rotavirus vaccine prevented 85% to 98% severe rotavirus illness episodes and prevented 74% to 87% of all rotavirus illness episodes, which was a significant reduction in both populations. Chronic obstruction to the lymphatic flow results in lymphatic varices, fibrosis, and proliferation of dermal and connective tissue. harveyi 0 99 0 99 50 0 1 100 5 1 15 45 99 95 50 50 50 0 From Abbott SL et al: Vibrio and related organisms. Culture Media An enriched selective agar, Campy-BAP (blood agar plate), is a commonly used medium to isolate C. Varicella is the primary infection and is highly contagious (Figure 29-10). tahir99 VRG 516 PART II Laboratory Identification of Significant Isolates • A rapidly growing colony exhibiting smooth swarming (as opposed to the waves observed with Proteus) and staining as thin rods with subterminal spores is likely to be C. The usual mode of transmission is ingestion of fecally contaminated water or food. dispar and E. This stain specifically stains melanin, causing phaeoid hyphae to appear brown, whereas hyaline hyphae stain pink to red. In a human genome of 3 billion “letters,” even one tenth of 1% translates into 3 million separate lettering differences, an important characteristic useful in forensic science but with related importance in diagnostic microbiology using the bacterial genome. What nonculture methods are used to diagnose Helicobacter pylori infections? The color of a yeast colony ranges from white to cream or tan, with a few species forming pink- to salmon-colored colonies. The organisms found in the mouth, nasopharynx, oropharynx, and nose, although similar, show some differences. Growth on Rice Grains. Poorly sporulating isolates of M. The hydrogen peroxide produced at this time diffuses from the cytoplasm into the phagosome. interrogans sensu lato and L. FIGURE 27-22 Conidia of Exophiala sp. meningosepticum and C. Two steps are involved in the utilization of lactose by a bacterium. For several decades, medical science has made advances in lifesustaining and life-lengthening treatments. 2 tahir99 VRG CHAPTER 1 Bacterial Cell Structure, Physiology, Metabolism, and Genetics Case in Point A 4-year-old girl had presenting symptoms of redness, burning, and light sensitivity in both eyes. Table 27-8 presents the predominant culture sites for recovery of the causative agents of fungal diseases. In North America and much of Europe, HHV-8 appears to be transmitted primarily through sexual contact, but studies in Africa and some Mediterranean populations suggest transmission by more casual means. EBV can be recovered from the oropharynx of symptomatic and healthy persons, who can transmit the virus to susceptible persons via infected saliva. EIEC isolates may be mistaken for nonpathogenic E. Edwardsiella The genus Edwardsiella is composed of three species: E. are important causes of zoonotic infections. The background fluid is black, and clear unstained halos are seen around individual yeast cells. Bacteria that are not acid-fast are blue. Colonies are intensely green and granular, with an abundance of conidia. (lactophenol cotton blue, ×450). Many clinical laboratories consider the isolation of C. The nucleus contains a large central karyosome that is surrounded by a halo. Compare the different methods used in the diagnosis of viral infections. marcescens d. 803. Which of the following strains is the most likely cause of the infection? Strains that phenotypically resemble V. pneumoniae is not associated with infections of the urogenital tract. are rapidly growing and form hyaline hyphae that give rise to yellow-green to green patches of conidia formed on clusters of tapering phialides (Figure 27-45). In HIV-positive persons, the seroprevalence can be as much as 20% to 50% higher than that of the surrounding healthy population. Confirm identification of equivocal results by subculture; repeat antisera tests and biochemical tests. fowleri. Therefore, gram-negative bacteria appear pink under the light microscope. Positive; can; negative 3. The Enterobacteriaceae have several key laboratory features in common, but as DNA studies on each of these organisms progress, classification of the members changes. putida have been documented, although rarely, as causes of UTIs, postsurgical abscesses, empyema, septic arthritis, and other wound infections. In anticipation of potential bioterrorism events, the microbiology laboratory must be equipped to participate in emergency preparedness programs. Gentamicin is used to treat systemic infections. At the end of the first week of infection, a maculopapular rash appears on the tahir99 VRG 708 PART II Laboratory Identification of Significant Isolates trunk and extremities, usually followed by the development of worsening nausea, vomiting, and diarrhea. Gastric aspirates should be obtained in the morning after an overnight fast. These adenoviruses can be identified but not serotyped by EIA. The QBC method, which has been used in the detection of malarial parasites, has also been adapted for the detection of Tsetse fly’s biting of human and ingestion of trypomastigotes Development in gut of fly Trypomastigotes in blood and cerebrospinal fluid Trypomastigote injection into human by bite Possible migration of amastigotes to choroid plexus of brain Epimastigote in salivary gland of fly Formation of metacyclic trypomastigote FIGURE 28-24 Life cycle of the agents of sleeping sickness (Trypanosoma brucei gambiense and Trypanosoma brucei rhodesiense). Characteristics of Shigella spp. LJ medium is the medium most commonly used in clinical laboratories. fennelliae, have been associated with human gastroenteritis, generally in immunocompromised patients. Blood and Tissue Roundworm Infections Trichinella spiralis. Trichinosis is the infection of muscle tissue with the larval form of T. The intermediate transmission cycle of yellow fever occurs in smaller villages in Africa. Other than fluid replacement, therapy is usually unnecessary. TABLE 22-3 Incidence of Anaerobes at Scott & White Memorial Hospital* Organism Group Bacteroides fragilis group Fusobacterium Prevotella-Porphyromonas group Clostridium Propionibacterium Peptostreptococci Veillonella *Data provided by author. Pedagogic Features As with the previous editions, each chapter is introduced by a Case in Point. In Wisconsin and Con necticut, the average annual incidence ranges from 24 to 58 cases/100,000 people. One year (all-cause) mortality has been reported to be as high as 23%. terreus, and A. Tortora GJ, et al: Microbiology: an introduction, ed 7, San Francisco, 2002, Benjamin Cummings. The life cycle is not completely known, and revisions continue to occur. If rapidly growing mycobacteria are suspected, smears should be stained with carbolfuchsin and a weaker decolorizing process used. Micrococcus spp. putrefaciens. infection of sea mammals with particular emphasis on isolates from Scotland, Vet Microbiol 90:563, 2002. After additional larval molts, the worms attach to the mucosa in the small intestine. A few dermatophytes have become adapted exclusively to human hosts and are termed anthropophilic. The presence or absence of spores, coupled with the Gram stain reaction and cellular morphology, can be helpful in making a presumptive identification of anaerobic bacteria and determining the appropriate identification tests to be performed. Specimens preserved in SAF do not stain well with trichrome and should be stained with iron hematoxylin. In 1996, RSV immune globulin was released for preexposure prophylaxis of susceptible patients; however, recommendations for its use are not clearly defined. For IFA, heat-killed or formalin-killed bacteria are fixed to a microscope slide. (Iodine wet mount, ×200.) strongyloidiasis and can delay diagnosis of the underlying infection. (lactophenol cotton blue, FIGURE 27-46 Alternaria sp. The culture methods using non-nutrient agar seeded with E. A number of modifications of the trichrome staining procedure have been developed to allow for the detection of a group of organisms known as the microsporidia. In front of each gene on the DNA strand is an untranscribed area containing a promoter region, which the RNA polymerase recognizes for transcription initiation. Bacterial species such as Yersinia pestis and Bacillus anthracis are pathogenic in nearly all situations; when these species are recovered in clinical samples taken from a body site, their clinical significance is well established. Criteria Less than 25 PMN cells per low-power field (LPF) and more than 10 epithelial cells or mixed bacteria per LPF must be present. Hospitalized patients often show colonization with gram-negative rods. Researchers are looking for more rapid methods involving nucleic acid amplification and the detection of resistance genes. Cladosporium spp. intestinal flagellate. Prevalence of infection varies according to socioeconomic levels and sanitary practices; infection is more common in poorly developed countries. Motile gram-negative anaerobes include some Campylobacter spp. In some anaerobic species, catalase is an inducible enzyme. BIBLIOGRAPHY Abbott SL, et al: Klebsiella, Enterobacter, Citrobacter, Serratia, Plesiomonas, and other Enterobacteriaceae. Rapid EIA kits that can be used with fresh or preserved fecal specimens and kits using monoclonal antibody to detect Giardia and Cryptosporidium organisms are also available. Under ordinary circumstances, microorganisms that are members of the microbiota do not cause disease and many actually can be beneficial. The onset of clinical illness is generally abrupt, with nonspecific, influenza-like constitutional symptoms such as fever, chills, headache, severe myalgia, and malaise. No animal reservoir or vector is known. pneu mophila is responsible for most cases of this illness. The mycelia are made up of many long strands of tube-like structures called hyphae, which are either aerial or vegetative. Some laboratories also prefer to inoculate a SBA plate in the same manner to be incubated in an ambient air incubator. The site of action is the cytoplasmic membrane. malariae are less severe than those caused by P. FIGURE 27-5 Bipolaris sp. Other Klebsiella spp. are fastidious, aerobic bacteria that do not grow on SBA and require L-cysteine for growth. Microscopically, erect sporangiophores are noted. Chest radiographs revealed progressive patchy lobar pneumonia that improved after administering erythromycin. CRM To my wife, Terri, who has given me constant support and encouragement, and whose love makes anything I do possible, and my parents, Gerald and Sherrie, who have always been proud of me—even though they have passed away, I know they still look over me. dermatitidis, C. Pigment is distributed throughout the cytoplasm except in P. 1001. Progress in molecular technology has diminished the frequency with which biochemical tests are routinely performed in the identification of mycobacteria. Sputum is the primary diagnostic specimen. Within each general type, there are nonselective formulations and formulations that have been made selective by the addition of antimicrobial agents. Many buds of various sizes can occur, or there may be only a few buds, giving the appearance of a so-called “Mickey Mouse cap” to the yeast cell. are often mucoid and can cause greenish discoloration of SBA. tahir99 VRG 592 PART II Laboratory Identification of Significant Isolates Dimorphism and Polymorphism The term dimorphism refers to the ability of some fungi to exist in two forms, dependent on growth conditions. Extrapulmonary complications, including cardiovascular, central nervous system, dermatologic, and gastrointestinal problems, are rare occurrences. At the present time, laboratories that offer PCR must conduct in-house verification and validation studies because FDA approval of products is limited to analytespecific reagents. Nosocomial transmission of CCHF virus has been reported. The occurrence of infections following construction c. 701 Human Herpesvirus 7 HHV-7 is in the genus Roseolovirus with HHV-6. Receiving available vaccines and

testing for diseases (e.g., for hepatitis B, chickenpox, influenza, meningococcus, tuberculin skin test [TST]) are steps to protect susceptible laboratory personnel. A nucleotide is formed when the 5′ carbon of the sugar and one of the nitrogenous bases attaches to the 1′ carbon of the pentose sugar. In clinical correlations with in vitro data, if 1% of a patient's bacilli are resistant to a particular drug, treatment fails. However, cultures are not often performed at this stage because the symptoms are nonspecific. Acid treatment of specimens contaminated with other bacteria, such as sputum, before inoculation enhances isolation of Legionella spp. The cyst is an environmentally resistant stage that is infective. The organisms, which originally multiply in the small intestine, move toward the colon, where they may be isolated 1 to 3 days after the infection develops. William Gary, Jr., Centers for Disease Control and Prevention, Atlanta, GA.) rarely reported but causes severe and sometimes fatal acute respiratory disease (ARD) in patients of all ages. The phagosome fuses with lysosomes, Skin surface TABLE Injury Diapedesis 2-6 Tissue Distribution of Monocytes/Macrophages Cell Name Monocyte Kupffer cell Alveolar macrophage Histiocyte Peritoneal macrophage Microglial cell Mesangial cell Macrophage Tissue Distribution Blood Liver Lung Connective tissue Peritoneum Central nervous system Kidney Spleen, lymph nodes Bacteria Chemotaxins PMNs Lumen of blood vessel FIGURE 2-2 Phagocytosis: chemotaxis migration of phagocytes. (Courtesy BD Diagnostic Systems, Sparks, MD.) FIGURE 19-13 H_2S–producing colonies of salmonellae growing on xylose-lysine-desoxycholate (XLD) agar. Anemia may develop if hemolysis is severe or prolonged. Staphylococcus aureus, Pseudomonas aeruginosa, MRSA, Escherichia coli c. The DFA test should be used only along with culture because the lack of sensitivity diminishes the clinical utility of a negative result, and false-positive results can occur even in experienced hands. Manjunath B: Fatal septicaemia due to Chromobacterium violaceum, West Indian Med J 56:380–381, 2007. The gastrointestinal tract population may be altered by antibiotics. Enteroinvasive Escherichia coli. EIEC differs greatly from EPEC and ETEC strains. Leptospirosis is still reportable in Hawaii, and from 1999 to 2008, Hawaii averaged 20 confirmed cases annually. Compare the diseases caused by B. Even with concentration techniques, the number of organisms observed on a smear will be considerably less than organisms seen on a smear from an individual with bacterial pneumonia. Painful oral mucosal lesions may develop, particularly in adults. Direct Detection In general, direct detection methods are not as sensitive as culture methods but can offer quick results to allow for rapid therapy. Scopulariopsis. Scopulariopsis spp. multivorum, J Clin Microbiol 50:202, 2012. The average mortality associated with DHF is 5%, accounting for 24,000 deaths each year. Persons living in these areas may acquire some level of immunity that increases resistance to future infection. The chlamydial outer membrane also contains lipopolysaccharide (LPS). Some false-positive test results represent patients who have had infectious mononucleosis and still have low levels of antibody. Proliferation of viruses is inhibited by interferon. The agar proportion and broth proportion methods are most commonly used. The clinical microbiologist must be able to recognize and identify the types of microorganisms found at the various body sites. FIGURE 28-40 Microsporidia spores identified by arrow (chro- motrope stain, ×1000). In the intermediate cycle, humans and monkeys are reservoirs and mosquitoes are the reservoirs and vectors for the high-morbidity, low-mortality outbreaks. The highest endemicity in the United States occurs tahir99 VRG CHAPTER 27 Medically Significant Fungi A 605 B FIGURE 27-29 A, Bone marrow aspirate stained with Giemsa showing the yeast cells of Histoplasma capsulatum var. The yeast or tissue state is seen in vivo or when the organism is grown at 37° C with increased CO_2. Filoviridae FIGURE 29-13 Electron micrograph of the coronavirus. In recent years, ingestion of wild game has led to infection by other species of Trichinella. canis is 72% positive. Antifungal therapy is not typically indicated, but the appearance of lesions may be diminished by treatment with antidandruff shampoos. trachomatis into 20 serovariants, or serovars (Table 24-3). Recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC), MMWR Recomm Rep 52(RR-10):1, 2003. Children are usually infected by ingesting fleas containing the larval stage. No vaccines are available for enteroviruses other than poliovirus; good personal and hospital hygiene and proper sanitation can reduce the incidence of enterovirus infections. coli is used as a primary marker of fecal contamination in water quality testing. (See also Procedure 7-1 in Chapter 7.) Gram-Positive Cell Wall. The gram-positive cell wall is composed of a very thick protective peptidoglycan (murein) layer. These five categories are sometimes collectively referred to as enterovirulent E. Clinical Infection. Clinically, the disease cannot be distinguished from bacterial meningitis. ■ Some species of the usual microbial flora may be opportunists, capable of causing disease in an immunocompromised host. difficile Eggerthella lenta A B FIGURE 22-13 Examples of fluorescence observed with longwave ultraviolet light. In the outbreak of infections caused by Acinetobacter spp., the laboratory may have cultured respiratory therapy equipment and water samples. Antibodies, especially secretory IgA, are found in mucous secretions of the respiratory, genital, and digestive tracts. Capsule removal is accomplished by boiling a suspension of the microorganism. ✓ Case Check 18-4 The child in the Case in Point at the beginning of this section was admitted to the ICU during the paroxysmal phase of pertussis. Discuss the pathogenesis of B. The modified Kinyoun procedure can be used for tissue if L. Because of these infection control implications, the laboratory must be prepared to offer diagnostic services or recommend laboratories that do offer those services for waterborne pathogens. 1, 22-mm square coverslip. oryzihabitans Burkholderia pseudomallei Odor Sweet Alcaligenes faecalis Myroides odoratus P. (Hematoxylin and eosin stain, ×100.) fluid. As the infant grows, the microbial flora eventually becomes similar to the flora seen in older individuals. When the organisms are ingested, they spread to the mesenteric lymph nodes, producing a generalized infection that is usually self-limiting. Some pathogenic organisms may establish themselves in a host without manifesting symptoms. Methylobacterium and Roseomonas The genus Methylobacterium contains 20 named species plus additional unnamed biovars; isolates produce a characteristic pink to coral pigment and can use methanol as a sole source of carbon and energy. 613 albicans dubliniensis glabrata guilliermondii krusei lusitaniae parapsilosis stellatoidea tropicalis Cryptococcus C. Positive glutamate dehydrogenase and EIA should be confirmed by an NAAT for toxin genes; some laboratories opt to use an NAAT. Reservoirs are soil and water; isolates are more commonly found in tropical and subtropical climates, in particular Southeast Asia and India. In the United States, most cases are found in the southeastern and south central states, as well as in the Mid-Atlantic states. What are the four most common nonfermentative, gramnegative bacilli isolated in the clinical laboratory? A few gram-negative–staining bacilli are also present. Many microorganisms possessing a capsule are highly virulent (as in the Case in Point at the beginning of the chapter) until removal of the capsule, at which point virulence becomes extremely low. None of the following tests alone is sufficient for proper identification, but when they are used together, accurate identification is often accomplished easily. Available at: 17/2/10-1109_article.htm. Enterobacteriaceae Eubacterium spp. Specimens that contain mucus and require digestion and decontamination are sputum, gastric washing, BAL, bronchial washing, and transtracheal aspirate. Centers for Disease Control and Prevention: 2010 sexually transmitted disease surveillance. trachomatis causes the chronic eye infection trachoma (Figure 24-3), the number one cause of preventable blindness in the world. Certain Clostridium spp. Why should you do both a thin film and thick film? An approved ELISA test from Meridian Diagnostics, Inc (Cincinnati, OH) is able to detect Stx in bloody stools, although not all patients have bloody stools. EIAs using monoclonal antibodies to detect soluble antigens of G. can be placed on Middlebrook agar plates. Conidia may remain clustered in balls at the phialide tips. If B. Serologic typing is best performed on a colony taken from a pure culture growing on nonselective media, such as an SBA plate, although TSI or MAC can be used for presumptive serologic identification. Similarly, extraintestinal infections, particularly among neonates, often require antimicrobial therapy. phlei M. A major concern regarding the potential risk of transmitting this organism is its ability to survive in cold temperatures; food refrigeration becomes an ineffective preventive measure. Despite the similarities between HHV-6 and HHV-7, their antigenic diversity is such that antibodies to one virus do not protect against infection from the other. pickettii, and symptoms resolved after cephalosporin and carbapenem were administered for are least 3 weeks. The organism binds to corneal epithelium and produces a protein that causes cell lysis. Serologic studies such as immunofluorescence assays can be used to detect circulating antibody (IgM and IgG). Direct staining (Giemsa or Wright) of peripheral blood smears or buffy coats for morulae can be used for diagnosing E. Most cases in this multistate outbreak were caused by Exserohilum rostratum, a common mold found in soil and on plants. Cytoplasmic Structures The nucleus of the eukaryotic cell contains the DNA of the cell in the form of discrete chromosomes (structures in the nucleus that carry genetic information; the genes). veronii biovar sobria has also been linked to cholera-like disease characterized by abdominal pain, fever, and nausea. The mortality rate for HPS is about 50%. In fermentation, the electron acceptor is an organic compound. The laboratory professional should look for squamous epithelial cells, bacteria without the cells of inflammation, food, or other debris. Noninvasive techniques, such as magnetic resonance imaging (MRI) and CT scanning, may be used in the diagnosis of patients suspected of having disseminated toxoplasmosis. Point of care tests using immunochromatographic techniques are being developed to assist with the rapid diagnosis of E. Penicillium. Penicillium spp. It is rare in humans but can produce severe local suppurative or acute pulmonary infections. Strict anaerobes also might require an environment that has a low oxidation-reduction (redox) potential. Prepare two smears for direct fluorescent antibody (DFA) test for B. Similar to Brucella spp., Francisella are also facultative intracellular pathogens. Polyclonal fluorescentlabeled conjugates for both B. The viruses replicate initially in the lymphoid tissue of the pharynx and gut. • Level 1. Koh SF, et al: Development of a multiplex PCR assay for rapid identification of Burkholderia pseudomallei, Burkholderia thailandensis, Burkholderia mallei, and Burkholderia cepacia complex, J Microbiol Methods 90:305, 2012. Enterobacter, Cronobacter, and Pantoea The genus Enterobacter is composed of at least 12 species. Anaerobic chambers contain a catalyst, a desiccant, anaerobic gas (5% hydrogen, 5% to 10% CO_2, and 85% to 90% nitrogen), and an oxidation-reduction indicator. Concern about disposal of hazardous mercury compounds has led to the development and evaluation of a number of alternative nontoxic fixatives. Necrosis in the gallbladder leading to necrotizing cholecystitis and necrosis of the Peyer's patches leading to hemorrhage and perforation of the bowel may occur as serious complications. NAATs are better assays for the diagnosis of C. An odor of roses (resembling phenylethyl alcohol) has been reported. Coccidioides spp. Purulence with red blood cells, neutrophils, protein background, and necrosis reflects acute inflammation. yeei is further characterized by the production of characteristic coccoid or O-shaped cells on Gram stain, the latter of which results from the presence of vacuolated or peripherally stained cells. Blood loss, ranging from 0.03 to 0.2 mL/worm/day, is primarily the result of the ingestion of blood by the adult worm. General Characteristics Molds and yeasts are very different morphologically, but some of the macroscopic characteristics used as aids in identifying molds can also be used to identify yeasts. Identification of B. The symptoms of intestinal salmonellosis, which may appear 8 to 36 hours after ingestion of contaminated food, include nausea, vomiting, fever, and chills, accompanied by watery diarrhea and abdominal pain. Patients bitten by mosquitoes carrying the yellow fever virus can develop an asymptomatic or acute infection involving fever, myalgia, backache, headache, anorexia, nausea, and vomiting. Diseases of major health concerns (e.g., smallpox) b. The laboratory diagnosis of Q fever can be made by direct immunofluorescence assays of infected tissue, immunohistochemistry. All bacteria that inhabit the human body fall into the heterotrophic group. Positive Voges-Proskauer b. The smooth, thin-walled macroconidia are produced in clusters or singly. The concept of using tribes in the classification of bacteria has been an effective way of placing species in groups based on similar biochemical features and is employed throughout this chapter. In six different years, from 1972 to 1995, influenza deaths in the United States exceeded 40,000. Prevotella spp. Together, these toxic compounds are detrimental to cell components such as proteins and nucleic acids. Cartwright EJ, et al: Transmission of Elizabethkingia (formerly Chryseobacterium meningosepticum) to tissue-allograft recipients: a report of two cases, J Bone Joint Surg Am 92:1501, 2010. The examiner must not be distracted by precipitated gram-positive stain, keratohyaline granules, or other artifacts. • Patients may remain infective and continue to shed oocysts for a time after the diarrhea ceases. These are some questions that might be addressed by the microbiology laboratory technologist while acting as a consultant to the infection control program. The outer membrane functions in the following ways: • It acts as a barrier to hydrophobic compounds and harmful substances. Occasionally, a few pink cytoplasmic dots, called Ziemann's dots, may be seen. Unless indicated, results are those at 4 days. In Brenner DJ, et al, editors: Bergey's manual of systematic bacteriology, ed 2, vol 2, New York, 2005, Springer-Verlag, p. Paecilomyces spp. Carrier Carrier state Commensalism Indigenous flora Opportunists Parasite tahir99 VRG Parasitism Pathogen Resident microbial flora Symbiosis Transient flora 23 24 PART I Introduction to Clinical Microbiology T he outcome from the interactions between host and pathogen is influenced by numerous factors. The physician believed that the respiratory distress was caused by allergies and prescribed albuterol microdiffusion inhaler, 2 puffs every 4 hours for 4 to 5 days. Most diseases produced by members of the Flavobacteriaceae are caused by Elizabethkingia meningoseptica. The two complementary sugar phosphate strands run in opposite directions (antiparallel), 3′ to 5′ and 5′ to 3′, similar to one train with its engine going one way alongside a caboose of a train going the opposite direction (Figure 1-13). Positive urea c. Lehman and Connie R. More than 14 common and uncommon genera were included in this study. In the Salmonella spp. Recently, a strain of C. Although such infections can occur in healthy hosts, the most serious cases are in immunocompromised individuals and those who have experienced a mild to severe injury to the infected site. Humans acquire the infection by ingesting metacercaria on freshwater vegetation such as bamboo shoots and water chestnuts. In addition, Acanthamoeba spp. falciparum, histidinerich protein 2. Colonization of the body by microorganisms begins at birth. Weakness and paralysis are the main features of botulism. Examination of specimens should begin with gross visual inspection and proceed to the level of magnification needed to identify the pathogen or determine that no pathogen is present. This type of search doesn't deliver consistent results, though it can be useful and delivers some results in map or satellite form.Brief History of Phone BooksThe first printed phone book was handed out in 1878 in New Haven. apple green structure. It is usually measured by the numbers of microorganisms necessary to cause infection in the host. To avoid aerosols, an alcohol-sand flask can also be used to clean the waxy culture material from the wire before flaming in a Bunsen burner Disposable sterile applicator sticks or plastic transfer loops are also recommended. pneumoniae involving immunocompromised hosts are wound infections, UTIs, liver abscesses, and bacteremia. A selective medium is often reserved for specimens in which heavy contamination is anticipated. parapertus sis are hemolytic. Clinical Infections Actinomycosis. Actinomycosis is a chronic, granulomatous, infectious disease characterized by the development of sinus tracts and fistulae, which erupt to the surface and drain pus that may contain so-called sulfur granules, dense clumps of bacteria that may be colored. Ehrlichia and Anaplasma are intracellular parasites of white blood cells: mononuclear cells and granulocytes, respectively. Patients may present with elevated liver enzyme levels, increased erythrocytic sedimentation rate, and thrombocytopenia. Grading or Classifying Materials Microscopic examination also immediately discloses that a specimen is unlikely to be helpful in diagnosis or culture management. This determines an organism's ability to reduce nitrate to nitrite or nitrogen gas. tahir99 VRG CHAPTER 22 Anaerobes of Clinical Importance 515 Organisms that produce proteolytic enzymes (proteases) have a completely clear zone, often quite narrow, around their colonies. During this time, the organisms are reproducing and beginning to enter the tahir99 VRG 648 PART II Laboratory Identification of Significant Isolates bloodstream. The incidence of generalized infection is higher among adults with underlying diseases, such as liver cirrhosis, diabetes, acquired immunodeficiency syndrome, leukemia, aplastic anemia, and other hematologic conditions. Staphylococcus spp. Petri dishes must be poured thicker than a standard medium for bacterial growth. moshkovkii is morphologically identical to E. Diphtheria toxin inhibits protein synthesis and affects the heart, nerve tissue, and liver. The educational role of the laboratory technologist once again becomes evident in teaching specimen collection, specimen transport, disinfection, and safety methods. Specimens taken out of the safety cabinet for transport to a decontamination area must be covered. parapertussis is detected a day or so sooner. Humans usually harbor only a single worm, which attaches in the jejunum and can reach a length of up to 10 m. Members of the B. When the results of fluorescent staining or agglutination are clear, confirmatory testing is not required. Mechanisms of Gene Transfer Genetic material may be transferred from one bacterium to another in three basic ways: • Transformation • Transduction • Conjugation Transformation Transformation is the uptake and incorporation of naked DNA into a bacterial cell (Figure 1-14, A). van Doorn HR, et al: Use of enzyme-linked immunosorbent assay and dipstick assay for detection of Strongyloides stercoralis infection in humans, J Clin Microbiol 45:438, 2007. Capsules are usually made of polysaccharide polymers, although they may also be made of polypeptides. The embryo develops a central cavity–like structure lined with a germinal membrane, from which brood capsules and protoscolices (hydatid sand) develop. Describe the types of infections that nonfermentative organisms cause and patients at high risk for infections. Proper characterization of the bacterial cells in human samples is critical in the correct identification of the infecting organism. Consequently, the genital organs—the vagina and cervix in females, and the penis in males—are the usual sites of inoculation. perfringens is the most common. The infection is potentially fatal, especially in young children and elderly adults in nursing homes. To comply with mandatory infectious disease safety policies, laboratory scientists must follow appropriate safety precautions. aeruginosa colonies is produced (Figure 21-5). All fungal elements appear black when Gomori methylene stain is used. EBO-Z continued to emerge periodically in the DRC (2003 and 2007), southern Sudan (2004), FIGURE 29-14 Transmission electron micrograph of the Ebola virus (×100,000). Symptoms include interstitial keratitis, bone and tooth deformities, eighth nerve deafness, neurosyphilis, and other tertiary manifestations. Few reports are available about the in vitro susceptibility of these species. The larger model BBL anaerobic jars and some of the commercially available bags and pouches, can be used to incubate biochemicalbased trays and strips if an anaerobic chamber is not available. It was also found in at least one case of uveitis, for which it was considered the potential pathogen. In general, fluoroquinolones alone or the synergistic combination of ciprofloxacin and cefotaxime display excellent in vitro activity against V. Usual Flora of the Gastrointestinal Tract The gastrointestinal tract comprises the esophagus, stomach, small intestine, and colon. Interlaboratory discrepancies have been noted. The clinical significance of these organisms is unknown; however, they have been isolated from patients with hospitalacquired infections. tarda, E. Historically, studies demonstrated that a large number of people were infected with an organism identified as E. A first-voided morning urine specimen is preferred. For a detailed discussion of DNA and molecular diagnostics, see Chapter 11. The microconidia are phagocytized by macrophages in the pulmonary parenchyma. 1880. Cell wall substance ? alpaca, organisms in the family Flavobacteriaceae are nonmotile. Glove boxes are fitted with airtight rubber gloves (Figure 22-6). The rickettsiae pass directly through the plasma membranes of infected cells into adjacent cells without causing damage to the host cells. Although some are easily distinguished from others, some aspergilli require extensive testing to determine a definitive identification at the species level. Vijayan VK: How to diagnose and manage common parasitic pneumonias, Curr Opin Pulmonary Med 13:218, 2007. Colonies of E. Cells that can take up naked DNA are referred to as being competent. To process the tissue or bone fragments in the laboratory, 1 mL of sterile thioglycollate broth is added to a sterile tissue grinder. Name the endogenous anaerobes commonly involved in human infections and the types of infections with which they are most often associated. Strict aerobic and facultative anaerobic bacteria that use oxygen have the enzymes superoxide dismutase and/or catalase to protect them from superoxide anions and their toxic derivatives, as shown in the following equations: TABLE 22-2 Endogenous Anaerobes Commonly Involved in Human Infections Infection superoxide dismutase $4\ O_2^- + 4H^+ \longrightarrow 2H_2O_2 + O_2$ catalase $2H_2O_2 \longrightarrow 2H_2O + O_2$ Superoxide dismutase converts the superoxide anion to oxygen and hydrogen peroxide. The trophozoite in Figure 28-3, B, contains an ingested RBC. Exotoxins can mediate direct spread of the microorganisms through the matrix of connective tissues and can cause cell and tissue damage. The life cycle (Figure 28-59) takes about 50 days after infection until adults are mature. Providencia FIGURE 19-7 Example of Proteus mirabilis swarming on sheep blood agar (SBA). On media with blood, a lavender-green discoloration of the agar may occur because of the proteolytic activity of the organisms. Respiratory Specimens Because many fungal infections have a primary focus in the lungs, lower respiratory tract secretions (e.g., sputum, transtracheal aspirates) and pleural lavage fluids are commonly 27-8 Predominant Culture Sites for Recovery of Causative Agents* Infection Blastomycosis Histoplasmosis Coccidioidomycosis Paracoccidioidomycosis Sporotrichosis Chromoblastomycosis Eumycotic mycetoma Phaeohyphomycosis Respiratory + + + + + Blood Bone Marrow + + Tissue + + + + *Organisms may be recovered from multiple sites in disseminated infections. Discuss the appropriate specimen, transport, and processing for maximum recovery of each organism. In the United States, the prevalence rate ranges from 0.4% to 4%, with most cases found in people living in Appalachia, in rural areas of the Southeast, or in immigrants from endemic areas. Epidemiology Members of the family Legionellaceae are found worldwide, occurring naturally in aquatic sources such as lakes, rivers, hot springs, and mud. salmonicida. Janda JM: Genus Plesiomonas. The teleomorph or sexual form of B. Centers for Disease Control and Prevention: Outbreaks of respiratory illnesses mistakenly attributed to pertussis—New Hampshire, Massachusetts, and Tennessee, 2004-6, MMWR 56:837, 2007. Originally discovered in Sapporo, Japan, in 1977, these viruses are detected by electron microscopy, molecular (e.g., RT-PCR), and/or immunologic methods (e.g., ELISA). RT-PCR and fluorescent monoclonal antibodies are currently being used for identification. Second, growth of colon microbiota is inhibited at this higher temperature. Examine smears with the ×16 and ×40 objectives of the fluorescent microscope equipped with a filter system comparable to a BG-12 exciter filter and an OG-1 barrier filter. Such a specimen would likely not provide useful information to the clinician. Figure 28-41 shows a generalized life cycle of the liver, lung, and intestinal flukes. Teleomorphs in this group of organisms are not observed in routine laboratory studies of patient isolates because dermatophytes are heterothallic, requiring the combination of two distinct mating types. FIGURE 28-14 Naegleria fowleri trophozoite. However, more recently, taxonomists have placed all organisms into three domains that have tahir99 VRG CHAPTER 1 Bacterial Cell Structure, Physiology, Metabolism, and Genetics replaced some kingdoms: Bacteria, Archaea, and Eukarya. are acquired through the respiratory tract via respiratory droplets or direct contact with infectious secretions. Digestion and Decontamination of Specimens To ensure optimal recovery of mycobacteria from clinical specimens, many specimens must be processed before inoculation onto culture media. The O antigens are designated by Arabic numbers. colonies is derived from the conidia. Gram-positive and gram-negative cells can lose their cell walls and grow as L-forms in media supplemented with serum or sugar to prevent osmotic rupture of the cell membrane. hydrophila. krusei. morganii Providencia Salmonella* P. The primary infection of the ductal epithelial cells in the glands results in cell death and inflammation. Laboratory Diagnosis Specimen Collection and Handling. Lesions of primary and secondary syphilis typically contain large numbers of tahir99 VRG CHAPTER 23 The Spirochetes spirochetes. These figures indicate that about one in six persons in the United States have HSV, and most infections are asymptomatic. Blastoconidia Pseudohyphae Candida sp. This potential relatedness is an additional epidemiologic tool that can be incorporated into the investigation of an outbreak. Biosafety level 3 conditions should be implemented when working with suspected F. Include labeling the 3′ and 5′ end. However, the cyclic nature of the disease probably best explains the dip in cases during 2005-2010, which was lowest in 2007. These arthroconidia are responsible for the darkening colony morphology. The positive PDA helps differentiate them from Alcaligenes spp. See the Gram stain discussion later in this chapter. Outbreaks in daycare centers and from environmental contamination of municipal water supplies have been reported. A second vaccine, Rotarix (RV1; GlaxoSmithKline, Middlesex, England), was approved in June 2008. Patients with hemoglobinopathies, such as sickle cell disease, hemoglobin C, and glucose-6-phosphate dehydrogenase deficiency, are somewhat protected against severe malaria because the parasite cannot replicate or, in some cases, exist in these RBCs. Treatment. Chloroquine remains the primary drug for prophylaxis and treatment of malaria. shigelloides appears to possess a much lower virulence potential than Shigella, with a low symptomatic carriage rate among humans. The cells are motile, with graceful flexuous movements in liquid. 8. What factors of Legionella can contribute to the colonization of human-made water supplies? Farming b. The CPE appears quickly and is readily identifiable. Bacteria generally ferment glucose preferentially over other sugars, so glucose must not be present if the ability to ferment another sugar is being tested. These microbes share some common characteristics with bacteria; they too can stain gram-positive and gram-negative. Clinical and Laboratory Standards Institute: Abbreviated identification of bacteria and yeast; approved guideline, ed 2, CLSI document M35-MA2, Wayne, PA, 2008, CLSI. The brown, barrelshaped egg is unembryonated when passed, 50 to 55 μm × 22 to 23 μm, with a thick wall and hyaline polar plugs at each end (Figure 28-58). Laboratory Diagnosis of Viral Infections Laboratories can provide different levels of services, based on the mission, financial resources, and need. Adults are tahir99 VRG 664 PART II Laboratory Identification of Significant Isolates Adults in specific organs Eggs in feces or sputum Fresh water Miracidia (first larval stage) release Fasciola hepatica adults in liver Fasciolopsis buski adults in intestine Penetration of or ingestion by snail Metacercariae encystment on aquatic vegetation— ingestion by humans Opisthorchis sinensis adults in liver First intermediate host Metacercariae ingestion in raw or undercooked fish Penetration of appropriate host Emergence of cercariae (second larval stage) from snail Heterophyes heterophyes, Metagonimus yokogawai adults in intestine Paragonimus westermani adults in lungs Metacercariae ingestion in raw or undercooked crabs Development into sporocyst and redial generations FIGURE 28-41 Life cycle of liver, lung, and intestinal flukes. One needs to be certain that all the suspected cases match the definition and that there is more than an expected number of cases. A likely to have an eccentric karyosome or uneven peripheral chromatin resembling that of Entamoeba coli. However, smears prepared directly from CSF, blood, and other body fluids or exudates from an uncontaminated site can be examined microscopically for the presence of gram-negative bacteria. pedrosoi but with more compact conidial heads Conidia are subglobose rather than ovoid. • Most produce H_2S; a major exception is Salmonella Paratyphi A, which does not produce H_2S. DFA tests are frequently used to identify the cyst of Giardia in a stool specimen. They show extensive size variation, from 3 mm to 10 m, generally require intermediate hosts in their life cycle, and are hermaphroditic. Some of these requirements are federally mandated by the CDC, whereas some may be designated by the state. The BACTEC system can be used for the antimicrobial susceptibility testing of M. kansasii and M. trichrome stain, the cytoplasm stains dark green and the central area may stain pale to intensely green, with the nuclei a dark purple-black. The four species and their respective serologic groups are depicted in Table 19-9. This procedure involves swabbing the person's perianal area with a tongue blade covered with cellophane tape (sticky side out). Accessed September 4, 2009. Because intestinal pathogens are usually acquired by ingesting BOX 2-3 Microorganisms Found in the Nose and Nasopharynx Common Less Common Staphylococcus aureus Staphylococcus epidermidis Diphtheroids (Corynebacterium spp.) Haemophilus parainfluenzae Streptococcus spp. tahir99 VRG 694 PART II Laboratory Identification of Significant Isolates three methods, the most commonly used by clinical virology laboratories is cell culture. Emerging and Reemerging Pathogens With the advent of terrorist activities worldwide, the microbiology laboratory has become an integral part of that area of the infection control program. Several grading or classification systems have been derived to aid the technologist in arriving at decisions relating to culturing the specimen or interpretation of growth from culture of a specimen. Diagnostic microbiologists traditionally emphasize placement and naming of bacterial species into three (occasionally four or five) categories: the family (similar to a human "clan"), a genus (equivalent to a human last name), and a species (equivalent to a human first name). They belong to the genus Rotavirus. This toxin produces damage to Vero cells (African green tahir99 VRG CHAPTER 19 Enterobacteriaceae monkey kidney cells)—hence the term verotoxin. Rats BIBLIOGRAPHY Baughman AL, et al: Establishment of diagnostic cutoff points for levels of serum antibodies to pertussis toxin, filamentous hemagglutinin, and fimbriae in adolescents and adults in the United States, Clin Diagn Lab Immunol 11:1045, 2004. parvum but larger, with an average size of 8 to 10 μm. Munoz P, Valerio M: Parasitic infections in solid organ transplant recipients, Infect Dis Clin N Am 24:461, 2010. Discuss the composition and efficacy of the vaccine for pertussis. It is the most commonly reported intestinal protozoan in the United States. Most of these cases are in travelers to endemic areas. Intracellular and extracellular forms should be noted. histolytica are available; the results are positive in more than 90% of patients with extraintestinal disease. The isolate grew on CHOC agar and SBA in 5% CO_2. They have been isolated from fish, processed meat, and poultry. Conidiophores are erect and can branch into several conidiogenous cells. They can be found as part of the microbiota of the oral cavity and GI and GU tracts. tuberculosis avium-intracellulare* bovis celatum gastri genavense haemophilum malmoense terrae complex ulcerans Rapid Growers M. After bacterial invasion through a break in the epidermis or penetration through intact mucous membranes, the natural course of syphilis can be divided into primary, secondary, and tertiary stages based on the clinical manifestations. Other types of laboratory support are often important as well. coli; HUS, hemolytic uremic syndrome; LT, labile toxin; MUG, 4-methylumbelliferyl β-D-glucuronide; NM, nonmotile; SMAC, MacConkey agar containing sorbitol; ST, stable toxin; UPEC, uropathogenic E. Each schizont produces a large number of merozoites. Strains causing lower UTIs and acute pyelonephritis in immunocompetent hosts are different from strains causing disease in the urinary tracts of individuals who are compromised either by urinary tract defects or by instrumentation such as placement of catheters. Ewingella was first thought to be related to Cedecea; however, DNA hybridization confirmed the placement of these organisms in separate genera. The immunohistochemical detection of rickettsiae is an established method for diagnosis of these infections. Carbolfuchsin (a red dye) is used as the primary stain (see Figure 1-6, C). hosts for T. Amies transport medium with charcoal (24-hour holding time) c. The development of immunologic competence depends on this flora. As these structures mature into spores, they develop a thick membrane and the polar tube. Originally considered protists, they have been reclassified as fungi or to a kingdom related to the fungi based on chitin present in the spore wall and rRNA sequencing. Because the two preparations are treated differently before staining, however, use of two slides may be more efficient. What type of host-parasite relationship does this represent? Moisture is necessary for growth, but spores and conidia survive in dry conditions for extended lengths of time. As seen during World War II, epidemic louse-borne typhus can recur even in developed countries when sanitation is disrupted. Edema secondary to increased vascular permeability reduces blood flow to the area and results in local necrosis. 501. Stool specimens contain enteric organisms as normal colon microbiota; in processing stool samples, laboratories may develop their own protocol for the maximum recovery of enteric pathogens. Symptoms of cutaneous mycoses include itching, scaling, or ringlike patches of the skin, brittle, broken hairs, and thick, discolored nails. That shift resulted in pandemic influenza, the Asian flu. Treatment involves a multiple antimicrobial regimen, including fluconazole, clarithromycin, and sulfadiazine. gergoviae is found in respiratory samples and is rarely isolated from blood cultures. The pathogenicity of E. aureus KVLB B. The toxin consists of two toxic A subunits and five binding B subunits. Two distinct forms of tinea capitis, gray patch ringworm and black dot ringworm, are caused by different species of dermatophytes. 817. Furthermore, biliary tract disease has been identified as a possible risk factor for bacteremia with this organism. When a human ingests the infective oocyst, sporozoites in the oocyst are liberated in the intestine, penetrate the intestinal wall, gain access to the circulation, and migrate to the organs. hominis has no role as a pathogen. longbeachae, and L. The number and sequence of amino acids in a polypeptide and the character of that particular protein are determined by sequence of codons in the mRNA molecule. coli subgroups associated with enteric disease (e.g., enterotoxigenic E. Phenol is irritating to the skin and hazardous to the eyes. After 18 to 24 hours incubation at 35° C, shiny, opaque, nonhemolytic colonies appear, with a slightly raised center and a smooth and entire edge. In a well-stained trichrome smear, the cytoplasm of protozoan cysts and trophozoites stains a blue-green, although Entamoeba coli often stains purple. To do it, you enter the house number and street in one search box and the city and state or zip code in the second search box before running the query. Cupriavidus paucula has been isolated from a number of aquatic environments and has been recognized as an opportunistic pathogen that can cause serious infections, including septicemia, peritonitis, abscesses, and tenosynovitis, most notably in immunocompromised patients. In Braunwald E, et al, editors: Harrison's principles of internal medicine, ed 16, New York, 2005, McGraw-Hill. It produces acids, alcohols, and gases. The most probable identification is: a. How does Coxiella burnetii differ from the Rickettsia spp.? Research studies using electro phoresis identified differing isoenzyme (zymodeme) patterns between organisms that caused clinical symptoms and those tahir99 VRG CHAPTER 28 Diagnostic Parasitology 633 Human ingestion of infective cyst in contaminated food or water Trophozoite Excysting of organism in intestine—asexual reproduction in colon Encyst Passing of trophozoite in liquid or soft stool —not infective Hepatic abscess Infective cyst in formed stool Erosion of intestinal wall by Entamoeba histolytica only To liver via circulatory system FIGURE 28-2 Generalized life cycle of intestinal ameba. The reaction occurs within the agar. The antigen used is a cardiolipin-lecithin complex made from bovine hearts. Patients are often misdiagnosed and treated for an extended period for bacterial sinusitis. Eggs and larvae stain red; however, they are often distorted or destroyed in the staining process. Van Gool T, et al: A simple and fast method to exclude high Plasmodium falciparum parasitaemia in travelers with imported malaria, Malaria J 10:300, 2011. Table 28-3 summarizes the characteristics of E. Many organisms bind to the buccal mucosa and tooth surfaces. The intestinal Apicomplexa include C. In addition, the specimens should not be refrigerated and, the amount of time they remain at room temperature should be minimized. They also are made of polysaccharides and Lophotrichous Polar Peritrichous FIGURE 1-4 Diagram of three flagellar arrangements that occur in bacteria. The occurrence of mutations can result in a change in the expected phenotypic characteristics of an organism and provides an explanation for atypical results sometimes encountered on diagnostic biochemical tests. The mode of transmission, incubation period, and infectiousness of C. tularensis is oxidase, urease, and satellite or X and V test-negative and weakly positive for catalase and β-lactamase activity. Bacteria such as E. The prokaryotic PM is made of phospholipids and proteins but does not contain sterols, in contrast to eukaryotic PMs (except for Mycoplasma). Again, the microbiology laboratory may provide insight into the hypothesis and its relationship to the control group. pylori has been primarily linked to gastric infections. High-volume air samplers are the preferred method for collection, although settle plates may also be used. There are more than 100 species of Lactobacillus. Does the specimen contain sulfur granules? Reagents that could distinguish between antibodies to HSV-1 and to HSV-2 were not previously available. This chapter is divided into three major areas: (1) clinically significant enteric species that cause opportunistic infections, (2) primary intestinal pathogens and their related human infections, and (3) methods of identification of these organisms. Neurocysticercosis, which is the most serious manifestation, is the causative agent for up to 10% of neurologic problems seen in developing countries. coli (Figure 20-7). often grow embedded beneath the surface of solid media; therefore, transferring colonies with a loop is ineffective. It is estimated that 24 of every 1000 children with RSV infection will be hospitalized. Once ingested, the spores germinate and the vegetative cells then colonize the colon and subsequently produce toxins. The immune response can be divided into two broad categories. hominis d. Previously, the genus Aeromonas resided in the family Vibrionaceae. tahir99 VRG CHAPTER 20 Vibrio, Aeromonas, Plesiomonas, and Campylobacter Species Clinical and Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing, 18th Informational Supplement, CLSI document M100-S18, Wayne, PA, 2008, Clinical and Laboratory Standards Institute. ureolyticus Bilophila wadsworthia Large (>1 mm) Translucent, pitting (some) Tiny, translucent Regular Tiny rods or coccobacilli Regular to filamentous Not done Negative Negative Fusobacterium nucleatum Ground-glass or breadcrumb Large, convex, black-gray No growth Translucent, with black center at 72 h No growth Positive Porphyromonas Small, translucent or opaque, brick red fluorescence on blood agar, no growth on KVLB Small, translucent or opaque, brick red fluorescence on blood and KVLB agar Small, translucent or opaque, brick red fluorescence on blood agar, no growth on KVLB No growth Fusiform, thin with pointed ends Tiny coccobacilli No growth Tiny coccobacilli No growth Tiny diplococci Prevotella intermedia Veillonella Positive Positive Negative Negative BBE, Bacteroides bile esculin; KVLB, Kanamycin and vancomycin with laked sheep blood. Pathogenic (disease-causing) bacteria are prokaryotic cells that infect eukaryotic hosts. However, with increased isolates of Acinetobacter that demonstrate resistance to most antimicrobial agents, including the carbapenems, their clinical significance when isolated from respiratory or urine specimens in a hospitalized immunocompromised patient cannot be dismissed routinely. In young children, abdominal pain is quite intense, and rectal prolapse may result from excessive straining. Close contact is required between the two cells. histolytica may have one to four nuclei, each with a small central karyosome and fine, evenly distributed peripheral chromatin. B, E. Epidemiologically, the Methylobacterium are isolated from soil, vegetation, sewage, water, and hospital nebulizers. coli; EHEC, enterohemorrhagic E. Serotypes have historically been identified by microscopic agglutination testing using sera of defined reactivity; however, other methods such as pulsed field gel electrophoresis and 16s rRNA DNA sequencing are also being investigated. FIGURE 22-5 Culture results that might be obtained from the primary isolation setup of a hypothetical wound specimen. More than 20,000 cases were documented during the 1980s, with the vast majority originating in Africa. As illustrated in the Case in Point at the beginning of this section, the relative importance of each factor depends on the microbe, route of infection, condition and genetic makeup of the host, and other factors yet to be clearly characterized. This agent is lethal for yeast and, although effective against the aspergilli, is not generally lethal. Growth is indicated by the amount of 14C-labeled CO_2 release, as measured by the BACTEC 460 instrument, or the amount of fluorescence or gas measured by the BACTEC MGIT 960, MB/BacT Alert 3D, or ESPII, respectively. The organism can be found in Africa and South and Central America; transmission occurs by the bite of the black fly (Simulium). The virus seemed to retreat into the jungles for the next 15 years. Clinical manifestations include muscular rigidity, usually in the jaw, neck, and lumbar region. lamblia and compares them with those of other flagellates. The microbial flora produces conditions at the microenvironmental level that block colonization by extraneous pathogens. As a general rule, pooled specimens collected over 12 to 24 hours are not recommended. It is estimated that 50% of the population worldwide is infected with H. Within the periplasmic space is a gellike matrix containing nutrient-binding proteins and degradative and detoxifying enzymes. Salmonellosis is a common cause of GI tract infections; 51,887 cases of salmonellosis were reported to the CDC in 2011, with an estimated incidence of 1.4 million. Various sites on the foot may be involved, but tinea pedis usually affects the soles of the feet and toe webs. Which of the following tests is most helpful in differentiating C. tahir99 VRG CHAPTER 28 Diagnostic Parasitology FIGURE 28-25 Trypanosoma trypomastigote in a blood smear (Giemsa stain, ×1000.) trypomastigotes. coli on MAC agar. Erythroid precursor cells contain a receptor for the virus, allowing viral infection and replication. difficult to stain with the Gram stain. It is far more important for a system to identify these organisms than to identify obscure anaerobes that are only rarely isolated from clinical specimens or involved in infectious processes. testosteroni infection and a total of seven bacteremias. Such granules are associated with actinomycosis, a condition caused by Actinomyces, Propionibacterium propionicum, and closely related organisms, such as P. Because many countries stopped counting individual cases, the actual number was much larger. In a retrospective study of 158 patients who had infections caused by nonfermenters other than P. Although larvae remain viable for many years, eventually the capsules calcify and the larvae die. The most bothersome contaminating materials are materials containing microorganisms that will grow in culture and potentially confuse the culture evaluation. Specimen Collection and Handling Specimens for culture and direct examination commonly include sputum, bronchoalveolar lavage, and bronchial washings. In addition, other enzymes from the lysosome have antimicrobial action. Incorrect or poor specimen collection can result in a false-negative diagnostic result. The organisms are called symbionts. The single species is L. Treatment with trimethoprim-sulfamethoxazole has been effective in eliminating diarrhea, but patients often show recurrence of infection when therapy is discontinued. Rickettsiaceae and Similar Organisms The genera Rickettsia and Orientia belong to the family Rickettsiaceae. Aureobasidium spp. will grow in nutrient broth with 0% NaCl (left), whereas neither will grow in nutrient broth with 6% NaCl (right). In addition, characterization of the MOMP has FIGURE 24-2 Elementary bodies and cells in Chlamydia trachomatis–positive direct specimen (×400). Alternative regimens for patients who are allergic to penicillin and not pregnant include doxycycline, tetracycline, and chloramphenicol. tahir99 VRG 686 PART II Laboratory Identification of Significant Isolates Gascon J: Epidemiology, etiology, and pathophysiology of traveler's diarrhea, Digestion 73(Suppl 1):102, 2006. (A, ×400; B, ×1000.) tahir99 VRG CHAPTER 28 Diagnostic Parasitology oval or spherical, 5 to 12 μm, and has up to four large nuclei (Figure 28-10, A). This binding stimulates the cell to synthesize enzymes that inhibit viral replication over several days. "Rose spots" (blanching, rose-colored papules around the umbilical region) appear during the second week of fever. The addition of antimicrobial agents to 7H10 or 7H11 makes the media more selective by suppressing the growth of contaminating bacteria. The vacuole stains dark brown in an iodine wet mount and appears empty in a permanently stained smear. Ambient air contains approximately 21% oxygen and 1% carbon dioxide. Fewer conidia are produced by zoophilic fungi than by geophilic species. Protein extracted and purified from the cell wall of culture-grown M. Proteus The genera Proteus, Morganella, and Providencia belong to the tribe Proteeae. The strains associated with diarrheal disease appear to be distinct from strains associated with neonatal sepsis or meningitis. Centers for Disease Control and Prevention: Emergence of Mycobacterium tuberculosis with extensive resistance to second-line drugs— worldwide, 2000-2004, MMWR 55:301, 2006. In addition, many laboratories screen for E. urethralis is nonmotile. The U.S. Centers for Disease Control and Prevention (CDC) reported 241 cases of human salmonellosis caused by Salmonella Typhimurium in 42 states by contact with African dwarf frogs in 2010-2011. The neutrophil membrane has various receptors, including receptors for the Fc portion of IgG1, IgG3, and the C3b component of complement. With Giemsa stain, the cytoplasm of the parasite stains bluish, and the chromatin stains red to purple-red. The carrier state may be terminated by antimicrobial therapy if gallbladder infection is not evident. Therefore, electron microscopy is rarely used in clinical laboratories and is more suited for large teaching or research institutions. What organism is the mostly likely cause of the infection? • Double zone of hemolysis on SBA plate incubated anaerobically, suggestive of C. Cytocentrifuge Preparations Cytocentrifugation is an excellent method for preparing nonviscous fluids such as CSF and bronchoalveolar lavage fluids. Although the Actinomyces are gram-positive, irregular staining can produce a beaded or banded appearance, much like that seen with Nocardia spp. Commonly encountered infections of this type are surgical wound (skin flora) infection, aspiration (oropharyngeal flora) pneumonia, perirectal (fecal flora) abscesses, and tuboovarian (vaginal flora) abscess. vaginalis lacks a cyst stage, and the trophozoite stage is infective through sexual contact. The swab should never be rubbed back and forth across the slide because important material on the opposite side of the swab might not be deposited, and smear elements could be broken up. The major way bacteria are classified in the diagnostic microbiology laboratory is by the Gram stain reaction. The virus spreads mostly through large-particle droplets and contact with fomites rather than through inhalation of small aerosols. algae requires NaCl (halophilic) and is asaccharolytic, whereas S. The number of bacteria present on the skin can be reduced by about 90% by scrubbing and washing, but they are not completely eliminated, and their numbers return to normal within a few hours. falciparum may simply attach to receptors that are integral parts of the red cell membrane itself. fetus Campylobacter concisus Campylobacter Campylobacter Campylobacter curvus gracilis hyointestinalis lari rectus Campylobacter sputorum biovar sputorum Helicobacter pylori Helicobacter bilis Helicobacter canadensis Helicobacter cinaedi Helicobacter felis Helicobacter fennelliae Associated with diarrheal disease and bacteremia in humans and in children with recurring gastrointestinal illness (abdominal cramps), endocarditis, and peritonitis Isolated from cases of human bacteremia and diarrhea Gastroenteritis Most common cause of bacterial diarrhea worldwide Gastroenteritis, bacteremia in immunocompromised patients Bacteremia in immunocompromised patients Involved in periodontal disease; has also been recovered from individuals with gastrointestinal illness Periodontal disease Periodontal disease Gastroenteritis Enteritis very similar to that caused by Campylobacter jejuni, also septicemia Gastroenteritis; associated with periodontal disease and has been recovered from patients with root canal infections and Crohn's disease Gastroenteritis Common cause of duodenal ulcers and type B gastritis; possibly a risk factor in gastric carcinoma Bacteremia Gastroenteritis Colitis and sepsis; recovered from blood of men who have sex with men and feces of children and adult females Gastritis and ulcers Proctitis, gastroenteritis, and sepsis, particularly in immunocompromised patients tahir99 VRG CHAPTER 20 Vibrio, Aeromonas, Plesiomonas, and Campylobacter Species Asia, and South America, the incidence is reported to be as high as 80% to 90%. Cercariae migrate from the snail into water. (Courtesy A.J. Horneman.) plesiomonads grow well in nutrient broth with 0% NaCl, but not in 6% NaCl. Conversely, most vibrios, specifically the halophilic species, cannot grow in 0% NaCl but thrive in 6% NaCl and even higher concentrations of NaCl (Figure 20-8). Chlamydiaceae General Characteristics As shown in Table 24-2, initial differentiation of the Chlamydia spp. Meamar AR, et al: Strongyloides stercoralis hyper-infection syndrome in HIV/AIDS patients in Iran, Parasitol Res 101:663, 2007. Note that frosted tape does not work because the fungi are not visible through this type of tape. This chapter discusses the major medically important parasites, their epidemiology and life cycle, and the clinical infections they cause. Testing should be done with positive and negative controls. Antisera kits usually consist of a polyvalent A through G, Vi, and individual antisera for serogroups A, B, C1, C2, D, E, and G. Mahon, Donald C. Infections caused by Shigella spp. Found worldwide, this isolate is common in the environment. Although the number of infections caused by these organisms is low compared with those caused by intestinal protozoans, they are very difficult to diagnose and treat and are associated with a high mortality rate. A direct susceptibility test may be performed, however, using clinical specimens that are positive on smear, provided that appropriate dilutions are made on the basis of numbers of AFB seen. Half-strength charcoal agar with horse blood and cephalexin (incubate 1 to 2 days at 35° C before transport to laboratory) 3. The swab is vortexed vigorously to remove the clinical material from the swab and then pressed firmly against the inner wall of the tube to remove as much liquid as possible. (unstained, ×450). Spread of the virus through airline travel by travelers from endemic areas highlights the vulnerability of large populations and, combined with a resurgence in Nigeria, has prompted vaccine development efforts, with some promising early results using a genetically engineered virus. There is no comparable method for detecting T. form brown to olive to black hyphae and conidia (Figure 27-48). In vivo, bacteria have a tendency to lower the redox potential at their site of growth. Anaplasmataceae Ehrlichia Ehrlichiosis was first noted in France in the 1930s when dogs infected with brown dog ticks became ill and died. Microbial Factors Contributing to Pathogenesis and Virulence Pathogenicity is the ability of a microbe to produce disease in a susceptible individual. The growth rate is rapid, with colonies covering the entire surface of the agar. Centers for Disease Control and Prevention: Multistate outbreak of Salmonella serotype Tennessee infections associated with peanut butter—United States, 2006-2007, MMWR 56:521, 2007. Petri dishes have the advantage of a larger surface area, but are more prone to dehydration because of the prolonged incubation necessary for the recovery of some fungi. coli strains in the large intestine. In contrast to many other Haemophilus spp., it is not considered part of the normal oral biota of humans. The female is 30 to 50 mm long, with a thin anterior and thick, straight posterior. RSV can produce classic syncytial formation in HEp2 or MKC cells. Stark D, et al: Irritable bowel syndrome: a review on the role of intestinal protozoa and the importance of their detection and diagnosis, Int J Parasitol 37:11, 2007. Reports exist of mortality rates of 5% to 10%, and perhaps even higher, resulting from S. The virus infects primarily children and adolescents and usually results in long-lasting immunity. tarda is the only recognized human pathogen. ureolytica. These leukocytes release mediators or cause other cell types to release mediators. Viruses are spread via aerosol inhalation, the 713 fecal-oral route, and fomites. Life cycle stages, as shown in Figure 28-36, develop under the brush border of the intestinal mucosal epithelial cells. pertussis B. maltophilia strains were excluded, genus level identification was 93% with MALDI versus 76% with API 20NE and 81% with Vitek-2. The organism was first seen using light microscopy in 1916. carinii was originally classified with the protozoa, but nucleic acid sequencing showed conclusively that the organism is a fungus. 375.) strains are phenotypically similar to toxigenic V. In the 1980s, cryptosporidiosis was identified as a major opportunistic infection in patients with AIDS. Disease caused by HSV infection is generally divided into two categories, primary (first or initial infection) and recurrent (reactivation of the latent virus). If immediate transport is not possible, the specimen may be refrigerated overnight. Small pieces of tissue can be placed in anaerobic transport tubes or vials containing PRAS medium to keep the tissue moist. • Motility is variable. Trypanosoma spp. The immune system response to infection is briefly discussed in this chapter to provide the reader with an appreciation of its role and complexity. Cholesterol has a stabilizing effect and helps keep the membrane fluid. These conditions prevent colonization by transient, possibly pathogenic organisms. Clinical Manifestations Cholera is an acute diarrheal disease spread mainly through contaminated water. The mumps strain, genotype G, was the same one that had been circulating in the United Kingdom since 2004, where more than 70,000 cases have been reported. About 60% of patients exhibit erythema migrans (EM), the classic skin lesion that is normally found at the site of the tick bite. Antibiograms and pathogen prevalence reports c. niger. Available at: . Many infections are completely asymptomatic or very mild. More than half of the strains of E. Approximately one in three adults will develop herpes zoster in their lifetime. falciparum has also demonstrated resistance to sulfadoxinepyrimethamine (Fansidar) and mefloquine (Lariam) in parts of South America, Southeast Asia, and Africa. The results of PCR-based assays have been published, but lack of a gold standard for comparison has made evaluation difficult. Immunofluorescencelabeled antibodies allow for direct visualization of virus infection, and some tests can amplify signals, which enhance sensitivity. tahir99 VRG CHAPTER 27 Medically Significant Fungi Antler hyphae Racquet hyphae Spiral hyphae 591 Rhizoids A B FIGURE 27-3 A, Specialized structures formed in vegetative mycelia by certain fungal species. Orientia FIGURE 24-10 The female head louse, Pediculus humanus, which is a vector for Rickettsia prowazekii, the agent of epidemic typhus (×40). fetus produces smooth, convex, translucent colonies. Aspergillus spp. B, T. In all species, eggs must reach water to mature, and all have a snail species as the first intermediate host. In addition, H. Other infections commonly associated with K. albicans currently reigns as the premier cause of yeast infection in the world. Unfortunately its overuse or misuse has resulted in the development of resistance, most notably with C. Although many have been isolated from humans, only three species are considered human pathogens. These categories are S (susceptible), I (intermediate), and R (resistant) for the candins. In the United States and most developed countries, the tertiary stage of disease is not often seen because most patients are adequately treated with antimicrobial agents before the tertiary stage is reached. Some species are phaeoid. Compare the characteristics of the Rickettsia and Coxiella and the diseases they cause. In addition, it is positive for catalase, DNase, esculin and gelatin hydrolysis, and lysine decarboxylase. NAG, N-acetyl-d-glucosamine; NAM, N-acetyl-d-muramic acid. coli strains involved in extraintestinal diseases. The rRNA is released from the cell after sonication. Smears from swabs are prepared by rolling the swab back and forth over contiguous areas of the glass slide to deposit a thin layer of sample material (Figure 7-1). infrequently penetrate the intestinal mucosa and disseminate to other body sites, it has been reported that 4% of severely ill hospitalized patients in Bangladesh have bacteremia caused by S. In 2011, the U.S. Food and Drug Administration (FDA) initiated a recall of povidone-iodine pads used for skin disinfection because they were found to be contaminated with E. The adult H. Microscopic evaluation reveals abundant arthroconidia formed from the vegetative hypha that occurs singly or may be branched (Figure 27-41). The organism was isolated from the stools of patients who were asymptomatic and from those who had GI symptoms such as abdominal pain or tenderness or diarrhea. Studies documented wide dissemination of influenza A (H5N1) throughout Asia as a result of migrating birds. influenzae with the X and V strip test. Some species release a characteristic fruity odor. baumanii. (Nomarski optics, ×450.) FIGURE 27-39 Chrysosporium sp. The anterior headlike segment of a tapeworm, or scolex, has suckers and, in some species, hooklets as a means of attachment to the intestinal mucosa. The cytoplasm is granular and vacuolated. Congenital Syphilis. Treponemes can be transmitted from an infected mother to her fetus by crossing the placenta. ureolytica are motile by means of peritrichous flagella—hence, the relationship to Alcaligenes and Taylorella. Jackson* CHAPTER OUTLINE ■ STERILIZATION VERSUS DISINFECTION ■ FACTORS THAT INFLUENCE THE DEGREE OF KILLING Types of Organisms Number of Organisms Concentration of Disinfecting Agent Presence of Organic Material Nature of Surface to Be Disinfected Contact Time Temperature pH Biofilms Compatibility of Disinfectants ■ METHODS OF DISINFECTION AND STERILIZATION Physical Methods Chemical Methods ■ DISINFECTANTS VERSUS ANTISEPTICS Alcohols Aldehydes Halogens Chlorine and Chlorine Compounds Detergents: Quaternary Ammonium Compounds Phenolics Heavy Metals Gases ■ ENVIRONMENTAL PROTECTION AGENCY REGULATIONS ON CHEMICAL SURFACE DISINFECTANTS ■ FOOD AND DRUG ADMINISTRATION REGULATIONS ON CHEMICAL SKIN ANTISEPTICS Hygienic Handwashing and Waterless Handrubs Surgical Hand Scrub and Waterless Surgical Handrubs Presurgical Skin Disinfection OBJECTIVES After reading and studying this chapter, you should be able to: 1. A heavy worm burden is more likely to result in complications. This neutral dye is a combination of basic thiazine dyes and acid eosin that attach to oppositely charged sites on proteins. Describe microbial classification (taxonomy) and accurately apply the rules of scientific nomenclature for bacterial names. szulgai exhibit activity with 14 days of incubation. If the causative agent suspected is a dimorphic fungus, cultures should also be incubated at 37° C. tuberculosis is detected on average in 9 to 14 days; NTM are detected in less than 7 days. Chronic infections are usually asymptomatic. Although not all patients suffer from chest pain, it is not uncommon for pneumonia-like symptoms to appear. cayetanensis vary based on age, infective dose, and host immune status. In practice, swabs are used more commonly than aspiration methods. Culture Media and Isolation Methods Mycobacteria are strictly aerobic and grow more slowly than most bacteria pathogenic for humans. Results were often too slow to be clinically useful and were perhaps even irrelevant. Biogroup 2 may also be indole-positive (50%). Tubes of Christensen urea agar are very lightly inoculated with the dermatophyte and held for 5 days at room temperature. The infection is usually transmitted during a vaginal delivery and is more severe when HSV-2 is involved. gondii (lacks antibodies to T. Phagocytic cells ingest and kill microorganisms, whereas activated complement components contribute to a wide variety of immunologic events, including promotion of attachment and engulfment of bacteria by neutrophils (opsonization) and attraction of neutrophils to sites of infection (chemotaxis). HIV is not highly contagious, and normal, social, nonsexual contact poses no threat to individuals. plymuthica, S. Members of this complex have been reported to cause endocarditis (specifically in IV drug abusers), pneumonitis, UTIs, osteomyelitis, dermatitis, and other wound infections resulting from the use of contaminated water. Lesions begin as scaling, painless papules and are followed by an erythematous rash that becomes hypopigmented with time. • Viruses are acellular (not composed of cells), lack cytoplasmic membranes, and are surrounded by a protein coat. Symptomatic congenital infection is characterized by petechiae, hepatosplenomegaly, microcephaly, and chorioretinitis. Secretory antibody, lactoferrin, and lysozyme all are produced by the host as a way to protect against infection. vivax in Southeast Asia and Papua New Guinea also show diminished response to treatment with chloroquine. Most strains of O. For expediency, all necessary biochemical tests should be set up at one time. Microfilariae can be isolated from the subcutaneous tissue, skin, and the nodule itself, but are rarely found in blood or lymphatic tahir99 VRG CHAPTER 28 Diagnostic Parasitology 683 Birth of live microfilariae via female Microfilariae in blood and lymphatics or subcutaneous tissue Adult worms in respective tissues Biting of human and ingestion of microfilariae by insect Larvae migration Larvae infection of human when insect bites Microfilariae development in insect Infective filariform larvae migration to insect salivary gland FIGURE 28-67 Generalized life cycle of microfilaria. It is motile with polar flagella and, as its name implies, produces a violet pigment about 91% of the time. Pseudomonas stutzeri (light yellow) and wrinkled colonies Pink Methylobacterium spp. The Biolog Microbial ID System (Biolog, Hayward, CA) is a microbial identification system that can be used manually or in fully automated format; the database contains over 2500 species of bacteria and yeast, including most of the clinically relevant nonfermenters. Shewanella putrefaciens and S. Describe anaerobic bacteria, including their sensitivity to oxygen, why they are sensitive to oxygen, and where they might be found in the environment and human body. In immunocompetent individuals, infection is asymptomatic; however, in immunocompromised patients, serious life-threatening pneumonia can develop. The INNO-LiPA Mycobacteria test (Innogenetics, Ghent, Belgium) is a PCR assay that targets the 16S-23S rRNA spacer region of mycobacteria and has been used to directly detect and identify M. The emergence of V. Diploid is the normal genetic makeup for eukaryotic cells. Identification of these organisms is more for academic interest than for the evaluation of their significance as causative agents of infection. The trophozoite is 6 to 14 μm long, with a prominent axostyle extending through the posterior of the organism, four anterior flagella, and an oval nucleus with a small karyosome. Size is a major determinant in differentiating E. The amino acids in the cross-linking tetrapeptides may vary among species. are listed in Box 18-2, and an identification algorithm is depicted in Figure 18-29. General Characteristics of Nonfermenters Nonfermenting gram-negative bacilli are grouped together because they fail to acidify oxidative-fermentative (OF) media when it is overlaid with mineral oil or fail to acidify triple sugar iron agar (TSIA) butts. When a delay between collection of the specimen and culturing the specimen is necessary, a transport medium is used. HBoV is closely related to the bovine parvovirus and canine minute virus, both members of the genus Bocavirus of the family Parvoviridae. The adults obtain nourishment by absorbing nutrients from tahir99 VRG 674 TABLE PART II Laboratory Identification of Significant Isolates 28-9 Comparisons of Intestinal Roundworm Eggs and Larvae Organism Average Size (μm) and Shape Other Identifying Features Ascaris lumbricoides Fertile 45-75 × 35-50, oval Bile-stained shell Bumpy, mammillated coat In one-cell stage when passed Some eggs may be decorticated (lack mammillated coat) Infertile 85-95 × 43-47, oval (some bizarrely shaped) Mammillated Thin shell Undifferentiated internal granules Enterobius vermicularis 50-60 × 20-30, oval, flattened on one side Colorless shell Usually embryonated with C-shaped larva Trichuris trichiura 50-55 × 22-23, barrel shaped Bile-stained, thick shell Hyaline polar plugs Unembryonated when passed Hookworm Egg 50-60 × 35-40, broadly oval Thin shell, colorless In four- to eight-cell stage when passed Rhabditiform larva 250-300 Long buccal capsule Inconspicuous genital primordium Filariform larva 500 Pointed tail Esophageal-intestinal ratio 1 : 4 Strongyloides stercoralis Rhabditiform larva Egg rarely seen; resembles that of hookworm 200-250 Filariform larva 500 Short buccal capsule Prominent genital primordium Notched tail Esophageal-to-intestinal ratio = 1 : 1 partly digested intestinal contents or by sucking blood. An indirect fluorescent antibody method has been reported for detecting C. perfringens type A is caused by a C. pneumoniae may be cultured on selected cell lines and visualized with fluoresceinconjugated monoclonal antibodies. doloda. Acid-fast bacteria retain the primary stain and are red. More than 150 cases of transfusion-transmitted babesiosis were reported between 1979 and 2009, but 122 cases (77%) occurred during 2000–2009. The small cells, when found in tissue (see Figure 27-29, B), resemble the blastoconidia of Candida glabrata, but they can be differentiated by fluorescent antibody (FA) techniques or culture. Acute infections with C. gonorrhoeae, and N. Antimicrobial Susceptibility Early diagnosis and antimicrobial treatment are important for preventing neurologic, cardiac, and joint abnormalities that can occur late in the disease. A number of species-related disease syndromes—A. Over 50% of cases reported occurred in individuals of Hispanic origin. Give the mechanism of action for each type of chemical agent commonly used in antiseptics and disinfectants. Subsequently, several others were identified. The presence of sphingophospholipids in the cell wall is unique to the group. Romano-Mazzotti L, et al: Outbreak of Ralstonia paucula pseudobacteremia in a paediatric accident and emergency department, J Hosp Infect 78:155, 2011. Penicillium marneffei Clinical Manifestations. Penicillium marneffei is unique among the Penicillium spp., being dimorphic. The H5N1 influenza virus appeared in flocks in parts of Asia in 2004 and again in 2005, resulting in large poultry losses in Japan, Cambodia, China, Vietnam, and nearby countries. With respect to laboratory technique in the isolation and identification of mycobacteria, discuss some causes of false-negative and false-positive results. Chromatoidal bars, when present, have a pointed, splintered appearance. ozzardi and M. They circulate until they reach the lungs or enter the liver, where maturation and pairing of female and male are completed. Courtesy Larry Stauffer, Oregon State Public Health Laboratory.) Points to Remember ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ The genus Haemophilus consists of gram-negative pleomorphic coccobacilli or bacilli. Each sporangiophore has a large columella on which merosporangia, containing stacks of sporangiospores, are formed (Figure 27-36). Enlarged mesenteric lymph nodes and inflamed ileum and appendix are common findings in cases of Y. For example, there are many different strains of S. mosselii and P. A trichrome stain can be performed on a smear made from a fresh stool specimen fixed in Schaudinn fixative or

from one that has been preserved in PVA. It occurs in the presence of oxygen. The remaining liquid suspension is used to inoculate media, as described earlier for an aspirate. PCR techniques are more sensitive and have shown that HPV is present in 95% or more of invasive cervical cancers, but the presence of the virus alone is not the sole factor in cancer development. audouinii does not grow but turns the rice grains brown. This section provides a practical overview of the following topics: • Sterilization and disinfection • Chemical and physical methods of disinfection and sterilization • Principles and application of each method • Common disinfectants and antiseptics used in health care settings • Principles and applications of disinfectants and antiseptics • Regulatory process of disinfectants and antiseptics Sterilization versus Disinfection The scientific use of disinfection and sterilization methods originated more than 100 years ago when Joseph Lister introduced the concept of aseptic surgery. The acids are identified based on their position on the chromatogram by their relative retention time as compared with a standard. When C. The toxic activity of endotoxin is found in the lipid A portion of the LPS. Nielsen S, et al: Desperately seeking diarrhoea: outbreak of haemolytic uraemic syndrome caused by emerging sorbitol-fermenting Shiga toxin-producing Escherichia coli O157:H-, Germany, 2009, Zoonoses Pub Health 58:567, 2011. LJ, Löwenstein-Jensen; SQ, semiquantitative. putrefaciens is nonhalophilic and saccharolytic. Because this flea infests a large number of domestic animals, it may be an important factor in the persistence of infection in urban areas. Symptoms last about 1 week and disappear as cercariae die and degenerate. davisae, C. Figure 27-55 presents a schematic guideline to assist in making a diagnosis of a mycosis. Amies transport medium with charcoal is appropriate for up to 24 hours. Enzyme-linked immunosorbent assay (ELISA) and latex agglutination tests detect the viral antigens in fecal material. Ideally, the mycobacteriology laboratory should be separate from the remainder of the laboratory and have a nonrecirculating ventilation system. caviae isolates to ampicillin. This form of yersiniosis has been reported more often in adults than in children. These agents can be purchased as strips inside foil pouches and opened inside the chamber. Iversen C, et al: Cronobacter gen. The egg is oval, colorless, thin-shelled, 50 to 60 µm long, and usually contains an embryo in the four- to eight-cell stage of cleavage (Figure 28-62). hermannii with E. are the most frequent cause of disease in bone marrow transplant recipients in addition to other cancer and transplantation patients. Conidia or hyphae from the dermatophyte in question are inoculated onto the water surface. The combination of lactic acid, phenol, and the blue dye kills, preserves, and stains the organism. Influenza A viruses are classified into subtypes using the two major surface glycoproteins hemagglutinin (H) and neuraminidase (N). † Some M. BIBLIOGRAPHY Baron EJ: Approaches to identification of anaerobic bacteria. What are the advantages and disadvantages of DFA testing for Legionella spp.? 900. histolytica and G. The mucosa is inflamed, and there is an influx of segmented neutrophils, macrophages, and lymphocytes. Laboratories often report the presence of the organism quantitatively, with the presence of more than five organisms/high-power field, and no other known enteric pathogens in symptomatic patients considered as evidence of B. Spinola S, et al: Immunopathogenesis of Haemophilus ducreyi infection (chancroid), Infect Immun 70:1667, 2002. Capnophilic organisms, such as Capnocytophaga, grow best when the concentration of carbon dioxide is increased to 5% to 10% in a CO_2 incubator. tahir99 VRG CHAPTER 2 Host-Parasite Interaction Connie R. Neither subspecies is isolated from the environment or GI tract; both are more commonly seen in tropical regions. They are all motile by means of a single polar flagellum. Note the pink precipitate surrounding the individual colonies. Patients manifest clinical symptoms of one disease but show a concurrent rise and fall in antibody titers to both Babesia spp. Identification of Mycobacterium spp. The case studies describe the clinical and laboratory findings associated with the patients, allowing students opportunities to correlate these observations with possible etiologic agents. americanus has a geographic distribution in Africa, Southeast Asia, and South and Central America and is endemic in rural areas of the southeastern United States. Their positive oxidase reaction (except for V. tahir99 VRG 506 PART II Laboratory Identification of Significant Isolates • Certain morphotypes might provide a presumptive identification of organisms and serve as a guide to media selection. LPS contains three regions—an antigenic O–specific polysaccharide, a core polysaccharide, and an inner lipid A (also called endotoxin). Although rare, systemic diseases caused by these fungi are frequently fatal and occur most often in the immunocompromised host, commonly those who have hematologic disorders or malignancies or are undergoing chemotherapy. Although some are moniliaceous (hyaline or light-colored), many are phaeoid, producing darkly pigmented colonies and containing melanin in their cell walls. Distortion of the organism may occur if time to preparation of the slide is longer than 1 hour, and organisms may be lost if the time exceeds 4 hours. haemolyticus. parahaemolyticus strains from clinical sources along the Pacific coast of North America. alpaca are pale yellow, becoming brown with age. are divided into three groups according to the types of clinical infections they produce. 697 Genital Herpes. Genital herpes infections are usually caused by HSV-2, although HSV-1 virus can cause as many as one third of the infections. hinzii appears to be an avian commensal. Numerous bacteria, viruses, and their products induce interferon production. This group of fungi produces olivaceous to black colonies that are initially yeastlike but become velvety at maturity. Amoxicillin c. vaginalis can also be detected in a wet mount of vaginal or urethral discharge. malayi, another nocturnal microfilarial species, is limited to the Far East, including Korea, China, and the Philippines. have been associated with a more chronic condition, granulomatous amebic encephalitis (GAE), especially in individuals with impaired cellmediated immunity. Certain microorganisms produce extracellular factors that appear to aid in infection, but the exact role of these factors is unknown. Clinical Infections. Initial symptoms of African sleeping sickness include a local inflammatory reaction with tenderness, edema, and erythema at the site of the insect bite. About 4% of clinical strains of P. However, its clinical significance is not yet established. The highest concentration of illness (7.6%) was in children younger than 2 years. A microarray assay for rapid subtyping influenza A virus isolates was developed that would be valuable in the event of an outbreak or pandemic. They show up as azurophilic granules on a Wright's stain. Macroscopic Examination The examination of an unpreserved stool specimen should include macroscopic (gross) and microscopic procedures. may blend into the background. coli Enterobacter — K/A H_2S K/A Hafnia Klebsiella Serratia — Salmonella* Shigella Aeromonas† E. In infection, the organism will be intimate with the purulence of necrotic debris. Identification The identification of members of the family Enterobacteriaceae can be accomplished in several ways. They are a diverse group of cells that can be classified into two major types—T (thymus-derived) cells and B (bone marrow–derived) cells—on the basis of cell surface markers. Persons who live in overcrowded conditions can acquire CMV at an early age. This stain detects any organism that contains chitin in its cell wall. Available at: 03-1107_article.htm. Susceptibility to the carbapenems is variable. Studies have shown a general resistance to the penicillin class of antibiotics, but penicillins combined with a β-lactamase inhibitor, as well as trimethoprim-sulfamethoxazole, are active. furnissii, V. enterica. aerogenes (% +) Sign %+ (2) (1.3) (94.2) (1.3) − + + − + (+)or− + 2 97 98 0 98 0 94.2 + + + − 96.7 93.4 95 0 (8) (1.5) K. (A, ×1000; B, Original magnification, ×1000.) FIGURE 28-18 Giardia lamblia cysts (trichrome stain, ×1000). The proglottid is wider than it is long, with a characteristic rosette-shaped or coiled uterus. lari) have similar clinical manifestations. Eggs are passed in the feces and require at least 14 days in warm, moist soil for embryonation to occur. What stains should be performed on the discharge or conjunctival scraping for microscopic examination? Investigators at the CDC Anaerobic Bacteria Branch have found that members of the genus Actinomyces are seldom obligate anaerobes. Symptoms vary according to the organ infected. An EIA for mannan, Platelia Candida (Bio-Rad), is available. Other members of the B. Isolates of both Alcaligenes and Achromobacter are found in water (e.g., swimming pools, tap water, dialysis fluids) and are resistant to disinfectants, such as chlorhexidine and quaternary ammonium compounds. However, this test is not as tahir99 VRG CHAPTER 20 Vibrio, Aeromonas, Plesiomonas, and Campylobacter Species TABLE 457 20-1 Clinical Infections Associated with Vibrio Species Species V. The number of reported cases has been increasing by over 5% annually since 1997. Biochemical Characteristics and Identification As noted, all the nonfermenters fail to ferment carbohydrates and thus will not yield acidic reactions in the anaerobic portion of media, such as TSIA or Kligler's iron agar (KIA). Available at: .gov/std/Gonorrhea/lab/Kden.htm. histolytica is reflected, however, in its ability to cause invasive intestinal amebiasis and extraintestinal amebic infections. are also divided into four major O antigen groups and must be identified by serologic grouping. In contrast to infections with the other herpesviruses, which are usually asymptomatic, varicella is generally clinically apparent. Allow to dry. This is called dissemination. Although their significance is unclear, isolates have been recovered from sputum, wounds, and blood. Sporangiospores are smooth and ovoid. At this time, the organisms may be isolated easily from the blood. The viremia of parvovirus B19 creates a risk for blood donors and fetuses. Fluorescent stain demonstrating the presence of staphylococci in a blood culture broth. Why are older bacterial cells more easily decolorized than younger colonies? Stains Staining imparts an artificial coloration to the smear materials that allows them to be inspected using the magnification provided by a microscope. With either stain, poor fixation of fecal material results in poorly staining or nonstaining organisms. Helicobacter pylori is strongly associated with gastric, peptic, and duodenal ulcers as well as with gastrointestinal carcinoma. bütschlii, which are 6 to 20 µm, show a vacuolated cytoplasm in a permanently stained smear (Figure 28-11). Gentamicin or chloramphenicol and cycloheximide are the antimicrobials usually included with fungal media. *Showing reactions of these species in different groups. These three factors can and do bind to the invading microorganism, resulting in the invading microorganisms being coated with one or more of these factors. FIGURE FIGURE 27-17 Trichophyton rubrum showing clavate- or peg- 27-16 Trichophyton mentagrophytes showing globose, teardrop-shaped microconidia (Nomarski optics, ×450.) shaped microconidia. They consist of the phyla Glomeromycota, Ascomycota, Basidiomycota, and the form division Fungi Imperfecti (Deuteromycota). Edwards JR, Peterson KD, Andrus ML, et al; NHSN Facilities: National Healthcare Safety Network (NHSN) Report, data summary for 2006, Am J Infect Control 35:290, 2007. It has, however, been implicated as a tahir99 VRG CHAPTER 25 Mycoplasma and Ureaplasma TABLE 25-3 Mycoplasma Species Indigenous to Humans Mycoplasma Species Usual Habitat Reported Frequency Colony Morphology* Very common Common Uncommon Uncommon Rare Common (with disease) Rare Uncommon Uncommon Large, fried egg Small, spherical Large, fried egg Large, fried egg Large, fried egg Small, spherical, granular Small, fried egg, spherical M. A single antibody titer greater than 1 : 32 is suggestive of acute illness in a symptomatic patient during an outbreak of psittacosis. Abdominal discomfort, loss of appetite, and colicky pains are caused by the presence of adults in the intestine. coli strains are generally associated with two kinds of human disease: diarrheal syndromes and UTIs. The two types of enteroadherent E. produce pycnidia, which appear as black fruiting bodies that are globose and lined inside with short conidiophores (Figure 27-50). Fresh isolates may produce conidia similar to those of B. jejuni and other enteric campylobacters is moist, runny looking, and spreading. Up to 95% of adults between 35 and 40 years of age have been infected. ✓ Case Check 4-1 The first Case in Point at the beginning of this section illustrates the importance of a safety program for the clinical laboratory. The hallmark of this phase is the sudden onset of severe, repetitive coughing followed by the characteristic "whoop" at the end of the coughing spell. Antibodies to the fimbriae of N. tetani, whereas one with subterminal spores is most likely C. Cysts often stain darkly and unevenly, giving an irregular appearance to the cyst. Again, the sensitivity is low (40%), and cross-reaction with other species has been noted. On BCYE agar, colonies appear convex and glistening. Two theories have been proposed concerning the introduction of syphilis to Europe. It may migrate to the tissues, where its half-life is less than 1 week. It includes all the characteristics that are coded for in the DNA of a bacterium and that have the potential to be expressed. Many multicellular parasites (e.g., tapeworms) may be 7 to 10 meters long (see Chapter 28). streptocerca are found in the skin. alginolyticus − + + + − − + V. Studies have shown that the virus persists in the salivary glands and has been isolated from stool specimens, but most evidence indicates that saliva is the most likely route of transmission. The treponemal antigens used are spirochetes derived from rabbit testicular lesions. were isolated were considered to represent true infection, primarily in immunocompromised patients. Moreover, Aeromonas spp. It is not, however, approved for use in clinical laboratories for diagnostic testing, Presumptive Identification Several key tests can aid in the initial identification of a Vibrio isolate. Larva Migrans. Two forms of larva migrans exist in humans: cutaneous (creeping eruption) and visceral. capsulatum can cause a progressive and potentially fatal disseminated disease. In general, most strains of V. Describe the colony morphology and microscopic characteristics of each organism. Colonies become visible on nonselective and differential media after 18 to 24 hours of incubation. No antimicrobial is completely effective against this infection. heimbachae. botulinum spores is the food most commonly associated with infant botulism. Protective mechanisms such as stomach acidity have been described as inhibiting colonization and initiation of disease; individuals with achlorhydria (deficiency of 19-4 Features of Pathogenic Escherichia coli Virulence Factors Relevant Disease Relevant Serotypes Laboratory Tests O55:NM O55:H6 O111:NM O111:H2 O114:NM O114:H2 O157:H7 O157:NM O26:H11 O104:H21 O111:H2 O111:H8 O113:H21 O118:H2 O124:H30 O143:NM O164:NM O6:NM O6:H16 O8:H9 O25:NM O27:NM O63:H12 HeLa cell adherence assay, DNA probes Uropathogenic E. Final determination of species may be made based on clinical symptoms and geographic area. Trophozoites, motile, rapidly growing parasite forms of some amebae or eggs of some helminths may disintegrate if not examined or preserved within a short time. generally result in an induration smaller than 10 mm. The virus is fragile and must be handled carefully. There are reports of eye infections caused by A. Ameba (Naegleria spp.) occur in various environmental situations, such as those associated with contaminated drinking water (e.g., hospitals, extended care facilities, prisons) or recreation water (e.g., swimming pools, whirlpools, lakes, streams). The classic PVA fixative, which consists of mercuric chloride (for fixation) and PVA (to increase adhesion of the stool to the slide), is used when a permanently stained smear will be made. Compare the procedures and uses of thick and thin blood smears for the identification of blood parasites. Procedures for Identifying Anaerobic Isolates FIGURE 22-9 Advanced Anoxomat Mark II System (Advanced Instruments, Norwood, MA) is a gas evacuation replacement system. jejuni, C. Gastroenteritis 5. The quickness and ease with which the method can be performed make it an ideal choice for the clinical laboratory setting. Endogenous anaerobes can contribute to an infectious disease in any anatomic site of the body if suitable conditions exist for colonization and penetration of the bacteria. produce green colonies (Figure 19-12). (The quenching with solution B may be excessive [1 : 4 dilution preferred].) RESULTS Yeast cells, pseudohyphae, and hyphae display a bright apple-green or blue-white fluorescence. For cold enrichment, fecal samples suspected of containing this organism are inoculated into isotonic saline and kept at 4° C for 1 to 3 weeks, with weekly subculturing to selective agar for Yersinia. Lactophenol cotton blue–stained slide of macroconidia and hyphae of the fungal dermatophyte Microsporum gypseum. Hair loss and scar tissue formation commonly follow. In deciding whether a particular organism that has been isolated is a pathogen, we also must consider the human host from whom the organism was isolated and whether that host has underlying disease that may affect susceptibility to infection. The rotavirus replicates in the epithelial cells in the tips of the microvilli of the small intestine. fragilis, P. Most (70%) C. The ideal media for use in the culture of anaerobes are those that have never been exposed to oxygen or have been exposed only briefly. Although mycetoma is an uncommon mycosis in the United States, the following species (in order A 599 of occurrence) are the most commonly incriminated agents: Pseudallescheria boydii, Acremonium falciforme, Madurella mycetomatis, M. The microscopic characteristics, epidemiology, and clinical infections caused by each organism are discussed. Box 22-2 lists indications of anaerobe involvement in infectious processes. These organisms produce infections resulting from ingestion of contaminated food or water or from other sources, which are discussed in this chapter. Gametocytes have a characteristic banana or crescent shape (see Figure 28-32, B). The ribosomes give the ER the rough appearance. carateum, is found in the tropical regions of Central and South America. Detection of B. Specimens must be kept cold but cannot be frozen. metschnikovii) separates them from the Enterobacteriaceae (excluding Plesiomonas shigelloides), and carbohydrate fermentation metabolism separates them from the oxidative Pseudomonas. Table 28-5 summarizes the characteristics of G. malariae, and P. The proglottid may be seen in human feces and is characterized by its pumpkin seed shape, twin genitalia, and the presence of two genital pores, one on each side of the proglottid. Diphyllobothrium latum. Diphyllobothrium latum, the fish tapeworm, is found worldwide in areas in which the population eats pickled or raw freshwater fish. Morris JG: Infections due to non-O1/O139 Vibrio cholerae, 2012. This association has earned it the more common name Kaposi sarcoma–associated herpesvirus. ✓ FIGURE 22-18 Double zone of hemolysis produced by Clos- tridium perfringens—inner zone of complete β-hemolysis and outer zone of partial β-hemolysis. enterocolitica, incorporates tahir99 VRG CHAPTER 19 Enterobacteriaceae 439 TABLE 19-10 Differentiation of Selected Species within the Genus Yersinia Test Y. pylori colonizes the stomach for a long time and can cause a low-grade inflammatory process, producing a chronic superficial gastritis. The population of the nasopharynx mirrors that of the nose, although the environment is different enough from the environment of the nose to select for several additional organisms. planticola is difficult to distinguish from K. Definitive identification depends on the biochemical reactions and serologic antigenic structures demonstrated by the particular species. The method and level of identification usually depend on the size and capabilities of the laboratory and whether the organism is from a mixed infection or from a sterile site such as the blood or other sterile body fluid—for example, synovial, peritoneal, or pleural fluid. Examine specimen on fluorescent microscope using the following set of filters: G 365, LP 450, and FT 395. Entamoeba histolytica is recognized as a true pathogen, whereas Blastocystis hominis still has questionable status as a pathogen. A more complete list of genera associated with subcutaneous phaeohyphomycosis is given in Box 27-1. Anaerobes vary in their response to oxygen. Intracystic bodies are seen around the interior of the organism. Because the infective aerosol dose is low, R. These organisms have developed methods to prevent being killed intracellularly. Eggs passed in packet of 15-25 Eggs embryonated, with six-hooked oncosphere when passed Eggs in feces Adults in intestine of human Eggs in water Coracidium release Ingestion by Cyclops (crustacean) Ingestion of Cyclops (crustacean) by fish Larvae release and maturation in intestine Procercoid larva in Cyclops (crustacean) Human ingestion of raw fish Plerocercoid larva development in tissue FIGURE 28-48 Life cycle of Diphyllobothrium latum. Mosquitoes continue to spread dengue virus throughout the world. The mortality rates for untreated patients can approach 40%, although mortality rates in treated patients are very low. Microbiological studies performed on the cerebrospinal fluid identified Cryptococcus neoformans. The transport container should also be unbreakable and able to withstand freezing and thawing. Smooth ER does not synthesize proteins, but it does synthesize phospholipids (similar to rough ER). Sorvillo FJ, et al: Deaths from cysticercosis, United States, Emerg Infect Dis 13:230, 2007. Staphylococcus, Peptostreptococcus, Stomatococcus spp. Sehulster L, Chinn RY; CDC; HICPAC: Guidelines for environmental infection control in health-care facilities. Cerebrospinal Fluid CSF and other sterile body fluids should be concentrated by centrifugation before inoculation. Disseminated strongyloidiasis, however, is not common in patients with advanced AIDS, despite their immunocompromised status. Diagnosis involves clinical symptoms, such as the presence of nodules, and microscopic identification of microfilariae. As with HME, staining of peripheral blood and buffy coats can be used to diagnose HGA. Laboratory Diagnosis. The diagnosis of babesiosis is made from a Wright- or Giemsa-stained thin blood smear. IgG often appears in the acute stage and will persist for life at lower titers. After the initial two large outbreaks, smaller outbreaks occurred. simiae Smooth Smooth Rough M. Table 3-6 lists examples of waterborne pathogens. The specificity for all five assays ranged from 96.4% to 100%. Organization Part I has remained the backbone of the textbook, providing important background information, Part II emphasizes the laboratory identification of etiologic agents, and Part III focuses on the clinical and laboratory diagnoses of infectious diseases at various body sites—the organ system approach. Symptoms are related to the phases of the fluke's life cycle. The IPCPs involved in the outbreak investigation must be consulted before routine culturing of environmental water is undertaken. † B. The amount of light emitted is related to the amount of hybridized probe. These antiviral drugs are effective only against influenza A. histolytica, G. Giemsa stain provides the best staining of the organisms and should be used on thick and thin films. cepacia complex isolates are intrinsically resistant to aminoglycosides and polymyxins; in addition many strains are resistant to several β-lactam antibiotics. A cysticercus can form in the vitreous or subretinal space of the eye. Conjugation Conjugation is the transfer of genetic material from a donor bacterial strain to a recipient strain (Figure 1-14, C). HSV is the most common cause of corneal infection in the United States. BOX 18-2 Common Phenotypic Characteristics of Legionella Species • • • • • • • A B C D Slow growth (3 to 5 days) Characteristic "ground-glass" colony morphology Lightly staining, gram-negative bacillus Requires L-cysteine for primary isolation No growth on unsupplemented sheep blood agar (SBA) Asaccharolytic Catalase or oxidase: weakly positive FIGURE 18-28 A, Legionella pneumophila colony on buffered charcoal yeast extract (BCYE) agar after 3 days of incubation, viewed with a dissecting microscope (×20). Influenza can also cause a fatal viral pneumonia. Citrobacter freundii c. Tissue Stains Common tissue stains used in the histology department for detection of fungal elements are the periodic acid–Schiff (PAS), Gomori methenamine–silver nitrate, hematoxylin and eosin, Giemsa, and Fontana-Masson stains. pertussis is inhibited by fatty acids, metal ions, sulfides, and peroxides, constituents found in many media. are transmitted to humans primarily via aerosolized particles. The parameters that should be considered by a laboratory in selecting a method to be used should therefore include not only cost but also diagnostic yield, patient population, relative incidence of the parasite in the area, and number of specimens to be processed. Coronaviridae The coronaviruses, in the family Coronaviridae, and the genus Coronavirus, are enveloped helical viruses with ssRNA. Tissue Amebae Free-living, thermotolerant amebae found in soil and water (e.g., freshwater sources, domestic water supplies, swimming pools) usually feed on bacteria in the environment but are known to be capable of causing human disease. 714. DFA assays are available for numerous viruses, including adenovirus, influenza viruses A and B, measles virus, parainfluenza viruses (PIVs) 1 through 4, and RSV from respiratory specimens, HSV-1 and HSV-2 and VZV from cutaneous lesion material, and cytomegalovirus (CMV) from blood. Other infections associated with lactobacilli include intraabdominal abscesses, meningitis, oral infections, and conjunctivitis. Other tests have too many limitations (e.g., polymerase chain reaction) or have not been adequately validated (e.g., urine antigen, CD57 lymphocyte). The pentose phosphate pathway is on the left, and the EntnerDoudoroff pathway is on the right. The text has been updated to reflect pathogens newly recognized in the past decade, present new applications of immunologic and/or molecular approaches to diagnose infections and identify infectious agents, and determine antimicrobial resistance in microorganisms. In general, a range that is considered acceptable in this delicate balance is between 2% and 5% of bacterially contaminated mycobacterial cultures. brucei rhodesiense, is characterized by a rapid course, often resulting in death within 6 months after the onset of symptoms. belong to one of four serogroups: A (S. Microscopic examination reveals hyaline hyphae giving rise to hyaline conidia directly from the vegetative hyphae. Colwell.) FIGURE 20-2 O/129 Susceptibility test for Vibrio sp. Most authorities advocate identification with conventional biochemicals, supplemented with additional NaCl, if warranted; alternatively, the rapid system identification should be confirmed at a reference laboratory or state public health department laboratory. tahir99 VRG CHAPTER 27 Medically Significant Fungi probability of isolating the causative agent as a result of overgrowth by contaminants. (A and B, Courtesy of Dr. Andrew G. xylosoxidans bacteremia demonstrated that it is a cause of nosocomial infection associated with the use of IV catheters; patients of advanced age and neutropenia are most susceptible to serious and sometime fatal (15%) illness. This method provides a way for organisms to obtain new combinations of biochemical pathways and copy with changes in their environment. is often associated with the use of long-standing intrauterine devices (IUDs). Some dermatophytes are known to also have teleomorphic stages, in which ascospores are the reproductive cells. moderate growth of oxidase-negative, non–lactose-fermenting organisms. Adults live in the small intestine and produce few symptoms. This occurs within 2 to 3 days and can last up to 4 weeks. Most strains are lysine decarboxylase (LDC) and o-nitrophenyl-β-Dgalactopyranoside (ONPG) positive, whereas most strains are ornithine decarboxylase (ODC) negative and fail to reduce FIGURE 21-10 Burkholderia cepacia on selective media. At autopsy, there will be evidence of trophozoites along with a purulent exudate, edema, and hemorrhagic and necrotic areas of infection in the brain. dispar and was thought to be a freeliving organism. During lysogeny, genes present in the phage DNA may be expressed by the bacterial cell. For optimal results, a paper towel or moistened cotton ball with 5 mL of water should be placed inside the pouch. mekongi. Assimilation tests identify which carbohydrates a yeast can use aerobically as a sole carbon source. Some patients with AIDS have been given aerosolized pentamidine as a prophylactic treatment. Antibody can be detected by Western blot. Adult worms mature in the deep connective tissue, and the gravid females migrate to the subcutaneous tissue. triviale Smooth Rough P = 91% − N = 9% S = 98% N = 99% −(99) R = 2% S = < 1% P = < 1% R = 61% P = 99% −(66) S = 39% S = 99% P = 99% −(99) S = 95% S = 97% −(99) R = 5% P = 3% 22-35 Rough M. Subsequent studies showed the adhesive property of EPEC strains, a characteristic not seen in ETEC or EIEC strains. Infections with H. Colonies that form tan to buff conidial masses are typical of fresh isolates, but this species tends to develop pleomorphic tufts of white sterile hyphae in aging cultures and after serial transfers. Another example involves the investigation of a potential measles outbreak involving an international flight from Russia to the United States. Comparisons between CSF and serum antibody responses can be helpful in potential cases of neurosyphilis. If a purged specimen is to be collected, it is recommended that a saline or phosphosoda purgative be used because mineral oil droplets interfere with identification of parasites, especially protozoan cysts, an infective dormant form resistant to environmental stress. The acidity of the vagina, resulting from the breakdown of glycogen by the resident flora, tends to inhibit transient organisms from colonizing. aeruginosa accounts for up to 6% of all bacteremias and as many as 75% of nosocomial bacteremias and is the third most common cause of gram-negative bacillary bacteremia, after E. Which of the following is (are) fluorescent stain(s) used in the detection of the mycobacteria? Left, Resistant; right, susceptible. Finally, it is important to remember that C. A number of EIAs are available to detect the presence of Giardia lamblia, Cryptosporidium spp., E. are phaeoid, septate fungi. The ameba can be converted to the flagellate stage by adding one drop of CSF sediment to 1 mL of distilled water and incubating it at 37° C. Because of the similarity in tissue damage, the infection may initially be confused with bacterial or herpes simplex viral infection, delaying treatment. The virus emerged almost simultaneously in Sudan. tahir99 VRG 692 TABLE PART II Laboratory Identification of Significant Isolates 29-2 Tests Available for Common Viral Pathogens and Specimens for Culture Body System Affected Antigen Detection Virus Isolation Serology Culture Specimens Adenovirus, herpes simplex virus (HSV), cytomegalovirus (CMV), influenza virus types A and B, parainfluenza virus, respiratory syncytial virus (RSV) Adenoviruses 40 and 41, rotavirus Adenovirus, coxsackie A, coxsackie B, echovirus, HSV, CMV, influenza virus types A and B, parainfluenza virus, RSV, reovirus, rhinovirus Adenovirus, coxsackie A virus, coxsackie B virus, echovirus, HSV, CMV, influenza virus types A and B, parainfluenza virus, RSV Nasal aspirate, nasopharynx (NP) or throat swabs, bronchoalveolar lavage, lung biopsy Adenovirus 40 and 41, coxsackie A, reovirus Stool, rectal swab Cutaneous HSV, adenovirus, varicella-zoster virus (VZV) HSV, adenovirus, coxsackie group A virus, coxsackie group B virus, echovirus, enterovirus, measles virus, VZV, reovirus, rubella virus, vaccinia virus Central nervous system HSV, mumps virus Ocular Adenovirus, HSV Genital HSV Coxsackie group A virus, Coxsackie group B virus, echovirus, enterovirus, poliovirus, HSV, mumps virus Adenovirus, HSV, coxsackie group A, Enterovirus HSV Adenoviruses 40 and 41, coxsackie A Hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV), hepatitis E virus (HEV), Epstein-Barr virus (EBV) HSV, adenovirus, coxsackie group B virus, dengue virus, echovirus, human herpesvirus 6 (HHV-6), measles virus, VZV, parvovirus B19, rubella virus, vaccinia virus Coxsackie group A virus, coxsackie group B virus, echovirus, poliovirus, HSV, HHV-6, mumps virus HSV, coxsackie group A virus HSV Respiratory tract Gastrointestinal Liver Methods in Diagnostic Virology The clinical laboratory uses four major methods to diagnose viral infections: • Direct detection of the virus in clinical specimens • Nucleic acid–based detection • Isolation of viruses in cell cultures • Serologic assays to detect antibodies to virus Each laboratory must decide which of these methods to offer based on the spectrum of infections encountered, population of patients served, and financial resources. tahir99 VRG 26 PART I Introduction to Clinical Microbiology BOX 2-2 Microorganisms Common Staphylococcus epidermidis Streptococcus mitis Streptococcus sanguis Streptococcus salivarius Streptococcus mutans Peptostreptococcus spp. Other tissues or fluids are generally acceptable when suspicion is high and should be tahir99 VRG CHAPTER 18 Haemophilus and Other Fastidious Gram-Negative Bacilli submitted in sterile, leakproof containers. Colonies will also exhibit an earthy odor; however, work should be done in a biological safety cabinet when B. Because of the low specific gravity of the AFB, a low centrifugal force has a buoyant rather than a sedimenting effect. microti and Borrelia burgdorferi (the causative agent of Lyme disease) because these two organisms use the same tick vector. haematobium eggs (see Figure 28-46, B) are elongated and 110 to 170 µm × 40 to 70 µm and have a terminal spine. Human Herpesvirus 8 Human herpesvirus 8, in the genus Rhadinovirus, can be detected in all forms of Kaposi sarcoma, including AIDSrelated, Mediterranean, HIV-1–negative endemic to Africa, and post-transplantation Kaposi sarcoma. Infections caused by V. These include Sabouraud dextrose agar (SDA), SDA with antimicrobial agents, potato dextrose agar (PDA) or the slightly modified potato flakes agar, and BHI agar enriched with blood and antimicrobial agents. Urine submitted specifically for fungal culture should be centrifuged and the sediment used to inoculate media. Bacillary dysentery caused by Shigella spp. It is ideally suited for specimen types in which bacterial infections are strongly suspected, but it may be used to characterize any specimen. After an incubation period of 7 to 10 days, there is an abrupt onset, with symptoms of sneezing, runny nose and cough, red eyes, and rapidly rising fever. are pleomorphic, weakly staining gram-negative bacilli that are ubiquitous in aquatic environments and infect humans through the respiratory route. *Each number is the percentage of positive reactions after 2 days of incubation at 36° C unless noted otherwise. DCL To my daughters, Kristina and Shellie, my sisters, Libby and Helen, and my brother, Demetrious and, in memory of my parents, Katherine and George, for their love and encouragement. Fish-eating mammals in endemic areas may also be infected. Clinical symptoms include malaise, fever, pneumonitis, and hepatomegaly. Cysts of G. lansingensis Blue-White L. Neutropenia is the single most predictive factor for developing aspergillosis. gergoviae or E. MERS-CoV is unlike any other coronavirus that infects humans. The column size is recorded as greater than or less than 45 mm. Many new molecular methods based on nucleic acid detection and amplification are being used in more high-complexity, full-service virology laboratories. burgdorferi sensu lato causes a spectrum of syndromes known as Lyme disease. In the past, when Acinetobacter spp. Second, the antibody-producing capabilities of human hosts vary widely. The K. However, there have been cases of bacteremia, without any known animal exposures. Lactobacilli in the urogenital tract of women offer a mutual association: the lactobacilli provide the host protection by preventing colonization of pathogenic species at that site while they derive nutrients from the host. White blood cells and red blood cells are typically absent from the stool. His two teammates were on doxycycline as malaria prophylaxis before and during the race. The slants are inoculated and incubated at room temperature for 48 hours. recurrentis and similar species cause relapsing fever. coli and C. Such cases have also been documented as progressing to necrotizing fasciitis and/or multiple organ system failure. Fats and vitamin B12 are two of the substances that may be incompletely absorbed in chronic infection, but macrocytic anemia is not common. For the major human pathogens, describe the mechanism of pathogenesis, clinical symptoms, treatment, and prevention. Several methods can be used to obtain the required environment for campylobacters. Once the DNA has been taken up, it can be incorporated into the bacterial genome by recombination. Vaccination of dogs and livestock has been effective in preventing disease but not the initial infection and leptospiruria. A small amount (2 mg) of feces is added to each drop and mixed well. Now, however, several FDAapproved, type-specific assays are available that differentiate antibody response to HSV. Hemorrhages at the site of attachment, however, also contribute to total blood loss. A common factor in infection is the report of recent swimming or other water-related activities in warm, artificial lakes or brackish or muddy water. A variety of approaches to the phenotypic identification of aerobic gram-negative bacilli are available. Identification of anaerobes by the use of conventional biochemicals is expensive and time-consuming and has been replaced largely in clinical laboratories by the systems described earlier. • Formaldehyde, 3% to 8%, or alkaline glutaraldehyde, 2%, is effective. ✓ Case Check 2-1 The patient in the Case in Point at the beginning of this section had surgery followed by a course of antibiotics. Young children might experience apnea, pneumonia, or both and require aid in maintaining a patent airway. infection may be influenced by several factors, including the organism's ability to enter, survive, and multiply within the host's cells, especially bronchoalveolar macrophages, and the ability to produce proteolytic enzymes. General Characteristics The family Enterobacteriaceae, often referred to as enterics, consists of numerous diverse organisms. Centers for Disease Control and Prevention: Multistate outbreak of E. The infection resolves within several weeks when the larva dies. Because many bacteria grow better in the presence of increased carbon dioxide, diagnostic microbiology laboratories often maintain their aerobic incubators at a 5% to 10% carbon dioxide level. coli is nonproductive because this organism will not feed on gram-negative bacteria. In the 1920s, Phoebus A. The cAMP causes the active secretion of sodium (Na), chloride (Cl^-), potassium (K^+), bicarbonate (HCO_3^-), and water out of the cell into the intestinal lumen. Over time, involved portions of the trunk develop diagnostically distinctive concentric rings of scaling tissue. The immune response to the organism after it attaches to and destroys alveolar cells is partly responsible for this radiographic pattern. veronii biovar veronii, A. Trichophyton mentagrophytes Trichophyton mentagrophytes makes microconidia and macroconidia. Clinically Significant Species Bordetella pertussis and Bordetella parapertussis Virulence Factors B. parahaemolyticus has an association with at least 30 different marine animal species, including oysters, clams, crabs, lobsters, scallops, sardines, and shrimp. Interpret the results of a hepatitis serologic profile. holmesii and B. stutzeri, which also produces a yellow pigment. 525. montelilii; sequence identification or MALDITOF is needed for their specific identification. Stained bacteria sometimes reveal the presence of granules in the cytoplasm (cytoplasmic granules). tahir99 VRG 690 TABLE PART II Laboratory Identification of Significant Isolates 29-1 List of Viruses Causing Human Disease, Based on Nucleic Acid Characteristics and Taxonomy Genome Strand dsDNA Family (Subfamily) Genus Species Adenoviridae Herpesviridae (Alphaherpesvirinae) Mastadenovirus Human adenovirus C Simplexvirus Varicellovirus Cytomegalovirus Roseolovirus Human herpesvirus 1, 2 Human herpesvirus 3 Human herpesvirus 5 Human herpesvirus 6 Human herpesvirus 7 Human herpesvirus 4 Human herpesvirus 8 Human papillomavirus Variola virus, vaccinia virus Hepatitis B virus Parvovirus B-19 Human Bocavirus Rotavirus A Colorado tick fever virus Lymphocytic choriomeningitis virus, Lassa virus Human astrovirus 1 California encephalitis virus Hantaan virus, Sin Nombre Crimean-Congo hemorrhagic fever virus Rift Valley fever virus Norwalk virus Sapporo virus SARS coronavirus Lake Victoria marburgvirus Zaire ebolavirus Yellow fever virus West Nile virus Hepatitis C virus Hepatitis E virus Influenza A virus Influenza B virus Influenza C virus (Betaherpesvirinae) (Gammaherpesvirinae) dsDNA, ssDNA ssDNA Papillomaviridae Poxviridae Hepadnaviridae Parvoviridae dsRNA Reoviridae ssRNA Arenaviridae Astroviridae Bunyaviridae Caliciviridae Coronaviridae Filoviridae Flaviviridae Hepeviridae Orthomyxoviridae Paramyxoviridae (Paramyxovirinae) (Pneumovirinae) Picornaviridae Rhabdoviridae Retroviridae Togaviridae Lymphocryptovirus Rhadinovirus Papillomavirus Orthopoxvirus Orthohepadnavirus Erythrovirus Bocavirus Rotavirus Coltivirus Arenavirus Mamastrovirus Orthobunyavirus Hantavirus Nairovirus Phlebovirus Norovirus Sapovirus Coronavirus Marburgvirus Ebolavirus Flavivirus Japanese encephalitis complex Hepacivirus Hepevirus (was "hepatitis E–like viruses") Influenzavirus A Influenzavirus B Influenzavirus C Respirovirus Morbillivirus Rubulavirus Henipavirus Pneumovirus Metapneumovirus Enterovirus Rhinovirus Hepatovirus Lyssavirus Lentivirus Sendai virus Measles virus Mumps virus Hendra virus Human respiratory syncytial virus Human metapneumovirus Poliovirus Human rhinovirus A Hepatitis A virus Rabies virus Human immunodeficiency virus 1, human immunodeficiency virus 2 Venezuelan equine encephalitis, eastern equine encephalitis, western equine encephalitis Rubella virus Alphavirus Rubivirus Viral Replication Viruses are obligate intracellular parasites, meaning that they must be inside a living cell and use the host cell machinery to replicate. Marciano-Cabral F, Cabral GA: The immune response to Naegleria fowleri amebae and pathogenesis of infection, FEMS Immunol Med Microbiol 51:243, 2007. Table 22-3 lists the anaerobes most frequently recovered from clinical specimens at a university medical center, including members of the B. At this stage, the infection is highly communicable because of the large number of organisms in the respiratory tract. Ideally, this procedure is performed in an anaerobic chamber. Proteus vulgaris c. fluorescens is positive. The sensitivity of the assay varies from 95% to 100%, depending on the species and species complexes. capsulatum (×1000). KOH with Calcofluor White A drop of calcofluor white (a fluorescent dye) can be added to the KOH preparation before adding a cover slip. lwoffii is much less virulent and, when isolated, usually indicates contamination or colonization rather than infection. If, however, cultures are not thoroughly decontaminated, overgrowth is a problem. Walker, PhD, MT(ASCP) Manager, Public Affairs American Society for Microbiology Washington, DC A. Presley SM, et al: Assessment of pathogens and toxicants in New Orleans, LA following Hurricane Katrina, Environ Sci Technol 40:468, 2006. 13. The CD4 molecule serves as a receptor for HHV-7 to infect T lymphocytes. Rinse with running tap water. Pet hamsters are also reservoirs. stercoralis rhabditiform larva. Organisms like Brucella spp. Two types of meronts develop—type I, which produce merozoites that infect other intestinal cells (asexual cycle), and type II merozoites, which progress into the sexual stage. concisus, C. Case in Point (reference range, 0.5% to 2%). Medical history revealed the mother to be a 17-year-old intravenous drug abuser with no prenatal care, who had a *My comments are my own and do not represent the views of the Health Resources and Services Administration of the Department of Health and Human Services. Eradication of the usual flora may have profound negative effects, yet many common infections are caused by members of the usual flora. Subcutaneous d. Light infections with T. nov., Int J Syst Bacteriol 33:336–343, 1983. • Were all the Salmonella spp. A B FIGURE 28-65 A, Strongyloides stercoralis rhabditiform larva, buccal capsule. Available at: http:// www.cdc.gov/foodborneburden/2011-foodborne-estimates.html. The stool should be delivered to the laboratory as soon as possible after collection or a portion placed immediately in a preservative. Immunocompromised patients suffer a prolonged course and can be symptomatic for as long as 4 months. Enterotoxin produced by certain Salmonella strains that cause gastroenteritis has been implicated as a significant virulence factor. Be certain that all suspected cases meet the definition. It is endemic in the tropics and subtropics, including Southeast Asia, Latin American, and sub-Saharan Africa. When direct fluorescent antibody (DFA) testing is used for endocervical or urethral specimens, the sensitivity is 80% to 85%. Marieb EN: Essentials of human anatomy and physiology, ed 8, San Francisco, 2006, Pearson Benjamin Cummings. baumanii species. Infections not involving the skin or deeper tissues just under the skin are termed systemic. In the United States, it is endemic in the Mississippi and Ohio River basins. Animal inoculation is extremely costly, used only as a special resource and in reference or research laboratories. Cryptosporidium spp. histolytica, is a nonpathogen. Abbott SL, et al: Vibrio and related organisms. While this is sometimes useful, it is usually 3 to 4 weeks after infection before these antibodies are produced, which may mean treatment is too late or not needed. Histologic preparations of the brain at autopsy show inflammatory lesions containing many segmented neutrophils, eosinophils, and trophozoites. DNA is also involved in the production of RNA. pneumoniae–specific IgM and IgG by MIF CF, Complement fixation; IgG, immunoglobulin G; IgM, immunoglobulin M; MIF, microimmunofluorescence. Identification of Clostridium Species An identification algorithm for some Clostridium spp. A double zone of hemolysis appears as an inner zone of tahir99 VRG CHAPTER 22 Anaerobes of Clinical Importance 519 Double zone of hemolysis on blood agar Yes No Clostridium perfringens* Lecithinase reaction on egg-yolk agar Positive Negative Lipase reaction on egg-yolk agar Lipase reaction on egg-yolk agar Positive Negative Positive Negative Clostridium novyi A Spot indole Clostridium sporogenes Use definitive methods outlined in Table 22-9 Positive Negative Rapid urease Gelatin hydrolysis Positive Negative Positive Negative Clostridium sordellii Clostridium † bifermentans Clostridium limosum Clostridium barati * Boxcar-shaped bacilli; lecithinase-positive; reverse CAMP (Christie, Atkins, and Munch-Petersen) test–positive; subterminal spores, but spores rarely observed † Produces chalk-white colonies on egg-yolk agar FIGURE 22-23 Identification of Clostridium spp. pneumoniae– and C. An additional test to separate aeromonads and plesiomonads from most vibrios is determining the ability to grow in the presence of NaCl. Aeromonads and 465 FIGURE 20-8 Both Aeromonas and Plesiomonas spp. Production of phenol oxidase is a feature differentiating C. • Subculture the isolate to BCYE agar with L-cysteine and to either SBA or BCYE agar without L-cysteine. The faint-staining, pleomorphic gram-negative bacilli can be found outside of and within macrophages and segmented neutrophils (Figure 18-26). Enteroviruses The enteroviruses found in the genus Enterovirus include the following: • Polioviruses 1 to 3 • Coxsackieviruses A1 to A23 • Coxsackieviruses B1 to B6 • Enteroviruses 68 to 104 (HEV A, HEV B, HEV C, and HEV D) • Echoviruses l to 33 • Parechovirus 1 to 4 These small naked viruses cause various conditions, including fever of unknown origin, aseptic meningitis, paralysis, sepsislike illness, myopericarditis, pleurodynia, conjunctivitis, exanthemas, pharyngitis, and pneumonia. Name and compare three biochemical pathways that bacteria use to convert glucose to pyruvate. A Gram-chromotrope stain will produce spores that are dark violet and demonstrate an equatorial band. Results of a white blood cell count of the cerebrospinal fluid were negative, the Gram stain was reported as "no organisms seen," and routine culture at 3 days was "no growth." The infant was still symptomatic at this time, and the pediatric infectious disease physician, after consultation with the microbiology laboratory, performed another spinal tap and ordered additional cultures. The patient also experiences headache, nausea, and chills. Points to Remember ■ B Bacterial chromosome Bacterial chromosome ■ Plasmid DNA containing the F factor entering the F– cell ■ ■ F– cell ■ F+ cell C ■ Bacterial chromosome FIGURE 1-14 Diagram of methods of gene transfer into bacterial cells. In the hospital-associated infections, most presented with pneumonia. heimbachae has yet to be isolated from any clinical specimens. expands. In addition, infants may contract the disease in utero and present with varying degrees of congenital problems. Influenza viruses grow in the amniotic cavity of embryonated chicken eggs and various mammalian cell tahir99 VRG CHAPTER 29 Clinical Virology culture lines, such as PMK and MDCK cells. Chapter 12 has been updated by incorporating discussions on the use of nanotechnology in drug delivery systems, and Chapter 39 includes a discussion of biomarkers in the diagnosis of septicemia. Working Solution PROCEDURE 0.1% calcofluor white containing 0.01% to 0.08% Evans blue as a counterstain. Escherichia coli c. Clinical Manifestations Gastroenteritis Unlike the genus Aeromonas, at least three well-documented outbreaks of diarrheal disease caused by Plesiomonas have occurred, two in Japan and one in Cameroon. The antigenemia test may prove helpful in assessing the efficacy of antiviral therapy. No growth on MAC agar is typical. In other bacteria, the peptidoglycan layer is exposed after other agents have damaged the cell wall (e.g., antibody and complement, hydrogen peroxide). tularensis subsp. Symptoms include drowsiness, seizures, loss of reflex activity, hemiparesis, headache, stiff neck, and personality disorders. Most of the positive reactions occur during the first 24 hours. Congenital syphilis affects many body systems and is therefore severe and mutilating. tahir99 VRG CHAPTER 27 Medically Significant Fungi Clinical Manifestations. Chromoblastomycosis is a chronic mycosis of the skin and subcutaneous tissue that develops over a period of months or, more commonly, years. Cow's milk has a greater buffering capacity and is less acidic. • Small, opaque colonies that are catalase- and indole-positive and stain as coryneform rods can be identified as P. Available at: . • Do not overinterpret the findings. Once acquired, H. This section discusses the role of each of the factors in hostpathogen interaction and how the host interacts with the microbe to prevent the onset of a disease process. It is an 80-nm spherical particle with two outer shells containing 12 RNA segments. Casalta JP, et al: Prosthetic valve endocarditis caused by Pseudomonas luteola, BMC Infect Dis 5:82, 2005. Explain. Spores are often not observed in Gram-stained smears of clinical specimens containing clostridia or in smears of colonies from an agar plate unless the culture has been incubated for many days. The anaerobic jar is evacuated to a pressure of 15 inches Hg at least twice and refilled each time with one of the following gas mixtures: 10% CO_2, 90% N_2; 5% CO_2, 10% H_2, 85% N_2; or 10% CO_2, 10% H_2, 80% N_2. As noted, a boxcar-shaped, gram-positive anaerobic bacillus that produces a characteristic double zone of hemolysis on SBA can be presumptively identified as C. • Is the infection monomicrobial or polymicrobial? coli on most ordinary enteric media. ozaenae has been isolated from nasal secretions and cerebral abscesses. veronii biovar sobria from medicinal leech therapy cases. Most cases are selflimiting, but antimicrobial therapy is indicated in severe and prolonged cases. The DFA test, discussed subsequently under Rapid Methods, also provides a useful method of confirming that an isolate is a Legionella species and for identifying the more common species and serogroups of the genus. When clinical symptoms persist and the organism cannot be demonstrated in the feces, or when the patient does not respond to treatment, a duodenal aspirate or the Enterotest has been used to detect the organism. HIV-1 is responsible for the AIDS pandemic, with about 34 million people living with HIV-AIDS at the end of 2011, with approximately 7400 new cases each day. strain produces a hollow surface appendage called a sex pilus, which binds to the recipient F− cell and brings the two cells in close contact. No male has been identified in intestinal infections. Preliminary Procedures When to Examine Primary Plates Recently, the emphasis on anaerobic bacteriology has shifted from full identification of all isolates to rapid, presumptive identification of the most commonly isolated anaerobes. World Health Organization: Global tuberculosis report, 2012. Animals such as armadillos and opossums may also be infected with the organism and serve as reservoirs for infection. Antigenic shift is only associated with influenza A. These daughter cells (2 to 5 µm) are connected by a narrow base, unlike the broad-based attachment in B. grow only on BCYE agar supplemented with L-cysteine. tahir99 VRG CHAPTER 19 Enterobacteriaceae Obesumbacterium Obesumbacterium proteus biogroup 2 is more closely related to Escherichia blattae than to other members of the family Enterobacteriaceae. mexicana complex—is characterized by lesions on the ear. After 72 hours of incubation, gray-white, raised colonies with a smooth appearance are visible. It is estimated that 100 to 200 million people are infected worldwide. This medium, which inhibits normal colon microbiota better than MAC agar, provides better opportunities to recover Y. Finegoldia magna is the most pathogenic of the anaerobic cocci and the one most often isolated in pure culture. Parvovirus B19 viremia can cause transient aplastic crisis, a self-limited erythropoietic arrest. immobilis organisms were also reportedly isolated from the blood and CSF of a 2-dayold infant. Symptoms of infection with C. Spirochetes vary in length and in the number of helical turns (not all helical bacteria are called spirochetes). This system provides many of the same tests as the conventional systems but in the form of a plastic strip or tray. The organism can alter surface antigens to evade host defenses. The effects of shigella toxin have been implicated as the mechanism responsible for the signs of disease, and it has been reported that the detectable toxin levels produced by S. Occasionally evident in direct smears from clinical materials, this capsule is sometimes helpful in providing a presumptive identification. tuberculosis complex rRNA in smear-positive and smear-negative respiratory specimens. The cardiovascular system is most susceptible. A chromatogram depicting the unknown organism's fatty acid composition and comparison to a database of fatty acids of known anaerobes are generated as a computer report. • What reservoirs may harbor them? East African sleeping sickness, FIGURE 28-23 Amastigotes of Leishmania spp., identified by arrows (Giemsa stain, ×1000.) caused by T. As of 2011, new cases of HIV in the United States were associated with the following: • Heterosexual contact (27%) • MSM (61%) • IV drug use (9%) • Heterosexual contact and IV drug use (2%) • Transfusions or mother to infant transmission (1%) HIV is a spherical virus, with a three-layer structure (Figure 29-16). Environmental cultures are usually to be avoided, except in: a. If so, is the morphology sufficiently characteristic to presume identification? Readily grows on most mycobacterial media c. The single agents of infection or agents causing classic infections are easily recognized by microscopy and require limited interpretations. Hair shafts are removed and microscopically examined in LPCB at weekly intervals for up to 1 month. When this occurs, extraintestinal amebiasis often results. Even, fine peripheral chromatin may be only slightly visible. Sometimes the surface antigens can change drastically, causing an tahir99 VRG 710 PART II Laboratory Identification of Significant Isolates antigenic shift, resulting in a new H or N antigen. Infected persons with dark skin tones typically scar from this infection because of the depth of the pustules. Flagella can be visualized microscopically with special flagellum stains. A facultative, noncapnophilic organism will grow on all plates, but a capnophilic aerobe should grow only on the CO_2-incubated chocolate agar plate. from highly B FIGURE 18-27 A, Nonselective buffered charcoal yeast extract (BCYE) agar plate inoculated with a sputum specimen. The focus of this chapter and this textbook is on microbes that are associated with disease. Viremia can result in the virus spreading from these locations to the spinal cord, heart, and skin. tuberculosis, are nonchromogenic or nonphotochromogenic. have been recognized for many years as the amebae most commonly associated with CNS invasion in humans. Factors That Predispose Patients to Anaerobic Infections Factors that commonly predispose the human body to anaerobic infections include trauma to mucous membranes or the skin, vascular stasis, and decreased oxygenation of tissue, leading to tissue necrosis and a decrease in the redox potential of tissue. It cannot be used as a test of cure because antibodies can persist for years after treatment. Thiophene-2-Carboxylic Acid Hydrazide. T2H distinguishes M. Trichomonas vaginalis. T. Antimicrobial susceptibility testing is not normally performed on B. Most infections occur from May to September, when outdoor activities peak and exposure to ticks is highest. Several substances serve as chemotactic agents. cepacia (Figure 21-10). The egg is the primary diagnostic stage. Compare the pneumonia caused by Mycoplasma pneumoniae with that caused by Streptococcus pneumoniae. Gram Smear Report The contaminating materials are quantitated as 1+ (light), 2+ (moderate), 3+ (moderately heavy), or 4+ (heavy). Rhizopus spp. Members of this genus are motile. B, Chartreuse fluorescence of Fusobacterium nucleatum. The domains Bacteria and Archaea include unicellular prokaryotic organisms. Laboratory scientists should be alert to the isolation and identification of Brevundimonas and consider that the isolate can be a significant pathogen. Similar infections resulting from this organism are uncommon in older children. IgG can cross the maternal placenta to the fetus, possibly conferring some protection in both the prenatal and the postnatal periods. Generally, person to person transmission does not occur with hantavirus. grow moderately rapid to rapidly and have a yeasty consistency. osloensis, M. Such discoloration may be caused by the pigment produced by pigmented species of Porphyromonas and Prevotella. parapertussis–like colonies with Gram stain and fluorescent antibody test or agglutinating antisera. To perform serologic grouping by slide technique, a suspension from a pure culture of the organism is prepared in sterile saline. The maculopapular patches eventually consolidate into larger areas of ecchymoses. Cellophane Tape Preparation. Cellophane tape preparations involve gently touching a piece of clear tape, sticky side down, to the surface of the colony and then removing it. Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen, and certain serovars are associated with trachoma, which can result in blindness. rettgeri, especially in immunocompromised patients, are particularly difficult to treat because of their resistance to antimicrobials. At this time, the patient experiences constipation rather than diarrhea. The genus Shigella is named after the Japanese microbiologist Kiyoshi Shiga, who first isolated the organism in 1896. Colony color is initially white, becoming gray to gray-brown with age. The CDC Pertussis Laboratory does not recommend incubation of specimens in the transport medium because of overgrowth of commensal biota and decreased yield of B. perfringens (Figure 22-18). tahir99 VRG 486 PART II Laboratory Identification of Significant Isolates In the United States, the members of B. Antibiotic Susceptibility Testing No antibiotic susceptibility testing is appropriate. On average, 70 cases/year were reported from 2001 to 2011. When fermentation occurs, a mixture of end products (e.g., lactate, butyrate, ethanol, and acetoin) accumulates in the medium. trachomatis infection rate in women to improved screening, increased use of nucleic acid amplification tests, better reporting, and ongoing high burden of disease. About 25% of adults carry the organisms on their skin, and about 7% carry the organism in their pharynx. It is spread by fomites or the oral-fecal route. In 2011, a total of 1,412,791 cases of genital infections were reported, but many infections are undiagnosed, and the Centers for Disease Control and Prevention (CDC) estimates that 2 to 3 million new cases occur annually in the United States. Within the M. In these cases, the organism is sequestered in the placenta and can compromise fetal development because it affects transplacental transport of nutrients. mirabilis is differentiated from P. This Kluyvera species resembles the colony morphology of Escherichia coli growing on MacConkey (MAC) agar. (Courtesy Edwin P. Fecal material is spread on a slide, reagent containing the antibody is added, and the specimen is examined with a fluorescent microscope for a characteristic Quality Assurance in the Parasitology Laboratory Quality assurance procedures in the parasitology laboratory are similar to those in other laboratory sections. Facultative anaerobes can grow either with or without oxygen. Indigenous Microbial Flora Nonpathogenic microorganisms compete with pathogens for nutrients and space. The egg is unembryonated when passed, operculated, and yellow-brown (Figure 28-49). In 2009, EHEC was the cause of a multistate outbreak linked to prepackaged cookie dough. No part of this publication may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without written permission of the publisher. This arm of the immune response is capable of being specific for distinct molecules, responding in particular ways to different types of foreign substances and developing memory, which allows for a more vigorous response to repeated exposures to the same foreign invader. ISBN 978-0-323-08989-0 (hardcover) I. Mahon, Connie R., editor of compilation. II. Lehman, Donald C., editor of compilation. III. Manuselis, George, editor of compilation. A, Brick red fluorescence observed with Porphyromonas asaccharolytica. vaginalis, a pathogen of the urogenital tract in men and women, causes trichomoniasis, one of the most common, nonviral, sexually transmitted diseases, with an estimate of 180 million cases worldwide. Survival rates and clinical outcomes are greatly improved with IV antiviral treatment. • Is there evidence of contamination by normal (resident) microbial flora? The viruses are spread through respiratory secretions, aerosol inhalation, and direct contact. Bacteria that grow best at high temperatures are called thermophiles (optimal growth at 50° to 60° C). Metacyclic trypomastigotes in the feces are scratched into the bite site and invade the bloodstream. Tellurite Reduction. Reduction of colorless potassium tellurite to black metallic tellurium in 3 to 4 days is a characteristic C + – FIGURE 26-17 Urease test. Although significant increases in serum IgA may occur in association with certain infections, the function of serum IgA is unclear, and few serologic tests for the diagnosis of infectious disease are designed specifically to detect IgA antibody. Lyme disease was first described after an outbreak among children in Lyme, Connecticut, in 1975. To further reinforce learning, identification tables, flow charts, and featured illustrations have been updated, and new ones have been added. With greater immunosuppression, the variety of organisms implicated in disease also tahir99 VRG 612 PART II Laboratory Identification of Significant Isolates spp. Kim DM, et al: In vitro efficacy of the combination of ciprofloxacin and cefotaxime against Vibrio vulnificus, Antimicrob Agents Chemother 49:3489, 2005. aureus (VRSA), vancomycinintermediate S. Recognize the initial clues to nonfermentative organisms isolated in clinical specimens. Well water, food, and food preparation sites were cultured. belli. brasiliensis, requires confirmatory identification, typically by DNA probe. A patient with a heavy worm burden may have diarrhea, colic, and loose stools, with a large amount of mucus. Based on the composition of the O antigen, V. conorii are considered potential bioterror agents. (Nomarski optics, ×450.) FIGURE 27-50 Phoma sp. Sarinas PSA, Chitkara RK: Brucellosis, Semin Respir Infect 18:168, 2003. Treatment with quinine sulfate and clindamycin is effective in eliminating the infection. This activation leads to phagocytic clearance and elimination of the bacteria. cephalosporins, and fluoroquinolones. Direct specimen examination offers one additional important advantage—it allows for immediate quality control of the specimen, revealing whether columnar epithelial cells are present. When the RBC ruptures, merozoites are released to invade other RBCs. Chromatin mass Ring-form trophozoite Red blood cell Malarial cytoplasm Chromatin Mature trophozoite Pigment granules Malarial cytoplasm Chromatin masses Immature schizont Pigment Merozoites Mature schizont Chromatin mass (loosely organized) Microgametocyte Pigment Cytoplasm Macrogametocyte Pigment Chromatin (condensed) FIGURE 28-28 Life cycle stages of malaria. tonsurans replaced it as the leading cause of scalp infection. A fourfold increase in verotoxinneutralizing antibody titer has been demonstrated in patients with HUS and in whom verotoxin or verotoxin-producing E. The largest barrier to widespread antifungal testing is the lack of established breakpoints for most of the agents. sonnei is also ONPG-positive. Most cases of Salmonella gastroenteritis are self-limiting. 5. Form a hypothesis. Hydrogen is explosive, and if the catalyst is not functioning properly, hydrogen gas will accumulate in the jar. mentagrophytes typically forms perpendicular penetration pegs in the hair shafts (Figure 27-57). They are usually susceptible to the aminoglycosides, polymyxin, and piperacillin, but are resistant to carbenicillin and SXT. The patient in the Case in Point was a bone marrow transplant recipient and would fall into the category of an at-risk group for development of infections with nonfermenters. caviae A. In the United States, most cases of leptospirosis disease result from recreational exposures. veronii biovar veronii A. However, some are fastidious, requiring special vitamins, amino acids, and hemin for adequate growth. A number of rapid detection tests are available that can detect toxin A, toxin B, an enzyme called glutamate dehydrogenase, or a combination of toxins A and B. Both of these pseudomonads are gram-negative, nonfermentative, oxidase-negative bacilli. Size 673 FIGURE 28-55 Dipylidium caninum egg packet (unstained, ×400). cholerae produces a powerful enterotoxin and is responsible for large numbers of epidemics and pandemics. Most geophilic fungi produce large numbers of conidia and therefore are among the most readily identified species. A number of manufacturers now market self-contained fecal concentration kits that do not use the solvent ethyl acetate. Right, Colonies of B. The term aseptate, meaning the absence of septations, has historically been used to describe the hyphae of the Zygomycetes. If the safranin is left on the slide for a prolonged period (minutes), the grampositive complex will be leached from the positive cells; however, failure to leave the safranin in place for sufficient time will result in failure to stain gram-negative bacteria and background materials. HGA is not a reportable disease in all states, so the number of cases is probably underreported. ■ Fecal-oral transmission is the route of infection for enteric protozoa. This relieves the concern that freshly inoculated plates will remain at room temperature in a holding system that may contain some oxygen. Before 1983, it was a member of the genus Pseudomonas; it was later reclassified as a member of the plant pathogen genus Xanthomonas. Sexual reproduction b. Several strains may exist, which could explain the differences in clinical presentation. These isolates are identified by the differences in their carbohydrate assimilation patterns and other secondary testing procedures. Trabulsiella Trabulsiella guamensis is the only species in this genus, and although it is very rarely isolated, it is biochemically similar to Salmonella. The mature trophozoite is small and compact and may have dark brown pigment. Note the dark pigmentation, which is caused by the presence of melanin in the cell wall. Yang CH, L YH: Chromobacterium violaceum infection: a clinical review of an important but neglected infection, J Chinese Med Assoc 74:435, 2011. Fungi are not plants and have no chloroplasts. recurrentis, B. 1. An isolate from a wound infection is oxidase-, catalase-, ornithine decarboxylase-, indole-, and urease-positive. Q fever is highly contagious and, as such, is considered a potential bioterror agent (see Chapter 30). GBS is believed to be an autoimmune disorder resulting from cross-reactivity of Campylobacter antibodies with the nerve ganglia. Typhoid fever occurs more often in tropical and subtropical areas, where international travelers are more likely to acquire the infection. It is also important to confirm biochemically the identification of MUG-negative or sorbitol-negative colonies as E. The antigenemia assay is specific, sensitive, rapid, and relatively easy to perform. The conidia are readily found in the environment and can be recovered in air-settling plates. Common observations can be coded so that they are consistent among observers. Among individuals infected with human immunodeficiency virus (HIV), as well as those receiving prolonged antibacterial therapy or other chemotherapeutic agents, thrush manifests as a serious infection capable of dissemination. However, electron microscopy is useful to detect noncultureable viruses, such as the Norwalk viruses in stool filtrates. Most patients infected with C. The Quantiferon-TB Gold assay (Cellestis, Carnegie, Victoria, Australia) and the T.SPOT.TB (Oxford Immunotec, Oxford, England) measure the cell-mediated immune response in whole blood samples to mycobacterial antigens. Adults lining in the veins can be killed with praziquantel but the drug does not affect the eggs in the tissue. The nucleus and cytoplasm divide to form individual organisms known as merozoites. Patients being treated with AMB may suffer from many adverse side effects, including fever, rigors, renal impairment, and possibly shock. Pigment on the reverse side of the colony or in the aerial mycelium can be noted but is not always helpful, especially with the phaeoid fungi. piechaudii has been isolated from the ear of a diabetic patient. Proteus mirabilis d. The bacteria should be exposed to room air for about 20 minutes before testing. Honey contaminated with the C. All species of the genus are urease-positive, and the nitrate reaction varies. Culture Identification Guidelines "New culture" using careful collection technique should be requested. mirabilis can lead to struvite kidney stones (calculi). Barakat FM, et al: Cryptosporidium

parvum infection rapidly induces a protective innate immune response involving Type I infection, J Infect Dis 200:1548, 2009. Laboratory Diagnosis Specimen Collection and Transport Vibrios are not fastidious, and only a few special collection and processing procedures are necessary to ensure the recovery of vibrios from clinical material. tahir99 VRG 426 PART II Laboratory Identification of Significant Isolates hydrochloric acid within the stomach) seem to be at higher risk than are normal individuals. These are most useful in epidemiologic studies. Baxt LA, Singh U: New insights into Entamoeba histolytica pathogenesis, Curr Opin Infect Dis 21:489, 2008. Consultation with the laboratory technologist often is sought when construction is anticipated. A mental inquiry checklist should be followed until the habit of searching a slide systematically is developed. If the DNA is a circular plasmid and the recipient cell is compatible, the plasmid can replicate in the cytoplasm and be transferred to daughter cells. The best example of the phenomenon is the teleomorph Pseudallescheria boydii, which has two anamorphs, Scedosporium boydii and Graphium sp. With primary cell lines, only minimal cell division occurs. Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. cinaedi has been isolated from the blood of patients with bacteremia and patients with HIV infection. The fifth and sixth steps are to form a hypothesis about the event and then to test that hypothesis. Agar-Based Media Serum albumin agar media, such as Middlebrook 7H10 and 7H11 agars, are prepared from a basal medium of defined salts, vitamins, cofactors, glycerol, malachite green, and agar combined with an enrichment consisting of oleic acid, bovine albumin, glucose, and beef catalase (Middlebrook OADC enrichment). Raoutella (Klebsiella) ornithinolytica (indole and ornithine decarboxylase-positive) and Raoutella (Klebsiella) planticola have been isolated from the urine, respiratory tracts, and blood of humans. Growth of a particular morphotype can be semiquantitated using terms such as light, moderate, and heavy or a coding system (e.g., 1+, 2+, 3+). neoformans (see Figure 27-53). Figure 19-10 illustrates the growth of S. (From Kumar V, Abbas AK, Fausto M: Robbins and Cotran pathologic basis of disease, ed 7, Philadelphia, 2005, Saunders.) Microbial biota, cilia, mucus Mucus, ciliated cells Skin, fatty acids, microbial biota Lower pH, urogenital tract, microbial biota Mucus and other secretions Saliva Gastrointestinal biota, gastric acid Emptying of the bladder FIGURE 2-5 Innate immune defenses located at different body sites. After the egg is ingested, larvae hatch in the duodenum, penetrate the intestinal wall, and gain access to the hepatic portal circulation. In contrast to DNA, RNA is single-stranded and short, not double-stranded and long, and contains the sugar ribose, not deoxyribose. In the United States, LACV infects as many as an estimated 300,000 persons annually; about 80 to 100 have severe disease of the CNS. The organism is often found concurrently with whipworm. Shown are the media and atmospheric conditions that would support the growth of various organisms contained in the specimen. The female has a straight posterior, and the male has a curved posterior. The average size of the cyst is 15 to 25 µm. Other family members may have been transiently infected because of waning immunity and served as the source of the organism. 57 BOX 3-1 Examples of Emerging and Reemerging Pathogens Emerging Pathogens • • • • Avian influenza virus Coronavirus (SARS) Viral hemorrhagic fever viruses West Nile virus Reemerging Pathogens • • • • • Anthrax (Bacillus anthracis) Botulism (Clostridium botulinum) Plague (Yersinia pestis) Smallpox virus Tularemia (Francisella tularensis) • How are these microbes spread? Anaerobic Utilization of Pyruvic Acid (Fermentation) Pyruvic acid is a key metabolic intermediate. The venereal transmission of syphilis was not recognized until the eighteenth century. It was first recognized as a pathogen in Japan in 1950, when it was the cause of a large food poisoning outbreak; even today it is the primary cause of so-called summer diarrhea in Japan. However, these mechanisms are effective primarily against extracellular bacterial pathogens, playing only a minor role by themselves in immunity to intracellular bacterial pathogens, viruses, and fungi. Available at: http:// www.cdc.gov/salmonella/wandsworth.htm. Such a situation can occur with specimens from patients who are on antifungal therapy, which may inhibit growth in vitro even though the infection can still be present in the patient. jandaei, and A. All pathogenic Borrelia are arthropod-borne. solium, are identical and must be reported as Taenia sp. duodenale. mucosalis and C. tertium have also been isolated from blood cultures from patients with serious underlying disease. Diagnostic schemes analyze each unknown microorganism for: (1) utilization of various substrates as a carbon source, (2) production of specific end products from various substrates, and (3) production of an acid or alkaline pH in the test medium. In Garcia LS, Isenberg HD, editors: Clinical microbiology procedures handbook, ed 3, Washington, DC, 2010, ASM Press, p. Anaerobic Cocci Most of the gram-positive anaerobic cocci were classified previously in the genus Peptostreptococcus, with the exception of Peptococcus niger, an infrequent isolate. A candle jar may be used if none of these systems is available. Trophozoites colonize the cecal area. The K antigen often masks the O antigen during bacterial agglutination testing with specific antiserum. Additionally, tears contain IgA and lysozyme. Hippurate hydrolysis d. The patient had no fever, and magnetic resonance imaging (MRI) ruled out spinal cord compression. Rhinoviruses are resistant to detergents, lipid solvents, and temperature extremes but are sensitive to a pH lower than 6. Cross walls of pseudohyphae are constricted and not true septations, whereas true hyphae remain parallel at cross walls, with no indentation. The illness is characterized by low-grade fever, malaise, vomiting, and diarrhea. Members of this genus are negative for urea and positive for lysine decarboxylase, H_2S, and indole and do tahir99 VRG CHAPTER 19 Enterobacteriaceae not grow on Simmons citrate. It might also be mild and go unnoticed by the patient. Ancillaries for Instructors and Students For this edition, we continue offering a variety of instructor ancillaries specifically geared for this book. When the F factor is integrated into the bacterial chromosome rather than a plasmid, there is a higher frequency of transfer of adjacent bacterial chromosomal genes. • Small, peaked, circular colonies appearing after 24 hours that stain as gram-positive cocci can be considered Peptostreptococcus spp. A B FIGURE 27-4 A, Phaeoacremonium sp. Once attached, they continue to ingest blood as a source of nourishment by secreting anticoagulants, platelet inhibitors, and substances that interfere with the factor VIIa tissue factor complex. (Giemsa stain, ×1000.) A B FIGURE 28-32 A, Plasmodium falciparum ring-form trophozoites. PROCEDURE 7-3 PROCEDURE 7-4 Calcofluor White Stain/Fungi-Fluor Kit* Rapid Modified Wright-Giemsa Stain PRINCIPLE PRINCIPLE Calcofluor white is a colorless dye that binds to cellulose and chitin. Which tickborne species of Borrelia is associated with a skin rash or lesion? Filtration of up to 5 mL of blood through a 5-µm Nuclepore filter (Nuclepore, Pleasanton, CA) can detect light infections. bieneusi and E. Yersinia enterocolitica Human infections resulting from Y. Invasion of the deeper layers of the intestinal wall is mediated by cysteine proteases that destroy collagen and fibronectin. Researchers in the United States tested bats from the Rocky Mountain region in 2006 and detected coronavirus RNA in two species of bats, Eptesicus fuscus and Myotis occultus. • The Gram stain may also reveal the presence of squamous epithelial cells that would suggest mucosal surface contamination during specimen collection. cholerae O1, but most lack the cholera toxin gene and appear to cause a milder form of gastroenteritis or cholera-like disease. All the amebae possess a trophozoite and cyst stage. Clinical Infections Mycoplasma pneumoniae Mycoplasma pneumoniae may cause bronchitis, pharyngitis, or a relatively common respiratory infection known as primary atypical pneumonia, or walking pneumonia. The symptoms include fever, headache, myalgia, nausea, vomiting, and rash. The SDD category indicates isolates that may be considered susceptible when highdose therapy, as opposed to standard therapy, is used. Double-Stranded RNA Viruses Reoviridae Rotaviruses Rotaviruses are naked viruses about 75 nm in diameter, with two protein layers surrounding the capsid. Increased resistance from the acquisition of plasmids is relatively uncommon in the United States, but there are reports of multiple resistant strains from Asia and Africa. enterocolitica morphologically resembles other Yersinia spp., appearing as gram-negative coccobacilli with bipolar staining. Some characteristics to note during the macroscopic examination are listed in Table 22-7. Prevalence ranges from close to zero in a study of Japanese blood donors to more than 50% in some parts of Africa. Hawaii Department of Health Communicable Disease Division: Communicable disease report, September/October 2004. It occurs primarily in North America and parts of Africa. This is one way resistance to antibiotics is acquired. Strict criteria for microbial morphotypes should be maintained. As with all specimens for microbiologic examination, aseptic collection is important. hominis has additional morphologic stages. Until the advent of molecular methods, culture of the organism from exudate was considered the most sensitive and specific method of detection, although an inoculum of 300 to 500 organisms is necessary. difficile. In Versalovic J, et al, editors: Manual of clinical microbiology, ed 10, Washington, DC, 2010, ASM Press, p. At the other extreme, M. flexneri), C (S. The heat-labile Vi (from the term virulence) antigen is a surface polysaccharide capsular antigen found in Salmonella serotype Typhi and a few strains of Salmonella serotype Choleraesuis. FIGURE 24-5 Inclusion body from ocular swab of a 7-day-old newborn who was discharged but then readmitted with fever, weight loss, lack of eating, and “fussiness.” At 3 days after delivery, Neisseria gonorrhoeae was isolated from the ocular discharge, although the patient had been given silver nitrate eye drops. The host produces antibodies against the “old” antigens, which are no longer effective because the organism now has “new” antigens that do not bind to antibodies made against the old antigens. Unlike tickborne disease, transmission of the louse-borne disease occurs when infected lice are crushed and scratched into the skin rather than through the bite of an infected arthropod. FIGURE 27-12 Microscopic appearance of Trichosporon species on lactophenol cotton blue preparation showing the presence of both blastoconidia and arthroconidia (×600). Gastric lavage may offer a diagnostic alternative only for those unable to expectorate sputum and for whom BAL might be contraindicated. avium as early as 5 to 8 days. Culture results b. Humans are the only known host for this organism, but other Cyclospora spp. Fluorescent Antibody Techniques Direct fluorescent antibody (DFA) techniques using monoclonal antibodies have been developed to detect Cryptosporidium oocysts in fecal specimens. A patient with a history of travel to Africa has fever and chills. Thus, metabolism can be regulated in the cell either by regulating the production of an enzyme itself (a genetic type of regulation, in which production of the enzyme can be induced or suppressed by molecules present in the cell) or by regulating the activity of the enzyme (via feedback inhibition, in which the products of the enzymatic reaction or a succeeding enzymatic reaction inhibit the activity of the enzyme). The SBA plate should also be examined for the presence of α- or β-hemolysis. In general, the clinical microbiology laboratory, unless serving a public health function, usually does not have a role in recovering C. Duration of the illness varies from less than 1 week to 3 weeks. Colonies are hyaline with a moderate growth FIGURE 27-38 Beauveria sp. Williams DT, et al: Severe hypokalemia, paralysis, and AIDS-associated Isospora belli diarrhea, J Emer Med 41:e129, 2011. Indications of Anaerobe Involvement in Human Disease Infectious processes involving anaerobes are usually purulent, with many polymorphonuclear leukocytes present. Human infections are rare but result in a vesicular, pustular febrile illness that is very similar to smallpox. Unlike other helminths, in which infection peaks in childhood and adolescence, hookworm burden often increases with age. TABLE Presumptive Identification of Gram-Positive Anaerobes Many gram-positive anaerobes can also be presumptively identified with simple procedures (Table 22-13). Note: The intensity of each stain may be altered by increasing or decreasing dips in solutions I and II. Neither M. fragilis Pigmented species of Prevotella S. Specimens from the throat, feces, rectum, CSF, and conjunctiva are recommended. Dutra WO, Gollob KJ: Current concepts in immunoregulation and pathology of human Chagas disease, Curr Opin Infect Dis 21:287, 2008. These organisms have no single characteristic that distinguishes them from the other members of the Enterobacteriaceae. Centers for Disease Control and Prevention: CDC responds to multistate outbreak of fungal meningitis and other infections, 2012. szulgai isolates are photochromogens. Unfortunately, resistance to AMB has been documented with P. Koplik spots, lesions on the oral mucosa consisting of irregular red spots, with a bluishwhite speck in the center (Figure 29-15), generally appear 2 to 3 days before the rash and are diagnostic. Gross examination of the specimen also reveals the consistency (liquid, soft, formed) of the stool sample. tuberculosis isolates produce free niacin (nicotinic acid) because this species lacks the niacin-connecting enzyme. Not all kits detecting E. Morganella morganii + Citrate Salmonella spp. A methylene blue oxidationreduction indicator strip is always added to the jar to verify that an anaerobic atmosphere has been achieved. Clostridia or their toxins usually gain access to the body through ingestion or open wounds that have become contaminated with soil. In the host with intact immune defenses, the infection is limited and is usually asymptomatic, with the only sequelae being areas of calcification in the lungs, liver, and spleen. Often, the same substance (e.g., glucose) is used as both the carbon source and the energy source. mosselii was first identified in 2002 during a study of strains of P. Clostridia initiate infection when spores are ingested by way of contaminated food or gain access to the body through open wounds contaminated with soil. At any point, the free-living cycle may revert and result in the production of infective filariform larvae. They have been isolated from irrigation fluids, anesthetics, nebulizers, detergents, and disinfectants. tahir99 VRG 528 PART II Laboratory Identification of Significant Isolates Chapin KC, et al: Comparison of five assays for detection of Clostridium difficile toxin, J Mol Diagn 13:395, 2011. Characteristic morphologic features of each fungal species are described. Although several genera of gram-negative anaerobic cocci are found in the endogenous microbiota, only Veillonella spp. The order includes the families Rickettsiaceae and Anaplasmataceae. Many laboratories also inoculate specimens onto a phenylethyl alcohol (PEA) or colistin nalidixic acid (CNA) blood agar plate. Sporothrix schenckii Clinical Manifestations. The most commonly seen presentation of S. FIGURE 26-10 Niacin test. A drop of India ink is mixed with a drop of sediment from a centrifuged CSF specimen, and the preparation is examined on high magnification (×400). (From Neidhardt FC, Ingraham M, Schaechter M: Physiology of bacterial cell: a molecular approach, Sunderland, MA, 1990, Sinauer Associates.) Mycobacterium spp. Enterococci, which have limited susceptibility to antibiotics and may require synergistic antibiotic action for killing 3. A strong granulomatous reaction with production of fibrous tissue around dead worms ensues. tahir99 VRG + + V. The patient had large right arachnoid cysts of congenital origin. A severe infection with gram-negative bacteria can lead to serious and often life-threatening situations. The knob may not be seen on all eggs, so size and lack of shoulders must be used to distinguish the egg from that of Paragonimus westermani. Leptospires are obligately aerobic and can be grown in artificial media such as Fletcher’s semisolid, Stuart liquid, or Ellinghausen-McCullough-Johnson-Harris (EMJH) semisolid media. The actual fungal structure must be seen before a positive preparation is reported. The most probable identity of this organism is: a. Detection of HBoV has improved with the development of sensitive and specific real-time PCR assays. The periplasmic space is absent in gram-positive bacteria. The remaining third ultimately develop tertiary or late syphilis, generally decades later. maltophilia is usually susceptible to SXT, so this is the drug of choice for most infections. Certain cultural characteristics can be used initially to identify clostridial isolates. canis and almost all other dermatophytes grow well and usually form many conidia, whereas M. A significant number of common clinical viruses can often be identified within 48 hours of inoculation, including HSV, influenza A and B viruses, PIVs 1 through 4, RSV, adenovirus, and many enteroviruses. The GasPak EZ Gas Generating Pouch System (BD Diagnostic Systems) can hold up to four plates but requires at least two plates per resealable pouch. Evaluate procedures such as the preparation of blood films, wet mounts, concentration methods, and staining methods for blood and tissue parasites. Diagnosis of chronic infection with T. Issues to Consider After reading the patient’s case history, consider: ■ The vaccination appropriate for a child this age that would have prevented this infection ■ Why the physician is concerned enough about the patient’s feeding and breathing to admit her to the ICU ■ Diagnostic tests that should be considered before antimicrobial therapy Adenylate cyclase toxin Bordet-Gengou potato infusion agar Catarrhal phase Convalescent phase Filamentous hemagglutinin (FHA) B Paroxysmal phase Pertussis Pertussis toxin (PT) Regan-Lowe transport medium Tracheal cytotoxin Whooping cough oth Bordetella pertussis and B. Pneumonic plague occurs secondary to bubonic plague or the septicemic form when organisms proliferate in the bloodstream and respiratory tract. The psychrophilic group consists of only one species, A. Woods GL, et al: Susceptibility test methods: mycobacteria, Nocardia, and other actinomycetes. pseudotuberculosis appears as a typical-looking plague bacillus. Symptoms begin about 24 to 48 hours after ingestion of contaminated seafood. The shell thickens at the end opposite the operculum (Figure 28-44). The O157 somatic antigen, which is usually the target in the commercial assays, can present a problem with regard to specificity because other enteric bacteria produce false-positive results. Although a few rapid and semiautomated identification systems can identify an isolate as belonging to the A. Microscopic Examination Clinical specimens can be examined for Bordetella spp. TABLE 24-2 Initial Differentiation of Chlamydiaceae Species Properties Inclusion morphology Glycogen in inclusions Elementary body morphology Sulfa drug sensitivity DNA relatedness (against C. Most of the outbreaks were associated with the consumption of contaminated fresh fruit, especially raspberries and strawberries imported from endemic areas of Central America. Laboratory Diagnosis. Careful examination of direct smear preparations of specimens for histoplasmosis frequently reveals the small yeast cells of H. In addition, typing genital lesions from children has been used to provide legal evidence supporting potential sexual abuse. This phenomenon occurs in fungi that are homothallic (ability of a single organism to undergo sexual reproduction without a mate). The Rickettsia, Orientia, Ehrlichia, and Anaplasma are typically transmitted to humans by the bites of arthropods. Diagnosis of tuberculous meningitis is extremely difficult. Peptoniphilus spp. With the exception of certain members (e.g., Klebsiella and sometimes Enterobacter) that produce characteristically large and very mucoid colonies, members of this family produce large, moist, gray colonies on nonselective media and are indistinguishable. The host cells must possess the necessary receptors for the adhesins. Examine each smear for 3 to 5 minutes. Environmental sources may be cultured during epidemiologic investigations, but this is normally a public health rather than a clinical or diagnostic function. Graves SR, Massung RF: Coxiella. capsulatum causes histoplasmosis, also known as reticuloendothelial cytomycosis, cave disease, spelunker’s disease, and Darling disease. noackiae have been isolated from human specimens. R, Resistant; S, susceptible (a zone of inhibition ≥ 10 mm is considered susceptible.); V, variable. Tears not only lubricate the eye but also wash foreign matter and infectious agents away from the surface. wet preparation. A swarming, gram-positive, anaerobic bacillus with terminal spores is probably C. B, Schistosoma haematobium egg. ✓ Case Check 27-2 When hyphal elements are seen in tissue sections, the disease is frequently attributed to Aspergillus species. pneumophila serogroup 1. Describe the laboratory diagnosis of relapsing fever and how it differs from the diagnosis of other spirochete diseases in the United States. Aspirated secretions are often preferable, but swabs are easier to use for collection. The delicate gram-positive filaments of Nocardia spp. kingii, and EO-1. There are 16 H antigens (H1 through H16), although human infections usually only occur with H1, H2, and H3. Stark DJ, et al: Dientamoebiasis: clinical importance and recent advances, Trends Parasitol 22:92, 2006. Some organisms evade phagocytic cell killing by releasing potent materials in tissues that kill phagocytes. Rifampin, miconazole, or fluconazole and azithromycin have been used in addition to amphotericin B. diminuta isolates are motile and possess a single polar flagellum, oxidize glucose, and are oxidase positive. Most Citrobacter spp. This methodology can circumvent many of the problems associated with specimen transport and bacterial cultivation; however, laboratories should use at least two DNA targets (e.g., IS481 and pxtS1) and may need to seek confirmation to avoid the mischaracterization of respiratory illness. Patients often report a flulike syndrome before the onset of diarrhea. Develop a laboratory protocol for the identification of the clinically significant yeast. In patients with AIDS whose cell-mediated immunity is compromised and whose CD4 count is less than 50 cells/mm^3, the infection is more likely to become fulminant or life-threatening or disseminate to extraintestinal sites. capsulatum, oval to cylindric, measuring 3 to 6 µm long, and may have a cross wall. rarely positive. Subsequent exposure to that antigen stimulates an increasingly effective and specific defense. His mother said that he sometimes ate dirt and always had a good appetite. dispar are morphologically identical organisms that differ in their effects on the host. The individual will enter a period of clinical latency; even though the virus is replicating rapidly in lymphoid tissue, virus is not detectable in the bloodstream, and the patient remains asymptomatic. tularemia infections. Diseases needing timely response (e.g., foodborne outbreaks) c. The newer tests use recombinant or affinity-purified, type-specific glycoprotein G1 or G2, giving the tests the ability to distinguish between HSV-1 and HSV-2. Because of the potential cross-contamination of AFB from one smear to another, careful attention should be paid to the staining technique. Antimicrobial Susceptibility Both Mueller-Hinton agar and broth contain sufficient salt to support the growth of Vibrio spp. Case in Point A 69-year-old diabetic woman hospitalized for an amputation complained of chest pain, a persistent cough, and shortness of breath. For many years this hypothesis was unproven; there was no way to differentiate the strains morphologically. Less commonly seen disease states include fixed cutaneous sporotrichosis, in which the infection is confined to the site of inoculation and mucocutaneous sporotrichosis, a relatively rare condition. It must also be collected in a manner that avoids prolonged exposure to oxygen and must be transported as quickly as possible to the laboratory under anaerobic conditions. Points to Remember ■ Protozoan cysts, helminth eggs, and helminth larvae can be identified on a wet mount preparation of a fecal concentrate. A color change suggests the presence of H. B, Modified acid-fast stain. influenzae type d. *Additional information about presumptive identification of aerobes and facultative anaerobes can be found in Clinical and Laboratory Standards Institute: Abbreviated identification of bacteria and yeast: approved guidelines, ed 2, CLSI document M35-A2, Wayne, PA, 2008, CLSI. Nuclear peripheral chromatin (DNA and proteins on the edge of the nucleus), karyosome (mass of chromatin in the nucleus), chromatoidal bars (dark-staining cytoplasmic inclusions of chromatin), and red blood cells (RBCs) stain a dark red-purple. The disease essentially occurs only in southern Texas and southern California in this country but continues to be a problem in areas of the world in which rats and their fleas are present in urban settings. To document infection, the mold must be seen in tissue and grown in culture. Accessed March 26, 2013. A total of 5387 cases and 243 deaths 709 were reported; 51% of all cases were described as neuroinvasive. Supporting laboratory findings and how they help establish the diagnosis ■ Methods for recovery of the suspected causative agent Key Terms Cell wall–deficient L-forms Pleuropneumonia-like organism (PPLO) T Nongonococcal urethritis (NGU) Primary atypical pneumonia T-strain mycoplasma his chapter discusses a group of organisms once thought to be viruses because of their size. Transmission is primarily fecal-oral, and clinical symptoms may be related to the different phases of the life cycle. PCR assays have also been described, but they too are not readily available. are opportunists, accounting for 1% to 3% of all nosocomial infections; they are second only to P. They are classified as prokaryotes (Greek: before kernel [nucleus]), having no mitochondria, endoplasmic reticulum (ER), or Golgi bodies. Serology may not be helpful unless paired sera are available. Issues to Consider After reading the patient’s case history, consider: ■ Risk factors for the retirees for pneumonia ■ Causes of community-acquired pneumonia ■ Empirical therapy options Key Terms Atypical pneumonia Buffered charcoal yeast extract (BCYE) 5. The virus is enveloped; its genome consists of seven ssRNA segments, lacking the gene coding for neuraminidase, as in influenza A and B viruses. Regardless of the preservative used, the ratio of three parts preservative to one part feces should be maintained for optimal fixation. freundii produce H_2S and some strains (50%) fail to ferment lactose, the colony morphology of C. pneumoniae–specific IgG does not reach diagnostic levels for 6 to 8 weeks. Sapoviruses (SaVs) and noroviruses (NoVs) are causative agents of human gastroenteritis. are straight to slightly curved rods, produce alkalinity in OF media, are catalase and oxidase positive, usually motile by multitrichous polar flagella, and reduce nitrate to nitrite. When a species varies in size and shape within a pure culture, the bacterium is pleomorphic. hydrophila complex, most are currently inadequate for identification to the species level. PRECAUTIONS The Gram stain reaction may vary from the expected in numerous wellrecognized circumstances. Identification to the species level is confirmed by biochemical reactions, absence of carbohydrate fermentation, use of potassium nitrate, assimilation of sugars, and urease positivity. Fusarium. Fusarium spp. Species identifications made with HPLC have been shown to agree well with biochemical and nucleic acid probe identifications. The characteristic lesion in the intestinal mucosa, a flaskshaped ulcer, is a result of lysis of the intestinal mucosa. rarely cause infections; most reports of disease involve chronic fungal sinusitis. putida has been associated with catheter-related sepsis in patients with cancer; isolation of P. gastri 25-42 22-37 22-37 22-37 33-37 25-35 22-37 25-40 30-45 Catalase +(99) +(96) +(85) +(93) −(64) +(91) +(97) +(91) −(67) −(85) +(92) −(79) +(99) +(95) +(81) +(99) +(55) +(95) +(99) +(99) − − +(99) +(93) +(69) +(96) +(99) −(99) −(99) + −(99) − −(99) −(99) − −(99) −(99) −(99) −(99) −(99) −(99) −(99) −(99) −(77) −(84) −(99) +−(99) +(83) Iron Uptake −(68) Growth on MacConkey +(76) −(99) −(84) N = 90% −(99) −(89) S = 10% −(99) N −(99) −(62) S S P S = 99% (99) 37° 25° R = 1% S = 99% −(99) +(57) R = 59% S = 41% S = 99% −(84) +(78) S S S = 80% N = 99% −(96) +(99) R = 20% S = 77% N = 95% −(99) −(51) R = 23% S = 4% P = 1% N = 99% −(66) +(99) S Rough Colony Morphology M. hyointestinalis can produce a dirty yellow pigment. Bloody diarrhea with associated necrosis of colonic mucosa is seen in patients with pseudomembranous colitis. Many of the kits use enzyme immunoassay (EIA) methodologies. The swab should be rolled back and forth across the slide to deposit the sample completely. The disease becomes apparent in individuals with dark complexions or in those who fail to tan normally. This is one reason that pharmaceutical companies have been so successful in developing effective antibiotics against bacterial pathogens but have been less successful in finding drugs effective against parasites, medically important fungi, and viruses, which are eukaryotic, similar to their human hosts. Dallas, PhD, D(ABMM), MT(ASCP)SM Assistant Director and Assistant Professor of Microbiology Department of Clinical Laboratory Sciences San Antonio, Texas tahir99 VRG xi This page intentionally left blank tahir99 VRG Preface W e welcome you to the fifth edition of the Textbook of Diagnostic Microbiology. *Test reactions listed as “+” or “−” followed by the percentage of strains reacting, as indicated. Hence, misdiagnosis of C. Two commonly used treponemal test methods are the Treponema pallidum–particulate agglutination (TP-PA) Test (Fujirebio America, Fairfield, NJ) and EIAs. The TP-PA test uses gelatin particles sensitized with T. Results, however, might be less reliable in children, making tuberculin skin testing recommended for children younger than 5 years. fragilis group, Porphyromonas and Prevotella spp., Fusobacterium spp., Clostridium spp., Propionibacterium spp., and the anaerobic cocci. The hexacanth embryo is contained within tahir99 VRG 670 TABLE PART II Laboratory Identification of Significant Isolates 28-8 Comparisons of Tapeworm Eggs Organism Average Size (µm) and Shape Other Identifying Features Diphyllobothrium latum 58-76 × 40-50, oval Inconspicuous operculum Small knob at end opposite operculum Unembryonated when passed Taenia spp. List the nutritional and environmental requirements for bacterial growth, and define the categories of media used for culturing bacteria in the laboratory. Some strains produce a fluorescent pigment. The Lake Victoria marburgvirus (formerly Marburg virus) is in the former genus, and the Ebola virus Zaire strain (EBO-Z), Ebola virus Sudan strain (EBO-S), Ebola virus Reston strain (EBO-R), and Ebola virus Tai Forest strain all belong to the latter. Although EIEC and Shigella spp. The slide should be air-dried and fixed with methanol, not heat-fixed. The Giemsa stain is used primarily to detect H. The agglutination test requires a larger quantity of bacteria, so subculture from the primary isolation plate is sometimes necessary. Dientamoeba fragilis, T. The sensitivity and time to positive culture is similar to the BACTEC 460TB. † Very pale yellow-green; young colonies may be negative. caviae from pediatric diarrhea, and A. Most clinical microbiology laboratories now use one of the commercially available biochemical-based or preexisting enzymebased minisystems for making definitive identifications, but it is important to remember that none of these will identify all the anaerobes that could potentially be isolated from clinical FIGURE 22-20 Appearance of Bilophila wadsworthia on Bacteroides bile esculin (BBE) agar. Organisms that stain lightly and may be difficult to identify are Entamoeba hartmanni, Dientamoeba fragilis, Endolimax nana, Chilomastix mesnili, and Giardia lamblia. Systemic manifestations include fever, lymphadenitis, hepatosplenomegaly, malaise, muscular pains, and diarrhea and vomiting. Because finding HPV in cervical tissue is TABLE not the sole predictor of invasive disease, there is some debate about whether it is useful to look routinely for the virus in cervical specimens. Laboratories should consider contacting physicians when unusual isolates are cultured from clinical specimens to determine if the workup should be more extensive in patients who have underlying risk factors. Chromoblastomycosis is caused by several infectious agents, namely Fonsecaea compacta, F. are frequently seen in mycotic keratitis. If large numbers of larvae are present during the lung phase of migration, the patient may have bronchitis, but unlike with Ascaris larvae, no host sensitization occurs. Many viruses produce distinctive and characteristic visual changes in infected cells, referred to as cytopathic effect (CPE). Dermatophytic infections usually involve a restricted region of the host; traditionally these diseases are named with respect to the portion of the body affected. Oral lesions should not be examined because numerous nonpathogenic spirochetes present in these specimens will lead to misinterpretation. The availability of culture reviews, which may result in the initiation of an outbreak investigation, was discussed earlier. It is often found in areas where step-down wells are used. Endotoxins are a constituent, the lipopolysaccharide (LPS), of the outer cell membrane of gram-negative bacteria exclusively. The stain is nonspecific and binds to chitin in the spore wall. jejuni and other enteric campylobacters grow optimally at 42° C. Fox LM, et al: Neonatal babesiosis: case report and review of the literature, Pediat Infect Dis J 25:169, 2006. Many state health departments and the CDC use this method to identify mycobacterial isolates. multocida, most frequently causes wound infections after cat or dog bites. cholerae O1 V. Campbell NA, Reece JB: Essential biology, San Francisco, 2001, Pearson Benjamin Cummings. It was first described in the western United States during the latter part of the nineteenth century. The resulting reaction causes the small lymphatics to become narrowed or closed, causing increased hydrostatic pressure, with subsequent leakage of fluid into the surrounding tissue. Microsporum gypseum b. Infections in adult women include urethritis, follicular cervicitis (leukorrhea hypertrophic cervical erosion), endometritis, proctitis, salpingitis, pelvic inflammatory disease (PID), and perihepatitis. Among the many organisms in the family Enterobacteriaceae, this chapter focuses only on the members that have been associated with human diseases. tahir99 VRG 59 60 PART I Introduction to Clinical Microbiology Case in Point Dr. Adams is a pediatrician at a busy metropolitan medical clinic. aeruginosa is still considered an opportunistic pathogen. Table 19-12 shows the differentiating characteristics of the species, biogroups, and enteric groups of the Enterobacteriaceae. Seminars, scientific articles and books, computer-based learning, and discussion with the infection control personnel are ways in which technologists can maintain their knowledge of infection control and laboratory techniques that can aid the infection control program. Chlamydia trachomatis C. The causative agents are organisms of the L. The microbiology laboratory is an integral part of that infection control activity. Therefore, readers may find both taxonomic classifications in published literature. Because infected humans can continue the transmission when bitten by uninfected mosquitoes, large outbreaks can occur from a single case of yellow fever. botulinum. The organism often stains faintly with trichrome stain. Centers for Disease Control and Prevention: Summary of reportable diseases—United States, 2010, MMWR 59:1, 2012. alginolyticus. The differential ability of the Gram stain makes it useful in microbial taxonomy. Therefore, SBA without ampicillin is preferred. However, nutritional needs vary greatly within this group. The spotted fever group includes a number of species generally recognized as human pathogens, such as R. Environmental Factors Influencing Growth Three environmental factors influence the growth rate of bacteria and must be considered when bacteria are cultured in the laboratory: • pH • Temperature • Gaseous composition of the atmosphere tahir99 VRG PART I Introduction to Clinical Microbiology Most pathogenic bacteria grow best at a neutral pH. Bacilli (plural of bacillus) may vary greatly in size and length from very short coccobacilli to long filamentous rods. Trimethoprim-sulfamethoxazole is also an alternative for treatment or prophylaxis. Failure to isolate certain organisms observed in the Gram-stained smear might indicate that problems exist with the anaerobic culture technique being used. C – + – FIGURE 26-16 Test for tellurite reduction. This medium distinguishes sucrose-fermenting strains from non–sucrose-fermenting strains. Among the gram-negative bacteria, the enterics have strong avidity and stain bright red; pseudomonads are less avid and stain moderately well. If the crystal violet is rinsed too vigorously before it is complexed with the iodine, it will wash away and leave poor or no staining of gram-negative organisms. perfringens, organisms are acquired through the consumption of contaminated food that has been improperly stored. It is mostly asymptomatic in the absence of secondary complications, such as bacterial infections, carcinomatous degeneration, and elephantiasis. to as swimmer’s itch. The organism has an incubation period of 5 to 10 days. Another important member of the family Flaviviridae is the dengue virus, which causes two distinct diseases, classic dengue fever (DF) and dengue hemorrhagic fever (DHF). The stomach is usually sterile, especially after ingestion of a meal, when gastric juices, acids, and enzymes, which help to protect the stomach from microbial attack, are produced. They were first identified by electron microscopy and were named for the distinctive club-shaped projections on their surfaces (Figure 29-13). Three Boston University scientists recently became infected through work unknowingly performed with a fully virulent strain. Aeromonas spp. Specimens obtained from the lung, pericardium, lymph nodes, bones, joints, bowel, or liver may be appropriate. Infections Caused by Leptospires Leptospires present in water or mud are most likely to enter the human host through small breaks in the skin or intact mucosa. tahir99 VRG CHAPTER 1 Bacterial Cell Structure, Physiology, Metabolism, and Genetics E F G H 13 FIGURE 1-6, cont’d E, Acridine orange stain. However, only about 10% of these individuals developed clinical symptoms or invasive disease. The piece of tissue or bone fragment is homogenized until a thick suspension is obtained. To visualize organisms, approximately 10,000 to 100,000 organisms/mL of specimen must be present. Cytomegalovirus Cytomegalovirus is in the genus Cytomegalovirus. Desiccants that change color when saturated are not recommended because of the fine powder they contain that can circulate throughout the chamber. This term was coined to separate several clinical infections caused by phaeoid fungi from those distinct clinical entities known as chromoblastomycosis. enterocolitica c. fragilis group. Women with this form of infection are often a major source of transmission to sexual partners. Ocular Herpes. A herpes simplex infection of the conjunctiva can manifest as swelling of the eyelids associated with vesicles. Helicobacter b. Short-term prophylaxis consisting of weekly doxycycline may be appropriate in high-risk groups with expected occupational exposure. bancrofti and B. There are 29 named arenaviruses; the family includes many species that cause hemorrhagic fever. parapaucimobilis are H_2Spositive by the lead acetate method, Simmon’s citrate positive, and DNase negative. At this point, the investigator seeks as much consultative assistance as possible. The egg is spherical to oval, measures 30 to 47 µm, and has a grayish color. Complications include secondary bacterial pneumonia. These methods have been reported to be tahir99 VRG 472 PART II Laboratory Identification of Significant Isolates reasonably sensitive and specific indicators of Campylobacter and H. Genetic tests circumvent the need to culture bacteria, providing a more rapid method of identifying pathogens. Endotoxin stimulates the fever centers in the hypothalamus. coli bacteremia in adults may result primarily from a urogenital tract infection or from a GI source. Discuss the epidemiology of leptospirosis in the United States. Patients with symptoms should be treated with analgesics and general supportive measures. Available at: . These strains are considered Kanagawa toxin-positive, whereas most environmental isolates are Kanagawa toxin-negative. Some patients develop pleural effusions, hypotension, and hemorrhaging. It identifies which amino acid will be in a specific location in the protein. Although some Mucorales are capable of sexual reproduction resulting in the production of zygospores, these structures are not routinely seen in clinical laboratories. This fungus grows well on most culture media, including those containing cycloheximide. During the course of a single infection, borreliae systematically change their surface antigens. difficile associated with hyperproduction of toxins was recovered from patients with severe and sometimes fatal cases of pseudomembranous colitis leading to bowel perforation and sepsis. If acid-fast bacteria are suspected, the acid-fast stain should be performed before an opinion is rendered. infection, including high-risk factors. Although fatty acid profiles can be identified manually, computerized, high-resolution GLC and specialized software programs are available to analyze cellular fatty acids of unknown bacteria and compare the results with patterns of known species. Sedimentation and flotation methods, both of which are based on the difference in specific gravity between the parasites and concentrating solution, are used to concentrate parasites into a small volume for easier detection. • A few hydrolyze urea slowly. The capsular antigen plays a significant role in preventing phagocytosis of the organism. In reduced conditions, the indicator, neutral red, turns yellow in the presence of an acid pH. In culture, conidia are only rarely produced. Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. Sputum purulence screens are not advised. Approximately 1.7 million HIV-AIDS deaths occurred in 2011, with about 2.5 million new cases diagnosed. tahir99 VRG 684 PART II Laboratory Identification of Significant Isolates Mansonella spp. M. and 5% to 10% O_2 and 5% to 12% CO_2 for Helicobacter spp. • Propionic acid fermentation: Propionic acid is the major end product of fermentations carried out by Propionibacterium acnes and some anaerobic non–spore-forming, gram-positive bacilli. Patients may also have evidence of leukopenia and neutropenia, thrombocytopenia, and elevated liver enzyme levels. To observe the typical motility, organisms should be suspended in Brucella or tryptic soy broth. Origin of Microbial Flora The fetus is in a sterile environment until birth. Because of this mechanism, arthropods are not only vectors for rickettsioses but also reservoirs. Approximately 50% of current male partners of women with a cervical chlamydial infection are also infected. In testing that tahir99 VRG 54 TABLE PART I Introduction to Clinical Microbiology 3-4 Steps of an Outbreak Investigation Steps 1. Verify diagnosis of suspected cases. Such a holding system, which may use a jar, box, or other small chamber, allows plates that have not been inoculated to be held under anaerobic conditions until needed. nov., comb. One disadvantage of these tests is that fresh unpreserved stool must be used. Discuss the most important human diseases caused by the Chlamydia, Chlamydophila, and Rickettsia, species and similar microorganisms. stercoralis can take one of three phases— direct, which is similar to that of hookworm; indirect, which involves a free-living phase; or autoinfection (Figure 28-64). The serotypes associated with these categories and the features associated with the intestinal infections produced by these strains are summarized in Chapter 34. CCFA is a selective and differential medium for the recovery and presumptive identification of C. • Viruses are mostly host or host cell specific. A mutation may be the result of a change in one nucleotide base (a point mutation) that leads to a change in a single amino acid within a protein or may be the result of insertions or deletions in the genome that lead to disruption of the gene or a frame-shift mutation, or both. 1962. The genus Providencia consists of five species: P. 24-1 Comparative Properties of Microorganisms Organisms Characteristic Typical Bacteria Chlamydiae Rickettsiae Mycoplasmas Viruses + − + + + + − + + + + − + + + + + + − − + + − + + − − + + + − + − + − − − − + − DNA and RNA Obligate intracellular parasites Peptidoglycan in cell wall Growth on nonliving medium Contain ribosomes Sensitivity to antimicrobial agents Sensitivity to interferon Binary fission (replication) +, Characteristic is present; −, characteristic is absent. The human T-lymphotropic viruses HTLV-1, HTLV-2, and HTLV-5 belong to the subfamily Oncovirinae. † Sometimes the type of interference listed has been described only in a particular type of phagocyte (polymorph or macrophage) from a particular host, but it generally bears a relationship to pathogenicity in that host. For this reason, it is necessary to set up screening tests to differentiate these organisms from stool pathogens. These organisms have a worldwide distribution and are major causes of morbidity, rather than mortality, in developing areas of the world. jejuni, other Campylobacter spp. milleri, S. Some virulence factors, such as capsules and toxins, are used by many organisms. frequently reported in Texas, Florida, Virginia, and California but has been reported as far north as Minnesota. immitis is encountered in the San Joaquin Valley region of California, whereas C. They appear large, moist, and gray on sheep blood agar (SBA), chocolate (CHOC) agar, and most nonselective media. freundii Ewardsiella tarda + – K/K (no change) A/A H_2S– – Motility (37° C) – + Motility (37° C) + – Enterobacter spp. The disease differs from the typical pneumonia caused by Streptococcus pneumoniae in that it is milder, has a higher incidence in young adults, and is not seasonal. are identical except for the fact that humans may also serve as intermediate tahir99 VRG CHAPTER 28 Diagnostic Parasitology 669 Rostellum and hooklets Suckers Scolex —means of attachment Neck—origin of proglottids Immature Mature (male and female sex organs) Strobila —all proglottids Gravid (producing eggs) FIGURE 28-47 Diagram of a tapeworm. Eye cultures confirmed the presence of Chlamydia trachomatis (Giemsa stain, ×600). Laboratory Diagnosis. The amastigote is the diagnostic stage in humans. Eosinophilia of 40% to 80% is common. Testing for these factors with X and V strips and the porphyrin test are important aspects of laboratory identification procedures. Published articles suggest that in some circumstances, pooling of the three formalinpreserved specimens gives a parasite recovery rate comparable to that of the individual examination of formalin-preserved stools. In the diagnostic microbiology laboratory, this classification is accomplished by testing each bacterial culture for various metabolic characteristics and comparing the results with those listed in established charts. Clinical Manifestations Lyme borreliosis is a complex disease that can generally be divided into three stages. As with malaria, cases of congenital babesiosis have been reported. Most adults demonstrate antibody against the virus, with a prevalence in the United States for adult women of 55% and adult men, 32%. The trophozoite is compact and may assume a characteristic band appearance, in which it stretches across the diameter of the RBCs (Figure 28-30, A). Although less frequently encountered, T. The virus had been transmitted to 24 other people through nosocomial transmission, casual contact, and sexual contact. perfringens, C. Although most do not produce pigment, C. Aeromonads are variable with regard to ticarcillin or piperacillin and the cephalosporins. This vocabulary must be shared by the microbiology and medical communities so that when tahir99 VRG Text continued on p. To obtain a culture-confirmed diagnosis of encephalitis, brain biopsy material is required. In 1969, the Lassa virus was isolated in Africa, which triggered the novel Fever, by John Fuller. Tubes of media with and without thiamine are inoculated with a tiny, mediumfree portion of the colony and observed for growth after 10 to 14 days. IgM cannot cross the placenta. On CCFA, C. Laboratory Diagnosis. The microscopic morphology of each of the agents is described in Table 27-2. neoformans polysaccharide antigen. However, Shigella spp. tahir99 VRG 668 PART II Laboratory Identification of Significant Isolates A B C FIGURE 28-46 A, Schistosoma mansoni egg. Late manifestations of the disease may be caused by the host’s immunologic response to the infection. The vector for the infection is Dermacentor andersoni, which has many hosts in nature, including deer, squirrels, and rabbits. pseudomallei is suspected. The third step is to find additional cases that may be added to the initial number of cases. After the miracidium penetrates a snail (the first intermediate host), sporocysts and then cercariae are produced during a 6-week period. parvum but is now recognized as a separate 659 species. The signs and symptoms along with the neonate’s history are suggestive of C. Specimens now used include bronchoalveolar lavage, transbronchial biopsy, tracheal aspirate, pleural fluid, and induced sputum. veronii (biovars sobria and veronii). multivorum and S. pneumoniae, increases with the length of hospital stay. Some studies suggest that the trophozoite may be transmitted to humans by ingesting a helminth egg, especially that of identified by arrow (trichrome stain, ×1000). Aigner BA, et al: Pseudomonas oryzihabitans cutaneous ulceration from Octopus vulgaris bite: a case report and review of the literature, Arch Dermatol 147:963, 2011. Both of these follow-up stains can be performed on the decolorized, Gram-stained preparation. Reagents and solutions must be properly labeled. It should take approximately 10 to 15 minutes to adequately examine selected areas. All grains or granules and the background should be examined carefully. hominis has come to prominence as a possible cause of diarrhea in humans, although controversy concerning its pathogenicity still exists because of the conflicting outcomes of numerous studies. Differences also exist in the processes of DNA synthesis, protein synthesis, and cell envelope synthesis and structure. Usually found in the GI tract of humans and animals, the genus Klebsiella consists of several species, including K. coli Citrobacter spp. paucimobilis infections include peritonitis associated with CAPD, septicemia, meningitis, leg ulcers, empyema, and splenic and brain abscesses. parahaemolyticus, P. Killing The phagocytosis of a particle triggers a significant increase in the metabolic activity of the neutrophil or macrophage. Describe how anaerobes, as part of endogenous microbiota, initiate and establish infection. 22-1 Classification of Microorganisms on the Basis of Their Relationship to Oxygen and Carbon Dioxide Category Requirement Examples Obligate anaerobe 15%-21% O_2 (as found in a CO_2 incubator or air) 5% O_2 Multiplies equally well in the presence or absence of O_2 Reduced concentrations of O_2 (anaerobic system and microaerophilic environments) Strict anaerobic environment (0% O_2) Capnophile 5%-10% CO_2 Mycobacteria, fungi Campylobacter, Helicobacter Enterobacteriaceae, most staphylococci Most strains of streptococci, Propionibacterium, Lactobacillus, some Clostridium Most Bacteroides spp., many Clostridium, Eubacterium, Fusobacterium, Peptostreptococcus, Porphyromonas Some anaerobes, Neisseria Obligate aerobe Microaerophile Facultative anaerobe Aerotolerant anaerobe Modified from Engelkirk PG et al: Principles and practice of clinical anaerobic bacteriology, Belmont, CA, 1992, Star. Naftifine is only used topically. Escherichia coli Providencia alcalifaciens Yersinia pestis Serratia spp. The risk of transmission is very low when the mother has recurrent herpes. After the fifth day, the infant was taken by her parents to the emergency department. Acridine orange is used to locate bacteria in blood cultures and other specimens where discerning bacteria might otherwise be difficult (see Figure 1-6, E). 611 FIGURE 27-49 Curvularia sp. The specific media vary somewhat from one laboratory to another and will depend on the specimen type; nevertheless, 5% sheep blood, MacConkey, and chocolate agar plates are usually included. The amount of organism required for testing in the case of MTB is a single colony of at least 1 mm in diameter or, in the case of MAC, a barely visible film of growth on the surface of the medium. Enterobacter aerogenes c. Boutonneuse fever is also clinically similar to RMSF. Infection can be acquired in utero, intrapartum (during birth), or postnatally (after birth). (Courtesy Jean Barnishan.) to Klebsiella, Enterobacter spp. These treponematoses are found in developing countries in which hygiene is poor, little clothing is worn, and direct skin contact is common because of overcrowding. Most known pathogens in this group form green- to tan-colored colonies. For example, the observation of a gram-negative bacillus, small and pleomorphic, from the spinal fluid of an infant implies that Haemophilus influenzae is the infecting agent. It fluoresces as a bright apple-green or blue-white, allowing visualization of fungal structures with a fluorescent microscope. Figure 28-31, A, shows trophozoites of P. Polymicrobial infections usually arise from displaced normal or altered flora, and culture yields the same species that can be isolated in culture from uninfected but contaminated specimens. It has been isolated from human diarrheal samples as well, but its role in disease is unknown. This opacity is actually in the medium and is not a surface phenomenon (see Figure 22-16). gladioli is negative for oxidase, although some strains are weakly positive. tonsurans, as well as Epidermophyton floccosum. pertussis infection. Richardson M, Lass-Flörl C: Changing epidemiology of systemic fungal infections, Clin Microbiol Infect 14:5, 2008. Clinically Significant Nonfermentative, Gram-Negative Bacilli The genus Pseudomonas accounts for the largest percentage of all nonfermenters isolated from clinical specimens. It is found in the southeastern United States, often as a co-infection with A. The nontreponemal tests detect reaginic antibodies that develop against lipids released from damaged cells. Serologic Tests In patients with a leptospiral bacteremia, IgM antibodies are detected within 1 week after onset of disease and may persist in high titers for many months. In addition, some may require an insect vector or intermediate host for completion of the life cycle. ✓ Case Check 19-1 The organism described in the Case in Point at the beginning of the chapter showed morphologic and biochemical characteristics of the Klebsielleae. microti, an intraerythrocytic parasite, morphologically resembles P. Mucin in the intestinal mucus competes for attachment to the lectin and protects epithelial cells from attack. However, outside their normal body sites, these organisms can produce serious extraintestinal, opportunistic infections, many of which are described in this chapter. Short peptides, each consisting of four amino acid residues, are attached to a carboxyl group on each NAM residue. The morphology of virions is helical, icosahedral (a geometric shape with 20 triangular sides), or complex. Mahlen, PhD, D(ABMM) Director, Clinical and Molecular Microbiology Affiliated Laboratory, Inc. The purposes of the digestion-decontamination process are as follows: (1) to liquefy the sample through digestion of the proteinaceous material; and (2) to allow the chemical decontaminating agent to contact and kill the nonmycobacterial organisms. The organisms invade the gallbladder and Peyer’s patches of the bowel. Infection spreads in the same way as typical seasonal influenza viruses, mainly through coughing and sneezing of infected individuals. Is the organism considered a pathogen? IgM levels may be detectable but are low. Macrogametocytes are often difficult to differentiate from mature trophozoites. However, complete biochemical testing differentiates Leminorella from Salmonella; Leminorella spp. All unstained elements, such as products of inflammation, are subsequently counterstained red by safranin dye. asteroides, T. A few drops of the specimen may also be inoculated into the bottom of a tube of enriched thioglycollate or cooked meat broth. Bacterial plaques that develop on teeth may contain 1011 streptococci per gram. Blood cultures were positive after 48 hours. Clinical Manifestations Campylobacter Several Campylobacter spp. This organism has different sugar assimilation patterns, notably rapid assimilation of trehalose, from those of C. The primary organisms associated with human outbreaks are C. The morphology of all trypomastigotes is similar (see Figure. falciparum infection However, the extended time and costly equipment required do not make the procedure cost-effective or efficient, especially for field work. This species, unlike the other two, has many mammalian reservoir hosts. The blood should be allowed to dry for at least 6 hours before staining. Ralstonia and Cupriavidus Ralstonia pickettii is the most common species of this genus isolated from humans. Once bacteria are attached, phagocytosis is less likely to occur. Cross-reactions between soluble and viral antigens can confuse interpretation of serologic results. (unstained, ×1000). malmoense M. alcalifaciens, is rarely isolated, and its pathogenicity also remains unproven, whereas P. To provide the best recovery of anaerobes, fresh plated media should be stored in an anaerobic chamber or holding system until used. Relapse and reinfections also seemed to occur with these infections. The lesions dry, crust over, and heal in 1 to 2 weeks. If a human ingests raw or undercooked meat containing the tissue cysts, the cyst wall is dissolved and bradyzoites are liberated. Because plesiomonads are often susceptible to ampicillin, any agar for detecting plesiomonads in clinical samples should not contain this antibiotic. H_2S, Hydrogen sulfide; TSI, triple-sugar iron agar; +, ≥90% positive reaction within 1 or 2 days; −, no reaction (≥90%) in 30 days; − or +, most strains negative, some cultures positive; + or (+), most reactions occur within 1 or 2 days, some are delayed; d, different reactions, + or −, most cultures positive, some strains negative. However, the incorporation of ampicillin in the blood agar may inhibit some A. Points to Remember ■ ■ ■ ■ ■ ■ ■ ■ Spirochetes are slender, flexuous, helically shaped bacteria. All species have two periplasmic flagella. In both cases, humans are the accidental host for nonhuman nematode larvae that are unable to complete their life cycle in humans. tahir99 VRG CHAPTER 19 Enterobacteriaceae FIGURE 19-10 Left, Lactose-negative appearance of Shigella sonnei growing on MacConkey (MAC) agar at 18 to 24 hours of incubation. Specimens from sterile body sites can simply be concentrated by centrifugation (if a large volume) and inoculated. Summerbell RC, et al: Trichophyton, Microsporum, Epidermophyton, and agents of superficial mycoses. It is oxidase and ADH positive like P. The kidneys and heart may also be affected. Cases are probably underreported because of the similarity to E. Patel G, et al: Strongyloides hyperinfection syndrome after intestinal transplantation, Transpl Infect Dis 10:137, 2008. However, disseminated Acanthamoeba infections have been treated with amphotericin B, pentamidine isethionate, fluconazole, ketoconazole, and cotrimoxazole (trimethoprim-sulfamethoxazole). Since then, the implementation of effective sterilization and disinfection methods remains crucial in the control of infections in the laboratory and health care facilities (nosocomial infections). 129 Cytocentrifuge Technique A cytocentrifuge with a closed bowl is preferred for microbiology. has alternating sexual and asexual reproduction stages. Also, special sterile receptacles containing a 50-mL centrifuge tube for sputum collection are available commercially. The first Mycoplasma was isolated in the late 1800s from a cow with pleuropneumonia. dermatitidis, is a heterothallic ascomycete that produces a teleomorphic state, Ajellomyces capsulatus, when mated with appropriate tester strains. Microscopic Examination Although direct demonstration of leptospires in clinical specimens during the first week of the disease by special stains, dark field, or phase contrast microscopy is possible, it is not recommended. The organism can also be transmitted via organ transplantation. In the United States, most cases occur in Texas and Louisiana. Regardless of the source, however, the specimen should consist of infected epithelial cells and not exudate. nonliquefaciens, M. One of the most widely recognized manifestations of C. are tiny gram-negative coccobacilli and might become elongated if recovered from media containing cephalexin. Hiransuthikul N, et al: Skin and soft tissue infections among tsunami survivors in southern Thailand, Clin Infect Dis 41:e93, 2005. (Courtesy Dr. Michael McGinnis.) tahir99 VRG 600 PART II Laboratory Identification of Significant Isolates A B FIGURE 27-19 Actinomycotic mycetoma showing fine-branching, filamentous rods in tissue sample (A), compared with the hyphal elements hematoxylin and eosin stain, (×1000) (B) seen in eumycotic infections (Gomori methenamine silver stain, ×1000.) TABLE 27-3 Description of Granules Seen in Eumycotic Mycetomas Fungus Pseudallescheria boydii Acremonium falciforme Madurella mycetomatis Madurella grisea Exophiala spp. It is therefore imperative to determine the oxidase activity of any suspicious Vibrio-like colony. Identification of Fungi Although the number of fungal species described exceeds 100,000, the number known to cause human disease is a small fraction of this number, and although the number of species that are routinely seen causing infection is quite low, new species are continually being implicated. Within the tribes, organisms are classified further into genera and species. Knowledge of the role of these organisms in the pathogenesis of an infectious disease is helpful in assessing their significance when isolated from clinical samples. sordellii, C. Conidia are produced from the phialides. When humans ingest raw or undercooked meat, the scolex in the cysticercus is freed, attaches in the human small intestine, and matures into the adult tapeworm within 10 weeks. The tissue exhibited marked necrosis, and gas pockets were noted in the tissue. HSV is one of the few viruses that can grow on rabbit kidney cells (Figure 29-2); therefore, it is a useful cell line for HSV detection. tuberculosis infection. Fungi grow optimally at these temperatures, whereas bacteria have a slower growth rate. Susceptibility to antimicrobial agents is not well known. cholera, V. These organisms have been isolated from human sources, especially sputum, and may be a rare cause of infection. Treatment of infected patients is primarily supportive and includes replacement of blood and clotting factors. Members of the order are rapidly growing organisms normally found in the soil. Molds are filamentous forms that can reproduce asexually and sexually. Mahon* CHAPTER OUTLINE ■ CHLAMYDIACEAE General Characteristics Chlamydia trachomatis Chlamydophila pneumoniae Chlamydophila psittaci ■ RICKETTSIACEAE AND SIMILAR ORGANISMS Rickettsia Orientia Anaplasmataceae Coxiella OBJECTIVES After reading and studying this chapter, you should be able to: 1. It is recovered as normal biota from a variety of sites, including skin, oral mucosa, and vagina. Patients with NPC often have elevated IgG and IgA anti-EA/D antibodies. Figure 2-5 shows examples of the innate tahir99 VRG 38 PART I Introduction to Clinical Microbiology Mast cell Fibroblast Macrophage CONNECTIVE TISSUE CELLS Smooth muscle Basophil Platelets VESSELS Polymorphonuclear leukocyte Lymphocyte Endothelium Monocyte Clotting factors, kininogens, and complement components Eosinophil Basement membrane CONNECTIVE TISSUE MATRIX Elastic fibers Collagen fibers Proteoglycans FIGURE 2-4 Components involved in acute and chronic inflammatory responses. Another stain that is often used to determine hyphal pigmentation in tissue is the Fontana-Masson stain. Describe the general characteristics of nonfermentative, gram-negative rods. In the state fair example, only a few of the affected individuals had cultures processed and results included in the investigation. Because these organisms grew within host cell vacuoles, they were reclassified into a new genus, Ehrlichia, in 1945. Superficial Mycoses Superficial mycoses are infections confined to the outermost layer of skin or hair. The eosinophil count is often elevated. parvum, C. Outbreaks have occurred in Angola (2005), Uganda (2007), and the Netherlands by Ugandan importation (2008). Bacteria can be made competent in the laboratory, and transformation is the main method used to introduce genetically manipulated plasmids into bacteria, such as E. Many different O antigens are present among the subspecies of Salmonella; more than one O antigen can also be found in a particular strain. Monkeys become sufficiently viremic to pass the virus to mosquitoes as they feed on the monkeys, thus keeping the transmission cycle active. • Direct plate count: By growing dilutions of broth cultures on agar plates, one can determine the number of colony-forming units per milliliter (CFU/mL). Antigenic Structures Salmonellae possess antigens similar to antigens of other enterobacteria. is made by observing characteristics of the infected RBCs and the malarial organism on a Wrightor Giemsa-stained peripheral blood smear. Aerial mycelia Key Terms Anamorph Arthroconidium Ascospore Ascus Blastoconidium Conidium Dermatophyte Dimorphic fungi Eumycotic mycetoma Germ tube Hyphae Macroconidium Microconidium Mold Mother Daughter cell cell Mycelia Mycoses Onychomycosis Polymorphic fungi Pseudo–germ tube Pseudohyphae Rhizoids Saprobe Sporangiophores Sporangiospores Synanamorph Teleomorph Yeast F ungi constitute an extremely diverse group of organisms and are generally classified as molds or yeasts. fragilis group are the most commonly isolated anaerobes from blood cultures. On SLW, et al: Genus Helicobacter. Microaerophilic Streptococcus spp., viridans streptococci, Staphylococcus spp. The patient experiences symptoms secondary to vasculitis, including decreased blood volume, hypotension, and disseminated intravascular coagulation. A case of prosthetic valve endocarditis was reported in the United Kingdom in 2009 that was successfully treated with piperacillintazobactam and ceftazidime, to which the isolate was susceptible. cepacia complex and B. except for B. Clinical Manifestations Classic pertussis or whooping cough resulting from B. These two components are unique to the gram-positive cell wall. Shigella The genera Shigella and Escherichia are so closely related according to molecular analyses that they should be a single genus. The PIVs are enveloped helical RNA viruses with two surface antigens, hemagglutininneuraminidase (HN) antigen and the fusion (F) antigen. terrigena. Therefore, the major use of EIA has been to detect parasite antigens in clinical specimens. Stool specimens should be collected in clean containers without any preservative and sent directly to the laboratory for processing. As predominant members of the GI biota, the organisms are often associated with peritoneal infections following disruption of the GI lining. In a thick film, the organisms may be difficult to identify, and there is no way to compare the size of infected and noninfected erythrocytes. Although the basic building blocks required for growth are the same for all cells, bacteria vary widely in their ability to use different sources of these molecules. Free or “naked” DNA is taken up by a competent bacterial cell. There are many ways by which microbial species evade phagocytosis. difficile infections in humans has been increasing in the United States and throughout Europe. jejuni is one of the most common causes of bacterial diarrhea worldwide. Many species often possess an intracellular pigment that can be pink or yellow (Figure 21-14). The cyst is approximately 15 to 20 µm, spherical, and double-walled, with the walls having a wrinkled appearance. Auramine-rhodamine b. Field diagnosis of infection is important in endemic areas, and a serologic method using a card agglutination test (CATT) and a micro-CATT have been developed. tahir99 VRG CHAPTER 22 Anaerobes of Clinical Importance • Translucent pitting colonies observed on the anaerobic blood agar plate, with no growth observed on BBE or KVLB agars, are characteristic of Bacteroides ureolyticus. Within the genus Chlamydia, four species were previously recognized— C. The mold phase is seen when the organism is grown at room temperature (22° to 25° C) in ambient air conditions. Many species of aspergilli have been implicated in human disease. A number of kits detect E. Cocci (plural of coccus) may occur singly, in pairs (diplococci), in chains (streptococci), or in clusters (staphylococci). Murine Typhus. The arthropod vector for R. Analyze the appropriate specimen collection procedures, staining methods, and culture techniques used in the mycology laboratory. Symptomatic patients may develop a fever only, whereas some patients develop meningoencephalitis. Vibrios can be differentiated from the similar genera Aeromonas and Plesiomonas by key biochemical and growth requirement characteristics, as shown in Table 20-2. Yersinia pestis The causative agent of the ancient disease plague still exists in areas where reservoir hosts are found. There has been significant resistance to amantadine seen in influenza A and H3N2, so it is not recommended for treatment. cholerae non-O1 strain producing epidemic disease. The urethra is colonized in its outermost segment by organisms found on the skin. Ewingella Ewingella americana is the only species of the genus Ewingella. enterocolitica from feces. Louse-borne typhus is still found commonly in areas of Africa and Central and South America where unsanitary conditions promote the presence of body lice. Not only can the assay be used to diagnose invasive fungal infections, but it has value in therapeutic monitoring. pylori being classified as a carcinogen. In many rapid identification systems, a numeric taxonomy is used in which phenotypic characteristics are assigned a numeric value and the derived number indicates the genus and species of the bacterium. If the test growth is less than 1% of the control growth, the organism is susceptible, and if greater, it is resistant. Uninfected cells that have been exposed to interferon are refractory to virus infection. tahir99 VRG CHAPTER 23 The Spirochetes A. parvum, and S. Leishmaniasis is a zoonotic infection in which dogs and rodents serve as the primary reservoir hosts for all species, and humans serve as an accidental host. Flat colonies are glabrous to leathery, wrinkled to folded, floccose to velvety, pink to beige to brown with a yellowish brown reverse, resembling those of B. Table 3-4 lists the steps that are followed in an outbreak investigation. The adult male measures 30 to 45 mm and has a thin anterior and a thick, coiled posterior. Some cases of malabsorption caused by inflammation and damage to the intestinal villi and a few cases of extraintestinal infection have occurred in immunocompromised patients. For example, for chronic dialysis centers, the CDC recommendations include performing bacteriologic assays of water and dialysis fluids at least once a month using standard methods. The clinical manifestations can include septicemia accompanied by mesenteric lymphadenitis, a presentation similar to appendicitis.

Conversion to the yeast or spherule form occurs when incubated at 35° C to 37° C on enriched media, with increased CO_2 (Table 27-5). bozemanii L. However, with the exception of heart tissue in cases of endocarditis, infected tissue contains low numbers of bacteria. Colors range from black to white and include yellow, brown, green, gray, pink,
beige, and tan. vulgaris P. These are referred to as obligate, or strict, anaerobes. schubertii A. Species within these genera are keratinophilic; that is, they are adapted to grow on hair, nails, and cutaneous layers of skin that contain the scleroprotein keratin. Types of Growth Media A laboratory growth medium whose contents are simple and
completely defined is termed minimal medium. By 2017, many states in the United States had even made it illegal to print phone books, according to TruthFinder. The procedure must be done as soon as the child arises in the morning. Many strains of P. maltophilia can quickly colonize the respiratory tract of hospitalized patients, in particular those
exposed to antimicrobial agents to which S. Cerebrospinal fluid Brain biopsy Characteristics Trophozoite Trophozoite Trophozoite, 10-12 µm Large karyosome Broad pseudopods Entry Nasal passage— olfactory nerve to CNS Fulminant (death within 1 wk of onset) Children to young adults, healthy (history of water activities in stagnant, warm water)
Trophozoite Trophozoite and cyst Trophozoite, 10-45 µm Spinelike pseudopod Cyst, 15-20 µm Wrinkled double wall Lungs and skin with hematogenous spread to CNS Slow and chronic Parameter Causative agent Stages in: FIGURE 28-13 Blastocystis hominis vacuolar form, identified by arrows (trichrome stain, ×1000). Additionally, the
Enterobacteriaceae, with few exceptions, share a common niche; they reside in the gastrointestinal (GI) tract. enterica, Shigella spp., and Yersinia spp., are considered true pathogens; that is, they are not present as commensal biota in the GI tract of humans. The incidence of resistance is much higher in nosocomial strains of P. Furthermore, it does
not cross-react with C. In this system, the specimen is added to the medium and incubated. vulnificus generally fall into two categories, primary septicemia and wound infections. Each malarial species has a typical number of merozoites, which may be used as an identifying characteristic. Each preparation should be covered with a no. Another
advantage to this type of slide is that it can be preserved indefinitely. Ngo V, Civen R: Babesiosis acquired through blood transfusion, California, USA, Emerg Infect Dis 15:785, 2009. Other components of the gram-positive cell wall that penetrate to the exterior of the cell are teichoic acid (anchored to the peptidoglycan) and lipoteichoic acid
(anchored to the PM). A large array of fungi produce human infections. or its affiliates tahir99 VRG YOU'VE JUST PURCHASED MORE THAN A TEXTBOOK! Evolve Student Resources for Mahon, Lehman and Manuselis: Textbook of Diagnostic Microbiology, 5th Edition, include the following: • Laboratory Manual Activate the complete learning
experience that comes with each textbook purchase by registering at REGISTER TODAY! You can now purchase Elsevier products on Evolve! Go to evolve.elsevier.com/html/shop-promo.html to search and browse for products. Reactions that become positive after 2 days are not considered. Enteric adenoviruses have a worldwide, endemic distribution,
and the number of cases increases during the warmer months. Allow excess to drain. Nitazoxanide, which is a relatively new agent, has been used for Giardia and Cryptosporidium infection in immunocompetent children and adults. Identify the major causes of fungal infections. HPV 1, 2, 3, and 4 are thought to infect universally all children and young
adults, with no significant consequences. The biochemicalbased multitest system is easier and faster to inoculate than a conventional system of plates and tubes. Most military personnel moving into areas of conflict receive the vaccination, with little to no ill effects. Thus, the fatty acid composition of a particular organism is relatively stable when
grown under specific growth conditions— medium, incubation temperature, and time. Serratia sp. Serologic testing is useful for epidemiologic studies for Campylobacter but is not recommended for routine diagnosis. Laboratory Diagnosis. M. nov., Cronobacter dublinensis sp. The organisms migrate to the insect gut and develop into epimastigotes,
which migrate to the salivary gland when mature. For this reason, GU swabs and voided or catheterized urine specimens are unacceptable for anaerobic bacteriology because recovery of these organisms would not distinguish whether they were present as pathogens or as endogenous microbiota. At the end of each chapter, readers will find Points to
Remember and Learning Assessment Questions to reinforce comprehension and understanding of important concepts. Fungal cultures are incubated at 30° C. with one or more of these organisms. However, there is a possible association between hemolysin production and virulence, known as the Kanagawa phenomenon. To recover all potential
pathogens, the clinical microbiology laboratory must use a variety of atmospheric conditions for culturing bacteria (Table 22-1). The cytoplasm is relatively clean but ingested red blood cells are seen. tahir99 VRG CHAPTER 4 Control of Microorganisms A. Disinfection and Sterilization Michelle M. Macrophages and neutrophils are the primary host
defense against the organism because the trophozoites are relatively resistant to the actions of host cytokines. Special flotation procedures have been used for the detection of specific organisms such as the Sheather tahir99 VRG CHAPTER 28 Diagnostic Parasitology sugar flotation method for Cryptosporidium spp. Because it is being reported in
regions previously free of Japanese encephalitis, including Australia, Japanese encephalitis virus is considered an emerging pathogen. HUS is characterized by low platelet count, hemolytic anemia, and kidney failure. This disease is characterized by discoloration or depigmentation and scaling of the skin and is caused by the yeast Malassezia furfur
complex. ■ N. Encapsulated strains of S. Of all childhood diseases for which universal vaccination is recommended, only pertussis incidence has increased in recent decades. In the United States, HSV encephalitis may account for up to 20% of all encephalitis cases. Two commercial kits are available in the
United States for culture identification, INDX-Campy (JCL) (Hardy Diagnostics, Santa Maria, CA, and Fisher Scientific, Hampton, NH) and Dry Spot Campylobacter test kit (Remel, Lenexa, KS). Countries with rates of polio considered to be endemic have decreased steadily since the program began. As of October 9, 2009, the WHO had received
reports of more than 375,000 human cases and 4525 deaths. Therefore, the traditional convalescent serum obtained approximately 14 to 21 days after onset does not contain MIF-detectable C. Discuss the use of serologic assays for diagnosing M. A modification of this procedure is more practical and permits retention of more of the fruiting structure.
are exceptions that do grow in the presence of 5% NaCl. Growth on MacConkey Agar. The Mycobacterium fortuitum-chelonae complex can grow on MacConkey agar without crystal violet, whereas most other mycobacteria cannot. infections, Infect Immunol 76:23, 2008. The respiratory viral panel assay from Luminex Molecular Diagnostics (Toronto)
claims 100% sensitivity and 98.2% specificity for hMPV in clinical specimens. Aspergillus fumigatus is the species most commonly isolated; other pathogenic species include A. are commonly found in a wide variety of aquatic environments, including fresh water, brackish or estuarine water, and marine or salt water. The microfilariae of M.
Alternatively, the ESwab transport system (Copan Diagnostics, Corona, CA; see Figure 22-3) which contains a swab transported in 1 mL of a prereduced Amies liquid that can maintain aerobic and anaerobic organisms for up to 48 hours at room temperature can be used. enterocolitica sepsis associated with the transfusion of contaminated packed red
blood cells has been reported. The reusable catalyst pellets can be rejuvenated after every use by heating in a 160° C oven for a minimum of 2 hours. All manipulations with cultures or specimens should be done with gloves and laboratory coats or gowns. Never blot a critical smear. These sclerotic bodies reproduce by dividing in various planes,
resulting in multicellular forms. Control measures include rodent elimination and drainage of contaminated waters. In adults, E. CMV is typically spread by close contact with an infected person. tuberculosis; other Mycobacterium spp. Antimicrobial Susceptibility Susceptibility testing of Legionella spp. They prefer and grow much better in an aerobic
environment; some do not grow in an anaerobic environment at all. The most recent methods, M44-A and M51-A, were developed as cost-effective procedures that could easily be incorporated into busy microbiology laboratories that routinely perform this type of antimicrobial testing for bacteria. Those strains with an MIC above the ECV have
potentially acquired mechanisms of resistance. chaffeensis have a rash; however, adults rarely experience a rash. Some nucleic acid–based assays using realtime PCR (RT-PCR) are becoming available from research laboratories that might be helpful in the future. Those considered most pathogenic are Brugia malayi, Wuchereria bancrofti, Onchocerca
volvulus, and Loa loa. Some Mycobacterium spp. Concentration Procedures The specific gravity of the tubercle bacilli ranges from 0.79 to 1.07. BV is most often diagnosed on clinical appearance and Gram staining of vaginal secretions that reveals a shift in the vaginal biota from predominantly gram-positive lactobacilli to a mixture of Gardnerella
spp. Genetic Recombination Genetic recombination is a method by which genes are transferred or exchanged between homologous (similar) regions on two DNA molecules. The formal steps in an outbreak investigation include: a. Table 19-9 shows the biochemical characteristics of Shigella spp. Conversion to the flagellate form occurs in 2 to 20
hours. Available at: . It is urease and PDA positive. Many laboratories, however, now use the latex agglutination test for cryptococcal antigen in place of the India ink examination. In fact, a multistate outbreak involving more than 100 individuals wearing soft contact lenses was reported in 2006. This higher incidence is attributed to poor sanitary
conditions, and it appears that infection occurs early in life. It is similar to E. Once the eggs have been deposited in warm, moist soil, the noninfective, feeding, first-stage rhabditiform larva develops within 1 to 2 days and feeds on bacteria in the soil. Although isolates may be susceptible in vitro to many antimicrobial agents, including SXT,
chloramphenicol, tetracycline, semisynthetic penicillins, and ceftazidime, the clinical response to therapy is usually slow, and relapses are common. Like oocysts of Cyclospora cayetanensis, the oocysts of C. Between 20% and 50% of deaths caused by P. Adults, measuring 15 to 35 cm long and about the diameter of a lead pencil, may be seen in stool
samples. pseudomallei is isolated from someone who has never traveled to an endemic area, its occurrence should be reported to local or state public health departments. If the isolates all had identical antibiograms (e.g., isolate 1) or if some of the isolates were different in their susceptibility patterns (isolate 2), the inclusion or exclusion from the
case definitions might have been affected. Describe the general characteristics of the mycoplasma and how they differ from other bacterial species. curvus, and C. typhi. Fimbriae (plural of fimbria) are nonflagellar, sticky, proteinaceous, hairlike appendages that adhere some bacterial cells to one another and to environmental surfaces. Similar to
brucellosis, tularemia is a CDC reportable disease, and about 100 cases are reported annually. However, only a few possible virulence features such as heat-stable and heat-labile toxins have been defined to explain this organism's association with enteric disease. The trophozoite is the motile feeding stage that reproduces by binary fission. Cover
smear with a piece of filter paper cut slightly smaller than the slide. Each specimen should be confined to a single collection in an individual collection container recommended by the laboratory providing the requested diagnostic service. Piedraia hortae Clinical Manifestations. Piedraia hortae is the causative agent of black piedra, an infection that
occurs on the hairs of the scalp. Long-term, costly therapy with terbinafine or itraconazole is considered the best treatment, but results are often unsatisfactory. What organism is often associated with lobar pneumonia in elderly hospitalized patients? Describe how viruses replicate. This multicolored sheen also can appear on the surface of the agar in
a narrow zone around the colony. Other techniques, such as high-pressure liquid chromatography (HPLC), have been used to distinguish mycobacterial species. fetus can be recovered in several routine blood culture media. What is the typical natural habitat of most nonfermenters? In addition, it has been associated with cases of otitis media,
meningitis, pneumonia, surgical wound infections, UTIs, peritonitis, and bacteremia. Malabsorption syndrome may occur. Chryseobacterium spp. Whenever possible, CSF specimens should be from large-volume spinal taps to increase diagnostic yield. Autotrophs obtain energy either photosynthetically (phototrophs) or by oxidation of inorganic
compounds (chemolithotrophs). Mycetomas occur in tropical and subtropical areas but are also seen in temperate zones. When P. gordonae, and M. Moreau-Gaudry V, et al: Three cases of post-cataract surgery endophthalmitis due to Rhizobium (Agrobacterium) radiobacter, J Clin Microbiol 50:1487, 2012. Robichaud S, et al: Prevention of laboratory-
acquired brucellosis, Clin Infect Dis 38:e119, 2004. Plesiomonas has been moved to the family Enterobacteriaceae but unlike the Enterobacteriaceae, it does not have the ability to produce gas from glucose, it is the only oxidase-positive member, and it is susceptible to agent O/129. This organism may be recovered from blood, tissues, and abscesses.
coli are motile and generally possess adhesive fimbriae and sex pili and O, H, and K antigens. Presence of the parasite in the insect's feces tahir99 VRG 650 PART II Laboratory Identification of Significant Isolates Development in gut Trypomastigote circulation in blood of host Reduviid bug's biting of human and ingestion of trypomastigote
Epimastigote Some amastigote transformation back to trypomastigotes Bursting of cell, which releases amastigotes Intestine of bug Metacyclic trypomastigotes Bite Feces Reinfection Feces deposition near site of bug bite Trypomastigote entry into bloodstream through bite Trypomastigote transformation into amastigote and multiplication
Trypomastigote entry into tissue cells (liver, heart, macrophage) FIGURE 28-26 Life cycle of Trypanosoma cruzi. A 20% increase in growth index is considered significant and rules out M. Differentiate between prokaryotic (bacterial and archaeobacteria) and eukaryotic cell types. feeleii L. The medium of choice is charcoal agar supplemented with
10% horse blood with or without 40 mg/L cephalexin. Convalescent serum contains protective antibodies. Centers for Disease Control and Prevention: Lyme disease: resources for clinicians: diagnosis, treatment, and testing, 2013. and risk factors related to high-risk occupational groups in Eritrea, Epidemiol Infect 129:85, 2002. There has been
controversy about the use of these vaccines because it is recommended that they be administered at a young age, ostensibly before sexual activity. More recently in the United States, there has been a series of outbreaks by various Salmonella serovars related to the ingestion of foodstuffs such as peanut butter, cantaloupe, puffed rice and wheat
cereals, corn and vegetable coated snacks, and raw tomatoes. Endolimax nana. The trophozoite of E. Nomenclature Nomenclature provides naming assignments for each organism in this textbook. However, some pathogenic species prefer a lower temperature for growth; when these organisms are suspected, the specimen plate is incubated at a
lower temperature. Penicillin 10. Case in Point A 25-year-old woman who received a bone marrow transplant as treatment for aplastic anemia presented with fever, chills, and malaise of about 2 days' duration. Typically, smallpox disease is characterized as a synchronous progressive rash accompanied by fever. Urine, Vaginal, and Urethral Specimens
Eggs of Schistosoma haematobium and E. In addition, fungal cell walls are made of chitin, whereas those of plants contain cellulose. Although it grows at 37° C, it has a preferential growth temperature of 25° C to 30° C. Growth of F. falciparum has a persistent liver phase, although recrudescence in untreated individuals with either of these organisms
may be the result of a continued subclinical erythrocytic infection. coli was initially considered a harmless member of the colon resident biota. Calcofluor white, a fluorescent stain used for the detection of fungi, can also be used to screen for microsporidia spores. Symptoms include a profuse watery discharge, nasal congestion, sneezing, headache,
sore throat, and cough. Heavy infections result in persistent diarrhea, anorexia, edema, ascites, nausea and vomiting, and/or intestinal obstruction. These viruses are not cytolytic but are associated with several leukemias, sarcomas, and lymphomas. Systemic Mycoses Systemic, or disseminated, mycoses are infections that affect internal organs or
deep tissues of the body. Children assume the major burden of this infection, with mortality as high as 30%; mortality in adults is much lower. 924. W. The diagnosis is made from the typical appearance of the lesion. The portal of entry is ocular or aspiration, with colonization of the oropharynx being a necessary event before infection. The invasive
properties of the organism are related to its ability to secrete multiple enzymes (e.g., phospholipase, sphingomyelinase), which facilitate tissue destruction and counteract the host immune response. Ab, Antibody; PMN, polymorphonuclear neutrophil. They are categorized by their locomotive structures: flagella (Latin: whiplike), pseudopodia (Greek:
false feet), or cilia (Latin: eyelash). tahir99 VRG Skin Mucus Bone + + + + + + + + + + + + 616 PART II Laboratory Identification of Significant Isolates CLINICAL SPECIMEN Culture Direct examination Tissue • Histology stain • Fluorescent antibody (selected organisms) Cerebrospinal fluid • India ink • Cryptococcal antigen Growth Skin, hair,
nails, and other selected specimens 30° C (suspect dimorphic) 22° to 25° C No growth Yeast Mold KOH/KOH with calcofluor white No hyphal Hyphal elements elements • Width • Hyaline vs. For example, two groups at increased risk for gram-negative rod pneumonia are diabetics and alcoholics. All the resident cases had an underlying medical
condition; two were hospitalized, but none died. tahir99 VRG 58 ■ ■ ■ ■ ■ PART I Introduction to Clinical Microbiology The microbiology laboratory supports outbreak investigations by providing consultative services, including epidemiologic correlation of isolates. Polar flagella may occur singly at one or both ends or multiply in tufts at one
tahir99 VRG 10 PART I Introduction to Clinical Microbiology end termed lophotrichous. Serratia The genus Serratia comprises S. In addition, the methodology among laboratories varies. B, Tissue phase of H. The ends are pointed and covered with a sheath. In addition, a more virulent strain BI/NAP1/027 (restriction endonuclease analysis group BI,
pulse-field gel electrophoresis type NAP1, and polymerase chain reaction ribotype 027) has emerged. An understanding of the microscopic and colony morphology of the Legionella spp., their nutritional requirements, the pathogenesis of infection, and the capabilities of various commercial laboratory tests enables clinical microbiologists to reliably
identify these microorganisms. Most anaerobe chambers can operate on lower quality gas. It can be activated from latency by various stimuli, including stress, caffeine, and sunlight. Both have three anterior lips with small, toothlike projections. Both form their conidia blastically (budding) like many yeasts; the parent cell enlarges and a septum forms
to separate the conidial cell. In the relationship where the organism benefits but there is no beneficial or harmful effect to the host, the association between organisms is called commensalism. In a wet mount it may be difficult to distinguish the large karyosome of E. Gram-negative archaeal cells, in contrast to the typical gram-negative bacterial lipid
membrane, have a layer of protein covering the cell wall and stain pink. Purulence, blood, and necrosis can be present if traumatic tissue damage occurs in the absence of infection. The reason for combining the two allows the species epithet to be used for a different species in another genus. Two very similar species that infect humans are C. What
environmental factors contribute to infection caused by Legionella spp.? Laboratory Diagnosis Culture Media Aeromonads grow readily on most media used for routine and stool cultures. Woeste, EdD Assistant Dean and Associate Professor College of Applied Science and Technology Illinois State University Normal, Illinois tahir99 VRG vii This page
intentionally left blank tahir99 VRG Contributors Annette W. A number of commercial rapid kits are available for the diagnosis of influenza in about 30 minutes. Patients with AIDS can develop encephalitis, nephritis, or keratoconjunctivitis. A multilayered outer sheath similar to the outer membrane of gram-negative bacteria completely surrounds the
protoplasmic cylinder (the cytoplasmic and nuclear regions are enclosed by the cytoplasmic membrane–cell wall complex and periplasmic flagella). 666. (Courtesy State Laboratories Division, Hawaii Department of Health.) are very close together, so the organism may appear to be a chain of cocci. Centers for Disease Control and Prevention: Child,
adolescent and “catch-up” immunization schedules. The devastating clinical scenario is the result of a powerful enterotoxin known as cholera toxin, or choleragen. Both P. All of the above BIBLIOGRAPHY Arias KM: Quick reference to outbreak investigation and control in health care facilities, Gaithersburg, MD, 2000, Aspen, CO. Specimen collection
only; no mycobacteriologic procedures performed; all specimens are sent to another laboratory • Level 2. trota is its unusual universal susceptibility to ampicillin. Direct examination of nasopharyngeal secretions by IF can give rapid results. The composition of the microbial flora is also affected by pH. The mycelia are placed in a drop of lactophenol
cotton blue (LPCB) on a slide and gently teased apart using the needles. This damage may be from toxins, either exotoxins or endotoxins, or from inflammatory substances that cause host-driven, immunologically mediated damage. Immunologic Diagnosis Parasites that invade tissue (e.g., E. mandrillaris can occur in immunocompetent and
immunocompromised hosts. The two variant, of the virus, A and B, are indistinguishable serologically, but variant B appears to be the cause of disease. urealyticum in infections of low-birth-weight and high-risk neonates. To do this, open your device and run a search for either a specific online directory, such as Telkom or WhitePages. The outbreak
was associated with a particular brand of contact lens solution and occurred typically in patients who wore lenses continuously without removal for several days at a time. Brugia malayi. B. only on swabs, two swabs are submitted. abortus, B. Septate and Phaeoid Saprophytes Alternaria. Although Alternaria spp. Clinical Infections Although all
Shigella spp. In temperate countries such as France, Canada, and the United States, most cases of sporotrichosis are associated with gardening, particularly with exposure to rose thorns (rose handler's disease) and sphagnum moss. pseudomallei is generally accurate in many of the commercial manual nonfermenter systems, although performance in
the Vitek-2 was shown to be unsuccessful. This type of atmosphere can be generated in culture jars or pouches using a commercially available microaerophilic atmosphere–generating system. Why are spore-forming organisms more resistant than non– spore-forming species? Available at: . flexneri. Malassezia furfur Clinical Manifestations. The M.
caviae tahir99 VRG 466 TABLE PART II Laboratory Identification of Significant Isolates 20-5 Differential Characteristics for Mesophilic Clinical Aeromonas Species Aeromonas Species Characteristic Esculin hydrolysis Voges-Proskauer Pyrazinamidase activity Arginine dihydrolase Fermentation Arabinose Cellobiose Mannitol Sucrose Susceptibility
Ampicillin Ticarcillin or Piperacilli Cephalothin Decarboxylase Lysine Ornithine Indole H_2S* Glucose (gas) Hemolysis (5% sheep RBCs) A. ✓ Case Check 28-1 The intermittent bouts of diarrhea in the young boy in the Case in Point could be caused by any number of organisms, from viral to bacterial to parasitic. indica tahir99 VRG 434 TABLE PART
II Laboratory Identification of Significant Isolates 19-8 Biochemical Differentiation of Selected Members of the Genus Salmonella Test Arabinose fermentation Citrate utilization Glucose gas production H_2S (TSI) Lysine decarboxylase Ornithine decarboxylase Rhamnose fermentation Trehalose fermentation S. Filarial Worms. Filarial worms are
roundworms of blood and tissue found primarily in tropical areas of the world. +, Positive; −, negative; R, resistant; RBC, red blood cells; S, susceptible; V, variable; V+, variable usually positive. schenckii has been implicated in pneumonia that is refractory to antifungal therapy. Compare the microscopic and colony morphology and results of
differentiating tests of anaerobic isolates. lwoffii is asaccharolytic. The stools produced by patients with cholera are described as rice water stools, and the number of stools, which are watery and contain numerous flecks of mucus, may be as many as 10 to 30/day. Light infections are usually asymptomatic; large numbers of worms may cause
abdominal pain, diarrhea, irritability, and headache. Patients who are given immunosuppressive drugs because they have received a transplant are more susceptible to infection. Russell, EdD, MLS(ASCP)SH Associate Professor and Program Director Program of Clinical Laboratory Science Department of Biomedical and Radiological Technologies
Medical College of Georgia Augusta, Georgia Linda A. Adaptations of this procedure using paddles with a sticky surface are commercially available. pneumoniae– specific IgM does not appear until 3 weeks after onset of symptoms, and often C. C, Bacterial conjugation. The physician expects the laboratory to affirm or reject the antibiotic choice made
after the presumptive diagnosis. Psychrophiles and thermophiles are found environmentally in places such as the Arctic seas and hot springs, respectively. Oil immersion should not be used on a wet preparation unless the preparation has been sealed with clear nail polish or vaspar. In addition, antigen titer corresponds to clinical outcomes; therefore,
this assay could be used to monitor patient response to treatment. The clinical manifestations resemble those caused by Chlamydophila pneumoniae. pulmonis, with emended description of P. PCR and viral load testing offer the most sensitive and specific means of diagnosing primary HHV-6 infection. The emergence results from increased spread of
the mosquito vectors, deforestation of Africa and South America, and increased travel by people into endemic regions. Reading the wet mount involves thorough examination of each coverslipped preparation at low power, starting at one corner and following a systematic vertical or horizontal pattern until the entire preparation has been examined.
Centers for Disease Control and Prevention: Vibrio parahaemolyticus infections associated with consumption of raw shellfish—three states, 2006, MMWR 55:854, Available at: preview/mmwrhtml/mm5531a5.htm. Compare the clinical diseases caused by Mycoplasma pneumoniae, Mycoplasma hominis, and Ureaplasma urealyticum. TABLE 27-4
Morphology of Systemic Fungi* Fungus Macroscopic Morphology Blastomyces dermatitidis Slow to moderate growth White to dark tan Young colonies tenacious, older colonies glabrous to woolly Histoplasma capsulatum Slow growth White to dark tan with age Woolly, cottony, or granular Coccidioides immitis, Coccidioides posadasii Rapid growth
White to tan to dark gray Young colonies tenacious, older colonies cottony Tend to grow in concentric rings Slow growth White to beige Colony glabrous, leathery, flat to wrinkled, folded or velvety Paracoccidioides brasiliensis Microscopic Morphology Oval, pyriform, to globose smooth conidia borne on short, lateral hypha-like conidiophores
Microconidia small, one-celled, round, smooth (2-5 µm) Tuberculated macroconidia large, round (7-12 µm) Hypha-like conidiophores Alternating one-celled, “barrel-shaped” arthroconidia with disjunctor cells Colonies frequently only produce sterile hyphae. fortuitum group Photochromogens Slow Growers M. Although a specialized medium is not
recommended for the detection of plesiomonads from stool specimens, and because certain strains are inhibited on eosin–methylene blue or MAC agar, the use of inositol brilliant green bile agar can enhance the isolation of plesiomonads. Black piedra is caused by Piedraia tahir99 VRG 594 PART II Laboratory Identification of Significant Isolates
hortae, and white piedra is caused by Trichophyton ovoides and T. pneumoniae and the more rapidly growing Mycoplasma hominis. pneumoniae causing a disease sometimes referred to as atypical pneumonia. Rectal prolapse can be seen as the result of repeated heavy infections in undernourished children. Salmonella typhi must have its capsular
(Vi) antigen removed for the technologist to observe agglutination with Salmonella somatic (O) antisera. are sensitive to SXT and the fluoroquinolones and resistant to aminoglycosides, clindamycin, and polymyxin B. Gaschet A, et al: Multiple brain abscesses caused by Pseudomonas luteola, Pediatr Infect Dis J 28:1144, 2009. The test is based on the
immunocytochemical detection of the 65-kDa lower matrix phosphoprotein (pp65) in the nuclei of infected peripheral white blood cells. It is recommended that at least 200 to 300 oil immersion fields be examined on either type of smear before the smear is considered negative. The exception is in pregnant women, in whom the dissemination rate
equals or exceeds that for men. For this reason, it may be advisable also to plate a medium without cephalexin. It is thin-shelled, measures 54 by 32 µm, and often is segmented. Examples are the K1 antigen of E. The genome and its protein coat together are referred to as a nucleocapsid. About one third of the total WNV cases were seen in Texas. The
positive oxidase activity separates it from other Enterobacteriaceae, sensitivity to the agent O/129 separates it from Aeromonas, and its ability to ferment inositol separates it from all Aeromonas and almost all Vibrio spp. nov., Cronobacter dublinensis subsp. Available at: . Fusobacterium spp. aureus P. tahir99 VRG CHAPTER 29 Clinical Virology
Kevin M. phenylpyruvicus has been isolated from urine, blood, CSF, and the genitourinary tract. The two mass spectrometry instruments for performing MALDI-TOF, the MALDI Biotyper (Bruker, Billerica, MA) and Vitek MS, were recently compared for the identification of 200 nonfermenting bacilli isolated from CF patients at the University of Iowa
Health Care. Available at: article/8/7/01-0501_article.htm. Physiology The vibrios are facultatively anaerobic, and all 10 clinically significant species are catalase- and oxidase-positive and able to reduce nitrate to nitrite, except for V. When the primary disease fails to resolve, pulmonary disease may ensue, with cough, weight loss, chest pain, and
fever. tahir99 VRG 583 584 PART II Laboratory Identification of Significant Isolates 1+ C – + 3+ 5+ FIGURE 26-11 Nitrate reduction test. The trypomastigote and amastigote (Leishman-Donovan body) are the diagnostic stages found in humans. chelonae, which is generally negative, from other rapid growers, which are positive. Patients with
secondary infection had a milder illness, and all survived the infection. Classically, the term immunity has been defined as a complex mechanism whereby the body is able to protect itself from invasion by disease-causing organisms. This human isolate became known as Mycoplasma pneumoniae. Enterovirus infections often cause mild nausea and
diarrhea except in neonates, in whom disease can be more severe because of the immaturity of their immune system. Others are capable of invading the central nervous system. Serologic and RNA sequence research have shown that the virus is found almost worldwide and that in some areas, most children have been exposed by the time they reach 5
years of age. Human neonatal lung is an example of a standard finite cell culture used in diagnostic virology. Endocarditis is the most common clinical disease caused by lactobacilli and has a high mortality rate (23% to 27%). The many different media available for the recovery of mycobacteria from a clinical specimen are variations of three general
types (Table 26-3)—egg-based media, serum albumin agar media, and liquid media. These isolates are opportunists that reside on the mucous membranes of humans and lower animals and can be isolated from the respiratory tract, urinary tract, and eyes. In addition, the diagnostic features that characterize these agents are presented. Superficial
antisepsis of the skin does not eliminate this organism, which may be found as a contaminant in culture specimens obtained by invasive procedures (e.g., blood, cerebrospinal fluid), as a result of contamination of the needle. luteola can be differentiated from P. If susceptibility tests are performed—and because of virulence should only be performed in
approved laboratories—CLSI recommends that a broth microdilution with Brucella broth and incubation at 35° C in ambient air for 16 to 20 hours be the method used. Gastric lavage has been reported to be better than BAL for the detection of mycobacteria in children. An India ink wet mount of Cryptococcus neoformans demonstrating the presence
of a capsule (arrow). pneumoniae pneumoniae subsp. falciparum gametocyte. Obsolete synonyms for H. fumigatus was linked to at least one case following the use of injectable steroids. The particle is taken into the cytoplasm and enclosed within a vacuole called a phagosome (Figure 2-3). ✓ Case Check 18-3 Legionella spp. Some species also form
diffusible subsurface pigments on a variety of media. A loopful of test organism is grown in 4 mL of urea broth at 37° C for 3 days. This method is more sensitive than a thick smear in demonstrating the presence of parasites, but a thin smear must still be made for definitive identification. The suspension is then cooled and retested with antisera A to G.
This can differentiate the organism from other pseudomonads as well, although this is not a test commonly used in clinical laboratories. Haemophilus influenzae, however, will grow on the anaerobic plate and the CO_2-incubated chocolate agar plate but not on the aerobically incubated SBA plate. Available at: . Available at:
preview/mmwrhtml/mm5312a5.htm. This step ensures that the rest of the investigation is based on a single definition. moshkovkii are morphologically identical, and specific immunoassay techniques must be performed to differentiate the pathogenic E. If C. Centers for Disease Control and Prevention: Reported tuberculosis in the United States, 2011.
Incubation Most laboratories routinely incubate fungal cultures at room temperature or at 30° C. Isolation of C. Laboratory Diagnosis There are numerous methods for the laboratory diagnosis of C. Local conditions select for organisms that are suited for growth in a particular area. The clinical manifestations seen are dependent on the age and
immune status of the infected person. Diarrhea caused by C. Whenever possible, body fluids, pus, or tissues should be submitted, but swabs are acceptable if they are transported in an appropriate holding medium, such as Cary-Blair, to prevent desiccation. streptocerca, and M. hMPV has also been documented to cause outbreaks in long-term care
facilities. Other rickettsiae pass directly through an uninjured cell. CMV produces a characteristic CPE, which can sometimes be seen in clinical specimens (Figure 29-7). It is important to note that any fungus is capable of disseminating from the primary site of infection in the immunocompromised host. maltophilia Pseudomonas luteola and P. On
CHOC agar or SBA plates, enteric bacteria produce large, grayish, smooth colonies. Full-service virology laboratories provide viral culture and identification using different mammalian cell cultures to support the growth of viruses from clinical specimens. falciparum (Figure 28-32, A) are more delicate than those of other species and often have two
chromatin dots. The most common tick vectors are Dermacentor variabilis (Figure 24-9) in the southeastern United States and Dermacentor andersoni in the western part of the country. upsaliensis, C. dermatidis, however, forms conidia at the tips of phialides (Figure 27-23). Clinical Manifestations After an incubation period of 2 to 15 days, a massive
spirochetemia develops and remains at varying levels of severity during the entire course of relapsing fever. The disease associations, coupled with differences in antimicrobial susceptibilities among the species, strongly suggest that conventional identification to the species level and antimicrobial susceptibilities using the Kirby-Bauer method should
be done on all clinical aeromonad isolates, if possible. jandaei, A. There is great variation among different lots in regard to performance, and not all Vibrio spp. Cytotechnologists and cytopathologists read Pap smears and look for koilocytes, cells with perinuclear clearing accompanied by an increased density of the surrounding rim of cytoplasm,
which are indicative of HPV infection. Gram-negative bacteria, which have a thinner layer of peptidoglycan and a different cell wall structure, are less affected by these antibiotics. Electron microscopy must be used to identify the species of the organism. The patient develops jaundice (hence, the name “yellow” fever) and bleeding from the mouth,
eyes, nose, stomach, or other areas. Nitrate reduction b. On the other hand, Petri dishes have a larger surface area for colony isolation and are easier to manipulate when making preparations for microscopic examination. jirovecii is the species most often recovered from humans. intestinalis both commonly infect the GI tract, and infection in
immunocompetent hosts is characterized by diarrhea, cramps, loss of appetite, and fatigue. vulgaris produces indole and is ornithine-negative. The ancient method of removal by rolling the worm a few inches at a time onto a stick is still practiced in some areas of the world. A shaking chill (the cold stage) of 15 to 60 minutes initiates the paroxysm and
is followed by a fever (hot stage) with headache, myalgia, and nausea and a temperature of up to 40° C. Johnson RE, et al: Screening tests to detect Chlamydia trachomatis and Neisseria gonorrhoeae infections—2002, MMWR 51(RR-15):1, 2002. • H antigen, or flagellar antigen—this is a heat-labile antigen found on the surface of flagella, structures
responsible for motility. immitis and C. Match the Shigella spp. coli; UTI, urinary tract infection. The rapid NF Plus (Remel, Lenexa, KS) provides identification by analysis of preformed enzymes; 70 medically important nonfermenters including members of the Burkholderia cepacia complex, can be identified in 4 hours. The ability to use one manual
system to identify enterics and nonfermenters may be attractive to a smaller laboratory, even if the database is limited, as it is in this system. agglomerans; however, they can be distinguished by a weak phenylalanine deaminase reaction; the fact that they are negative for potassium cyanide (KCN), gelatin, lysine, ornithine, and motility; and their lack
of yellow pigmentation. DNA probes for EIEC more recently have become commercially available. ■ Case in Point A premature male infant in the neonatal intensive care unit, who weighed 1.5 lb at birth (low birth weight), developed signs of meningitis, and a lumbar puncture was performed. With other roundworms, the egg would be ingested; with
tapeworms, there could possibly be a history of eating raw or undercooked meat. Again, the incidence of human disease was low, but the mortality rate was high, although it is possible that some mild cases went undiagnosed. 1612. Patients typically present with a nonproductive cough, fever, headache, and myalgia. neoformans, the most notable
pathogen in this genus, has become a major cause of opportunistic infection in patients with AIDS. All secondary lesions of the skin and mucous membranes are highly infectious. vermicularis, infection is common in children and is diagnosed by finding eggs on a cellophane tape preparation. coli are considered normal fecal microbiota, several strains
(diarrheogenic E. Approximately 80 people in 31 states were affected. Eggs are broadly oval, 80 to 115 µm × 48 to 60 µm, with a flattened operculum and slight shoulders. Learning objectives and a list of key terms are also found at the beginning of each chapter. Bifidobacterium spp. Except for Salmonella, Shigella, and Yersinia, they can be resident
microbiota if confined to their natural environment. 15. Like the agent for RMSF, this rickettsia is tick-borne, and its reservoirs include ticks and dogs. The antimicrobial agent of choice for the treatment of pertussis is: a. Because these systems do not depend on enzyme induction or bacterial growth, the results are available in about 4 hours. What is
psittacosis or ornithosis? A, Acid; G, gas; H_2S, hydrogen sulfide; K, alkaline; LIA, Lysine-iron agar; R, deamination (red slant); TSI, triple sugar iron. If malarial stippling is present, it appears as discrete pink-red dots. Laboratory Diagnosis. After inhalation, the barrel-shaped arthroconidia, which measure 2.5 to 4 µm × 3 to 6 µm, round up as they
convert to spherules. sonnei − + + + V − − − V − + + A B C D From Farmer JJ, et al: Enterobacteriaceae: introduction and identification. The size and shape of the oocyst serve as the identification characteristics in this examination. coli is the most documented virulence factor associated with neonatal meningeal infections. pylori is also recognized
as a major cause of type B gastritis, a chronic condition formerly associated primarily with stress and chemical irritants. aureus from another patient is susceptible to penicillin, the two isolates are considered to be different strains of the same species. Also from August 2010 through June 2011, the CDC documented 109 cases of Salmonella
Typhimurium from clinical and teaching laboratories. Some state health laboratories and the CDC perform EIAs to detect anti-SNV IgM and IgG antibodies, which may provide some useful information on infection. Centers for Disease Control and Prevention: Anaplasmosis: statistics and epidemiology. They are biochemically similar to Hafnia but differ
primarily by yielding negative Voges-Proskauer test results. In contrast, during reinfection, a CF antibody change is not detected, but by MIF, an IgG titer of 1 : 512 or more can appear within 2 weeks. When grown on Sabouraud dextrose agar, colonies usually form a rustcolored pigment on the colony's reverse. baumannii are often resistant to many
antimicrobials, including penicillins, first- and second-generation tahir99 VRG CHAPTER 21 Nonfermenting and Miscellaneous Gram-Negative Bacilli FIGURE 21-8 Acinetobacter baumannii on Gram stain. • A source of energy (adenosine triphosphate [ATP], for performing cellular functions). pneumoniae is the most common cause of bacterial
pneumonia. The small intestine contains few microorganisms; organisms that are present are usually from the colon. They do not form spores or capsules and are motile by monotrichous or two to five lophotrichous flagella. Colony morphology on nonselective media, such as SBA or chocolate (CHOC) agar, is of little value in initial identification.
Members of the genera Aeromonas, Plesiomonas, and Vibrio have many similar characteristics. when isolated in the clinical laboratory. Basidiomycetous molds are being recovered in increasing numbers in the laboratory, but their clinical significance is not clearly understood. These organisms usually grow well on most laboratory media, including
MAC agar. Despite some limitations, these rapid tests represent an important step forward in timely diagnosis of Legionella infections. They may serve as opsonins, enhancing phagocytosis, or they may fix complement and neutralize the infecting organism. are found worldwide and are often associated with soil or decomposing organic matter. The
laboratory must not only quickly learn how to identify these agents in human infections, but also collaborate with the infection control program in dealing with these agents in the human population. World Health Organization: Leprosy: Situation and trends, 2013. tsutsugamushi to the human host is followed by an incubation period of approximately 2
weeks. The sensitivity of serologic tests is reported to be 75% to 80%, with a specificity of 90% to 100%. *Vibriostatic agent (2, 4-diamino-6, 7-diisopropylpteridine). 627. Fusarium spp. Plesiomonas will not grow on TCBS agar but will grow on CIN, a selective agar for Yersinia spp., as opaque (non–mannitol-fermenting) colonies with an opaque apron.
To identify an isolate, the clinical microbiologist first must determine whether the isolate belongs to the family Enterobacteriaceae. Campylobacter, Helicobacter, Gastrobacillum, Borrelia, Leptospira, Treponema spp. 1853. tuberculosis isolates should consider sending isolates to a reference laboratory for susceptibility testing. rettgeri, P. found in
asymptomatic persons. However, patients with disease may not show an increase in titer for 8 weeks or longer after symptoms begin. Engelkirk PG, et al: Principles and practice of clinical anaerobic bacteriology, Belmont, CA, 1992, Star Publishing Company, Inc. The scolex, which is 2 to 3 mm long, is elongated and has two sucking grooves, one
located on the dorsal surface and the other on the ventral surface. Complete recovery may require weeks or months. The oxidase test should always be performed using young growth from an SBA plate. Available at: . cruzi, transmitted by the triatomid bug, also known as the reduviid or kissing bug (Triatoma sp. Plasmodium ovale. Plasmodium ovale,
the least commonly seen species, resembles P. The microbiology laboratory interacts with the infection control program by providing: a. Unlike the case with RMSF, the face may also be affected by a rash. (e.g., Campylobacter concisus, C. schenckii is commonly recovered from the soil and is associated with decaying vegetation. Once fertilization
occurs, a zygote forms and develops into an immature oocyst that is passed in the stool. Many of the older, traditional diagnostic methods were slow and cumbersome and required significant expertise because they were primarily based on cell culture, serology, and microscopy (both bright field and electron). Despite the progress made and
significant advances that have occurred in their control, prevention, and treatment, infectious diseases remain a major threat to human health. The CDC, in BOX 26-3 Levels of Service as Defined by the American Thoracic Society and College of American Pathologists Extent of Service as Defined by the American Thoracic Society • Specimen
collection only; no mycobacteriologic procedures performed; all specimens sent to another laboratory • Acid-fast stain, inoculation, or both; identification by a reference laboratory • Isolation and definitive identification of Mycobacterium tuberculosis; preliminary grouping of nontuberculous Mycobacterium spp., with definitive identification at a
reference laboratory • Definitive identification of all mycobacterial isolates with assistance in the selection of therapy, with or without drug susceptibility testing College of American Pathologists Levels of Service FIGURE 26-7 Septi-Chek biphasic media system for acid-fast bacilli. produce a pigment, which can be a helpful feature in the
identification of the mycobacteria. Currently, the most useful serologic application for the diagnosis of histoplasmosis appears to be the combination of complement fixation and immunodiffusion tests, in which rising titers in serial dilutions are considered significant. The isolates are positive for oxidase and indole. Rare cases of bacteremia, keratitis,
and endophthalmitis caused by M. A predominantly cellassociated virus, HSV produces a focal CPE (in which adjacent cells become infected) and plaques, or clusters of infected cells. Cats c. In the United States, fewer than 50 cases of C. Infection was associated with consumption of raw dough. The body is under constant surveillance by these and
other phagocytic cells. trachomatis infection; however, cell culture usefulness has been limited because of the inherent technical complexity, time and specimen handling requirements, expense, and labile nature tahir99 VRG CHAPTER 24 Chlamydia, Rickettsia, and Similar Organisms TABLE 543 24-7 Appropriate Specimens for Detection of
Chlamydial Infections Clinical Manifestation, Site of Infection Inclusion conjunctivitis and trachoma Urethritis Epididymitis Cervicitis Salpingitis Lymphogranuloma venereum Infant pneumonia Sexually transmitted disease, male sex partner Specimen Site, Type Comments Conjunctival swab, scraping with spatula or tears Urethral swab Epididymis
aspirate Endocervical swab Fallopian tube (lumen) or biopsy Bubo or cervical lymph node aspirate Throat swab, nasopharyngeal aspirate, or lung tissue Urine Specimen collection in neonates is difficult Psittacosis Chlamydophila pneumoniae pneumonia or pharyngitis Sputum, lung tissue Sputum, throat swab, or lung tissue Sexually transmitted
disease, result clarification Rectal, vaginal swabs In males, >4 cm; do not use discharge Remove exudate first. EIA tests are often less sensitive than cell cultures or IF tests, so negative results are confirmed with cell culture or IF or nucleic acid–based tests. In patients with AIDS, a latent infection can reactivate and cause encephalitis or pneumonia.
This medium is prepared in screw-capped containers and is inoculated by streaking the surface and submerging the swab in the agar and leaving it in place. Symptomatic B. Rapid detection with DFA is about 60% to 70% sensitive compared with culture and can lack specificity. Available at: . Most have been recovered from sputum, blood, and
wounds. or EIEC infections. malariae have similar distributions throughout Africa and tropical countries. One drop of bacterial suspension is added to each drop of antisera for a direct agglutination assay. In some patients, it manifests as granulomas, whereas in others it develops into nodules, ranging from pea- to walnutsized. akari, the reservoir is
the common house mouse, and the vector is the mouse mite Liponyssoides sanguineus. Reports have shown that all E. Adults, which may range in size from 2 to 50 cm, live in human lymphatics, muscles, or connective tissues. Verkooyen RP, et al: Reliability of nucleic acid amplification methods for detection of Chlamydia trachomatis in urine: results
of the first international collaborative quality control study among 96 laboratories, J Clin Microbiol 41:3013, 2003. The carrier state may be acute (short-lived or transient) or chronic (lasting for months, years, or permanently). No toxins or virulence factors have been identified. Individuals in the acute phase may be asymptomatic or have local
symptoms, including the presence of a chagoma (ulcerative skin lesion at the site of the insect bite) or unilateral edema around the eye (Romaña sign) if the bite is on the ocular conjunctiva. Infection confers lifelong immunity. Even then, the halophilic species might grow poorly, if at all, and they often are confused with other genera, such as
Aeromonas. Humans ingest the plerocercoid larvae by eating raw or undercooked fish. Surgical wounds are ideal points of inoculation, allowing saprobes to become opportunistic agents of disease. A nonfeeding, infective, filariform larva develops within 1 week. Evaluation of stains made from stools preserved with these compounds has shown varying
quality of the stained preparations. freundii from Salmonella. (Nomarski optics, ×450.) tahir99 VRG 608 PART II Laboratory Identification of Significant Isolates sometimes confused with Aspergillus on initial examination. PCR assays to detect T. Esculin hydrolysis is the best test to differentiate the two species; approximately 88% of B. When
sputum is not obtainable, bronchoscopy may be performed, at which time samples such as bronchial washing, bronchoalveolar lavage (BAL), or transbronchial biopsy specimens are obtained. usually comprise the largest portion of the vaginal biota. This structure prevents attachment of the organism to the neutrophil's membrane, which must occur
before ingestion can take place. The cysticercus is oval, translucent, and about 5 to 18 mm in size. B, Examples of positive and negative results. Conversely, in developing countries, S. The eggs are infective with a third-stage larva within several hours of being laid. Fruiting structures, as well as conidial arrangement, are better observed by this
method. The New World complex includes Tacaribe, Junin, Machupo, Amapari, Cupixi, Parana, Latino, Pichinde, Tamiami, Flexal, Guanarito, Sabia, Oliveros, Whitewater Arroyo, Pirital, Bear Canyon, Allpahuayo, Catarina, Pampa, Skinner Tank, and Chapare viruses. Porphyromonas sp. The incidence of Pontiac fever in the general population is
unknown. Establishing at least a limited clinical virus isolation capability in routine laboratories can be justified, providing qualified personnel and space are available. The illness usually subsides within 72 hours. Viruses have DNA or RNA, but never both, in contrast to prokaryotes and eukaryotes. The vagina contains a large population of organisms
as part of the indigenous flora. If legionellosis is suspected, for example, the laboratory needs to have standard procedures for recovering this microbe. Describe the general characteristics and structures of fungi. Whereas most immunocompetent individuals do not have serious infection, the most serious forms of the infection are found in HIV
positive and transplant patients. Although the catheter was a necessary procedure in the medical treatment of the individual, its use may result in an infection. Pseudomonas, Clostridium LPS, lipoprotein Anergy of miliary tuberculosis Anthrax, plague, Clostridium Brucella, Salmonella, Neisseria, Staphylococcus, Pseudomonas Capsules, M protein, Ig-
binding proteins, gonococcal pili Salmonella typhi Mycobacteria Gram-positive cell wall, smooth LPS, polyanionic capsules Catalase, superoxide dismutase, carotenoid pigments Mycobacterium bovis, Legionella pneumophila Streptococcus pneumoniae, Streptococcus pyogenes, Staphylococcus aureus, Pseudomonas aeruginosa Inhibition of attachment
and ingestion Inhibition of metabolic burst Inhibition of degranulation Resistance to permeability-inducing cationic protein Resistance to oxidative attack Escape from phagosome Destruction of phagocyte Sialic acid capsules Cleavage of C3b in empyema fluids CoIV plasmid Bacteroides fragilis capsule Adapted from Gotschlich EC: Thoughts on the
evolution of strategies used by bacteria for evasion of host defenses, Rev Infect Dis 5:S779, 1983. Empirical treatment with macrolides must begin immediately and not wait for culture confirmation. sonnei outbreaks in daycare centers in several states. TABLE 705 CCHF virus causes a high-mortality infection in humans. are strictly aerobic, and they
appear as gram-negative coccobacilli or even gram-negative cocci on Gram stain (Figure 21-8). westermani), the egg may be found in sputum; for S. Because no subjective interpretations are required, the identifications are objective and highly reproducible. The mycobacteria have a unique cell wall and require special stains to visualize the
microorganisms. The adults live in the distal bile ducts. Methylobacterium extorquens Burkholderia mallei Sphingomonas paucimobilis Brevundimonas vesicularis Sphingobacterium spiritivorum Oxidizers Bordetella pertussis Brucella canis Brucella sp. Coverslip, or rinse and dry. All species, except for V. Antigen detection kits have replaced
microscopic examinations in some hospital laboratories. The hybrid-bound acridine ester is available to cause a chemiluminescent reaction, resulting in the emission of light. Although other organisms may grow on CCFA, their colonies are smaller and do not resemble the characteristic colonies of C. Bordetella spp. Variola virus belongs to the genus
Orthopoxvirus; other members of the genus include vaccinia virus (the smallpox vaccine strain), monkeypox, cowpox, and other poxviruses. In the immunocompromised patient, the bradyzoites are released from the cyst and become active tachyzoites that invade multiple organs, resulting in disseminated infection. With this procedure, the oocysts
appear as magenta-stained organisms against a blue background. Macrophages also originate in the bone marrow. Plates incubated in an anaerobic chamber can be examined at any time without exposing the colonies to oxygen. The resulting nucleotide sequence is compared to known sequences in public databases such as GenBank, a repository of
sequences maintained by the National Institutes of Health. Points to Remember ■ ■ ■ ■ ■ ■ Legionella spp. Designated early on as E. Stain for 5 minutes without additional heating. Citrobacter freundii d. tahir99 VRG CHAPTER 18 Haemophilus and Other Fastidious Gram-Negative Bacilli 415 C. Francisella spp. The genus Proteus consists of at
least four species. pestis is the causative agent of plague, a disease primarily of rodents transmitted to humans by fleas. Because many pathogenic fungi grow slowly, any delay in processing compromises specimen quality and decreases the B FIGURE 27-54 A, Pneumocystis jirovecii cysts (silver stain). One sachet is added to each pouch and is
activated on exposure to air, similar to the procedure above. Pseudallescheria boydii. The anamorphic form of P. Poor hygienic conditions are important in perpetuating these infections. Serologic studies indicate that the rate of infection often varies from country to country but, overall, up to one third of individuals worldwide may be infected. Drug
susceptibility tests may be performed on agar-based media without altering drug concentrations, which occurs with egg-based media. Organisms causing subcutaneous mycoses belong to a variety of genera in the form class Hyphomycetes. Unfortunately, the gel can be overwhelmed and become saturated without the users knowing. malmoense, M.
immobilis is a nonmotile, oxidase-positive, oxidative diplococcus. parvum and C. A month or more after the onset of illness, IgG antibodies can be detected in some patients. It takes about 30 to 45 minutes to obtain an anaerobic environment. Lehman, EdD, MT(ASCP), SM(NRM) Associate Professor Department of Medical Laboratory Sciences
University of Delaware Newark, Delaware tahir99 VRG ix x CONTRIBUTORS Steven D. Patients with HPS have a 3- to 5-day febrile prodrome, with fever, chills, and myalgia. Prolonged incubation time and increased CO_2 also play a role in culture success. 658. Outbreaks of Lake Victoria marburgvirus are noticeably rare, but some have been fierce.
Available at: http:// www.cdc.gov/mmwr/preview/mmwrhtml/mm6053a1.htm. Sugar assimilations also vary among the species (see Table 27-7). HHV-6 has been associated with the childhood disease roseola, which is also called roseola infantum, exanthem subitum, and sixth disease, reflecting its role as the sixth childhood rash. (1-3)-β-D-Glucan
Detection (1-3)-β-D-Glucan is present in the cell wall of most yeasts and molds, including Pneumocystis, with the exception of the tahir99 VRG 622 PART II Laboratory Identification of Significant Isolates mucoraceous molds. vulnificus. Many anaerobes produce sufficient growth after only 24 hours of incubation. Most clinical microbiologists
inoculate stool samples on highly selective media, such as HE or XLD agars, in addition to regular MAC and SMAC agar for E. As the organism causes the body's defense mechanisms to respond via inflammation, directed migration of phagocytes occurs (chemotaxis) (Figure 2-2). H. Although the organism is most commonly encountered in
immunocompetent patients, it may be considered an opportunistic infection in patients with AIDS. Lactobacilli are widely distributed in nature and foods, as well as in normal biota in the human mouth, GI tract, and female genital tract. marinum and M. Drop or place the sample onto the surface of a labeled glass slide. The Case in Point describes
significant and repeated exposure risk that should be reported to the primary health provider on presentation. is marked by penetration of intestinal epithelial cells after attachment of the organisms to mucosal surfaces, local inflammation, shedding of the intestinal lining, and formation of ulcers after epithelial penetration. In contrast to opaque egg-
based media, clear agar-based media can be examined using a dissecting microscope for early detection of growth and colony morphology. Moellerella is positive for citrate, methyl red, lactose, and sucrose. In human infections, the earliest stage is the ring-form trophozoite (Figure 28-28), in which the organism has a prominent, red-purple chromatin
dot and a small blue ring of cytoplasm surrounding a vacuole. These and other questions arise among ancillary health care personnel and the laboratory technologist needs to be prepared to respond. A microhematocrit tube is coated with the fluorescent dye. Humans become infected when fleas defecate on the surface of the skin while feeding. The
poem represented the compendium of knowledge at the time regarding the disease. Nucleic acid–based assays are also used for the detection of influenza viruses, with the most common method being RT-PCR. The trophozoite ranges from 20 to 45 µm in diameter and has a single nucleus, with a central prominent endosome. Box 21-2 summarizes
characteristics common to some gram-negative nonfermenters. None appeared to have symptoms of a bloodstream infection. Determination of staining characteristics, based on differences in cell wall structure, is the first step in bacterial classification. ulcerans M. The promastigote is taken up via receptor-mediated phagocytosis by a macrophage.
All of the above 4. “Thin” specimens of fluids such as urine, cerebrospinal fluid (CSF), and transudates should be dropped but not spread on the slide. tahir99 VRG 440 PART II Laboratory Identification of Significant Isolates A B C FIGURE 19-11 A, Blue-violet pigment of Kluyvera spp. Specimens from normally sterile sites, such as blood, CSF,
synovial fluid, and biopsy tissue from deep organs, do not require decontamination. are easily recovered in blood culture systems. This preservative, however, is not routinely used for permanently stained smears. Difficulty in swallowing results from muscular spasms in the pharyngeal area. fonticola have also been isolated from human sources.
Humans b. Two species of hookworm, Necator americanus (New World) and Ancylostoma duodenale (Old World), infect humans. First, serologic assays measure the tahir99 VRG 696 PART II Laboratory Identification of Significant Isolates host response rather than directly detecting the virus. When hyphae are not seen in tissue, the positive culture
may be caused by a contaminant and conversely, when hyphae are seen in tissue but not grown in culture, the causative agent cannot be determined. Sphingomonas Sphingomonas paucimobilis can be isolated from many water sources, including swimming pools, as well as from hospital equipment and laboratory supplies. eggs.” Gravid proglottids
may be seen in the stool specimen and can be used to differentiate the two species. Epidemic V. Give the major characteristics (including size) that you would use to identify eggs of the following organisms: Taenia spp., Ascaris lumbricoides, Trichuris trichiura, and hookworm. The level and pattern of virulence is more like the multifactorial patterns of
the various E. See Chapter 29 for a further discussion of viruses. The most important exotoxin is exotoxin A; this exotoxin functions similarly to diphtheria toxin by blocking protein synthesis. Pneumocystis jirovecii is an important pathogen of patients with AIDS. lactaridi subsp. TABLE dermatitidis to the broad-based bud yeast form (Nomarski optics,
×1250.) FIGURE 27-26 Mold phase of Blastomyces dermatitidis grown on potato flakes agar (Nomarski optics, ×1250.) 27-6 Summary of Systemic Mycoses Fungus Ecology Clinical Disease Tissue Form Blastomyces dermatitidis Mississippi and Ohio River valleys Large yeast (8-12 µm) Broad-based bud Histoplasma capsulatum* Ohio, Missouri, and
Mississippi River valleys Bird and bat guano Alkaline soil Semiarid regions— southwest United States, Mexico, Central and South America In soil Primary lung Chronic skin-bone Systemic, multiorgan Primary lung Asymptomatic Immunodeficient hosts prone to disseminated disease Primary lung Asymptomatic Secondary cavitary Progressive
pulmonary Multisystem Primary lung Granulomatous Ulcerative nasal and buccal lesions Lymph node involvement Adrenals Coccidioides immitis, C. Chlamydophila psittaci Chlamydophila psittaci is the cause of psittacosis among psittacine birds, also known as ornithosis or parrot fever. GM tahir99 VRG This page intentionally left blank tahir99 VRG
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Children's Hospital Spokane, Washington Nicholas Moore, MS, MLS(ASCP) Laboratory Director Kindred Healthcare, Inc. Immunity is short-lived, and B. Seven people died. However, in contrast tahir99 VRG 430 TABLE PART II Laboratory Identification of Significant Isolates 19-6 Diagnostic Features of Enterobacter cloacae, Enterobacter
aerogenes, and Klebsiella pneumoniae subsp. The collected tissue sample is placed onto Christensen's urea medium and incubated at 37° C for 2 hours. All isolates had the same molecular fingerprinting; environmental sources were presumed to have caused the contamination, although none were found. All species reduce nitrates to nitrites; A. are
transmitted by a wide variety of wild and domestic animals. Pulsed-field gel electrophoresis might be performed to: a. The consequences of working up improper or incorrectly collected or transported specimens places a burden on the laboratory and may provide misleading results to the physician. more efficiently. ETEC c. The host cell ruptures, and
spores are released to penetrate other host cells to repeat the reproductive cycle or be tahir99 VRG CHAPTER 28 Diagnostic Parasitology 663 pathologic consequences and severity of infection are related to the number of adults present, commonly referred to as the worm burden. can cause several types of diarrhea and a variety of extraintestinal
infections that can lead to septicemia, meningitis, hemolytic uremic syndrome, and death. A high infective dose (106 to 1010 organisms) is necessary to initiate disease in an immunocompetent host. However, P. Acinetobacter organisms can appear as gram-positive cocci in smears made from blood culture bottles. Although order and tribe may be
useful for the classification of plants and animals, these taxa are not always used for the classification of bacteria. Approximately tahir99 VRG CHAPTER 28 Diagnostic Parasitology 3 million to 5 million new cases are reported in the United States each year, with up to two thirds of these in women 15 to 24 years of age. One problem with serologic
assays is the ubiquitous nature of many of the opportunistic fungi. The zygote migrates to the salivary glands of the tick, where sporozoites are formed. Zbinden R, von Graevenitz A: Actinobacillus, Capnocytophaga, Eikenella, Kingella, Pasteurella, and other fastidious or rarely encountered gram negative rods. There are a number of more recently
identified pseudomonads that can be mistakenly identified as P fluorescens by commercial phenotypic identification systems. Indirect fluorescent antibody tests and EIAs using T. Some strains of B. Only a few cases were reported initially, but 5 of the 11 human cases were fatal. Most of the cases were reported in Midwestern states among previously
vaccinated persons 18 to 25 years of age. tuberculosis that are rough often exhibit a prominent patterned texture referred to as cording (curved strands of bacilli); this texture is the result of tight cohesion of the bacilli. McNabb CHAPTER OUTLINE ■ CHARACTERISTICS OF VIRUSES Structure Taxonomy Viral Replication ■ LABORATORY
DIAGNOSIS OF VIRAL INFECTIONS Specimen Selection, Collection, and Transport Appropriate Specimens for Maximum Recovery Methods in Diagnostic Virology ■ DOUBLE-STRANDED DNA VIRUSES Adenoviridae Herpesviridae Papillomaviridae Poxviridae ■ SINGLE-STRANDED DNA VIRUSES Parvoviridae ■ DOUBLE-STRANDED RNA
VIRUSES Reoviridae ■ SINGLE-STRANDED RNA VIRUSES Arenaviridae Bunyaviridae Caliciviridae Coronaviridae Filoviridae Flaviviridae Orthomyxoviridae Paramyxoviridae Picornaviridae Retroviridae Rhabdoviridae Togaviridae ■ HEPATITIS VIRUSES Hepatitis A Virus Hepatitis B Virus Hepatitis D Virus Hepatitis C Virus Hepatitis E Virus Other
Hepatitis Viruses ■ PRIONS ■ ANTIVIRAL THERAPY OBJECTIVES After reading and studying this chapter, you should be able to: 1. Box 3-1 lists examples of emerging pathogens and reemerging pathogens. The next day the clinician was informed that the gram-negative bacillus was an oxidase-positive, nonlactose fermenter. Current CDC guidelines
highly recommend both zanamivir and oseltamivir as primary treatments in confirmed cases of influenza. Subungual is the most common form of onychomycosis, described as lateral, distal, or proximal. Sterile water, 30 to 60 mL, is instilled orally or via nasogastric tube aspiration. It is the only true pathogen in the genus. The first is known as innate
or natural immunity with little or no specificity, and the second, called adaptive or specific, is highly specialized. The addition of phosphate buffer to digested specimens results in greater isolation of mycobacteria. (May “glide”) Oligella spp. The S stands for Svedberg units, which refer to sedimentation rates (unit of time) during high-speed
centrifugation. In some forms of ringworm, a persistent allergic reaction, dermatophytid, is manifested in the formation of sterile, itching lesions on body sites distant from the point of infection. TABLE 18-8 Differential Characteristics of Bordetella Species Infecting Humans Characteristics Growth on Charcoal–horse blood Blood agar
MacConkey agar Catalase Oxidase Urease production Nitrate reduction Motility B. Young Actinomyces colonies are frequently spider-like or wooly, tahir99 VRG TABLE CHAPTER 22 Anaerobes of Clinical Importance 521 22-16 Characteristics of Some Clinically Encountered Gram−Positive Non–Spore-Forming Anaerobic Bacilli 48-h Colony (1 mm)
growing on a BBE plate at 24 hours can presumptively be called a member of the Bacteroides fragilis group. • What does mold growing on wet drywall look like? Rapid development of a blue or green color indicates a positive test (production of indole from the amino acid tryptophan), whereas a pink or orange color indicates a negative test.
Interpreting these mixed cell cultures is sometimes more difficult, but this is easily learned and well worth the effort. cepacia on PC agar are white, surrounded by a pink-red pigment. Describe the disease states associated with each group of organisms. With the Giemsa stain, the organism appears round, and the cyst wall is barely visible.

bronchiseptica is an opportunistic human pathogen, causing respiratory and wound tahir99 VRG 416 PART II Laboratory Identification of Significant Isolates infections. When the rash is present, however, it usually occurs on the trunk and extremities. Haemophilus, Legionella (thin with filaments), Actinobacillus, Bordetella, Brucella, Francisella, Pasteurella, Capnocytophaga, Prevotella, Eikenella spp. Methylobacterium mesophilicum and M. Obligate aerobes require oxygen for growth. pseudotuberculosis is about 90%. Occasionally, aerial mycelium form, producing colonies that resemble those of C. Whereas the recovery of etiologic agents in cultures has remained the gold standard in microbiology in determining the probable cause of an infectious disease, the increase in our capabilities for microbial detection and identification can be attributed to the advances in molecular diagnostic techniques and how they are applied in clinical laboratories. Therefore, species identification should be made from a thin film because the characteristics of the parasite and the RBCs can be seen. pertussis is fastidious and requires special collection and transport systems and culture media. brasiliensis produces a variety of mold morphologies when grown at 22° C. Clinical studies that took place after the emergence of the human immunodeficiency virus (HIV) provided the impetus to explain the discrepancy. The eggs, however, are identical. vermicularis is more common than co-infection of D. N-Acetyl-L-cysteine. A combination of a liquefying agent, such as N-acetyl-L-cysteine (NALC) or dithiothreitol, plus NaOH is commonly used. † Includes V. However, increased laboratory awareness of the existence of Plesiomonas, coupled with the knowledge of previously outlined recreational, immunologic, and gastronomic risk factors, has resulted in a gradual but steady increase in the number of reported cases, mostly in adults. Dermatophytoses are caused by Trichophyton, Microsporum, and Epidermophyton species. The type strain was isolated from vacuum-cleaner contents on the island of Guam when environmental indoor dirt samples were being collected. If the host or the infectious agent undergoes a mutation that changes the structure of the adhesin or the host receptor, adherence is not likely to take place, and the virulence of the infectious agent is affected. Following incubation, the aerotolerance test plates are examined for growth. require preformed growth factors, X and V factors, present in blood. schenckii as small, cigar-shaped yeast (Figure 27-24, A). Because the virus is extremely fragile, recovering it from cultures is difficult. The symptoms are usually less severe in recurrent disease. This method is used to prepare a standard inoculum for antimicrobial susceptibility testing. Some eggs, termed decorticated, lack the mammillated outer coat. More than 60% of the cell wall is lipid, and the major lipid component is mycolic acid, which is a strong “hydrophobic” molecule that forms a lipid shell around the organism and affects its permeability. Aspiration of the cyst contents usually reveals the presence of protoscolices. Spores are described as terminal when it is located at the end of the bacterial cell and subterminal when the spore is found at a location other than the end of the cell. Lactobacillus spp. A number of RT-PCR, Taqman, and nucleic acid sequence–based amplification assays have been used for successful confirmation of WNV. The delay would further expose anaerobes to molecular oxygen, increase the likelihood of specimens drying out, and decrease the probability of recovering anaerobes. Unfortunately, no cure has been found for the common cold. terrigena have been reported to cause nosocomial bacteremia. Only genital warts, caused by the human papillomavirus, is a more common sexually transmitted disease in the United States. Serologic methods may be useful to detect current infection if the individual is from a nonendemic area. vietnamienesis, B. oryzihabitans has been isolated from the eye of one patient with postoperative endophthalmitis. The classic illness caused by EHEC produces a watery diarrhea that progresses to bloody diarrhea with abdominal cramps and low-grade fever or an absence of fever. If a child has recovered from confirmed pertussis, additional doses of pertussis vaccine are unnecessary. A newborn usually acquires the infection in the birth canal just before or during delivery, when the mother’s vagina is heavily colonized. They are temperature-sensitive in that in temperate climates in which water temperatures exceed 20° C, as in the summer months, vibrios can easily be isolated from water, suspended particulate matter, algae, plankton, fish, and shellfish. multivorans. Isolates can be identified by FA and EIA methods, along with nucleic acid tests. fragilis closely resembles that of the amebae, but electron microscopy studies of ultrastructure, small-subunit ribosomal RNA gene analysis, and lack of a cyst stage indicate that it is closely related to the trichomonads. as well as some monomorphic yeasts, Candida albicans and Cryptococcus neoformans. Antimicrobial Susceptibility Although most cases of Aeromonas-associated gastroenteritis are self-limited, antimicrobial therapy is often indicated. Four types (1 through 4) of PIVs can cause disease in humans. Urease activity can also be detected by the urea breath test, which is reportedly sensitive and specific and is recommended for monitoring therapy. The disk results for C. It results from sequestration of neutrophils in capillaries of the lung and other organs. coli or Esch. A positive result must be considered preliminary and should be verified, because antigen detection methods may give a false-positive result when used in low-prevalence (50% of adults Attack rate highest between the ages of 6 and ≈25 yr, often focusing on college-age students No seasonal incidence; epidemics have been reported every 4-6 yr Reinfection common TABLE Clinical Estimated to account for approximately 6%-10% of outpatient and hospitalized pneumonia; 90% of infections are asymptomatic or mildly symptomatic. Salmonella infection has been observed among two different groups: (1) young children, who experience fever and gastroenteritis with brief episodes of bacteremia, and (2) adults, who experience transient bacteremia during episodes of gastroenteritis or develop symptoms of septicemia without gastroenteritis. Available at: . dysenteriae, and even S. Feder H, et al: HACEK endocarditis in infants and children: two cases and a literature review, Pediatr Infect Dis J 22:557, 2003. Once the physical and chemical barriers to infection have been penetrated, nonspecific mechanisms of innate immunity become operational. Jacobs RF: Tularemia. It has been observed that most clinical V. Chlamydia trachomatis b. This increase can be attributed to advances in medicine that have prolonged life expectancy, especially for those with chronic diseases. Details on how to seek permission, further information about the Publisher’s permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions. The list of enzymes found within the lysosomes is long—more than 20 enzymes, including proteases, lipases, RNase, DNase, peroxidase, and acid phosphatase. This procedure allows facultative organisms present on the medium to reduce the O_2 tension created by the candle to a more suitable concentration for campylobacters. Positive phenylalanine deaminase d. The organism then responds to the slightly alkaline pH in the small intestine and completes the process in the duodenal area, in which the presence of carbohydrates and bile stimulates trophozoite growth. In immunocompromised individuals, H. Laboratory Diagnosis. Identification of the tachyzoite or pseudocysts with bradyzoites in tissue is very difficult, because no single organ is invaded. Inhibitory Tests NAP. NAP (p-nitroacetylamino-β-hydroxypropiophenone) is a precursor in the synthesis of chloramphenicol. Doxycycline, atovaquone-proguanil (Malarone), or quinine use has been reestablished in some cases of multidrug-resistant P. paucimobilis isolates demonstrate variable resistance to antimicrobial agents, although most are susceptible to the aminoglycosides, tetracyclines, chloramphenicol, and SXT; susceptibility to the third-generation cephalosporins (e.g., ceftazidime, ceftriaxone, ceftizoxime), and fluoroquinolones varies. These organisms, collectively referred to as microsporidia, are obligate intracellular parasites common to invertebrates and other animals. This presentation is known as rhinocerebral zygomycosis. Match the species of Shigella to their appropriate serogroup. Include the following in your discussion: causative organism, population usually infected, route of infection, clinical symptoms, and method of diagnosis. Jansen A, et al: Clinical picture: rabbit’s revenge, Lancet 3:348, 2003. Serologic screening of high-risk populations should be performed, and to avoid congenital syphilis, pregnant women should have serologic examinations early and late in their pregnancy. A percentage of infections in surgical sites or procedures d. It appears to be the only one of the drugs tested that has even partial effectiveness in immunocompromised individuals. difficile is a common cause of health care–associated (nosocomial) infection. In some individuals, especially those heavily infected with S. Among other uses, the catalase test is valuable in differentiating aerotolerant strains of Clostridium (catalase-negative) from Bacillus (catalase-positive). The mature cyst has eight nuclei; the immature cyst may have one or two large nuclei, with a large glycogen vacuole. Medically Important Parasitic Agents Medically important parasites can be found in phyla representing single-celled organisms, such as the protozoa, and complex, multicelled organisms, such as tapeworms and roundworms. It has also been implicated in diarrheal disease among travelers. mentagrophytes. Pyrazinamide is also to be considered. in particular, have been associated with large epidemics and pandemics. catarrhalis can be readily isolated. Clostridia can be grouped according to the location of the endospore within the cell. Accessed August 14, 2012. The combination of broth-based growth for detection and DNA hybridization identification using the probe technology allows rapid recovery and identification. Blindness, the most serious complication, results when microfilariae collect in the cornea and iris, causing hemorrhages, keratitis, and atrophy of the iris. hominis, a human-adapted pathogen, was once thought to be a genotype of C. Becton Dickinson (Franklin Lakes, NJ) manufactures the Crystal enteric nonfermenter system, which includes a database of 24 taxa of nonfermenters, consisting of 10 different genera. A drop taken from a few millimeters below the surface is examined by dark field microscopy for tightly coiled, rapidly motile spirochetes, with hooked ends. Reller ME, Dumler JS: Ehrlichia, Anaplasma, and related intracellular bacteria. Production of the infective stage (sexual reproduction) takes place in the tick. The porphyrin test for H. Strains that cause UTIs produce factors that allow them to attach to the urinary epithelial mucosa. neoformans, H. The review of health care–associated infections in laboratory personnel c. Figure 28-12 demonstrates a trichromestained cyst of I. piechaudii, are usually susceptible to SXT, piperacillin, ticarcillin, ceftazidime, and quinolones (although variability occurs). They usually have been added to the specimen in the course of collection from or through a nonsterile area. Furthermore, CLSI antimicrobial susceptibility studies should always be determined by the standard Kirby-Bauer disk diffusion method. maltophilia, and Burkholderi cepacia complex. However, because V. Tape preparations should be read within 30 minutes and then discarded. They are somewhat resistant to chlorine and iodine, but are susceptible to desiccation and heating to 50° C. All three of these organisms are now classified as A. Legionnaires’ Disease Legionnaires’ disease typically manifests in three major patterns: (1) sporadic cases, which are most common and usually occur in the community; (2) epidemic outbreaks, characterized by short duration and low attack rates; and (3) nosocomial clusters, occurring in compromised patient populations. Although they are almost always encountered as agents of human disease, the infections are seldom inflammatory. D. neoformans is a significant pathogen of immunocompromised hosts, such as the patient with AIDS from the Case in Point. hominis) form colonies with slightly raised centers giving the classic fried egg appearance (Figure 25-1). Available at: . If the deposit is too heavy, a portion of the material may be smeared to produce a thin area. are based on the enzymes that the organisms possess, the substances that their metabolism produce, and the inhibition of growth on exposure to selected biochemicals. fetus has been isolated most frequently from blood cultures and is rarely associated with gastrointestinal illness. Other enteric Campylobacter infections (those caused by C. The virus is shed in large quantities in the stool and can cause nosocomial outbreaks in the absence of good hygiene. On differential and selective media used primarily to isolate intestinal pathogens, shigellae generally appear as clear, non–lactose-fermenting colonies. Lab Med 39:38, 2008. The two countries continue to defend maintaining these stocks on the grounds that further study is needed for the production of better vaccines and as countermeasures to bioterror. (Nomarski optics, ×1250.) Although many such kits are available for yeast identification, the API 20C yeast identification system (bioMérieux) is probably the most commonly used. The bile disk may be added to the anaerobic subculture plate whenever the Gram stain reveals the isolate to be a gram-negative bacillus. Shigella Similar serogrouping procedures may be used in the serologic testing for Shigella. An easy approach is to take a well-isolated, NLF colony and perform a screening battery of tests consisting first of an oxidase test and the inoculation of lysine iron agar (LIA) and TSI agar slants (Table 19-11). Cetrimide acts as a detergent and inhibits most bacteria; the medium also enhances the production of the two pigments produced by P. Bethany J, et al: Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm, Lancet 367:1521, 2006. These newly described rickettsiae were tahir99 VRG 550 PART II Laboratory Identification of Significant Isolates sensitive (29%). so that atypical organisms are not misidentified. If recognition or suspicion of such pathogens does not occur from smear or history, the isolation of certain pathogens will not be made, and diagnosis will be delayed or missed. The modern vaccine, ACAM2000, is manufactured by Sanofi Pastuer Biologics Co. (Canton, MA). Isolates of Clostridium difficile, for example, have to be tested for toxin production. The bacterial genes may be transferred when the phage infects a new bacterium. The main groups at risk for infection are travelers returning from endemic areas, hikers who drink untreated water from streams, and children in daycare centers. The organism produced dark pink colonies on MAC agar and had the following biochemical results: triple sugar iron, acid over acid with gas; phenylalanine deaminase-negative; sulfide-indolemotility agar, H_2S-negative, indole-positive, and motile; ureasenegative; and citrate-negative. There are many species of Ralstonia, and definitive identification may require other than phenotypic methods, although there are even some problems with those. In dysentery caused by S. mexicana complex, the cause of New World cutaneous leishmaniasis, is found in South and Central America. septicum (80% to 95% of the cases) Peptostreptococci, Porphyromonas spp., Fusobacterium spp. nana is B FIGURE 28-5 A, Entamoeba hartmanni Identified by arrow (trichrome stain). The patient’s leg was painful, bluish, and edematous. The trophozoite enters the nasal cavity through inhalation of contaminated water or soil. The application of PCR and chromatography methods has become routine in many laboratories. Middlebrook 7H9 broth and Dubos Tween albumin are nonselective liquid media used for subculturing stock strains, picking single colonies, and preparing inoculum for in vitro testing. denitrificans Weeksella zoohelcum Pseudomonas diminuta Pseudomonas mallei Flavobacterium gleum Flavobacterium indologenes Ralstonia pauculus Ralstonia gilardi Comomonas acidovorans Chryseobacterium meningosepticum Flavobacterium brevis Pseudomonas mesophilica Flavobacterium odoratum CDC group EF-4a CDC group EF-4b Achromobacter biovar 1, 2, or Vd-1 CDC WO-2 CDC Group EO-2 Chryseomonas luteola Moraxella phenylpyruvica Pseudomonas pickettii Agrobacterium radiobacter Flavobacterium multivorum Flavobacterium spiritivorum Pseudomonas paucimobilis Definitive identification of every nonfermenter is timeconsuming and can be costly. Primary and secondary pulmonary sporotrichosis and extracutaneous and disseminated forms of the disease may also occur. The helix is a double strand twisted together, which many scientists refer to as a “spiral staircase” (resembling the handrail, sides, and steps of a spiral staircase). The chromatin mass often is set eccentrically in the organism. vivax Enlarged (to ×2) Schüffner’s dots P. grow rapidly and usually form flat, granular to velvety colonies in shades of tan, brownish gold, or mauve. cholerae but fail to agglutinate in O1 antisera are referred to as V. tahir99 VRG CHAPTER 20 Vibrio, Aeromonas, Plesiomonas, and Campylobacter Species ✓ 461 Case Check 20-2 It is important to use selective media and key biochemical reactions to differentiate Vibrio from Vibrio-like organisms. pertussis infection occurs after exposure to the organism through the respiratory tract and a 1- to 3-week incubation period, usually 7 to 10 days. Porphyromonas spp. burgdorferi sensu lato cause Lyme disease (Lyme borreliosis). Candida sp. Sporozoites attach to receptors on the intestinal mucosal border, penetrate cells, and mature into trophozoites. Common Stains Used for Microscopic Visualization Stains that impart color or fluorescence are needed to visualize bacteria under the microscope. faecalis is the species usually seen in clinical specimens and has been isolated from the blood of patients with and without septicemia. Animals such as beavers may serve as reservoirs and can be a source of infection for backpackers who drink from streams or rivers. (often polymicrobial) Porphyromonas spp., F. These are questions that the infection control program must ask. These agents, such as zanamivir (Relenza) and oseltamivir (Tamiflu), are more expensive than amantadine but provide coverage against influenza A and B infections. Many clinicians rely on ruling out other tickborne diseases to diagnose Colorado tick fever. ozaenae (% +) Sign %+ (% +) d − + − d − − or + − − or + − or (+) 0 0 97.7 0 30 0 40 6 25 0 (14.8) d d − 50 30 0 − or + − or + 18 39 (32.4) (6.3) (11) (9.4) (61.3) Modified from Ewing WH: Edwards and Ewing’s identification of Enterobacteriaceae, ed 4, East Norwalk, CT, 1986, Appleton and Lange. The nucleus has a small central karyosome and is surrounded by fibrils that curl around the cytostome to give a so-called shepherd’s crook appearance. Centers for Disease Control and Prevention: Two cases of visceral leishmaniasis in U.S. military personnel—Afghanistan, 2002-2004, MMWR 53:265, 2004. The pneumococci have proliferated to high numbers, and the lung is responding with increased mucus and fluid release. Both microgametocyte and macrogametocyte are infective for the female mosquito when she takes a blood meal. aeruginosa are β-hemolytic on sheep blood agar (SBA) and will produce flat spreading colonies with a characteristic metallic sheen. Salmonella Typhi does not have a known animal reservoir; humans are the only known source of infection. Thus the overall sensitivity of the acid-fast smear varies from 20% to 80%, depending on the extent of the infection. Describe the difference between chromoblastomycosis and eumycotic mycetoma. These bacteria are killed by the enzyme alone. • Except for Klebsiella, Proteus, and some Enterobacter isolates, none has remarkable colony morphology on laboratory media. Laboratory Identification Laboratory Levels or Extents of Service A change in the distribution of mycobacterial laboratory testing led to the development of levels of service by the ATS and extents of service by the College of American Pathologists to maintain quality of service. In some species, antler, racquet, rhizoids, or spiral hyphae are formed (Figure 27-3, A). Lung Flukes. Organisms of the genus Paragonimus usually infect tigers, leopards, dogs, and foxes. They are rarely, if ever, part of the normal host microbiota but can easily colonize hospitalized patients, especially those who are immunocompromised. Fertile Ascaris eggs are oval, measure 45 to 75 µm × 35 to 50 µm, and have a thick hyaline wall surrounding a one-cell stage embryo. moshkovkii. EIA kits are commercially available and have sensitivities and specificities comparable to those of IFA. Identifying Characteristics. Members of the Pseudomonas fluorescent group, which includes P. Haemophilus spp. All except Myroides and Sphingobacterium are indole positive, a distinctive characteristic among this family. They are not as closely related to the other members of Enterobacteriaceae, but they do qualify as members of the family. Their average size range is smaller than that of most other parasitic organisms, and they must be distinguished from artifacts and cells that appear in the clinical specimen. In the soil, the rhabditiform larvae develop into filariform larvae, which are infective for humans by direct penetration. In 2012, there was a report of eight neonates in one neonatal ICU who had B. Citrobacter spp. Viral Isolation In clinical virology, isolating viruses is still the gold standard against which all other methods are compared. Lesions usually occur on mucous membranes after an incubation period of 2 to 11 days. Available at: .who.int/dracunculiasis/en. Fungi that appear to be regularly associated with this condition include Exophiala spp. parahaemolyticus is found in aquatic environments, but appears to be limited to coastal or estuarine areas, despite a halophilic, or salt-loving, requirement of 1% to 8% NaCl. V. GLC of cellular fatty acids may be required to definitively place isolates within a genus. Other rapid, commercially available tests include the CLOtest Rapid Urease Test (Kimberly-Clark/Ballard Medical Products, Neenah, WI), Hp Fast Rapid Urea Test (GI Supply, Camp Hill, PA), and a paper strip test by PyloriTek (BARD, Murray Hill, NJ). Immunofluorescence can be a valuable tool to detect various viral agents directly in clinical specimens. A chronic state can develop, with mild symptoms of pruritus and scanty discharge. Colonies of M. fluorescens, P. histolytica trophozoites but usually are not seen. These viruses are distinguished using two major structural proteins, the matrix protein (M) and nucleoprotein (NP). Micrococcus, Staphylococcus, Peptostreptococcus spp. Recurrent, or reactivation, HSV infection usually occurs on the border of the lips at the junction of the oral mucosa and skin. Left, BBL Port-A-Cul with prereduced gel (BD Diagnostic Systems, Sparks, MD.). The enteroinvasiveness of EIEC has to be demonstrated for definitive identification. This phenomenon can make it difficult to determine the serotype. The tahir99 VRG 436 PART II Laboratory Identification of Significant Isolates Percentage of patients with positive cultures TABLE Serum agglutinins 100 90 Test 80 70 19-9 Biochemical and Serologic Differentiation of Shigella Species Mannitol fermentation ONPG Ornithine decarboxylase Serogroup Blood Stool 60 50 40 S. These viruses replicate initially in the gut of the arthropod vector and eventually appear in the saliva. Describe the microscopic characteristics you would use to differentiate the oocysts of Cyclospora cayetanensis and Cryptosporidium parvum. Molecular Methods Molecular methods for parasite identification are still primarily limited to reference laboratories such as those at the CDC. enterica, are found in infections with symptoms identical to those of Salmonella infections and may be transmitted to humans from pet turtles, snakes, and fish. coli is the most significant cause of UTIs. Salmonella and Shigella are enteric pathogens and are not considered normal fecal biota. No water is produced in response to the anaerobic atmosphere generation, so plates in bags can be more easily viewed. Direct Microscopic Examination of Specimens Direct examination of clinical material for fungal elements serves several purposes. dysenteriae type 1, particularly among undernourished children during epidemic outbreaks. A ×40 or ×60 lens is preferred for scanning, and a ×100 lens is used for final evaluation. They are able to gain access to the central nervous system (CNS) by inhalation into the upper respiratory tract followed by penetration of the nasal mucosa, which allows them to travel along the olfactory nerve to the brain, or by hematogenous spread from the lungs or skin lesions. incenata, described in 2005. To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein. Species identification may be difficult because most anthropophilic species produce few conidia. Syncephalastrum. Syncephalastrum is rarely implicated in human disease but has been documented in cutaneous infections. Malik P, et al: Emergence and re-emergence of glanders in India: a description of outbreaks from 2006-2011, Vet Ital 38:167, 2012. Isolates can be found contaminating sterile hospital fluids and, as such, may be isolated from specimens such as urine, nasopharynx, abscesses, wounds, and blood, usually as colonizers or contaminants. Isolated cases of endophthalmitis have been reported to be caused by Empedobacter brevis following cataract surgery. A vaccine effective in controlling the disease is available. Enterovirus infections are more prevalent in areas with poverty, overcrowding, poor hygiene, and poor sanitation. They produce a characteristic CPE, with swollen cells in grapelike clusters. The most clinically significant species belong to the phylum Glomeromycota in the order Mucorales and include the genera Lichtheimia (formerly Absidia), Mucor, Rhizomucor, and Rhizopus. gladioli has been recovered from the blood and tissue of immunocompromised patients, particularly CF patients who have had a lung transplant. The rash can extend to the palms of the hands and soles of the feet but normally does not affect the face. choleraesuis, and S. These organisms are usually susceptible to ampicillin, tetracycline, chloramphenicol, erythromycin, and the aminoglycosides, but resistant to penicillin and cefazolin. Protective clothing (boots and gloves) should be worn in situations involving possible occupational exposure to leptospires. If the patient has undergone a barium enema, stool examination should be delayed for 7 to 10 days, because barium obscures organisms when specimens are examined microscopically, even after concentration procedures. Sarcoidosis, cardiovascular relationships (?) and bronchitis but is rarely accompanied by sinusitis. mentagrophytes, these are seldom seen in clinical isolates. Now viruses are classified in orders, families, genera, and species based on genome type (RNA or DNA), number of strands in the genome (ds or ss), morphology, and presence or absence of an envelope. Extraintestinal Infections E. The following section describes tests, some rapid (results in about 1 hour or less), that are of value in presumptively identifying many of the anaerobes commonly encountered in clinical specimens. Hyaline (moniliaceous) hyphae are nonpigmented or lightly pigmented, whereas phaeoid (dematiaceous) hyphae are darkly pigmented (Figure 27-5) because of the presence of melanin in the cell wall. nucleic acid by PCR from nasopharyngeal swabs is a primary rapid diagnostic strategy. Because patients with formed stools are rarely positive for CDAD, EIA and NAATs should not be performed on these types of specimens. Walker, Connie R. East African sleeping sickness is a zoonosis, with game animals serving as important reservoirs of T. Improperly transported specimens should be rejected. However, granules are not formed, thus not qualifying this infection as a mycetoma. Good infection control measures, including adequate chlorination of swimming pools, prevent adenovirus infection, such as adenovirus-associated conjunctivitis. Humans inhale the aerosolized virus or contract the virus directly through breaks in the skin. aeruginosa, P. freundii, C. Thin areas should be selected and examined under oil immersion (×100 objective) for identification of organisms. akari, R. Research supports the association of B. This fungus has a worldwide distribution. Newer lateral flow immunochromatographic tests provide results in 15 minutes. acnes colonize the deep sebaceous glands. Assimilation patterns can be determined from methods such as an automated identification system or various manual procedures and commercial kits. Katz AR, et al: Leptospirosis in Hawaii, USA, 1999–2008, Emerg Inf Dis 17:221, 2011. Unfortunately, these methods can only confirm a diagnosis in convalescent specimens and offer little help in diagnosing acute infections that could guide antimicrobial therapy. The VersaTrek Culture System (Trek Diagnostics, Cleveland, OH) is based on the detection of pressure changes in the headspace above the broth medium in a sealed culture bottle resulting from gas production or consumption during the growth of microorganisms. Fungi are eukaryotic; they possess a true nucleus, with a nuclear membrane and mitochondria. chaffeensis, and no currently available serologic test can distinguish these agents. Long-term immunity is not developed after an acute infection, and reinfection can occur. The structure of the cell envelope and enzymes of archaea (Greek: ancient, origin from the earliest cells) allows them to survive under stressful or extreme (extremophiles; lovers of the extreme) conditions. Clinical disease is uncommon in very young children and older adults. The NAATS detected 34.7% more cases of CDAD than the non-NAAT methods. It is rare not to be able to find more than one because in infection organisms are usually distributed throughout the specimen. The infections occur in crowded urban areas where rodents and their mites exist. Most of these tahir99 VRG CHAPTER 20 Vibrio, Aeromonas, Plesiomonas, and Campylobacter Species 459 A2 Cholera toxin B A1 B B K+ Na+ INTESTINAL LUMEN - HCO_3 H_2O B B K+ A2 B B GM1-ganglioside receptor B Cell-membrane H_2O Na+ A1 HCO Gs 3 K+ cAMP ADPR Stimulatory Na+ H_2O cAMP AC AC cAMP H_2O Gs cAMP active Inhibitory inactive Gi AC AC AC AC ATP ATP ENTEROCYTE ATP ATP FIGURE 20-3 The action of cholera toxin. The cytoplasm is basophilic. Infections were also passed to the victims’ families, often during a process in which the intestines of infected deceased males were cleaned to prepare the bodies for funerals. The ELISA method seems to be more accurate than previously used latex agglutination assays. Boyce JM, Pittet D: Guideline for Hand Hygiene in Health-Care Settings. Stools from patients with symptomatic amebiasis caused by Entamoeba histolytica often demonstrate blood. ✓ Case Check 27-1 Unlike most mold fungi, Fusarium spp. In 1988, 125 countries reported endemic rates; by 1999, that number had decreased to 30 and to only 3 in 2013: Afghanistan, Nigeria and Pakistan. Educating people about sexually transmitted diseases, including the proper use of barrier contraceptives, reporting each case of syphilis to the public health authorities for contact investigation, and treating all sexual contacts of persons infected with syphilis are cornerstones of syphilis control efforts. (Figure 18-27). Since 1990, when it was first linked to human illness, this free-living ameba has caused more than 200 cases, with a mortality of more than 95%. Association for the Advancement of Medical Instrumentation: Water treatment equipment for hemodialysis applications, ANSI/AAMI RD62-2001, Arlington, VA, 2001, American National Standards Institute. Thermally dimorphic fungal species associated with human disease include Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum var. Latency within 1 year of infection is referred to as early latent, whereas latency greater than 1 year is late latent syphilis. Cover smears with carbolfuchsin, and stain for 5 minutes. A discussion on forensic microbiology has been included in Chapter 30, Agents of Bioterror. Analyze the diagnostic methods appropriate for the detection and identification of mycoplasmal and ureaplasmal infections. Frequently Encountered Anaerobes and Their Associated Diseases Taxonomically, anaerobic bacteria encountered in human clinical specimens may be divided into gram-negative and gram-positive genera. Points to Remember ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ Most species in this chapter are found in fresh, estuarine, or marine water. coli K1 were higher than the fatality rates for infants infected with non-K1 strains. can be recovered from the sinuses or other organs during disseminated disease. PRECAUTIONS RESULTS Blood cells stain as with Wright stain. Dermatophytes that primarily inhabit the soil are termed geophilic. Both strains are tested in parallel by adding a standardized inoculum to media containing twofold serial dilutions of the drug. catarrhalis, M. Available at: . Mixed cell lines have been developed by combining two cell lines susceptible to certain types of viruses, such as respiratory or enteric viruses. The somatic O antigens and flagellar H antigens are the primary antigenic structures used in serologic grouping of salmonellae. Nasopharyngeal swab specimens should be plated directly onto culture media or transferred to an appropriate transport system at the bedside. Campbell L, et al: Evaluation of the OSOM Trichomonas rapid test versus wet preparation examination for detection of Trichomonas vaginalis vaginitis in specimens from women with a low prevalence of infection, J Clin Microbiol 46:3467, 2008. In flotation methods, the organisms are suspended at the top of a high-density fluid. The morphology initially may be confused with that of P. Once the egg is ingested, the hexacanth embryo is released into the intestines, penetrates the intestinal wall, and enters the Eggs in feces Ingestion by specific intermediate host Hexacanth embryo release in intestine of host Penetration of mucosa to tissue Taenia solium only Development into cysticercus Human ingestion of eggs Cow Taenia saginata Embryo released in intestine Hog Taenia solium Development into adult To tissue Scolex release Cysticercus in eye, brain, muscle, bone Attachment to intestine FIGURE 28-50 Life cycle of Taenia spp. usual intestinal flora also carry out various metabolic degradations and syntheses that appear to play a role in the health of the host. isolates recovered were all the same serogroup. coli, also appeared gram-positive, which is an unacceptable result and indicative of an error in performing the Gram stain procedure. Oropharyngeal candidiasis is easily diagnosed by direct smear and culture. Isolation after the first 2 weeks does not confirm congenital infection. The Vitek-2 and MicroScan (Siemens, Newark, DE), afford automated approaches to the identification of a variety of bacteria and yeasts that include many of the clinically relevant nonfermenters. campylobacters may appear as long spirals or ‘S’ or seagull-wing shapes. caviae as FIGURE 20-6 Aeromonas hydrophila exhibiting β-hemolysis on sheep blood agar. Ortega YR, Sanchez R: Update on Cyclospora cayetanensis, a foodborne and waterborne parasite, Clin Microbiol Rev 23:218, 2010. DAEC may be associated with UTIs and diarrheal disease. Serologic Testing Serologic diagnosis of pertussis can be used to study outbreaks and to document seroconversion after immunization or infection. For example, a biopsy specimen can first be placed into a sterile screw-capped tube containing sterile saline, which is then placed in one of these bags. The envelopes are of host origin but contain virus-encoded proteins. The trachoma biovar includes serovars A through K. However, V. Biopsy of muscle tissue and identification of the encapsulated, coiled larva is the definitive diagnostic method. The undulating membrane extends the length of the organism and is joined to the body along the costa. Because serologic tests for LAC virus are not offered in most laboratories, and because this disease has very low mortality (about 1%), definitive diagnosis does not often occur. The organisms remain localized to the respiratory tract, but toxins and other virulence factors are produced and have systemic effects. Patients with DF develop fever, headache, myalgia, and bone pain (resulting in the nickname “breakbone fever”). When symptoms occur, the disease is acute and febrile; a maculopapular rash appears as the fever resolves. Bacterial overgrowth may contribute to symptoms. In addition to serogroup 1, L. cholerae. ✓ Case Check 28-2 The patient in the Case in Point shows several characteristics associated with hookworm infection. From April 15, 2009, to July 24, 2009, the CDC reported a total of 43,771 confirmed and probable cases of H1N1 infection in the United States. To prevent the dispersal of infectious aerosols into the laboratory area, all potentially infectious materials should be tightly covered when they are outside the biological safety cabinet. Figure 28-37 shows an acid-fast stain of Cryptosporidium spp. Under certain conditions, a temperate phage can be induced, the phage DNA is excised from the bacterial genome, and a lytic state occurs. Salmonella Members of the genus Salmonella produce significant infections in humans and in certain animals. gondii causes a tissue infection that is usually asymptomatic in immunocompetent hosts. bronchiseptica is unpredictable, and tests should be performed, although the organism is usually susceptible to the aminoglycosides. Specimens from large muscles, such as the deltoid and gastrocnemius, should be stained for histologic examination. Because of this, the WHO has stepped up efforts to eradicate this disease in endemic areas. Causative agents are identified on the basis of characteristic structures, such as arrangement of conidia and the manner in which conidia are borne. No growth occurs on MAC agar. The administration of antimicrobial agents can shorten the duration of diarrhea and thereby reduce fluid losses. Vomiting and diarrhea can cause rapid loss of fluids and fatal dehydration. The anemia seen in malaria is the result of erythrocyte lysis, removal of the infected cells, and in some cases immune clearance of erythrocytes coated with immune complexes of malarial antigen and antibody. These three genera include the causative agents of important human diseases such as syphilis, zoonoses (transmitted from animals to humans) such as leptospirosis, and vector-borne diseases such as Lyme borreliosis or Lyme disease and relapsing fever. However, O serogrouping could not differentiate these E. He touched his mustache while talking on the telephone, as he habitually does when he speaks. Distilled water and saline seem to inhibit motility. tahir99 VRG α-Methyl-D-glucoside Fermentation Erythritol Fermentation Esculin Hydrolysis Melibiose Fermentation D-Arabitol Glycerol Fermentation Mucate Fermentation Tartrate, Jordan’s Acetate Utilization Lipase (Corn Oil) DNase (25° C) Nitrate Nitrite Oxidase, Kovacs’ ONPG Test Yellow Pigment D-Mannose 0 0 0 0 0 5 0 95 0 0 0 90 0 10 0 98 0 0 0 0 0 0 100 0 0 0 0 0 7 7 100 0 0 0 100 0 0 0 100 0 0 0 0 0 0 0 0 0 0 0 0 0 100 0 100 0 0 0 90 0 20 0 100 0 0 0 0 15 50 15 85 0 0 0 0 0 0 0 0 0 0 100 0 0 30 95 0 100 0 0 30 0 0 85 91 0 89 93 97 82 100 100 25 0 0 55 55 0 7 0 0 0 0 0 60 0 0 50 50 0 100 0 0 30 27 0 40 0 0 25 30 9 100 50 0 0 0 0 0 0 0 85 91 0 0 0 0 90 91 0 75 98 27 100 50 100 0 0 0 0 0 0 0 0 60 0 0 0 0 0 60 100 0 0 1 0 0 100 0 80 0 60 0 0 0 0 0 0 1 0 78 0 0 0 50 0 0 0 0 0 0 0 0 0 0 70 0 100 0 55 0 60 0 15 0 100 50 95 50 100 40 90 80 98 0 100 0 0 0 0 0 0 0 0 1 1 1 0 0 0 0 0 0 0 0 0 0 0 100 0 0 0 0 0 100 0 0 0 100 70 2 10 0 3 0 0 0 0 0 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0 0 10 100 0 0 0 100 0 0 0 100 0 0 0 100 0 95 100 97 95 98 0 0 0 1 0 45 1 0 10 90 70 50 10 0 1 0 0 0 0 0 100 95 0 100 5 0 0 0 95 0 10 10 0 0 D-Galactose Cellobiose Fermentation 0 Tyrosine Utilization Trehalose Fermentation 0 Fermentation D-Xylose 0 Fermentation Maltose Fermentation 0 Fermentation L-Rhamnose 0 Fermentation Raffinose Fermentation 0 Fermentation L-Arabinose 0 Fermentation 0 myo-Inositol Fermentation 0 Citrate, Christensen’s 449 CHAPTER 19 Enterobacteriaceae D-Sorbitol Continued tahir99 VRG 450 Gelatin Hydrolysis (22° C) Growth in KCN D-Glucose, Acid D-Glucose, Lactose Fermentation D-Mannitol Dulcitol Fermentation Salicin Fermentation Adonitol Fermentation 0 0 100 90 0 0 100 0 0 0 98 3 0 97 0 0 0 100 0 1 0 100 0 0 0 0 0 13 3 15 0 0 0 0 0 0 100 0 95 0 99 0 55 0 0 0 0 0 4 0 5 0 100 0 100 100 95 0 0 0 0 94 0 2 0 0 0 0 0 0 0 0 0 100 0 0 97 0 0 0 20 100 100 0 75 75 100 0 100 9 91 1 93 93 90 1 20 98 98 0 100 60 30 0 0 0 0 0 0 60 100 50 100 50 60 100 0 0 25 45 50 0 0 40 40 94 80 20 100 100 0 99 0 100 0 100 0 0 5 100 0 0 0 75 0 95 0 0 0 0 0 0 0 0 0 2 0 88 100 0 0 80 100 97 100 100 92 100 80 100 0 0 2 0 5 0 0 0 0 0 0 0 15 5 0 0 0 0 0 0 0 0 0 0 0 0 0 60 60 75 70 80 77 20 5 95 0 0 0 0 0 0 0 0 0 0 62 0 97 0 100 0 10 0 0 0 92 0 0 0 62 0 0 0 0 0 50 0 100 100 0 85 100 0 100 100 0 0 100 50 0 0 100 100 0 0 0 0 0 70 0 50 0 0 0 0 50 100 100 30 0 0 0 0 10 0 0 0 97 Urea Hydrolysis 90 H_2S (TSI) 0 94 0 0 0 55 0 0 0 0 0 0 0 0 90 0 0 100 0 0 0 0 0 0 0 0 0 0 0 0 0 100 100 100 0 100 100 55 97 91 0 70 95 95 90 90 99 97 90 95 65 17 30 70 0 100 0 0 50 0 0 85 18 2 0 2 0 0 5 0 0 2 98 0 0 0 0 50 100 100 0 0 100 0 40 0 80 0 95 0 95 0 100 0 92 0 80 0 0 0 0 100 100 100 100 100 100 0 0 79 75 55 0 0 100 100 15 100 100 97 21 100 10 98 100 2 99 99 4 100 96 60 0 100 0 70 100 100 0 98 50 19 0 100 13 97 97 0 45 55 100 40 80 100 100 100 30 100 99 100 100 0 1 0 97 0 0 0 1 100 100 3 1 1 95 100 0 2 1 99 100 0 0 98 0 100 100 0 0 100 80 0 0 0 0 0 0 5 0 1 0 0 0 0 94 99 0 0 0 0 55 13 0 0 99 0 0 0 0 0 0 0 100 100 100 100 100 100 100 100 100 0 0 0 20 0 20 0 92 0 100 0 15 0 20 0 70 0 25 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 8 0 0 0 0 100 85 100 85 30 0 100 85 100 0 80 90 100 100 80 0 100 0 100 0 0 100 100 100 0 0 50 0 0 0 100 0 100 0 0 100 100 0 50 0 100 100 100 100 100 25 100 100 100 0 0 0 0 0 60 30 90 25 0 0 0 0 0 100 0 0 0 0 0 100 80 0 0 0 88 2 3 0 0 94 0 0 0 0 0 0 0 0 0 2 5 5 5 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 2 0 10 0 0 0 0 0 0 0 0 0 5 0 0 0 0 0 25 0 5 100 0 8 100 100 0 30 0 0 15 92 0 100 0 0 100 5 0 100 100 0 100 0 85 100 30 0 60 0 100 0 0 0 100 75 0 0 0 0 0 0 0 100 100 100 Fermentation Motility 0 0 Sucrose Fermentation Ornithine Decarboxylase 0 90 Gas Arginine Dihydrolase 95 0 Malonate Utilization Lysine Decarboxylase 10 Citrate (Simmons) 0 100 Voges-Proskauer Salmonella serotype Pullorum Salmonella serotype Typhi Serratia entomophila Serratia ficaria Serratia fonticola Serratia liquefaciens Serratia marcescens Serratia marcescens biogroup 1 Serratia odorifera biogroup 1 Serratia odorifera biogroup 2 Serratia plymuthica Serratia rubidaea Shigella boydii Shigella dysenteriae Shigella flexneri Shigella sonnei Tatumella ptyseos Trabulsiella guamensis Xenorhabdus nematophilus Yersinia aldovae Yersinia bercovieri Yersinia enterocolitica Yersinia frederiksenii Yersinia intermedia Yersinia kristensenii Yersinia mollaretii Yersinia pestis Yersinia pseudotuberculosis Yersinia rohdei Yersinia ruckeri Yokenella regensburgei (Koserella trabulsii) Enteric group 58 Enteric group 59 Enteric group 60 Enteric group 68 Enteric group 69 Methyl Red Organism Phenylalanine Deaminase 19-12 Biochemical Reactions of Named Species and Unnamed Groups of the Family Enterobacteriaceae—cont’d Indole Production TABLE PART II Laboratory Identification of Significant Isolates 0 0 5 40 18 23 0 0 0 0 20 5 40 35 8 40 0 0 0 20 100 95 100 100 0 100 0 0 80 100 98 100 100 100 100 97 100 0 100 100 0 0 100 0 0 100 0 100 0 0 100 0 91 100 100 0 97 5 0 95 40 0 92 30 From the Centers for Disease Control and Prevention, Atlanta, GA. Clinically, S. Unlike the bronchiolitis caused in children, adults often develop pneumonia. During budding, part of the host cell plasma membrane surrounds the viral capsid and becomes the viral envelope. Enzyme-labeled oligonucleotide probes have been reported to detect ETEC in fecal specimens, but this method is undergoing further testing to determine its efficacy. It is estimated that 4500 cases of V. The organism lyses and phagocytizes the cell. Burkholderia mallei Burkholderia mallei causes glanders, a respiratory tract zoonosis primarily affecting livestock such as horses, mules, and donkeys. The cyst is round, 10 to 30 µm in diameter, and has a single nucleus. cryocrescens growing on MAC agar. White piedra is endemic in tropical areas of South America, Africa, and parts of Asia. In general, infections caused by P. Enteroviruses can be cultured from the pharynx immediately before the onset of symptoms and for 1 to 2 weeks afterward; they can be isolated from the feces for as long as 6 weeks afterward. Endotoxins, in contrast to exotoxins, do not have tahir99 VRG CHAPTER 2 Host-Parasite Interaction enzyme activity, are secreted in only very small amounts, do not have specificity in their activity on host cells, are not very potent, and are not destroyed by heating. After extraction, the methyl esters are analyzed by GLC. Diagnostic laboratories routinely incubate cultures for bacterial growth at 35° C. Doxycycline and amoxicillin are equally effective in treating early stages of Lyme disease without complications. Some viruses, such as respiratory syncytial virus (RSV), become much more difficult to recover, even a few hours after collection. The mortality rate is about 15% for patients ill enough to need hospitalization. The nature of the agent was a mystery, because no bacteria were apparent on direct examination or on culture. Before routine use of the vaccine in children, an estimated 4 to 5 million cases occurred annually. The physician ordered a stool culture for bacterial pathogens and an ova and parasite (O & P) examination. Public health settings b. In most patients, the acute infection manifests as a self-limiting diarrhea, with malaise, cramps, nausea, and abdominal tenderness after an incubation period of 12 to 14 days. donovani complex. Nonspecific staining methods are most useful for examination of specimens from normally sterile sites. Fusobacterium nucleatum Fusobacterium necrophorum (pleomorphic) Yeast, Fungi, and Algae Yeast Small Medium With capsules Thick-walled, broad-based bud Histoplasma, Torulopsis spp. F. Finite cell cultures can divide, but passage is limited to about 50 generations. Laboratory Diagnosis. Direct microscopic examination of cutaneous and mucosal lesions demonstrates the characteristic yeast cells. Microgametocytes (male) have pale blue tahir99 VRG 652 PART II Laboratory Identification of Significant Isolates Ingestion during blood meal Zygote Exflagellation Gametocytes Fertilization Ookinete Formation of gametocytes infective in mosquito Oocyst Gut wall of mosquito Rupture Mature trophozoite Mature schizont Immature schizont Sporozoites Migration to mosquito's salivary gland Early trophozoite Erythrocytic cycle Mosquito’s biting of human and injection of sporozoite Red blood cells invaded Release of merozoites Via bloodstream to liver cells Schizogony Exoerythrocytic cycle FIGURE 28-27 Life cycle of Plasmodium spp. Clinically important Zygomycetes include Rhizopus, Mucor, Mycocladium, Cunninghamella, and Syncephalastrum. The latter manifestations were observed among patients who had underlying illnesses, such as malignancies and liver disease. SXT and the fluoroquinolones seem to work well clinically. CNS involvement includes seizures and encephalopathy. aeruginosa. They have been occasionally isolated from human clinical specimens, including wound infections, bacteremias, feces from patients with acute gastroenteritis, and septicemia, especially from immunocompromised patients. Frequently, when fungal hyphae are being described, they are referred to as septate or sparsely septate. histolytica. The virus is transmitted by a mosquito vector between birds and humans. These procedures were used extensively in the early days of anaerobic bacteriology, but because of cost, safety, and time commitment, they have been largely abandoned for more rapid identification procedures. afzelii have been confirmed in Asia, and all three species have caused disease in Europe. • Heterolactic fermentation: Some lactobacilli use this mixed fermentation pathway, of which, in addition to lactic acid, the end products include carbon dioxide, alcohols, formic acid, and acetic acid. The genus Hepatovirus includes the hepatitis A virus (HAV). When a primary care provider is confronted with an infection that may be caused by a parasite, the patient’s symptoms and clinical history, including travel history, are significant data to be gathered and shared with the laboratory scientist. They are isolated in specimens from hospitalized patients such as urine, feces, sputum, and wounds. Virulence Factors. Pseudomonas aeruginosa can produce a variety of factors that lend to its pathogenicity, such as endotoxin (lipopolysaccharide [LPS]), motility, pili, capsule, and several exotoxins—proteases, hemolysins, lecithinase, elastase, and DNase. Dysentery was characterized by the presence of blood, mucus, and pus in the stool. Gloves are always to be worn when handling blood or body fluids. In most Wright-stained smears, the nucleus and kinetoplast and parts of the undulating membrane are well stained, but only the suggestion of a flagellum can be seen at the anterior end. The development of an infection control risk assessment 2. are occasionally found as part of the normal skin biota and can also be isolated from animals and soil. Although a few reference laboratories perform mating tests with known tester strains, this procedure is not regularly performed in clinical laboratories. Men infected with T. The primary and secondary lesions are usually papules that often go unnoticed. Many patients with HME may experience asymptomatic infection. that may have broken through on selective charcoal–horse blood). Severe hypotension occurs within 30 minutes after exposure. Patients with latent infections constitute an important reservoir for the organism. haemophilum Rough (Needs hemin) M. Secondary bacterial infections can result from scratching. Not only is this agent lethal for fungi, but it is also toxic for patients. Phagocytosis Pili Respiratory burst True pathogen Virulence Zoonoses T he relationship that exists between the human host and the microbial world is exceedingly complex. The signs and symptoms of EBV include sore throat, fever, lymphadenopathy, hepatomegaly, splenomegaly, and general malaise. The advantage of this direct assay is a quicker susceptibility report—that is, within 2 to 3 weeks of culture, rather than 5 to 7 weeks or longer. Which of the following genera is typically microaerophilic? Giardia lamblia, also known as Giardia intestinalis or Giardia duodenalis, originally was considered the only pathogenic B FIGURE 28-15 A, Balantidium coli trophozoite. gondii or E. Finegoldia spp. solium (Figure 28-50). 7. Institute control measures. The susceptibility of B. The background of the sample being evaluated should be described in sufficient detail to convey the composition of the material. Biopsy and culture of the site are important to distinguish this infection from a much more serious nonfungal disease, melanoma. Entamoeba coli. Entamoeba coli is a commonly found intestinal commensal transmitted by the ingestion of cysts in fecally contaminated food or water. The specificity of the test is enhanced when paired sera from patients with symptoms of legionellosis are tested. Never use fewer than three dips of 1 full second each. Flagella that occur on all sides of the bacterium are peritrichous. cepacia grows on MAC agar, but selective media containing antimicrobials to reduce the growth of P. Trophozoites, schizonts, and gametocytes may be seen in the blood smear. When infection with EBV occurs in adolescence, it presents as infectious mononucleosis 35% to 50% of the time. (Engelkirk PG, et al: Principles and Practice of Clinical Anaerobic Anaerobic Bacteriology, Belmont, CA 1992, Star Publishing Company, Inc. Lesions enlarge with time, usually with most inflammation occurring at the advancing edge of the lesion. Microbes commonly encountered in health care–associated infections in hospitals are: a. histolytica from the nonpathogenic E. Although complete immunity to malaria does not exist, individuals in endemic areas develop antibodies against the asexual stages, which helps reduce the parasite load and severity of illness. The family most closely resembles the family Coxsiellaceae. Both Marburg and Ebola viruses share a common morphology and similar genome and structural proteins. When BAL was compared with three morning gastric lavage specimens, diagnosis of childhood pulmonary TB could be made in 10% versus 50% of children, respectively. This process is called invasion. The ends may be square or rounded. properties and host ranges. The cell wall of gram-negative microorganisms is composed of two layers— the inner peptidoglycan layer and an outer membrane. The two biogroups also have different bacteriophage susceptibility patterns. The mature schizont contains 6 to 12 merozoites (see Figure. Colonies may appear bluish on MAC agar. Invariably the patient has a history of recent aquatic exposure or a water-associated traumatic injury to the infected site. Comments Inappropriate specimens should be rejected. Classification/Taxonomy Taxonomy (Greek taxes: arrangement; Greek nomos: law) is the orderly classification and grouping of organisms into taxa (categories). Primary cell cultures are obtained from tissue removed from an animal. Culture of CSF is usually not productive. Documented S. Burkitt lymphoma is a malignant disease of the lymphoid tissue seen most commonly in African children. Antidiarrheal agents are also restricted in cases of salmonellosis because these agents may encourage adherence and further invasion. trachomatis has been divided into three biovars—trachoma, lymphogranuloma venereum, and mouse pneumonitis (renamed C. Some of the newer nuclear assays are becoming easier to perform and less costly, so it is expected that they will be used more frequently in clinical laboratories. Anaerobic chambers allow all steps in the processing of a specimen to be performed in an oxygen-free environment. When the infiltrate is examined, it is found to contain cells from the alveoli and plasma cells. Note that this term should not be used until the causative agent has been identified as a dermatophyte. Identification of Plasmodium spp. Diagnosis can also be made by direct antigen detection, NAATs, and isolation in cell cultures. The hazard of working in a mycobacteriology laboratory is minimal, however, when the laboratory is well designed, appropriate equipment is available, and precautions are followed closely. FIGURE 28-60 Ascaris lumbricoides egg, fertile (unstained, ×400). A coverslip is carefully placed over the drop, and the slide is examined with a light microscope. Treatment of foodborne botulism involves the use of antitoxin and supportive care. fluorescens from blood culture bottles in asymptomatic patients has been responsible for clusters of pseudobacteremia, probably related to contaminated catheters and catheter-related devices. coli — Aeromonas*† E. It is important to note, however, that they are usually identified on the basis of characteristic asexual structures. ‡ Colistin, 10 µg. Jackson, PhD Senior Microbiologist U.S. Food and Drug Administration Center for Drug Evaluation and Research Silver Spring, Maryland James L. asymbiotica; and P. Filariform larvae may be identified in the sputum of patients with hyperinfection. Major symptoms associated with lung habitation are nonspecific and often include persistent cough, chest pain, and hemoptysis. require a microaerophilic and capnophilic environment. Continued tahir99 VRG 132 PART I Introduction to Clinical Microbiology PROCEDURE 7-2 Acid-Fast Staining of Mycobacteria—cont’d Kinyoun Stain 1. This was the first V. In 2012, 49 cases, 47 in the United States and 2 in Canada, of human infections caused by Salmonella Infantis from dry dog food were reported. Naegleria fowleri and Acanthamoeba spp. S. There are an additional 20 taxa of miscellaneous gram-negative bacilli included in their database. What is a carrier? Accessed June 7, 2013. The infection usually does not require therapy other than relief of symptoms, such as anti-inflammatory agents for painful joints and generalized aches. Although prophylaxis with VORI is effective in decreasing infections by Aspergillus spp., it is also feared that an increase in zygomycete infections might be attributed to this same agent. clostridioforme, routinely stain gram-negative. This bacterium produces lower respiratory tract infections in humans. (Nomarski optics, ×450.) FIGURE 27-52 Ulocladium sp. organism (Figure 28-56) is characterized by migration of the female out the anus at night to lay eggs in the perianal area. parvum, however, immediate person to person transmission is unusual because these oocysts require about 1 to 2 weeks outside the body to mature and become infective. CHOC, modified Thayer-Martin, and buffered charcoal yeast extract (BCYE) agars and MuellerHinton and tryptic soy broths may be used. Immunosuppressed patients, particularly those with leukemia or lymphoma and those undergoing chemotherapy, may suffer from a serious primary infection or reactivation of a latent infection that can manifest as a fulminating encephalitis and result in rapid death. and Cryptococcus neoformans. BOX 2-1 Microorganisms Found on the Skin Common Less Common Candida spp. Isolates that reproduce sexually, either by forming ascospores or basidiospores, are truly yeasts. Microorganisms usually do not multiply in the esophagus and stomach but are present in ingested food and as transient flora. fragilis. The names beaver fever and backpacker’s diarrhea have been used to describe the condition in this group of people. 22-5 Potential Virulence Factors of Anaerobic Bacteria Potential Virulence Factor Polysaccharide capsules Adherence factors Clostridial toxins, exoenzymes Collagenases Cytotoxins DNases Enterotoxins Hemolysins Hyaluronidase Lipases Neurotoxins (e.g., botulinum toxin, tetanospasmin) Phospholipases Proteases Possible Role Anaerobes Known or Thought to Possess Promotes abscess formation; antiphagocytic function Fimbriae, fibrils enable organisms to adhere to cell surfaces Bacteroides fragilis, Porphyromonas gingivalis B. Individual structures absorb a different amount of safranin, so some have prominent staining (strong avidity), and others are weakly stained (low avidity). These organisms possess polar, sheathed flagella when grown in broth, but they can produce peritrichous, unsheathed flagella when grown on solid media. cloacae and E. Influenza A virus remains one of the most crucial health problems worldwide. are the urine antigen detection test and DFA test. The A2 subunit then facilitates the entrance of the A1 subunit. An evacuation replacement system similar to that used to obtain a strict anaerobic condition can also be used. Entamoeba histolytica Giardia lamblia Cryptosporidium spp. The mode of transmission and the number of infecting organisms in the inoculum play a role in the clinical features of the infection. Viruses Viruses are the smallest infectious particles (virions); they cannot be seen under an ordinary light microscope. Staphylococcus aureus Enterococcus spp. Often patients are able to produce sputum for several days after bronchoscopy; these samples should be collected and examined. pneumoniae seems to be considerably more labile than C. Trichosporon spp. dysenteriae 20 10 0 1 2 3 4 5 6 7 8 Weeks of infection FIGURE 19-9 Culture and serologic diagnosis of typhoid fever. In 2003, a multistate outbreak of monkeypox occurred in the United States. Accessed November 2, 2012. Quantization of the risk of working with an infectious agent is difficult. hermannii from foodstuffs such as raw milk and beef, the same sources as E. Roundworms are characterized by the presence of two sexes and a life cycle that may involve larval migration throughout the body. A liquid stool specimen or purged specimen may contain motile protozoan trophozoites; hence, purged specimens should be examined immediately (within 30 minutes) after passage to ensure motility of the organisms. The spirals are regular and angular, with 4 to 14 spirals per organism (Figure 23-3). Endospores should not be confused with the reproductive spores of fungi (see Chapter 27). multivorum has been isolated from the blood of patients with septicemia and from those with peritonitis. Factors that may play a role in pathogenicity include reduced phagocytosis in the host, a soluble hemolysin produced by some virulent strains, cellmediated sensitivity to leptospiral antigen by the host, and small amounts of endotoxins produced by some strains. veronii biovar veronii is moderately resistant to tobramycin. adecarboxylata, which can have a yellow pigment but only on initial isolation. The inhalation of only a few arthroconidia produces primary coccidioidomycosis. Pseudohyphae (Figure 27-62) are produced when the blastoconidia germinate to form a filamentous mat. Microorganisms such as P. The fungus can be isolated from cutaneous lesions, which are frequently present in infected patients. Psychrophilic nonmotile strains are not considered human pathogens. A fourfold increase in IFA titer to at least 1 : 128 from the acute serum phase (obtained within 1 week of onset of symptoms) to the convalescent serum phase (3 to 6 weeks later) is evidence of recent infection. However, about 15% enter a systemic toxic phase in which fever reappears. • With a few exceptions, no indole is produced from tryptophan. Incubating the suspected isolate in aerobic and anaerobic environments determines the actual atmospheric requirements of the organism. Pathogens exhibit an ability to penetrate and grow in tissues. oxytoca Sign %+ (% +) Sign %+ (% +) Sign + − − or + + + − + + + + or (+) 95.4 0 10 98 98 0 98 92.5 90 88.5 (0.1) + + − + + − + + + nd + + − or + 96 98.7 30 + or − + or − 64.4 67.1 (0.6) (0.2) (0.1) (9.2) (1) + + +or− %+ 90 99 20 96 95 0 99 98 93 97 100 55 nd nd K. Although motile organisms such as Trypanosoma and microfilariae (larval form of filarial worms) can be detected on a wet preparation of a fresh blood specimen under low- and high-power magnification, identification is made on the basis of characteristics seen on a permanently stained smear. The infection manifests in females as vesicles on the mucosa of the labia, vagina, or both. A Immunofluorescent monoclonal antibody stains are commercially available and are widely used. The cytoplasm may occasionally contain chromatoidal bars composed of ribonucleic acid. The larvae coil and become encapsulated. Thus, the result of an encounter with the antigen is an expanded clone or clones of activated lymphocytes. Specimens can be homogeneous or heterogeneous and may contain pathogens evenly distributed throughout the specimen or limited to one visual field. vivax macrogametocyte. vulgaris by the indole and ornithine decarboxylase tests. What presumptive identification methods are currently used to identify Legionella spp. Species are abbreviated “sp.” (singular) or “spp.” (plural) when the species is not specified. If the worm is broken during an attempt to remove it, the patient may experience a severe inflammatory reaction and secondary bacterial infection. There are specific methods and breakpoints available for many of the nonfermenters in the CLSI standards. Pigs have receptors for both avian and human influenza viruses, as well as swine influenza viruses, and can be co-infected with all three types of viruses. pertussis have been reported to be inhibited at 40 mg/L or greater. This assay is offered at major U.S. reference laboratories and is the preferred method for determining viral loads. However, additional smears can be made and examined if the likelihood of contamination is high. The information obtained from these studies has resulted in the reclassification of some bacteria. Clamp connections occur at the septations in the vegetative hyphae and are easily visible with light microscopy. Depending on the species present, the amastigote (obligate intracellular form) can be detected in tissues such as skin, liver, spleen, and bone marrow. Bordetella bronchiseptica Pseudomonas oryzihabitans CDC group EF-4b Brucella sp. In the United States, this is a major cause of sterility. Abundant, single-celled, tear-shaped sympoduloconidia are formed on sympodulae, which taper extremely from a rather swollen base (Figure 27-38). H_2S, Hydrogen sulfide; KCN, potassium cyanide; TSI, triple sugar iron; +, ≥90% positive within 1 or 2 days; (+), positive reaction after ≥3 days (decarboxylase tests: 3 or 4 days); −, ≥90% no reaction in 30 days; + or −, most cultures positive, some strains negative; − or +, most strains negative, some cultures positive; d, different reactions, +, (+), −; +w, weakly positive reaction. Unlike many of the arboviral infections, SLE is milder in children than in adults; older patients have the greatest risk of serious illness and death. indigenous to humans are listed in Table 25-3. endemic relapsing fever infection. The diseases are contracted generally by the inhalation of infectious conidia. The laboratory must be consulted before environmental surface cultures are undertaken to ensure that proper procedures and media are used. Testing procedures for the detection of ESBLs are described in Chapter 13. Larvae migrating through the lungs can cause an immune response in the host characterized by asthma, edema, pneumonitis, and eosinophilic infiltration. 3. Specimens should never be frozen. freundii can be isolated in diarrheal stool cultures, and although it is a known extraintestinal pathogen, its pathogenic role in intestinal disease is not established. Surgical specimens or sterile site specimens may also be inoculated into a broth medium such as thioglycollate or prereduced chopped meat to serve as a backup source of culture material. Strong evidence suggests that Campylobacter infection plays a role in GBS, an autoimmune disorder characterized by acute paralysis caused by damage to the peripheral nervous system. tarda C. Collection and Preparation. Blood taken directly from a fingerstick is the ideal specimen for a malarial smear because it tends to give the best staining characteristics. in patients with a variety of clinical manifestations. In patients with AIDS, the diarrhea may persist several years, with malabsorption of fats and vitamin B_{12} and cachexia as additional complications. Staphylococcus, Streptococcus, Enterococcus spp. It presents the role of the microbial flora at each body site in the host immune defense and as a source of opportunistic infections. The nucleolus is the site of rRNA synthesis. Infections acquired later in pregnancy may result in the child being asymptomatic at birth but developing complications, especially ocular problems, later in childhood. pallidum causes syphilis. Some anaerobes are killed almost immediately in the presence of oxygen (e.g., Clostridium novyi). *Biohazard precautions; consider organisms such as Francisella spp. This is because every set of three bases (known as a codon) specifies a particular amino acid, and when the reading frame is askew, the codons are interpreted incorrectly. Several of these DFA tests also contain a monoclonal antibody that identifies Cryptosporidium. As with all members of the family Herpesviridae, reactivation of latent infections can become clinically significant in immunocompromised individuals. These methods are more expensive than the modified acid-fast procedure but demonstrate greater sensitivity, especially when only rare oocysts are present. An expectorated sputum sample was plated. Most isolates of C. The mycoplasmas adhere to the epithelium of mucosal surfaces in the respiratory and urogenital tracts and are not eliminated by mucous secretions or urine flow. An alternative drug is doxycycline. Transovarian transmission can occur in the tick, allowing the life cycle to persist without an intermediate host. Gram-stained smears of the exudate were positive for gram-negative bacilli and gram-positive cocci. Alternatively, hemin (X) strips or disks used for the identification of Haemophilus spp. Treponema reproduce via transverse fission, whereas Leptospira and Borrelia divide by the more common binary fission. Immunofluorescent staining of CSF with monoclonal antibody is available in some reference laboratories for detection of the trophozoite. That same day, after 4 days of incubation, the patient’s blood cultures were positive with a yeastlike colony. It differentiates sucrose-fermenting (yellow colonies) species (Figure 20-4) such as V. Accessed March 17, 2013. Another third remain latent for life but have reactive serology. This increase in CO_2 reduces the oxygen concentration to 15%, which is still sufficient to allow aerobic organisms to replicate. baumanii, including endophthalmitis, conjunctivitis, and corneal ulcerations. At least 16 species of mollicutes have been isolated from humans. Accumulation of niacin, detected as nicotinic acid, is the most commonly used biochemical test for the identification of MTB. Figure 21-2 illustrates typical biochemical and morphologic characteristics for many of the more common nonfermentative, gram-negative bacilli. Treponema pallidum subsp. Cronobacter (Enterobacter) sakazakii typically produces a yellow pigment and has been documented as a pathogen in neonates causing meningitis and bacteremia, often coming from powdered infant formula. Lam CS, et al: Disseminated strongyloidiasis: a retrospective study of clinical course and outcome, Eur J Clin Microbiol Infect Dis 25:14, 2006. For example, a solution that has been filtered through a certain pore-size filter (1 mm in size For Microscopic Examination Wet preparation (direct or sedimented) Cytocentrifuged (direct or presedimented) Smear 1. Drop 2. Pellet 3. Rolled 4. Imprint (touch preparation) Fluids or semisolids Clear or slightly turbid fluids Clear or slightly turbid fluids Pus or fluid Tissue homogenate Swab rinse Blood culture Dilute specimen Swabbed material Tissue FIGURE 7-1 Smear preparation of a sample collected on a swab. The apocrine sweat glands in these areas secrete substances metabolized by the skin bacteria, releasing odorous amines. Disk results are interpreted as shown in Table 22-12. Figure 28-33, A, shows the small, compact, ringlike trophozoites of B. (Courtesy Anaerobe Systems, Morgan Hill, CA.) 22-13 Presumptive Identification of Gram-Positive Anaerobes* Identification Clostridium difficile C. Microscopically, E. A good patient history combined with proper selective screening agar (e.g., HE, XLD, and SMAC agars) can be very helpful in a timely and accurate identification of enteric pathogens associated with diarrheal disease. Tissue should be gently minced before inoculation. (gram-variable bacilli) and mixed gramnegative anaerobes. All these functions connect the microbiology laboratory safety program and infection control program. They have good sensitivity in detecting low levels of P. All members of the Streptococcus genus and many members of the Lactobacillus genus ferment pyruvate using this pathway. This makes tahir99 VRG CHAPTER 1 Bacterial Cell Structure, Physiology, Metabolism, and Genetics 9 CH_2OH O (NAG) OH CH_2OH (NAM) CH_2OH O (NAG) OH O O NH CH_2OH C O O CH_3 (NAM) O O NH OH CH_2OH C O O O O CH_3 (NAG) NH NH HC CH_3 OH C O C O C O O CH_3 L-Alanine NH CH_3 HC CH_3 C O D-Glutamate C O CH_3 Meso-diaminopimelate L-Alanine D-Alanine D-Glutamate Meso-diaminopimelate D-Alanine FIGURE 1-3 Diagram that

demonstrates the structure of the peptidoglycan layer in the cell wall of Escherichia coli. The acids are separated in the column and recovered in order, based on their chemical and physical properties. Sodium Polyanethol Sulfonate Disk. A sodium polyanethol sulfonate (SPS) disk aids in the identification of anaerobic gram-positive cocci. Nuns in the hospital routinely reused syringes without sterilizing them. Acidified alcohol is used as a decolorizer, and methylene blue is the counterstain. Humphrey, Centers for Disease Control and Prevention, Atlanta, GA.) infection rate can be as high as 50%. Plasmodium falciparum. Although identified as having a tertian life cycle, P. The virus, which is relatively fragile, can also be recovered from the urine and CSF. The poxviruses are the largest viruses (250 × 350 nm), and the smallest human virus is the poliovirus, which is 25 nm in diameter. Size is an important diagnostic criterion for parasites, and use of a properly calibrated ocular micrometer ensures accurate measurement of organisms. Flavobacteriaceae Balaneatrix, Bergeyella, Chryseobacterium, Elizabethkingia, Empedobacter, Myroides, Weeksella, Wautersiella, Sphingobacterium spp. Vibrio 4. As this structure enlarges, the nucleus of the parent cell undergoes mitosis. There was a pseudobacteremia cluster involving five patients, four adults in the emergency department and one neonate in ICU. Discuss rapid assays useful in the detection of Legionella spp. Eggs of the beef tapeworm, T. With some organisms, the invasion is localized and involves only a few layers of cells. Growth patterns should be observed, serologic tests repeated, and additional biochemical tests performed (Table 18-8) to confirm the identification. Mitochondria are the main sites of energy production. They invade cells by attaching to surface proteins on nucleated cells, replicate intracellularly within a parasitophorous vacuole formed by the host cell plasma membrane, cause cells to rupture, and release tachyzoites to invade other cells. The pouch is sealed by closing the zipper part of the pouch and then incubated at the appropriate temperature. The presence of endosymbiotic bacteria of the genus Wolbachia have been linked to stimulation of the host immune response and may contribute to the inflammatory tissue reaction. Never put immersion oil on a smear until it is completely dry. tahir99 VRG 470 PART II Laboratory Identification of Significant Isolates Incubation at 37° C allows the recovery of Campylobacter spp. Acid-fast staining is used to identify Saccharomyces, a yeast, and coccidian parasites, such as Cystoisospora belli (formerly known as Isospora belli), Cryptosporidium, and other coccidia-like bodies. Anal pruritus is caused by migration of the female worm to the perianal area. If no granules are present, the material may be plated directly onto the media. Skin infections present as a relatively painless nodule. A control plate is set up in each test of drugs so that the number of colonies on the test quadrants can be counted and compared with the number on the control quadrant. often fluoresce red, but the fluorescence is dependent on the culture medium. falciparum on blood smears. Karanis P, et al: Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt, J Water Health 5:1, 2007. Finally, one drop of material is spread evenly over an alcohol-cleaned glass slide for Gram staining. A reappraisal of host-cell parasitism by Cryptosporidium, Trends Parasitol 24:509, 2008. The eighth step, which comes after the development of interventional strategies, is to evaluate the effectiveness of the interventions. She had been coughing with increased severity, resulting in posttussive vomiting with some choking. In contrast to the O antigens, flagellar antigen proteins are heat labile. Whether an organism is gram-positive (blue or purple) or gram-negative (pink or red) is an important first step in identifying bacteria and in determining appropriate antimicrobial therapy. psittaci should be strongly suspected. Adherence to surfaces d. In these cases, lysozyme acts with the other agents to cause death of the infecting bacteria. Two common conidiogenous cells are the phialides and annellides. malariae schizont. are Butzler medium and Skirrow's medium. However, prolonged administration has resulted in side effects such as nasal irritation, ulceration, and bleeding. The first arenavirus found to cause hemorrhagic fever was the Junin virus, which causes Argentine hemorrhagic fever. Almost any Clostridium spp. Hopkins H, et al: Comparison of HRP2- and pLDH-based rapid diagnostic tests for malaria with longitudinal follow-up in Kampala, Uganda, Am J Trop Med Hyg 76:1092, 2007. Discuss the serologic response to B. Usual Flora of the Mouth The mouth contains large numbers of bacteria, with Streptococcus being the predominant genus. coli strains. Clostridia cause classic diseases such as tetanus, gas gangrene (myonecrosis), botulism, and food poisoning (foodborne intoxication). Toxoplasmosis can present with a wide range of clinical symptoms and complications that are often related to the immunologic status of the patient. If influenza virus is present, the RBCs will adsorb or stick to the infected cells. Investigate to determine whether additional cases exist. capsulatum (Nomarski optics, ×1250.) circulating antibody, along with FA microscopy to detect viable or nonviable fungal elements in tissue sections. ascorbata (the most common clinical isolate), K. The cyst is the infective stage, and the trophozoite is the stage that causes tissue damage. oxytoca, K. aeruginosa is isolated from a sterile site such as a blood culture, most laboratories would provide this information as rapidly as possible so that clinicians have the in vitro information they can use in developing a treatment regimen for their patient. Vibrio hollisae is now classified in the new genus Grimontia as G. † Mostly considered contaminants. Klebsiella spp. It is now recognized as an important human pathogen associated with a wide range of clinical syndromes, including UTIs, diarrheal diseases, and central nervous system infections. Gram-positive clostridia are always susceptible to vancomycin. Appropriate cultures on a specific Yersinia medium at 25° C should be performed in diarrheal outbreaks of unknown etiology. Although asymptomatic infections can occur, infections are usually characterized by rapid onset of malaise, fever, myalgia, and often a nonproductive cough. The ECV is an endpoint that can be used to evaluate a given MIC when clinical breakpoints are not available. Because Actinomyces spp. burnetii is generally not transmitted by arthropods, although it is known to infect more than 12 genera of ticks and other arthropods. pseudotuberculosis − + d + − + − − d − − − − − + − − − + − + + − + + − + + − − − − − − − − − + − − or +* − or +* + or − + − − + + + − Indole Methyl red Voges-Proskauer 25° C 37° C Motility 25° C 37° C β-Galactosidase Christensen urea Phenylalanine deaminase Ornithine decarboxylase Acid produced from Sucrose Lactose Rhamnose Melibiose Trehalose Cellobiose Modified from Washington J: Laboratory procedures in clinical microbiology, ed 2, New York, 1985, Springer-Verlag. Eventually lymphopenia results, with the greatest loss in the CD4+ T-cell population. Although associated with the common wart, some HPV types are linked to cancer, including cervical cancer. pathogens, and the infections they cause are called opportunistic infections. The infection is self-limiting and usually resolves in several weeks in an immunocompetent host. ✓ Case Check 2-3 In the Case in Point at the beginning of this section, meningitis caused by Cryptococcus neoformans was diagnosed in a patient with advanced human immunodeficiency virus (HIV). During the competition, he had completed various events, including mountain biking, caving, climbing, jungle trekking, swimming, and kayaking in fresh and salt water. To ensure isolation of both opportunistic and fastidious pathogens, laboratories must provide appropriate transport media, such as Cary-Blair, Amies, or Stuart media. fluvialis Group 5 − + + + − − + V. Surface Structures That Promote Adhesion to Host Cells and Tissues Most infectious agents must attach to host cells before infection occurs. Catalase Test. To perform a catalase test, a plastic, disposable inoculating loop or wooden applicator stick is used to place some of the colony onto a small area of a glass microscope slide. Antigenic drifts occur with all three types of influenza: A, B, and C. Table 19-1 lists the tahir99 VRG 422 TABLE PART II Laboratory Identification of Significant Isolates 19-1 Classification of Selected Species within the Family Enterobacteriaceae Tribe I. Escherichieae Genus Escherichia Shigella II. Edwardsielleae Edwardsiella III. Salmonelleae Salmonella IV. Citrobacteriaceae Citrobacter V. Klebsielleae Klebsiella Species Tribe coli albertii blattae vulneris fergusonii hermanii dysenteriae flexneri boydii sonnei tarda liquefaciens hoshinae ictaluri enterica bongori freundii koseri (C. FIGURE 21-1 Grouping of nonfermenters based on eight possible results. Ewing WH: Edwards and Ewing's identification of Enterobacteriaceae, ed 4, New York, 1986, Elsevier. are thin, gram-negative bacilli that may show size variation from 2 to 20 µm in length. Wade W, Kõnönen E: Propionibacterium, Lactobacillus, Actinomyces, and other non–spore-forming anaerobic gram-positive rods. Much of the respiratory biota has been eliminated. The genus name followed by the word species (e.g., Staphylococcus species) may be used to refer to the genus as a whole. The prodromal phase of the paroxysm involves headache, bone pain, nausea, and/or flulike symptoms. For example, the isolation of oral anaerobes from a brain abscess may suggest invasion from the oral cavity, perhaps caused by poor dentition. The addition of zinc detects nitrate and results in a pink color change in a true-negative reaction. Microscopic morphology indicated gram-negative fusiform bacilli. Vials of merthiolate-iodine-formalin (MIF) can also be used to preserve trophozoites, cysts, larvae, and helminth eggs for wet mount or concentration procedures. Sutton SA, Brandt ME: Fusarium, and other opportunistic hyaline fungi. This mechanism, known as the immune system, consists of numerous cells and protein molecules that are responsible for recognizing and removing these foreign substances. cepacia complex often produces a weak, slow, positive oxidase reaction. Growth Range (°C) 22-45 3 Days M. Many of the nonfermentative, gram-negative bacilli tend to be resistant to multiple classes of antimicrobial agents. Young children can have false-negative results with the heterophile test; performing an EBV-specific antibody test on these individuals is appropriate. More recently, there has been an increase in the influenza variant H3N2v, which is a nonhuman influenza virus normally found in swine. Nucleic acid amplification tests (NAATs), such as the Illumigene C. LT consists of two fragments (A and B), which follow the A/B model of bacterial toxins, where A is the enzymatically active portion. Wounds b. tahir99 VRG 32 PART I Introduction to Clinical Microbiology Adherence protein Fibrilla (e.g., M protein) Pilus Protein F Lipopolysaccharides Non-pili adhesins Lipotechoic acid Capsule Outer membrane Peptidoglycan Peptidoglycan Inner membrane Cell membrane GRAM-NEGATIVE GRAM-POSITIVE FIGURE 2-1 Surface bacterial structures that are involved in the pathogenesis of disease. grimontii and L. curvus. Embryonated eggs are rarely used; isolation of influenza viruses is enhanced in embryonated eggs, but this is generally accomplished more easily in cell culture. Isolates are ubiquitous in the environment, being found in water, sewage, and plant materials; they are very common in the hospital environment, where they can be found contaminating blood-drawing equipment, disinfectants, transducers, and other equipment. An inoculating loop or wooden applicator stick is used to transfer some growth from the culture plate onto the saturated filter paper. FIGURE 28-44 Paragonimus westermani egg (unstained, ×400). Unlike the tuberculin skin test, these assays are not affected by BCG vaccination and do not cross-react with antigens from most other mycobacterial species. Although the zinc sulfate method yields less fecal debris in the finished preparation than the FES method, the zinc sulfate causes operculated eggs to open or collapse. ficaria, tahir99 VRG CHAPTER 19 Enterobacteriaceae A 431 B FIGURE 19-6 A, Serratia marcescens growing on CHOC agar (left) and showing brick-red pigment when grown on MacConkey (MAC) agar (right). These lesions may be associated with prolonged disabling pain that can remain for months, long after the vesicular lesions disappear. Formalin can be used when a wet mount or concentration procedure (sedimentation or flotation) will be performed. The lesion resolves in several months and provides some level of immunity (incomplete) against future infection. A small opening at the posterior, the cytopyge, is used to expel the contents of food vacuoles. Escherichia coli b. It is also thought to be a cause of dandruff. (Courtesy Anaerobe Systems, Morgan Hill, CA.) 22-14 Presumptive Identification of Gram-Negative Anaerobes* Identification Colony Morphology on Blood or KVLB Agar Colony Morphology on BBE Cellular Morphology Spot Indole B. However, PCR assays are generally regarded as more sensitive than cultures and DFA assays. To grow obligate anaerobes, the laboratory needs to use oxygen-free growth conditions. The lysosomes release their contents into the phagosome, a process called degranulation. inkin. A 10-year analysis of 54 cases of A. When the louse feeds on another human, it defecates, and the infected feces are scratched into the skin, just as in murine typhus. Flocked swabs (Copan, Murrieta, CA) tend to have higher sensitivities when attempting to recover respiratory pathogens. carinii is the species most commonly found in rats, and P. Patients present with high fever, possibly disseminated skin lesions and, in some patients, fungemia. There are estimates that 50,000 to 100,000 immigrants to the United States are chronically infected and could be capable of causing transfusion- or solid organ transplant-transmitted Chagas disease. Transfer of DNA then occurs. Serology also tends to be retrospective; several weeks are often required to demonstrate a diagnostic response. The bacteria enter the lymph nodes near the genital tract and produce a strong inflammatory response that often results in bubo formation and subsequent rupture of the lymph node. This usually requires aseptic inoculation of anaerobic and aerobic blood culture bottles. Interpretation is also difficult because of passive transfer of antibodies, such as in transplacental or transfusion transmission. Immediate processing of these samples is important. are straight to slightly curved bacilli of varying lengths, from short rods to long filaments. The tissues of these structures are normally sterile as a result of this protective action. The perianal area is touched with the sticky side of the tape or paddle. enterocolitica have caused sporadic cases of mesenteric lymphadenitis in humans, especially in children, and generalized septicemic infections in immunocompromised hosts. This organism is found worldwide, with the greatest prevalence in hot, humid, and tropical locations. +, 90% to 100% positive; V, variable, 11% to 89% are positive; −, negative, 0% to 10% positive; NG, no growth, possibly because the NaCl concentration is too low, even when 1% NaCl is added. Differentiate the role of the pili from the role of the flagella. Although these organisms were suspected of causing human infections earlier, campylobacters were TABLE not established as human pathogens until sensitive isolation procedures and media were developed. You suspect that a yeast isolated from the oral cavity of a patient with HIV infection is Candida albicans. Compare the virulence factors of the Escherichia coli strains pathogenic for the gastrointestinal tract and the E. Interestingly, M. Wudhikarn K, et al: Transfusion-transmitted babesiosis in an immunocompromised patient: a case report and review, Am J Med 124:800, 2011. Cases of septic arthritis can also occur in patients who had asymptomatic salmonellosis. Microscopic Examination. Organisms are too thin to be observed by bright field microscopy, so spirochetes are illuminated against a dark background. Tinea favosa, or favus, begins as an infection of the hair follicle by Trichophyton schoenleinii and progresses to a crusty lesion made up of dead epithelial cells and fungal mycelia. The Binax NOW (Binax, Inc, Portland, ME) is a 15-minute immunochromatographic assay that detects soluble antigen from L. These kits can detect the presence of C. The NoVs (Figure 29-12) are the most common cause of infectious gastroenteritis in the United States, accounting for as many as 23 million cases annually. FIGURE 24-3 Conjunctival scarring and hyperendemic blind- FIGURE 24-4 Inguinal swelling and lymphatic drainage caused ness caused by Chlamydia trachomatis in ocular infections. Anaerobes have special nutritional requirements for vitamin K, hemin, and yeast extract, and all primary isolation media for anaerobes should contain these three ingredients. Poor prognostic factors associated with P. vesicularis has been reported as a cause of meningitis, infective endocarditis, and infections in a CAPD patient; it was also isolated, with unknown significance, from urine, and eye specimens. Antigen detection in tissues such as bone marrow, liver, and spleen has been described. It is the primary mechanism in the host defense against extracellular bacteria and numerous viruses and fungi. burgdorferi. Layer filter paper with carbolfuchsin stain. pneumoniae is recognized as the third most common cause of infectious respiratory disease. Extended exposure to oxygen results in cell death for strict anaerobes and cessation of growth for oxygen tolerant anaerobes. In addition to routine testing, an immunoassay for Giardia lamblia or Cryptosporidium may be requested on specimens from immunocompromised patients with diarrhea or children in daycare settings who are symptomatic. Eggs are produced within 6 to 8 weeks after skin penetration by the filariform larva. The medium is then inoculated with a portion of the acid-treated specimen. Most pathogenic mycobacteria are slow growers, taking up to several weeks for isolation on artificial media. typhi can also be transmitted to humans directly from the flea bite itself. Some pathogens do not grow in culture and are usually unsuspected. The typical budding yeast measures 15 to 30 µm in diameter with multipolar budding at the periphery, resembling a mariner's wheel (Figure 27-31). Others do not break down carbohydrates at all and are inert or biochemically inactive; they are referred to as nonoxidizers or asaccharolytic. Those caused by bacteria are referred to as actinomycotic mycetomas, and those caused by fungal agents are referred to as eumycotic mycetomas. This enzyme hydrolyzes the bond between the sulfate group and aromatic ring structure in compounds with the formula $R–OSO_3H$. Rickettsia spp. Fix with alcohol. xenopi c. A typical Jarisch-Herxheimer reaction and exacerbation of cutaneous lesions can occur within hours following treatment. The interferon produced binds to the surface receptors on noninfected cells. Future studies will likely lead to the naming of many other species. Centers for Disease Control and Prevention: 2010 Sexually transmitted diseases surveillance: chlamydia, 2011. Fothergill, MA, MBA, MT(ASCP), CLS(NCA) Assistant Professor Department of Pathology Technical Director, Fungus Testing Lab University of Texas Health Science Center at San Antonio San Antonio, Texas Wade K. tahir99 VRG 494 PART II Laboratory Identification of Significant Isolates Henry DA, Speert DP: Pseudomonas. Nonfermentation of mannitol produces a colorless, translucent colony. What morphology would you see if you inoculated the colony onto cornmeal agar? Symptoms, which can mimic those of cryptosporidiosis or isosporiasis, include anorexia, weight loss, abdominal cramping and bloating, vomiting, lowgrade fever, and nausea. Clinical disease varies from mild upper respiratory tract to acute lower respiratory tahir99 VRG CHAPTER 29 Clinical Virology infection and includes fever, a nonproductive cough, sore throat, wheezing, congestion, shortness of breath, and lethargy. meningoseptica in appearance, but motility and nitrate reduction should help differentiate Balneatrix from Elizabethkingia (negative for both characteristics). Variation exists in atmospheric growth conditions, temperature requirements, and cell structure. The child was pale, listless, and had a protuberant abdomen. oryzihabitans Pseudomonas cepacia Tan (occasionally) P. 8. Evaluate effectiveness of control measures. The onset of symptoms depends on the number of organisms ingested; the larger the inoculum, the shorter the incubation period. Spores are visualized microscopically as unstained areas in a cell with the use of traditional bacterial stains (Gram) or by using specific spore stains. Concentration Techniques. Concentration techniques are designed to concentrate the parasites present into a small volume of fluid and remove as much debris as possible. At least one of the nuclei shows evidence of the thin nuclear membrane. Japanese encephalitis virus is a major cause of encephalitis in Asia and is the most common cause of arboviral encephalitis in the world. They are attached to the rough ER. The infection can be diagnosed using PCR, immunohistochemistry, and IgM-capture ELISA. Ascospores are formed within a saclike structure known as an ascus. On SBA, colonies may be β-hemolytic or nonhemolytic. Some patients are asymptomatic, whereas others develop acute or chronic infections. If agglutination fails, the suspension must be heated to remove the capsular antigen that may be present, and subsequently the agglutination test procedure is repeated. Home care settings d. Available at: mmwr/preview/mmwrhtml/mm5728a4.htm. Although they stain grampositive, mycobacteria have a modified cell wall called an acid-fast cell wall. The neck is directly behind the scolex. + – Serratia spp. histolytica infection presents in several ways—asymptomatic colonization, amebic dysentery (colitis), and extraintestinal amebiasis. The precise mechanisms whereby anaerobic bacteria cause disease are not always known. These conidia are formed by fragmentation of fertile hyphae as opposed to being formed by conidiogenous cells (Figure 27-7). As described in the text, antibiotic use may alter the usual flora of nearly any body site. Pseudomonas luteola and Pseudomonas oryzihabitans The natural habitat of Pseudomonas luteola and Pseudomonas oryzihabitans is unknown, although members of both genera have been isolated from soil and water. saginata is less than 5 mm long and has four suckers, whereas that of T. (unstained, ×650). Because of the permanent absence of a cell wall, they were originally grouped under the general term cell wall–deficient bacteria. Figure 19-11 illustrates the colony characteristics of Kluyvera spp. Positive stains reveal bright, yellow-orange bacilli against a dark background. A continuous flow of mucus emanates from the membranes lining the nasopharynx, which traps particles and microbes and sweeps them to the oropharynx, where they are either expectorated or swallowed. Infection is initiated following inhalation of fungal conidia. After 3 days of uneventful hospitalization, the patient was discharged from the hospital with a 10-day regimen of prophylactic antimicrobial therapy. The infection may be diagnosed by various methods, including radiography to detect calcified cysts, ophthalmoscopic examination of the eye to detect cysticerci, imaging techniques (CT and MRI) to locate larvae in the brain, and biopsy and histologic staining of tissue. Diagnosis of ETEC infection is made primarily by the characteristic symptoms and the isolation of solely lactose-fermenting organisms on differential media. The patient had been otherwise healthy until 2 weeks ago, when he began to experience fatigue and felt slightly swollen lymph nodes. dysenteriae type 1 are higher than those produced by other Shigella spp. The most important risk factors in affected individuals were immunosuppression, hospitalization, especially in an ICU, and the presence of a central venous catheter. (Courtesy Syva Microtrak, Palo Alto, CA.) separated C. capsulatum var. These infections usually represent displacement of environmental, skin, oropharyngeal, gastrointestinal, or vaginal flora into tissues, with subsequent infection. Laboratories should have agglutinating sera for these groups and report isolates as Salmonella groups (A through E1). Within the next few days, 10 members of the retiree party and several other passengers were acutely ill with pneumonia and required medical evacuation to a hospital. Immunocompromised patients with previous M. Some species exist as free-living tahir99 VRG 423 CHAPTER 19 Enterobacteriaceae TABLE 19-2 Biochemical Characteristics of Tribes of Enterobacteriaceae Tests or Substrate H_2S (TSI agar) Urease Indole Methyl red Voges-Proskauer Citrate (Simmons) KCN Phenylalanine deaminase Mucate Mannitol Escherichieae Edwardsielleae Citrobacteriaceae Salmonelleae* Klebsiellae Proteeae† Yersiniae − − + or − + − − − − d + or − + − + + − − − − − − − + or − (+w) or − − or + + − + + or − − + − − + − − d + − − or (+) − − + + + − + or − + + + + + − d + + − − or − or − or − − + + or − + − − − − or + + + Modified from Ewing WH: Edwards and Ewing's identification of Enterobacteriaceae, ed 4, East Norwalk, CT, 1986, Appleton & Lange. Proto zoan cysts, helminth larvae, and helminth eggs, however, can be detected using this method. In most cases, however, tests for antibody serve only as epidemiologic markers, especially in endemic areas. Cornmeal Agar 37° 42° 45° Pseudo Hyphae True Hyphae Arthroconidia Cycloheximide Urea Nitrate + + + + + + + + + + − + + + + − + + + − + − − + − + + + + − + + + + + + + + + − − − − − + − − − − − − − − − − R R S R S V S S V − − − − V − − − − − − − − − − − − − − − − + + − − V − − − − − + − − + − − + S S R + + + + − − +, Positive; −, negative; R, resistant; S, sensitive; V, variable. Figure 28-29 shows several stages of P. (A, ×1000; B, ×400.) tahir99 VRG 638 PART II Laboratory Identification of Significant Isolates whereas others have not. Although P. 885. 472. If patients who develop yellow fever from the intermediate cycle travel to larger cities and are bitten by mosquitoes, they can trigger an outbreak of urban yellow fever. The proper word for the name of a species is an epithet. This is especially important if delays in transport of the specimen are expected. Double or blurred vision, impaired speech, and difficulty in swallowing are also commonly seen. flexneri S. These bacilli are usually oxidase-positive, grow on MAC agar, and if they are motile, it is by means of peritrichous flagella. To determine the true Gram stain reaction of the isolate, special potency disks can be used. Box 26-2 lists the types of clinical samples acceptable for mycobacteriologic culture. The lesions are seldom inflamed, but luster and color of the hair shaft may be lost. These organisms are resistant to digestive tract enzymes for about 6 hours, during which time they convert to tachyzoites and invade the intestinal wall. eosinophilia, an inflammatory reaction, secondary bacterial infection, and fibrosis in the biliary ducts. *H_2S from gelatin-cysteine-thiosulfate media, not the conventional TSI (triple sugar iron agar) media. The infection is divided into three phases—acute, intermediate (latent), and chronic. Knowledge of microbial structure and physiology is extremely important to clinical microbiologists in three areas: • Culture of organisms from patient specimens • Classification and identification of organisms after they have been isolated • Prediction and interpretation of antimicrobial susceptibility patterns Understanding the growth requirements of a particular bacterium enables the microbiologist to select the correct medium for primary culture and optimize the chance of isolating the pathogen. lumbricoides. Mycobacterium species stained with the acid-fast fluorescent auramine-rhodamine stain. Histoplasmosis occurs worldwide. tahir99 VRG CHAPTER 23 The Spirochetes Binding factor H allows for complement evasion and immune system suppression and might explain, in part, why IgM antibody does not peak for 3 to 6 weeks. tahir99 VRG 534 PART II Laboratory Identification of Significant Isolates throughout the body, and infect almost any organ system. The material may not be grossly visible after staining because of a low protein or cell count. Because they are morphologically identical, molecular evaluation is required to differentiate C. Individuals who are hospitalized for several days may become colonized in the upper respiratory tract by gram-negative bacteria, particularly members of the Enterobacteriaceae. Research into this seeming contradiction has shown that individuals with severe immunosuppression may harbor larval stages that are more likely to develop into free-living females or males rather than into filariform larvae. No one medium is likely to support the growth of all anaerobic bacteria; however, CDC blood agar provides the best recovery. One of the two sections on the reverse side of the paddle contains a modified egg-based medium for differentiating M. cinaedi and H. A biphasic media system for the detection and isolation of mycobacteria, Septi-Chek AFB (Figure 26-7), is available commercially (BBL Septi-Chek AFB, Becton Dickinson Diagnostic Systems). This is one of many systems available for rapid definitive identification of commonly isolated anaerobes. Fermentation is an anaerobic process carried out by both obligate and facultative anaerobes. The course of yaws resembles that of syphilis, but the early stage lesions are elevated, granulomatous nodules. However, M. Louse-borne fever is transmitted via the body louse, Pediculus humanus, and humans are the only reservoir. most likely to be encountered in the clinical laboratory are V. pneumoniae include sputum, bronchial lavage fluid, nasopharyngeal aspirates, throat washings, and throat swabs (Table 24-7). Most cases are self-limiting, but in the pediatric, geriatric, and immunocompromised populations, supportive therapy and antimicrobials are often indicated. tropicalis, and C. pneumoniae) Natural hosts Major human diseases Number of serovars Chlamydia trachomatis Chlamydophila pneumoniae Chlamydophila psittaci Round, vacuolar + Round + 10% Humans Sexually transmitted diseases Trachoma Lymphogranuloma venereum 20 Round, dense − Pear-shaped − 100% Humans Pneumonia Pharyngitis Bronchitis 1 Variable shape, dense − Round − 10% Birds, lower animals Pneumonia FUO FUO, Fever of unknown origin; +, characteristic is present; −, characteristic is absent. albicans will be positive, and other species can yield FIGURE 27-59 Germ tube production by Candida albicans. The test uses lyophilized T. gordonae szulgai† scrofulaceum xenopi‡ Rapid Growers M. are asporogenous, gram-negative rods that measure approximately 0.5 to 0.8 µm in diameter by 1.4 to 2.6 µm in length. Antimicrobials can reduce the number of organisms present. Centers for Disease Control and Prevention: Summary of notifiable diseases—United States, 2011, MMWR 60(53):1, 2013. Suspicious areas are evaluated using the 100 oil objective of the microscope. The MB/BacT Alert 3D System (bioMérieux, Durham, NC) uses a colorimetric carbon dioxide sensor in each bottle to detect bacterial growth. This genus is discussed in Chapter 20 with the biochemically similar genera Vibrio and Aeromonas. (subspecies VI). ascorbata has small zones. A B FIGURE 20-1 A, Microscopic morphology of Vibrio sp. On the fifth day after her discharge, she noticed the presence of creamy white, cheesy material on her tongue and buccal cavity. Epidemiology Leptospirosis is a zoonoses primarily associated with occupational or recreational exposure. The collection should take place first thing in the morning, before the individual uses the bathroom or has bathed. military personnel. The test is most useful in distinguishing M. A fungus that reproduces sexually is known as a teleomorph. Points to Remember ■ ■ ■ ■ ■ ■ ■ Nonfermenters will not acidify the butt of TSIA or KIA. pneumoniae adherence. grow more rapidly in liquid medium, and it can be used for both primary isolation and subculturing. If the patient cannot produce sputum, a nebulizer may be used to induce sputum. The diagnosis is made by finding the egg in feces or, occasionally, in duodenal aspirates. Proteins perform several important functions of the membrane. On selective and differential media used primarily to isolate enteric pathogens (e.g., MAC), salmonellae produce clear, colorless, non–lactosefermenting colonies; colonies with black centers are seen if the media (e.g., HE or XLD) contain indicators for H_2S production. should be incubated at 35° C in ambient air for a minimum of 7 days. Approximately 20% of Americans have HSV-2 infections. It contains all the information needed for cell growth and replication. Annual antibiograms, lists of reportable diseases, pathogens recovered in certain hospital units, isolates recovered from certain sites, and blood culture contamination rates are examples of reports that might be requested. Hairs infected with fungi such as Microsporum audouinii fluoresce when a Wood's lamp is shone on the scalp. If serologic assays are to be performed, reference sera available from the Laboratory of Pertussis (FDA, Bethesda, MD) should be used for quality control. trota and a moderate number of A. Name the four strains of the genus Treponema that are pathogenic for humans. *Colonies are exposed to long-wave ultraviolet light (366 nm). These strains are found to adhere to HEp2 cells, packed in an aggregative "stacked-brick" pattern on the cells and between the cells by means of fimbriae. If definitive identification is considered necessary for epidemiologic purposes or for academic reasons, such as for publication of an unusual case report involving the organism, clinical laboratory scientists should consider sending the isolate to a reference laboratory that is better equipped to achieve these identifications in a costeffective manner. pneumophila, contains 16 serogroups. In the United States, most cases occur in the Northeast and upper Midwest, with cases peaking in the summer. fragilis group B. A good history is paramount in evaluating bird exposure, incubation time, and disease process. List common opportunistic infections and other indicators of acquired immunodeficiency disease syndrome. The cyst of E. Sequencing of the 16S rDNA gene has been used most recently to reclassify many of these organisms into new genera. BIBLIOGRAPHY Abbott SL: Klebsiella, Enterobacter, Citrobacter, Serratia, Plesiomonas, and other Enterobacteriaceae. Table 24-6 provides the predictive values for isolation, detection, and identification methods. HSV encephalitis is also associated with immunocompromised patients. FIGURE 22-17 Positive lipase reaction on egg yolk agar. Consequently, anatomic sites colonized with mixtures of organisms, such as those found on mucosal surfaces, frequently provide conditions favorable to the growth of obligate anaerobes. Sarma S, et al: Elizabethkingia meningosepticum: an emerging cause of septicemia in critically ill patients, J Lab Physicians 3:62, 2011. hominis may present with diarrhea, abdominal pain, bloating, anorexia, and flatulence. This organism degenerates within hours after excretion, and rapid preservation of the stool is important. Infection worldwide is estimated at close to 1 billion, and in the United States, hookworm is the second most commonly reported helminth infection. Ig, Immunoglobulin; IgA, immunoglobulin A; LPS, lipopolysaccharide. Vibrio The genus Vibrio resides in the family Vibrionaceae and encompasses more than 110 validly published species, although to date only 10 of these species have been found in human clinical specimens. audouinii appear cottony white and generally form little or no pigment on the reverse. likely to be encountered in the clinical laboratory, V. simiae, bacillus of Calmette-Guérin (BCG) strain of M. most often isolated from clinical specimens. In a study that compared biochemical methods with MALDI-TOF using a 16S rRNA gene sequencing tahir99 VRG Nonoxidizers Oxidizers tahir99 VRG Nonoxidizers Acinetobacter lwoffii Bordetella parapertussis Brucella canis Stenotrophomonas maltophilia Oxidizers Brucella sp. Blastoconidia are the characteristic budding yeast forms usually seen on direct mounts of yeasts. Describe the four major approaches to treat anaerobe-associated diseases—antimicrobial therapy, surgical therapy, hyperbaric oxygen, and administration of antitoxins. Compare the general characteristics of the major phyla of human parasites. The host's immune response may be reduced or altered because of suppression by immunosuppressive drugs, chemotherapy, or radiation. Glutamate dehydrogenase is not a virulence factor, but it is an enzyme frequently associated with C. Eggs of most of the tapeworms contain a hexacanth embryo or oncosphere (tapeworm embryo with three pairs of hooks that is infective for the intermediate host). Some can replicate with a minimal number of nutrients present, whereas others require enriched nutrients to survive. coli remains as a clinically significant isolate in blood cultures from adults. Leishmania-containing macrophages invade the bone marrow. Most strains of E. hepatica, light infections produce few or no symptoms. The edges of the coverslip can be sealed with clear nail polish or vaspar (1 : 1 mixture of petroleum jelly and paraffin). xylosoxidans Achromobacter xylosoxidans var. growing rapid and form cottony, dirty white colonies that become mousy brown to gray with age. Streptococcus pneumoniae Moraxella catarrhalis Haemophilus influenzae Neisseria meningitidis Moraxella spp. Using enzyme immunoassay (EIA) methods, H. TABLE 2-4 Differences Between Bacterial Exotoxins and Endotoxins Characteristic Organism type Part of or secreted from organism Chemical nature Exotoxins Endotoxins Gram-positive and gram-negative Part of and secreted from organism Simple protein Gram-negative Stability to heating (100° C) Detoxification by formaldehyde Neutralization by homologous antibody Biologic activity Labile Protein-lipidpolysaccharide Stable Detoxified Not detoxified Complete Partial Individual to toxin Toxicity compared with strychnine as 1 100 to 1,000,000 Same for all toxins 0.1 Modified from Braude AI, Davis CE, Fierer J, editors: Infectious diseases and medical microbiology, Philadelphia, 1986, Saunders. Biseriate species possess a supporting structure called a metula. In addition, he had suffered a minor wound following handling shellfish that rapidly developed into septic shock. Identification is based on different colony colors, depending on the breakdown of chromogenic substrates by the different species. For example, a patient who lives in a nonendemic area for a parasite tahir99 VRG CHAPTER 28 Diagnostic Parasitology 631 has recently traveled to an endemic area and now shows symptoms, but the organism has not been detected in a clinical specimen. The antibody and complement components are termed opsonins. The azoles are important because they exhibit reasonable activity against the fungi while maintaining fewer side effects. Int J Syst Evol Microbiol 55:519, 2005. Points to Remember ■ ■ ■ ■ ■ ■ ■ ■ Infections caused by Bordetella spp. Typically, transmission involves inhalation or ingestion of the infective eggs. Direct transmission through fomites is also possible. Infected patients are ill for as long as 7 days, and convalescence may require more than 2 weeks. The positive oxidase test should differentiate Shewanella from the family Enterobacteriaceae (except for Plesiomonas). It also stimulates interferon production and causes changes in carbohydrates, lipids, iron, and sensitivity to epinephrine. The rash can present as macular, papular, follicular, papulosquamous, or pustular and is unusual in that it can also occur on the palms and soles. Each of these domains is divided into kingdoms based on the similarities of RNA, DNA, and protein sequences. Other stages mature in the venules and capillaries of the major organs. Chloroplasts are the sites of energy production. Because it is difficult to recover adults or larvae in a stool specimen, the diagnosis is often based on clinical symptoms and the patient's history. Electron microscopy shows the same general features as are seen with the treponemes—long, periplasmic flagella (15 to 20/cell) coated with sheaths of protoplasm and periplasm. A high incidence of disease also results from the use of immunosuppressive drugs in patients with malignancies or organ transplants. Sharma S, et al: Microsporidial keratitis: need for increased awareness, Surv Ophthalmol 56:1, 2011. They are variable in the oxidase reaction, often weak to negative, but isolates are catalase and urease positive. After the removal of the invading object, the injured tissue is repaired. vivax mature schizont. The primary risk factor for serious neuroinvasive disease is age older than 50 years. tahir99 VRG CHAPTER 29 Clinical Virology Herpesviridae The herpesviruses belong to the family Herpesviridae. albicans are not inhibited by routine antibiotics that are prescribed for bacteria and may proliferate and cause infection in circumstances of prolonged antibiotic therapy. coli, one of the best-studied members of the Enterobacteriaceae, is a member of the normal bowel biota but can cause urinary tract infections (UTIs), septicemia, wound infections in healthy individuals, and meningitis in neonates. Routine ova and parasite examination will not detect the spores in a fecal specimen. coli O157:NM (NM indicates nonmotile) has been seen increasingly in European outbreaks, so relying on sorbitol as a single test to identify O157 strains is unwise. Maturation through the trophozoite and schizont phases results in the production of merozoites. pneumoniae, K. lumbricoides in children may hamper growth and development. difficile is being seen with increasing frequency in outpatients who have received antimicrobial therapy. pneumoniae antibody. The other members of the genus Yersinia are found in water, soil, and lower animals; isolates occasionally have been found in wounds and the urine of humans. Cerebrospinal fluid, sterile fluids, expectorated sputum or bronchoalveolar lavages, and wounds and exudates are routinely stained directly. Pigs are the natural host for this organism, and humans serve as accidental hosts. An IgG titer of 1 : 16 or higher but less than 1 : 512 is evidence of past infection or exposure. that are inhibited at 42° C. This immune response includes the production of IL-2 and IFN-γ. lamblia include irritation and damage to the mucosa, as well as interference with absorption of nutrients, fats, and fat-soluble vitamins. Adult worms are encapsulated in fibrous tumors in human subcutaneous tissues. Any of the aforementioned systems perform adequately to identify these four groups. Rhodotorula Rhodotorula spp. Unless a patient regains some cellmediated immunity, mortality is certain because of the ability of these fungi to grow and continue to invade, despite antifungal therapy. Virulence Virulence is the relative ability of a microorganism to cause disease or the degree of pathogenicity. 986. The most important risk factors in mortality in C. These bacteria have become extremely well adapted to their arthropod hosts. Rahnella Rahnella aquatilis is the name given to a group of water bacteria that are psychrotolerant, growing at 4° C. If cryptosporidiosis is suspected, a smear for staining with a modified acid-fast stain or by a fluorescent procedure should be prepared. Risk to an individual increases with the frequency and type of organism and level of contact with the agent, as demonstrated by the Case in Point cases at the beginning of this chapter. Warnock DW: Antifungal agents. When grown at 37° C on suitable media, B. Vascular stasis prevents oxygen from entering a particular site, which results in an environment TABLE 499 conducive to growth and multiplication of any anaerobe that might be present at that site. If the material is still too thick, repeat the first three steps with another (third) glass slide. 1822. Compare and contrast prokaryotic and eukaryotic cytoplasmic and cell envelope structures and functions. The ingestion of unpasteurized milk is also a risk factor. Giemsa staining gives the best morphologic detail but is a time-consuming procedure. The alternative compounds do not contain PVA, so the smears dry faster. The rate in males was 233.7. The CDC attributes the higher rate in women and the continued increase in the reported national C. A transport medium is a holding medium designed to preserve the viability of microorganisms in the specimen but not allow multiplication. The Amplicor assay is approved for use with smearpositive respiratory specimens. parahaemolyticus reported before 1996 were associated with various serotypes; however, after 1996, a pandemic strain of V. Figure 28-2 illustrates a generalized life cycle for amebae and the extraintestinal phase of E. coli O157:H7, Aeromonas, Campylobacter, Vibrio, and Plesiomonas shigelloides. As the age at the time of infection increases to young adulthood, a corresponding increase occurs in the ratio of symptomatic to asymptomatic infections. All sputum specimens should be collected into a sterile, screwtop container. This places the influenza viruses into three genera within the family: Influenzavirus A, Influenzavirus B, and Influenzavirus C. The use of tribes in classifying the members in this family was proposed by Ewing in 1963 and has been continued and extended. phenylpyruvicus, are predominantly P. They are motile by means of a polar flagellum. The life cycle stages seen in peripheral blood are usually limited to the ringform trophozoite and gametocyte. are ubiquitous in the environment in soil, water, and foodstuffs. Albendazole has been used to treat infections with Encephalitozoon spp., but most other microsporidial organisms are resistant to this and other drugs. Clinically Significant Species Agents of Superficial Mycoses Superficial mycoses are fungal diseases that affect only the cornified layers (stratum corneum) of the epidermis. If ingested RBCs are present in the trophozoite, the organism can be reported as E. contain long-chain fatty acids called mycolic acids, which may be detected by chromatography. Because these systems are monitored continuously, bottles are incubated in the instrument for the entire monitoring period. Photosynthesis produces glucose from carbon dioxide and water. Syphilis was first recognized in Europe at the end of the fifteenth century, when it reached epidemic proportions. The EB has an outer membrane similar to that of many gramnegative bacteria. After about 4 hours, the string is brought up, and part of the mucus adhering to the surface is stripped off and examined on a wet mount for motile trophozoites. Many strains exhibit resistance to carbapenems; carbapenemases have been reported throughout the United States. α-Toxin is a lecithinase (phospholipase C) produced by all strains of C. Concentration centrifugation speeds must be at least 3000 × g to maximize recovery. They resemble E. In many studies, gene amplification for HSV in CSF approaches 100% sensitivity. EPEC b. There are two mechanisms of antigenic shift. While circulating, the organisms can resist antibodymediated destruction by continuous antigenic variation. The unusual practice of moistening culturette swabs with tap water prior to the collection of microbiology samples led to an outbreak of C. Infected children display many clinical symptoms similar to infections caused by RSV, influenza virus, and PIV. recently instituted use of a mass spectrophotometric method for identification of gram-negative bacilli, and the final identification of the isolate was reported a few hours later, with an antimicrobial susceptibility performed thereafter. They should be used only when aspiration of material is not possible and a biopsy specimen is not available. The colony morphology of S. The M. (weak fermenters) Sphingomonas paucimobilis Pseudomonas (Chryseomonas) luteola Pseudomonas oryzihabitans Sphingobacterium spp. Rhizoids, typical of some Mucorales, are absent in Mucor spp. Isolates can be identified by hemadsorption inhibition, IF, and EIA tests. tahir99 VRG 498 PART II Laboratory Identification of Significant Isolates Although many different species of anaerobes can be isolated from human clinical specimens, the number of species routinely isolated is relatively small. All Shigella spp. The report includes statistical values, or similarity indices, which are based on deviations in the unknown organism's fatty acid composition from the known profiles contained in the database. Ulocladium spp. arizonae Salmonella enterica subsp. After 24-hour incubation at 35° C, aeromonads appear as large, round, raised, opaque colonies with an entire edge and a smooth, often mucoid, surface. It is reminiscent of the complete clearing seen with β-hemolytic organisms on SBA plates. pneumoniae is the most commonly isolated species and has the distinct feature of possessing a large polysaccharide capsule. Within 24 to 48 hours, a faint macular rash develops on the body. Cetrimide agar is a selective and differential medium for the identification of P. Each agent has a fairly well-defined endemic area. The two major types of cell walls are grampositive and gram-negative types. Although many people with S. Microscopically, care must be taken to avoid confusion between Paecilomyces and Penicillium spp. coli BBE B. The goal of the laboratory is to give accurate information to the clinical team so that they can best evaluate and interpret the results of the culture. VZV infection is usually diagnosed on the basis of characteristic clinical findings. In addition to the neonatal population, E. lwoffii, the glucose-negative, nonhemolytic strain. are either opportunists or not primary human pathogens. 5. Young cysts may show discrete glycogen masses that stain light brown in an iodine wet mount, but in the more mature cyst, the glycogen is diffuse. The incubation period is usually 2 to 3 days but may range up to 2 weeks. The rash occurs more commonly in children than in adults, lasts as long as 2 weeks, and can recur after exposure to heat and sunlight. The mycelial phase of the systemic dimorphic fungi, B. Influenza virus commonly does not exhibit a well-defined CPE. Centers for Disease Control and Prevention: Sexually transmitted diseases treatment guidelines, 2010, MMWR 59(RR-12):1, 2010. pestis Providencia sp. Most infected individuals demonstrate no clinical symptoms; others have vague GI symptoms, including nausea and vomiting and intestinal irritation. Cultures from other sites may provide additional significant information. Both Candida albicans and C. Koneman EW, et al: Color atlas and textbook of diagnostic microbiol ogy, ed 7, Philadelphia, 2006, Lippincott. oxytoca produces infections similar to those caused by K. Evaluate the available assays for the laboratory diagnosis of C. Strain to strain variation in texture may be noted within a species. are similar, the strains can be identified as possibly related. They are usually associated with polymicrobial infections but can occasionally be recovered from blood cultures and can cause infections following orthopedic surgery. In laboratories not equipped with anaerobic chambers, holding systems may be used (see later, "Inoculation Procedures"). Members of the genus Sphingobacterium at one time were regarded as members of the genus Flavobacterium. Paracoccus yeei Pseudomonas luteola Psychrobacter phenylpyruvicus Ralstonia pickettii Rhizobium radiobacter Sphingobacterium multivorum Sphingobacterium spiritivorum Sphingomonas paucimobilis Old Name Achromobacter xylosoxidans var. hartmanni cysts measure 5 to 10 µm; those of 10 µm or larger are identified as E. ovale infections generally do not have the range of complications seen with other species. All aerotolerance test plates should be incubated at 35° C for up to 48 hours. In turn, any special media indicated for species identification can be inoculated immediately. richardii. Used with permission. Eukaryotic cells contain several membrane-enclosed organelles. Influenza C is capable of causing mild upper respiratory illness in humans. Describe the way each physical agent controls the growth of microorganisms. Wet Mount Preparations. The direct wet mount of unpreserved fecal material is primarily used to detect the presence of motile protozoan trophozoites in a fresh liquid stool or from sigmoidoscopy material. In addition, a derivative of the biotype El Tor was responsible for causing a major epidemic in Bangladesh in 1992. Loa loa. Infection with L. Adults of both species are small (1 to 2 mm) and delicate. Specimens are usually collected from a warm, moist environment that is low in oxygen. Figure 28-38 shows the characteristic appearance of an acid-fast stain of the oocyst of C. Fader, PhD, D(ABMM) Section Chief, Microbiology/Virology Laboratory Scott and White Memorial Hospital Baylor Scott & White Health Assistant Professor Department of Pathology Texas A&M University College of Medicine Temple, Texas Amanda T. The affinity of microorganisms for a specific site depends on the ability of the organisms to resist the antibacterial effects of substances such as bile, lysozyme, or fatty acids. (A from Engelkirk PG et al: Principles and practice of clinical anaerobic bacteriology, Belmont, CA, 1992, Star.) Porphyromonas and Prevotella and fades completely if the colonies are exposed to air for 5 to 10 minutes. All of the above 5. Sore throat, dry cough, and earache are accompanying symptoms. Many patients with GBS test positive for antibodies to Campylobacter, with an estimated 1 in 1000 individuals diagnosed following a Campylobacter infection. coli strains possessing the capsular K1 antigen were isolated from neonates with septicemia or meningitis. albicans may multiply and cause diarrhea or possibly infections in the mouth or vagina, as illustrated in the Case in Point presented at the beginning of this section. vivax. No FDA-approved laboratory tests are available for the identification of a hantavirus infection; however, some European tests are being evaluated. hartmanni, and the organisms may be misidentified. bovis is susceptible to lower concentrations of T2H than MTB. mirabilis and P. Antigenic Structures The genus consists of four species that are biochemically similar. salamae Salmonella serotype Choleraesuis Salmonella serotype Gallinarum Salmonella serotype Paratyphi A Methyl Red Organism Urea Hydrolysis 19-12 Biochemical Reactions of Named Species and Unnamed Groups of the Family Enterobacteriaceae—cont'd Indole Production TABLE PART II Laboratory Identification of Significant Isolates 0 0 0 0 0 0 0 2 15 0 100 1 100 2 97 0 15 0 0 0 0 0 2 0 0 0 0 0 0 0 78 0 0 0 50 1 0 0 0 0 0 98 0 0 0 0 92 0 95 0 98 98 0 15 0 0 100 0 93 0 30 95 0 94 0 0 95 0 94 100 0 0 100 99 99 0 0 99 0 0 94 0 0 30 0 0 85 0 0 6 94 100 100 70 99 99 0 97 0 100 0 100 0 100 0 100 0 100 0 0 100 100 0 100 0 100 0 1 95 100 10 5 15 100 35 0 35 0 0 2 50 10 98 100 100 100 94 0 0 100 99 15 1 100 0 50 100 0 0 0 0 2 5 88 100 0 94 0 0 0 0 0 2 100 0 98 99 0 0 99 70 99 99 0 1 95 100 99 85 5 100 1 0 0 1 100 0 95 95 1 0 98 70 97 95 0 0 0 100 96 1 1 100 96 0 0 0 100 0 98 100 2 0 100 70 100 98 0 95 0 100 100 0 0 98 0 60 5 0 100 0 89 100 0 0 0 100 67 100 100 0 0 0 100 100 22 0 100 67 0 0 2 100 0 100 100 0 0 100 90 100 98 2 0 95 100 100 1 1 100 90 5 0 0 100 0 50 0 0 95 55 100 95 0 0 0 100 95 0 0 98 5 0 0 0 100 0 0 100 0 0 90 10 1 0 0 0 0 100 0 0 0 100 90 0 0 0 100 0 0 0 0 0 15 95 95 0 0 0 100 99 0 0 100 90 0 0 25 10 From the Centers for Disease Control and Prevention, Atlanta, GA. bacterial species in the family Enterobacteriaceae and their respective tribes; Table 19-2 shows the biochemical features that differentiate the tribes. However, they have the disadvantage of trying to accommodate many different hand sizes into one pair of gloves. The basal body, or kinetosome, is a small structure located at the base of cilia or flagella, where microtubule proteins involved in movement originate. Many strains also produce a fruity, grapelike odor caused by the presence of 2-aminoacetophenone. C, Acid-fast stain, carbolfuchsin-based. Characteristically, during the first week of the disease, the patient develops a fever accompanied by malaise, anorexia, lethargy, myalgia, and a continuous dull frontal headache. When smears are stained with Giemsa or Wright stain, the yeast cells are commonly seen within monocytes and macrophages, occurring in significant numbers, as shown in Figure 27-29, A. The liberation of phenolphthalein causes a pH change in the presence of sodium bicarbonate, indicated by the formation of a pink color change. Nonpathogenic Intestinal Flagellates Chilomastix mesnili and Trichomonas hominis are intestinal nonpathogens that must be differentiated from pathogenic flagellates. Another nondermatophytic superficial infection is tinea nigra. Smallpox, anthrax, and plague are agents that have been learned about but have not been identified by the average microbiology laboratory in many years. The two dilutions provide a set of plates that should be countable (i.e., 100 to 300 CFU on the control plate). Danesh J, et al: Chlamydia pneumoniae IgA titres and cardiovascular heart disease: prospective study and meta-analysis, Eur Heart J 23:371, 2002. Typically, serologic assays are the only laboratory tests performed for the diagnosis of rickettsial diseases. Rinse slide with tap water. Develop an algorithm for the diagnosis of pertussis from specimen collection to identification of the causative agent. fowleri, Acanthamoeba spp., and Balamuthia mandrillaris are free-living amebae that can infect humans. The primary pathogens, which include S. hydrophila complex, which includes A. Trichophyton rubrum d. The polioviruses tend to infect the nervous system and can cause paralysis in a small percentage of infected patients. farciminosum causes epizootic lymphangitis in horses and mules. meningoseptica. B, Schüffner's stippling of P. produce a spectrum of symptoms ranging from mild upper respiratory tract infections to pneumonia. Obligate anaerobes must be grown in an atmosphere either devoid of oxygen or with significantly reduced oxygen content. The time required for one cell to divide into two cells is called the generation time or doubling time. Several pathogens (H. Lau SK, et al: Matrix-assisted laser desorption ionization-time of flight mass spectrometry for rapid identification of Burkholderia pseudomallei: importance of expanding databases with pathogens endemic to different localities, J Clin Microbiol 50:3142, 2012. The clinical manifestations of shigellosis vary from asymptomatic to severe forms of the disease. fewer than 7 days, or slow growers, producing colonies in more than 7 days. All of the antibody molecules derived from a single clone of B cells are of a single specificity (recognize a unique antigen), identical to the receptor molecule on the original activated B cell. Negative; cannot; negative b. The organism may cause a vitamin B_{12} deficiency, especially in persons of northern European descent, and long-term infection may lead to a megaloblastic anemia. Kassutto S, Doweiko JP: Syphilis in the HIV era, Emerg Infect Dis 10:1471, 2004. distasonis, are among the most common species of bacteria isolated from human feces. fowleri is capable of destroying and ingesting erythrocytes and other cells, including nerve cells. Lehman George Manuselis tahir99 VRG xv This page intentionally left blank tahir99 VRG Contents PART I INTRODUCTION TO CLINICAL MICROBIOLOGY 1 Bacterial Cell Structure, Physiology, Metabolism, and Genetics, 2 2 Host-Parasite Interaction, 23 3 The Laboratory Role in Infection Control, 47 4 Control of Microorganisms, 59 5 Performance Improvement in the Microbiology Laboratory, 93 6 Specimen Collection and Processing, 111 7 Microscopic Examination of Materials from Infected Sites, 126 8 Use of Colony Morphology for the Presumptive Identification of Microorganisms, 169 9 Biochemical Identification of Gram-Negative Bacteria, 181 10 Immunodiagnosis of Infectious Diseases, 198 11 Applications of Molecular Diagnostics, 226 12 Antimicrobial Agent Mechanisms of Action and Resistance, 254 13 Antimicrobial Susceptibility Testing, 274 PART II LABORATORY IDENTIFICATION OF SIGNIFICANT ISOLATES 14 Staphylococci, 314 15 Streptococcus, Enterococcus, and Other Catalase-Negative, Gram-Positive Cocci, 328 16 Aerobic Gram-Positive Bacilli, 349 17 Neisseria Species and Moraxella catarrhalis, 372 18 Haemophilus and Other Fastidious Gram-Negative Bacilli, 390 19 Enterobacteriaceae, 420 20 Vibrio, Aeromonas, Plesiomonas, and Campylobacter Species, 455 21 Nonfermenting and Miscellaneous Gram-Negative Bacilli, 474 22 Anaerobes of Clinical Importance, 495 23 The Spirochetes, 529 24 Chlamydia, Rickettsia, and Similar Organisms, 537 25 Mycoplasma and Ureaplasma, 552 26 Mycobacterium tuberculosis and Nontuberculous Mycobacteria, 563 27 Medically Significant Fungi, 589 28 Diagnostic Parasitology, 625 29 Clinical Virology, 688 30 Agents of Bioterror and Forensic Microbiology, 727 31 Biofilms: Architects of Disease, 752 PART III LABORATORY DIAGNOSIS OF INFECTIOUS DISEASES: AN ORGAN SYSTEM APPROACH TO DIAGNOSTIC MICROBIOLOGY Upper and Lower Respiratory Tract Infections, 765 Skin and Soft Tissue Infections, 804 Gastrointestinal Infections and Food Poisoning, 835 Infections of the Central Nervous System, 854 Bacteremia and Sepsis, 868 Urinary Tract Infections, 884 Genital Infections and Sexually Transmitted Infections, 901 39 Infections in Special Populations, 933 40 Zoonotic Diseases, 942 41 Ocular Infections, 955 32 33 34 35 36 37 38 Appendices A B C D Selected Bacteriologic Culture Media, 977 Selected Mycology Media, Fluids, and Stains, 994 Answers to Learning Assessment Questions, 997 Selected Procedures, 1019 Glossary, 1026 Index, 1048 tahir99 VRG xvii This page intentionally left blank tahir99 VRG PART I Introduction to Clinical Microbiology tahir99 VRG CHAPTER 1 Bacterial Cell Structure, Physiology, Metabolism, and Genetics George Manuselis, Connie R. Describe the factors and mechanisms by which the human host is protected from microbial invasion. Hall CHAPTER OUTLINE ■ GENERAL CHARACTERISTICS OF NONFERMENTERS Clinical Infections Biochemical Characteristics and Identification ■ CLINICALLY SIGNIFICANT NONFERMENTATIVE, GRAMNEGATIVE BACILLI Pseudomonas Fluorescent Group Pseudomonas Nonfluorescent Group Acinetobacter Stenotrophomonas maltophilia Burkholderia Moraxella, Oligella, and Psychrobacter ■ LESS COMMONLY ENCOUNTERED NONFERMENTATIVE, GRAM-NEGATIVE BACILLI Alcaligenes and Achromobacter Brevundimonas CDC Groups EO-3, EO-4, and Paracoccus Chromobacterium Comamonas and Delftia Flavobacteriaceae Methylobacterium and Roseomonas Ralstonia and Cupriavidus Shewanella Sphingomonas OBJECTIVES After reading and studying this chapter, you should be able to: 1. Rhinovirus infections occur throughout the year, but their incidence increases in the winter and spring. influenzae, N. This organism is saccharolytic, which may cause it to resemble a lactose-fermenting organism. Detecting Aspergillus-specific galactomannan by enzymelinked immunosorbent assay (ELISA) in patient samples has demonstrated high sensitivity and specificity. xylosoxidans is usually resistant to aminoglycosides, ampicillin, first- and second-generation cephalosporins, chloramphenicol, and fluoroquinolones; but usually are susceptible to piperacillin, third-generation cephalosporins, carbapenems, and SXT. Asexual reproduction is characterized by the presence of sporangiophores and sporangiospores. chelonae. The adult female can occasionally be seen in this preparation. Formed stools are unlikely to yield motile trophozoites. The three major subgroups of V. Of the reported cases, 5011 people were hospitalized, and 302 deaths occurred. Many restriction enzymes with various recognition sequences have now been isolated from various microorganisms. coli is a gram-negative bacillus forming nonswarming, β-hemolytic colonies and spot indole-positive. Stoute JA: Complement receptor 1 and malaria, Cell Micribiol 13:1441, 2011. The parasite develops as a promastigote in the gut of the insect and migrates to the salivary glands tahir99 VRG CHAPTER 28 Diagnostic Parasitology 647 Multiplication of amastigotes intracellularly Ingestion of amastigote by sand fly Promastigote phagocytosis by macrophage and transformation into amastigote Transformation of amastigote into promastigote in midgut of fly Fly's biting of human and regurgitation of promastigote FIGURE 28-22 Life cycle of Leishmania spp. Herpes Simplex Virus Encephalitis. HSV encephalitis is a very rare but devastating disease, with a mortality of about 70%. With the advent of chemotherapy, radiation therapy, and diseases such as acquired immunodeficiency syndrome (AIDS) that affect the immune system, the line between pathogen and saprobe has been blurred. Figure 21-3 481 FIGURE 21-3 Gram stain of bronchial specimen positive for Pseudomonas aeruginosa (×1000). Stains most commonly used in the diagnostic laboratory are listed in Table 7-2. Other organisms, such as Corynebacterium diphtheriae, do not spread beyond their initial site of infection, yet the disease they produce is serious and often fatal. scrofulaceum M. Most agents of dermatophytoses live freely in the environment, but a few have adapted almost exclusively to living on human tissues, and they are rarely recovered from any other source. B, Mucorales hyphae in tissue appears sparsely septate. growing on sheep blood agar (SBA). This is because these viruses are so closely associated in a clinical laboratory as a result of their common tissue tropism. Rash on the palms of the hands occurs rarely. coli O157:H7. pestis–specific DNA probe for plague surveillance has been studied. An example of a chronic carrier state is found in post–Salmonella typhi infection. Cutaneous Mycoses Dermatomycoses are defined as fungal diseases of the keratinized tissues of humans and other animals. kansasii b. Laboratory tests then must demonstrate the rate of resistant organisms. This disadvantage can be overcome by the use of the gloveless anaerobic chamber (Figure 22-7). Counterstain with methylene blue for 30 seconds. zatmanii are the two species usually isolated in clinical specimens; they prefer a lower temperature (25° C to 35° C), produce distinctive large vacuolated pleomorphic rods, and are oxidase positive and motile, with a polar flagellum. lapagei, C. Individuals with ulcerative sexually transmitted infections (e.g., syphilis, genital herpes, chancroid) are at the greatest risk. L. The processing laboratory can test any growth on the transport medium directly for Bordetella spp. What types of infections do nonfermenters cause? Alternaria spp. Although genetic typing has established relatedness based on nucleic acid similarities and is taxonomically correct, serogroup-based nomenclature continues to be preferred by scientists and physicians. are the second most isolated fungus after Candida spp. The system allows for higher yields and shorter recovery times for mycobacteria than conventional blood culture methods. List and define five methods used by epidemiologists to subdivide bacterial species. Although lipids and fatty acids are bactericidal to most bacteria, Propionibacterium spp. Both HEp2 and A549 were developed from cancer tissue obtained from patients during treatment. pertussis. burnetii is an obligate intracellular parasite, it develops within the phagolysosomes of infected cells. Less than 10% of the rapidly growing mycobacteria may be acid-fast; they may not stain at all with fluorochrome stains. Yersinia pseudotuberculosis Yersinia pseudotuberculosis, similar to Y. vaginalis, a pathogen of the genitourinary tract in men and women, is also discussed in this section. TTACGGACAAC: _______________ 13. When the easily identified S. Includes bibliographical references and index. Swabs Swabs, in general, are not appropriate for anaerobic culture. ; cm. A portion should also be placed in a fixative such as PVA, so that permanent stained smears for definitive identification can be made. In cases of B. In some cases, NoV can be detected in stool samples collected 2 weeks after recovery. In addition, Y. Centers for Disease Control and Prevention: CDC estimates of foodborne illness in the United States, 2011. (Courtesy Dr. Steven Dallas, University of Texas Health Science Center, San Antonio, TX.) FIGURE 22-10 Anaerobic pouch. The life cycle of Pneumocystis sp. Because microbes in the air can be incriminated in HAIs, cultures of the air can be a component of air quality investigations. It is acquired by person to person contact and is rarely transmitted by sexual intercourse. • Augment visual identification of microbes by use of specific stains, including antibody or gene-directed probes. Streptococcus, Peptostreptococcus spp. Is there a specified antibiotic for treatment of infections with this agent? The yeast form of Histoplasma capsulatum takes up some of the heme pigment in the medium and becomes light tan, with a grainy, wrinkled texture. Encephalitis is usually caused by HSV-2 in neonates and HSV-1 in older children and adults. Fermentation is less efficient in energy generation than respiration (oxidation) because the beginning substrate is not completely reduced; therefore, all the energy in the substrate is not released. Currently, four methods are used for determining antimicrobial susceptibility of M. Mature colonies usually become tan to brown to lavender. They belong to a few serotypes and are resistant to the antibacterial activity of human serum. Rupture of the cyst liberates large amounts of foreign protein (allergin) that may elicit an anaphylactic response. Although not all medical treatment facilities provide full virology services, these laboratories can still provide information about viral infections using rapid tests that detect specific viruses in clinical specimens. A selective medium containing sheep red blood cells and the antimicrobials colistin and nalidixic acid; used primarily to suppress the growth of any facultative, gram-negative bacilli (e.g., Enterobacteriaceae) that might be present in the clinical specimen, especially swarming Proteus spp. tahir99 VRG 432 TABLE PART II Laboratory Identification of Significant Isolates 19-7 Differentiating Characteristics of Selected Species of Proteus, Providencia, and Morganella Test Indole Methyl red Voges-Proskauer Simmons citrate Christensen urea H_2S (TSI) Ornithine decarboxylase Phenylalanine deaminase Acid produced from Sucrose Mannitol Salicin Adonitol Rhamnose Maltose Xylose Arabitol Swarms Proteus penneri Proteus mirabilis Proteus vulgaris Providencia alcalifaciens Providencia stuartii Providencia rettgeri Morganella morganii − + − − + − (70%) − + + − or + + or (+) + or (+) + + + + + − d + + − + + + − + − − − + + + − + − or + − − + + + − + + − − + + + − − + − + + + − − − − − + + − + d − − − − − + − + + − d − − + + or (+) − + d − − + − − − − − d d − − − − − − − d + d + + or − − − or + + − − − − − − − − − − − Modified from Washington J: Laboratory procedures in clinical microbiology, ed 2, New York, 1981, Springer-Verlag. fast-growing bacterium such as E. Available at: . What risk factors are associated with Borrelia spp. When the infected copepod is ingested by a fish, the larva leaves the fish's intestine and invades the flesh, where it develops into a plerocercoid larva, which consists of a scolex with a thin, ribbon-like portion of tissue. When present, symptoms depend on the organ affected and size and number of cysticerci present. Motility Test. Motility may be determined by wet mount using either very young (4 to 6 hours old) broth cultures or 24- to 48-hour-old colonies on agar. The last edition of Bergey's Manual of Systematic Bacteriology (Vol. Classification by Cellular Type: Prokaryotes, Eukaryotes, and Archaeobacteria Another method of classifying organisms is by cell organization. The traditional biochemical tests to perform for identification include the following: tahir99 VRG Text continued on p. Maturation is completed in the intestine. Prevention includes either or both antimicrobial treatment and a simple surgical procedure on the eyelid. aureus isolated from one patient is resistant to penicillin and another S. Colonies are varied and can be smooth or wrinkled, dry or moist, creamy or velvety in appearance. An increase in age brings with it a decrease in the effectiveness of the immune response. Because Legionella spp. Stool c. nana are easily transmitted among children, because an intermediate host is not required. pestis is a gram-negative, short, plump bacillus. dysenteriae), B (S. With others, it involves deep tissues; for example, the gonococcus organism is invasive and may infect the fallopian tubes. The optimal

decontamination procedure requires an agent that is mild and yields growth of mycobacteria while controlling contaminants. marinum, M. The parasite-infected RBCs demonstrate sticky knobs that mediate adhesion to the endothelial cells of the capillary walls. Between the outer membrane and the inner membrane and encompassing the thin peptidoglycan layer is an area referred to as the periplasmic space. The second step occurs inside the cell and requires the enzyme β-galactosidase to break the galactoside bond, releasing glucose, which can be fermented. Before these products were developed, only laboratories that could prepare their own reagents performed testing. colonies are visible usually within 3 to 5 days. When S. pylori is strongly associated with gastric and duodenal ulcers and has been implicated in cases of gastric carcinoma. are not members of the normal GI microbiota, and all Shigella spp. A Luminex assay to detect and type or subtype 20 different viral pathogens within 5 hours has also been described. It is thought that the virus was introduced into the country by rodents imported from Africa (Gambian rats). The biphasic media system is sensitive and does not require the use of radioactivity and a CO_2 incubator. The onset is rapid (within 3 to 7 days after ingestion of the oocyst), but the infection is self-limiting. As many as one third of all college-age women are infected with HPV and most only develop subclinical infections. They originate when a human accidentally becomes the intermediate host for the parasite. They may act as enzymes, hormone receptors, pore channels, and carriers. The objective is to separate the representative sample from the contaminated sample before culture or culture evaluation. Diphtheroids (Corynebacterium spp.) Cytomegalovirus Staphylococcus spp. Letesson JJ, et al: Fun stories about Brucella: the “furtive nasty bug,” Vet Microbiol 90:317, 2002. These adult roundworms give birth to liveborn larvae referred to as microfilariae. stercoralis are not usually present in the stool; the typical diagnostic stage is the rhabditiform larva. Relapsing fever is the only spirochetal disease in which the organisms are visible in blood with bright field microscopy. A DFA combination reagent for G. Urine can also be cultured and is most productive after the first week of illness. In a wet mount, the cilia are seen propelling the organism with a rotary motion. play an important role in the health of the female vaginal tract in that they help protect the host from urogenital infections. All specimens should be transported to the laboratory immediately after collection. These include the ability to do the following: screen large volumes of specimens, obtain objective results, have test results available in 3 to 5 hours, and use various specimen types. At least six nonpathogenic species have been identified in the normal microbiota, and they are particularly prominent in the oral cavity. Air contains approximately 21% oxygen and 1% carbon dioxide. To reduce the risk of nosocomial spread, testing hospital personnel and infants with upper respiratory tract infections for RSV, isolating RSV-infected infants, following good handwashing and personal protective equipment practices, limiting visitation, and organizing patients and staff members into cohorts are recommended. marcescens and S. Sexual and asexual reproduction occur in the intestine of the cat, whereas only asexual reproduction occurs in humans and other intermediate hosts. Available at: preview/mmwrhtml/mm5648a2.htm. This medium is identical in composition to the transport medium of Regan and Lowe except that it contains agar at full strength. In a wet mount, the trophozoite has a characteristic jerky motility, and the motion of the flagella and undulating membrane may be seen. The organism now exists in large numbers in the bowel and may be isolated from the stool. Autotrophs occur in environmental milieus. FIGURE 28-68 Wuchereria bancrofti microfilaria. conorii also causes Kenya tick typhus, South African tick fever, and Indian tick typhus. pneumoniae and H. When mixed with a positive serum on a disposable card, the black charcoal particles clump together with the cardiolipin-antibody complexes. The patient had a history of intravenous drug abuse, chronic diarrhea for 112 years, Kaposi sarcoma for 2 years, and pancytopenia for several weeks. The larva is released in the small intestine and undergoes several molts before maturing into an adult worm in the cecum. coli O groups have shown remarkable cross-reactivity with O antigens from other members of the Enterobacteriaceae, most notably Shigella spp. Centers for Disease Control and Prevention: Investigation of outbreak of infections caused by Salmonella Agona. Several of the more than 150 genera and 1200 species (Microsporidium, Nosema, Tubulinosema, Vittaforma, Encephalitozoon, Pleistophora, Brachiola, Trachipleistophora, Anncaliia, and Enterocytozoon) have been implicated in human infections. Lactobacilli metabolize glycogen in vaginal epithelial cells to maintain a low pH, creating an environment that is inhibitory to many organisms. Accessed February 21, 2013. Slide Culture. Slide cultures are useful for demonstrating the natural morphology of fungal structures and for encouraging conidiation in some poorly fruiting fungi. Agents of Opportunistic Mycoses The terms saprobe and saprophyte have been used to describe free-living microorganisms in the environment that are not typically of concern in human disease. An advantage of this procedure is that the conidial arrangement is retained. tahir99 VRG 471 CHAPTER 20 Vibrio, Aeromonas, Plesiomonas, and Campylobacter Species TABLE 20-8 Biochemical Tests to Differentiate Campylobacter, Arcobacter, and Helicobacter Species Species Campylobacter jejuni subsp. Campylobacter spp. audouinii, a species that typically forms few spores. Serologic tests such as direct agglutination and indirect fluorescent assays can be used to detect antibodies in individuals from nonendemic areas. Colonies grow slowly and are grayish brown, becoming grayish violet. jandaei, display strong β-hemolysis (Figure 20-6). If the fetus is exposed to the infection early in the pregnancy, more severe complications are likely to result. Allergic reactions to Aspergillus spp. nana from the karyosome of E. Brill-Zinsser disease, also called recrudescent typhus, is seen in patients who previously had louse-borne typhus. pertussis or B. Edelstein PH: Legionella. Table 22-4 summarizes the variety of endogenous anaerobes that may be found at specific body sites. Nevertheless, P. Compare the pathogenesis of H. Fever can be as high as 41° C. Agents of Systemic Mycoses Organisms that cause classic systemic fungal diseases have historically been categorized together because they share several characteristics, such as mode of transmission, dimorphism, and systemic dissemination. These are the basic building blocks of DNA (Figure 1-12). The rounded, thick-walled cyst (Figure 28-15, B) averages 45 to 75 µm. The diagnosis of CMV infection is best confirmed by isolation of the virus from normally sterile body fluids, such as the buffy coat of blood or other internal fluids or tissues. Count the number of acid-fast bacilli seen on the smear and report as follows: No. Acid-Fast Bacilli Report 1-20 21-80 81-300 >300 Number seen Few Moderate Numerous Kinyoun and Ziehl-Neelsen Stains Mycobacteria stain red, whereas the background material and non– acid-fast bacteria stain blue. Important clinical genera of the Actinobacteria include Actinomyces, Bifidobacterium, Eggerthella, Mobiluncus, and Propionibacterium. Microscopic characterization is followed by observing the metabolic biochemical differences between organisms that form the basis for most bacterial identification systems in use today. Only a few bacterial species, such as Streptococcus pneumoniae, Neisseria gonorrhoeae, and H. Many of the infectious processes involving anaerobes are polymicrobial, consisting of mixtures of obligate anaerobes or mixtures of obligate or aerotolerant anaerobes and facultative organisms. More than 1.2 million people were living with HIV-AIDS in the United States in 2011, with 20% unaware that they were infected. The reaction occurs on the surface of colonies and surrounding medium. The growth of M. On CHROMagar Salmonella (CHROMagar Co, Paris, France), Salmonella isolates produce mauve-colored colonies (Figure 19-14) owing to the activity of an esterase on a patented substrate. Patients may be asymptomatic or symptomatic; the severity of the symptoms is related to the worm burden, host’s nutritional status and age, and duration of infection. Fortunately, most infections involve only the superficial epithelial layer and heal completely with treatment. pseudomallei septicemia provides a definitive identification of this pathogen in a nonendemic setting in which systemic melioidosis might not otherwise be suspected. At least six other species are recognized: B. The fluorescence result of the test would be __________. The infection easily spreads hematogenously, and it is not uncommon to find multiorgan system involvement, including the brain, liver, heart, and bone. • Rupture of thin-walled oocysts in the intestine creates the potential for continual autoinfection. Adenovirus is shed in secretions from the eyes and respiratory tract. However, the cuffs for the arms might not accommodate everyone, allowing air to leak around the arms of smaller individuals. During gross examination, intact worms or proglottids (tapeworm segments) can be seen on the surface of the stool. Neither P. Spores stain pink-red and may have a diagonal or equatorial band that helps distinguish them from bacteria or yeast. coli and Klebsiella pneumoniae. Immunodiagnosis of Fungal Infections Immunodiagnostic assays to aid in the identification of fungal isolates and diagnosis of infections have been available for several years, although false-positive reactions caused by crossreactivity have been noted. Almost 90% of infections are asymptomatic. Chromatography is rapid and highly reproducible, but the initial equipment cost is high. mendocina is motile by means of a single polar flagellum, it oxidizes glucose and xylose, and is nonproteolytic and does not hydrolyze starch. It does not distinguish between live and dead cells. All fecal specimens should be routinely screened for Salmonella, Shigella, and Campylobacter (see Chapter 20). veronii biovar sobria, or A. In addition, Tatumella isolates are slow growing, produce tiny colonies, and are relatively nonreactive in laboratory media. Repeat until excess red dye is removed. Available at: mm5626a1.htm. Sporadic cases of infection in the United States in nonimmigrants have been reported because several of the insect vectors exist there. These are available from BD Diagnostic Systems, Mitsubishi, Oxoid (Hampshire, England), Hardy Diagnostics (Santa Monica, CA), and others. When bacteria use other sugars as a carbon source, they first convert the sugar to glucose, which is processed by one of three pathways. If a microorganism requires a relatively high infective dose but the disease it causes is often fatal, we tend to think of the microorganism as highly virulent. An updated procedure manual; controls for staining procedures; and records of centrifuge calibration, ocular micrometer calibration, and refrigerator and incubator temperatures should be available. vulnificus V. Protists (protozoans) of clinical importance are named similar to animals; instead of divisions, one uses phyla (plural of phylum), but the names of the others remain the same. A much higher number of room air changes per hour can cause problems of air turbulence within the biological safety cabinets. trachomatis infections in adult men include nongonococcal urethritis (NGU), epididymitis, and prostatitis. Aliquots of the extraction solutions are used to analyze volatile (ether-extracted) and nonvolatile (chloroform-extracted) acids. It has been seen in infections following keratoplasty, myocarditis in a heart transplant patient, and infection associated with CAPD. In addition, diphtheroids and M. pallidum infection? Simple stains are directed toward coloring the forms and shapes present, differential stains are directed toward coloring specific components of the elements present, and diagnostic antibody or DNA probe– mediated stains are directed specifically at identification of an organism. +, Most strains positive; −, most strains negative; B, brown; F, fluorescein; G, green; Y, yellow. The toxin initially binds to the GM1 ganglioside receptor on the cell membrane via the B subunits. is an example of a phaeoid fungus. Fermentation and Respiration Bacteria use biochemical pathways to catabolize (break down) carbohydrates and produce energy by two mechanisms— fermentation and respiration (commonly referred to as oxidation). Create an algorithm for the serologic diagnosis of human immunodeficiency virus infection. The base extends out of the sugar. The natural reservoir has not been unequivocally established, although the organism has been recovered from the soil and from some natural environments. Agents responsible for these mycoses are organisms commonly found in nature, encompassing many genera of Hyphomycetes, Coelomycetes, and Ascomycetes. rhodesiense. Taxonomy involves three structured, interrelated categories: classification/taxonomy, nomenclature, and identification. The nucleocapsid is enclosed by a matrix shell to which an envelope of host cell origin is attached. Almuzara MN, et al: First report of an extensively drug-resistant VIM-2 metallo-β-lactamase-producing Brevundimonas diminuta clinical isolate, J Clin Microbiol 50:2830, 2012. Although it was not confirmed to be SARS, the signs and symptoms indicated a high likelihood that this was the cause of this outbreak. are gram-positive, highly pleomorphic bacilli, which may appear on Gram stain as a coccoid or spiral-shaped organism. Heukelbach J, Feldmeier H: Epidemiological and clinical characteristics of hookworm-related cutaneous larva migrans, Lancet Infect Dis 8: 302, 2008. Conidiophores are erect, sometimes branched, with metulae bearing one or several phialides on which oval to ovoid conidia are produced in long, loose chains (Figure 27-43). rickettsii, R. These two anamorphs are synanamorphs to each other. tahir99 VRG CHAPTER 28 Diagnostic Parasitology Mating of adults in intestine 677 Maturation in soil Passage of eggs in stool Complete maturation in intestine Human ingestion of infective eggs Migration up trachea, over epiglottis Breaking of larvae into alveoli of lungs Release of larvae into intestine and penetration of intestinal wall Into circulation Swallowed FIGURE 28-59 Life cycle of Ascaris lumbricoides. Because viral shedding is usually greatest during the early stages of infection, the best specimens are those collected as early as possible, which in many infections is even before symptoms occur. neoformans from other Cryptococcus spp. Gardnerella vaginalis Mycobacteria, antibiotic-affected lactobacilli, and corynebacteria Anaerobic morphotypes, antibiotic-affected cells Actinomycetes, Nocardia, Nocardiopsis, Streptomyces, Rothia spp. Early symptoms are followed by necrotic inflammation of the small intestines, at times leading to bowel perforation. coli, enterohemorrhagic E. japonicum, an acute serum sickness–like illness (Katayama fever) occurs during the initial egg laying period. The lipid A moiety is responsible for producing fever and shock conditions in patients infected with gram-negative bacteria. salivarius, L. pneumoniae detection, but it is rarely used today. Patients are previously healthy individuals who complain of flulike symptoms of fever, headache, and myalgia that last 2 to 5 days and then subside without medical intervention. Conidia have angular cross walls and, in some species, echinulate surfaces. necrophorum. Pyoverdin is water-soluble and fluoresces under short-wavelength ultraviolet light. The KOH breaks down the keratin and skin layers to see fungi present 617 in the specimen more easily. Serovars L1, L2, L2a, L2b, and L3 are associated with lymphogranuloma venereum (LGV), an invasive urogenital tract disease. Lung histopathology shows cytomegalic pneumocyte containing characteristic intranuclear inclusions, hematoxylin and eosin (×1000). Media for the isolation of B. The azoles FLU, ITRA, and VORI are categorized as SDD (susceptible dose-dependent), and R. boydii c. difficile Certain Clostridium Certain Clostridium C. Table 28-1 presents some of the more common preservatives and their appropriate use. White piedra occurs on the hair shaft and is characterized by a soft mycelial mat surrounding hair of the scalp, face, and pubic region. When water is added to the GasPak envelope, two gases are generated, carbon dioxide and hydrogen. in immunocompromised patients. mycetomatis produce conidia from the tips of phialides, but many remain sterile. Interaction with the infection control program strengthens the establishment of prevention and control strategies. ‡ Except for suction curettings or other specimens collected via a double-lumen catheter. pneumoniae carbapenemase. wound infections, septic arthritis, conjunctivitis, and UTIs. Isolates in vitro are usually susceptible to the aminoglycosides, SXT, ampicillin polymyxin, tetracyclines, fluoroquinolones, and thirdgeneration cephalosporins (e.g., ceftazidime) but resistant to chloramphenicol and the first- and second-generation cephalosporins. Four activities must occur for phagocytosis to take place and be effective in host defense: (1) migration of the phagocyte to the area of infection (chemotaxis), (2) attachment of the particle to the phagocyte, (3) ingestion, and (4) killing. braziliensis) or within cells of a bone marrow aspirate or liver or spleen biopsy specimen (L. Daroy ML, et al: Identification of unknown ocular pathogens in clinically suspected eye infections using ribosomal RNA gene sequence analysis, Clin Microbiol Infect 17:776, 2011. It slowly ferments lactose, producing a “delayed” positive fermentation of lactose with the formation of pink colonies on MAC agar only after 48 hours of incubation. Bacterial motility c. EO-3 and many EO-4 isolates have a yellow nondiffusible pigment. Specimens should be centrifuged in aerosol-free safety carriers, and the tubes should be removed from the safety carriers only inside the biological safety cabinet. Under normal conditions, they are very effective, and the air moving into and out of the lungs is sterile. It was first reported throughout Mexico and forced the closings of schools and cancellation of sporting events. Other organ systems affected by SARS infection include the spleen, lymph nodes, digestive tract, urogenital tract, CNS, bone marrow, and heart. (From Joklik WK et al: Zinsser microbiology, ed 20, Norwalk, CT, 1992, Appleton & Lange.) tahir99 VRG CHAPTER 1 Bacterial Cell Structure, Physiology, Metabolism, and Genetics BOX 1-1 Three Pyruvate Major Metabolic Pathways EMP Glycolytic Pathway (see Figure 1-9) • • • • • 17 Major pathway in conversion of glucose to pyruvate Generates reducing power in the form of $NADH_2$ Generates energy in the form of ATP Anaerobic; does not require oxygen Used by many bacteria, including all members Enterobacteriaceae Acetyl–CoA CO_2 2H of Pentose Phosphate (Phosphogluconate) Pathway (see Figure 1-10) • Alternative to EMP pathway for carbohydrate metabolism • Conversion of glucose to ribulose-5-phosphate, which is rearranged into other 3-, 4-, 5-, 6-, and 7-carbon sugars • Provides pentoses for nucleotide synthesis • Produces glyceraldehyde-3-phosphate, which can be converted to pyruvate • Generates NADPH, which provides reducing power for biosynthetic reactions • May be used to generate ATP (yield is less than with EMP pathway) • Used by heterolactic fermenting bacteria, such as lactobacilli, and by Brucella abortus, which lacks some of the enzymes required in the EMP pathway Citrate Oxaloacetate cis–Aconitate Malate Isocitrate 2H Aerobic Utilization of Pyruvate (Oxidation) The most important pathway for the complete oxidation of a substrate under aerobic conditions is the Krebs or tricarboxylic acid (TCA) cycle. With some organisms, such as Salmonella spp., the disease or organisms spread to distant sites (organs and tissues). disease-causing agents. veronii, and P. tuberculosis be tested for susceptibility to isoniazid, rifampin, ethambutol, and streptomycin. The Puumala hantavirus is the most common member of this genus in Europe and causes a mild form of HFRS, called nephropathia epidemica. The heavy mixture of sizes and types of colonies makes the task of isolating and identifying colonies very difficult without the enhancement obtained with a dissection microscope. Cells of the cardiac muscle and skeletal muscle are most commonly infected. avium complex Best CA, Best TJ: Mycobacterium smegmatis infection of the hand, Hand 4:165, 2009. The length of incubation is related to the distance from the injury to the central nervous system. Attachment is facilitated by the binding of specific antibodies to the microorganism. Cesarean section or suppressive antiviral therapy at delivery significantly reduces the risk of transmission. When tissue is collected, histologic evaluation may reveal caseating or noncaseating granuloma formation with the presence of multinucleated 577 giant cells. This observation was made simultaneously with the recognition of gastroenteritis in men who have sex with men, in which S. Clostridium perfringens is shown here. The urease activity of P. because the organisms are intracellular. Figure 19-3 illustrates the mucoid appearance of K. As soon as all plates are examined, those requiring additional incubation are returned to an anaerobic system and incubated for an appropriate period before reexamination. pertussis possesses various virulence factors that play a role in the pathogenesis of disease. Not only are diverse sites on the host involved in infections, but also certain species cause distinctive lesions. The bubonic form, the most common, usually results from the bite of an infected flea. Chapters devoted to anaerobic bacterial species, medically important fungi, parasites, and viruses affirm the significance of these agents. If the microbial flora is eliminated, such as in patients receiving antimicrobial therapy or some types of chemotherapy, resistant or more pathogenic species may be able to establish infection. A serious side effect of procedures, such as organ transplantation and cancer chemotherapy, is the short- or long-term insult to the host defenses. The egg is 29 to 35 µm long, embryonated when passed, flask-shaped, and operculated, with prominent shoulders at the operculum and a knob at the opposite end (Figure 28-43). 1995. Antibodies of the immunoglobulin M (IgM) class account for 10% to 15% of serum immunoglobulins. In 2007, SARS-CoV antibodies were detected in 47 of 705 (6.7%) South African and Democratic Republic of the Congo (DRC) bat The family Filoviridae includes two genera, Marburgvirus and Ebolavirus. Gastrointestinal Tract Of the estimated 500 to 1000 species of bacteria that inhabit the human body, approximately 300 to 400 live in the colon. Primary risk factors for acquiring the infection include cleaning a cat litter box, gardening without gloves, and eating raw or undercooked meat or unwashed vegetables and fruit. However, in the United States and other developed countries, they are seldom regarded as major causes of disease. Clinical infections include asymptomatic pulmonary disease and allergic manifestations. 28-25); therefore, a complete patient history is necessary to determine the species present. Martin-Carnahan AJ, Joseph SW: Genus Aeromonas stanier 1943, 213AL. In Zaire, a patient treated at a village hospital for a bloody nose probably introduced the virus into the hospital, where it was then transmitted nosocomially and via contact with infected individuals at home. He had lost 25 lb, was experiencing bowel incontinence, and had been 688 tahir99 VRG CHAPTER 29 Clinical Virology perianal region. The members of the genus Rickettsia have not been grown in cell-free media but have been grown in the yolk sacs of embryonated eggs and several monolayer cell lines. Lymphogranuloma Venereum. C. Species resistant to aminoglycosides, penems, and cephems, such as the Bacteroides fragilis group and Pseudomonas aeruginosa tahir99 VRG 136 PART I Introduction to Clinical Microbiology 4. B. Pathogenesis of Infection ■ MICROBIAL FACTORS CONTRIBUTING TO PATHOGENESIS AND VIRULENCE Pathogenesis Virulence Ability to Resist Phagocytosis Surface Structures That Promote Adhesion to Host Cells and Tissues Ability to Survive Intracellularly and Proliferate Ability to Produce Extracellular Toxins and Enzymes ■ HOST RESISTANCE FACTORS Physical Barriers Cleansing Mechanisms Antimicrobial Substances Indigenous Microbial Flora Phagocytosis Inflammation Immune Responses ■ MECHANISMS BY WHICH MICROBES MAY OVERCOME HOST DEFENSES ■ ROUTES OF TRANSMISSION Airborne Transmission Transmission by Food and Water Close Contact Cuts and Bites Arthropods Zoonoses tahir99 VRG CHAPTER 2 Host-Parasite Interaction 29 OBJECTIVES After reading and studying this chapter, you should be able to: 1. Waterskiing, wakeboarding, or other activities that increase the chances of forceful entry of water into the nose may facilitate infection. It is a major protozoan pathogen for humans, causing an estimated 40 to 50 million cases of colitis and hepatic abscesses annually. Aerial mycelia extend above the surface of the colony and are responsible for the fuzzy appearance. Chagas disease is a zoonotic infection found primarily in rural areas of Mexico and Central and South America. The disease is often fatal in animals. Enterics, pseudomonads Devitalized clostridia or bacilli Vibrio, Campylobacter spp. In cases of dehydration, fluid replacement therapy may be indicated. Large numbers of hyaline conidia are generated in the pycnidium and flow out of a small apical pore. Then, find the search box and enter the name of the person or business you want to call.Results to ExpectIf you’re searching in the Telkom directory, expect to find the name, address and phone number of the party you want to call, if they have a listed number. Issues to Consider After reading the patient’s case history, consider: ■ How the patient’s history could relate to the current symptoms ■ What information is obtained from the laboratory and MRI results ■ What information provided helps to determine the most likely cause of the patient’s symptoms Key Terms Antigenic drift Antigenic shift Arboviruses Capsid Cell cultures Continuous cell cultures Cytopathic effect Diploid Envelope Hemagglutinin C Heteroploid Koplik spots Nucleocapsid Obligate intracellular parasites Primary cell cultures Prions Syncytia Tissue culture Vaccinia virus Virion linical virology is a challenging and exciting field of clinical microbiology. Chest radiographs can be normal or show a diffuse interstitial infiltrate. stuartii, P. hoshinae, and E. As the organism spreads to the blood vessels in the dermis, damage occurs to the endothelium. are endogenous biota of the oral cavity, many cases of actinomycosis can be seen in the maxillary region, with draining sinuses in the neck and thorax. Phaeoid yeasts are associated with several species of the polymorphic fungi and are discussed elsewhere in this chapter. jejuni. Another outbreak of EBO-R occurred in monkeys at a Texas quarantine facility in 1996. Biopsy of these nodules reveals fungal elements that are easily recovered in culture. The conjugate binds to antigens on the cell surface, and the antigen-antibody complexes are detected using a fluorescence microscope. Since 1997, large outbreaks of HFMD caused by EV71 have been reported, mostly in children in East and Southeast Asia, with some recent cases seen in Cambodia and China that killed hundreds. aeruginosa strains produce mucoid colonies caused by the overproduction of alginate, a polysaccharide polymer (Figure 21-4). histolytica, and Entamoeba dispar. In 2006, a single dose-attenuated vaccine for shingles, Zostavax (Merck,, Whitehouse Station, NJ), was approved, which uses an attenuated VZV. posadasii. Although clinical manifestations are similar for both types, the cause must be determined before appropriate therapy is begun. The consequence of accumulation of cAMP is hypersecretion of both electrolytes and fluids into the intestinal lumen, resulting in watery diarrhea similar to cholera. TABLE 26-3 Mycobacterial Culture Media Medium American Thoracic Society Löwenstein-Jensen (LJ) Petragnani Middlebrook 7H9 Middlebrook 7H10 Middlebrook 7H11 Middlebrook 7H12 (BACTEC 12B) Composition Inhibitory Agents Fresh whole eggs, potato flour, glycerol Fresh whole eggs, defined salts, glycerol, potato flour Fresh whole eggs, egg yolks, whole milk, potato, potato flour, glycerol Defined salts, vitamins, cofactors base, with pyruvate, albumin, catalase, glucose Defined salts, vitamins, cofactors, oleic acid, albumin, catalase, glycerol, glucose Middlebrook 7H10 with 0.1% casein hydrolysate 7H9 Broth base, casein hydrolysate, bovine serum albumin, catalase, 14C-labeled palmitic acid Malachite green (0.02%) Malachite green (0.025%) Malachite green (0.052%) Middlebrook 7H13 (Bactec 13A) 7H9 Broth base, casein hydrolysate, bovine serum albumin, catalase, 14C-labeled palmitic acid, SPS, polysorbate 80 Gruft (modification of LJ) Fresh whole eggs, defined salts, glycerol, potato flour, RNA Mycobactosel (BBL, Becton Dickinson Diagnostic Systems) LJ Fresh whole eggs, defined salts, glycerol, potato flour Middlebrook 7H10 (selective) Defined salts, vitamins, cofactors, oleic acid, albumin, catalase, glycerol, glucose Mitchison’s selective 7H11 Middlebrook 7H10 with casein hydrolysate Malachite green (0.00025%) Malachite green (0.0001%) Polymyxin B Amphotericin B Nalidixic acid Trimethoprim Azlocillin Polymyxin B Amphotericin B Nalidixic acid Trimethoprim Azlocillin Malachite green Penicillin Nalidixic acid Malachite green Cycloheximide Lincomycin Nalidixic acid Malachite green Cycloheximide Lincomycin Nalidixic acid Carbenicillin Amphotericin B Polymyxin B Trimethoprim lactate SPS, Sodium polyanethol sulfonate. Cryptococcus spp. These viruses have many similarities—they rarely cause human infections, they cause infections with high mortality rates, and they have unknown reservoirs in nature, although human infection can result from contact with infected monkeys. Laboratory Diagnosis Contemporary laboratory diagnosis of pertussis generally employs culture isolation with or without PCR or DFA testing. Infections in immunocompromised patients include infections of the blood, CSF, and organs. Electron microscopy has been used in several epidemiologic studies but is not routinely used as a clinical tool. Electron microscopy examination of stool samples can be used; however, this method is not very sensitive and is usually restricted to large reference or research laboratories. Accessed September 4, 2012. Some bacilli are curved. Microscopy. Bright field light microscopy is best for poxviruses because all other virus particles are too small to be seen. A colorogenic substrate is used so that a positive reaction produces a yellow end product that can be read spectrophotometrically. During oxidation-reduction reactions that occur during normal cellular metabolism, molecular oxygen is reduced to superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) in a stepwise manner by the addition of electrons, as shown in the following equations: In a world in which oxygen abounds, anaerobes are found only in specific ecologic niches. Media containing additives that inhibit the growth of some bacteria but allow others to grow are called selective media (e.g., MacConkey agar [MAC] selective for gram-negative bacteria while inhibiting gram-positive bacteria and colistin–nalidixic acid [CNA] selective for gram-positive bacteria while inhibiting gram-negative bacteria). The last reported case of smallpox in the United States was in 1949, and the last case of smallpox worldwide was in Somalia in 1977. Sejvar JB, et al: Leptospirosis in “Eco-Challenge” athletes, Malaysian Borneo, 2000, Emerg Infect Dis 9:702, 2003. tahir99 VRG 702 PART II Laboratory Identification of Significant Isolates Papillomaviridae Papillomas, or warts, are caused by HPVs, which belong to the genus Papillomavirus in the family Papillomaviridae. The disease is endemic in areas of rural Latin America, Asia, and Africa and is reemerging as a zoonosis in the United States as a result of immigration from endemic areas. Other species of helicobacters, including H. Tissue samples must be kept moist. mirabilis does not produce indole from tryptophan and is ornithine-positive, whereas P. The most common clinical form is ulceroglandular, in which an ulcer forms at the site of inoculation and is followed by an enlargement of the regional lymph nodes. dysenteriae type 1 shigellosis. H antigen or flagella typing is usually performed in a state or reference laboratory that provides epidemiologic information in outbreaks. In foodborne disease caused by C. In most situations when an organism is engulfed by macrophages, lysosomal contents are released into the phagocytic vacuoles, and the organism is killed. Skin must be cleaned with 70% isopropyl alcohol before sampling. and Delftia spp. The LaCrosse virus (LACV) and California encephalitis virus cause encephalitis, and the CrimeanCongo hemorrhagic fever (CCHF) virus infects the vascular endothelium and liver. In OF media, most isolates are nonoxidative and produce a deep blue color at the top, except for A. In lower respiratory tract infections of patients with CF, most P. Processing and culture interpretation of such nonrepresentative specimens may lead to delayed treatment because of a false-negative culture or to inappropriate antibiotic treatment owing to a false-positive culture from growth of normal biota (normal flora) or antibiotic-altered flora. Although virus particles cannot be visualized, the CPE can be detected in cell scrapings from infected sites by bright field microscopy. ovale to prevent relapses of these infections. With the introduction in 2006 of a human-bovine rotavirus vaccine (RV5; RotaTeq, Merck), a delay in the onset of rotavirus season from mid-November to late February was seen. The serovars causing LGV are able to survive inside mononuclear cells. As the adults thread themselves through the intestinal mucosa, inflammation develops. There is early migration of the neutrophils, but phagocytosis of the diplococci is limited. Often the best indication of a possible Vibrio infection is the presence of recognized factors, such as the following: • Recent consumption of raw seafood (especially oysters) • Recent immigration or foreign travel • Gastroenteritis with cholera-like or rice-water stools • Accidental trauma incurred during contact with fresh, estuarine, or marine water or associated products (e.g., shellfish, oyster or clam shells, fishhooks) Microscopic Morphology Vibrio spp. The vaccine is expected to give lifelong immunity. This insertion is thought to be mediated by a series of molecular interactions between the tip of the polar tube and receptors on the cell membrane. Wright stain has a shorter staining period, but the color intensity for the differentiation of parasites is not as good as that with Giemsa stain. Immunodiagnosis of Mycobacterium tuberculosis Infection Skin Testing The tuberculin skin test has been used for many years to determine an individual’s exposure to M. This action can result in loss of an observable characteristic, such as the ability to ferment a particular sugar. India had remained free of glanders in livestock for more than 8 years when in 2006-2007, outbreaks occurred in which 164 horses were found positive in eight Indian states. (From Ryan KJ: Vibrio and Campylobacter. Yersinia pestis b. Botulism toxin and C. Therefore, the presence of spores should be confirmed using one of the modified trichrome stains. Legionnaires’ disease is a febrile disease with pneumonia that is often associated with travel; Pontiac fever is a milder febrile disease without pulmonary involvement. coli). Specimens should never be stored at –20° C because this temperature facilitates the formation of ice crystals, which will disrupt the host cells and result in loss of viral viability. from groups 1, 5, and 6 are summarized in Table 20-4. Infants younger than 6 months old, who are too young to be fully vaccinated, continue to be at highest risk for severe disease. These particular anaerobes should be suspected as participants in any infectious process occurring in the oral cavity and in suspected cases of aspiration pneumonia. The mortality rate in immunocompromised patients is over 80%; the usual causes of death are complications resulting in respiratory failure. The colonies were nonhemolytic, slightly adhered to the surface of the media, and had a slight yellow appearance when removed. Slides for DFA testing may be prepared directly from swab specimens or after expression of the material from the swab to a solution of 1% casein hydrolysate. New diagnostic tests have been developed that are based on identifying unique RNA or DNA sequences present in each bacterial species. Transmission is about 50% for a mother who has a primary infection. Cyclospora cayetanensis. Cyclospora cayetanensis is a foodborne and waterborne organism causing endemic and epidemic diarrheal disease. Taxonomy There are over 100,000 named fungal species and an estimated 1 to 10 million undiscovered species. All these organisms are transmitted by the fecal-oral route—ingestion with contaminated food or water or by direct contact with fecal material containing infective oocysts. fowleri does not stain well with Gram stain, but motile trophozoites can be seen in a wet mount of the CSF. Visual recognition can lead to a request for stool examination for parasite load and prompt treatment. Simultaneously, a maculopapular rash appears on the hands, feet, and buttocks, followed by bullae on the soles of the feet and palms of the hands. Stuart medium can be used to maintain the viability of the organisms if a delay in processing is anticipated. Discuss the possible roles of M. These tests, in contrast to antibody tests, provide information about current infection. One type of interferon, interferon-gamma, plays an especially important role in the immune response. Usual Flora of the Skin Normal skin has numerous mechanisms to prevent infection and protect the underlying tissue from invasion by potential pathogens. cepacia are nonwrinkled, and this trait may be used to differentiate isolates from P. Acinetobacter lwoffii Acinetobacter baumannii NONMOTILE NONMOTILE, COCCOBACILLI PIGMENTED, INDOLE-POSITIVE OxidaseNONFERMENTERS negative Elizabethkingia meningosepticum 480 Oxidase Pyocyanin / / Maltose / / 42° C / / / Fluorescein Glucose Xylose Mannitol Lactose Esculin Urea DNase ONPG / / / / / / / Indole Motility Flagella H_2S N_2 gas / Pigment Y B B / Growth on MacConkey Y Flagella 1, Polar monotrichous >1, Polar tuft (>1 flagellum) P, Peritrichous FIGURE 21-2, cont’d tahir99 VRG CHAPTER 21 Nonfermenting and Miscellaneous Gram-Negative Bacilli Today, methods that include MALDI-TOF and a variety of sequencing tools are becoming more popular because of their ability to identify rapidly and accurately many more nonfermenters than can be done with phenotypic methods. The MGIT 960 system (Becton Dickinson Diagnostic Systems) is a nonradiometric microbial detection system that uses a modified Middlebrook 7H9 broth in conjunction with a fluorescence quenching-based oxygen sensor to detect growth of mycobacteria. Certain O serogroups of EPEC were identified in the late 1960s and 1970s as a cause of diarrhea, but only certain H antigenic types within each O serogroup were connected to the intestinal infections. In women, the diagnosis is typically made by finding the trophozoite in vaginal discharge or occasionally in urine; in men, the trophozoite is seen in urine or prostatic secretions. Chlamydiae are deficient in energy metabolism and are therefore obligate intracellular parasites. It is usually isolated from cold-blooded animals and the environment, but there was a report of 18 cases of human enteritis caused by S. damsela,, and most V. Forming and testing a hypothesis, performing PFGE, and calculating an infection rate d. Inoculate charcoal–horse agar (with cephalexin). The most common Pasteurella species isolated from humans, P. microti is the most common. Nasal sinus specimens obtained surgically can be plated directly to media containing antimicrobial agents except for cycloheximide, which can inhibit some fungi. Important microfilaria morphologic characteristics include the presence or absence of a sheath (the remnant of the egg from which the larva hatched) and presence and arrangement of nuclei in the tail. Mumps Virus Mumps virus is related to the PIVs and is in the genus Rubulavirus. FIGURE 27-48 Cladosporium sp. The resistance ratio method compares the resistance of the test organism with that of a standard laboratory strain. Pertussis is one of the most highly communicable diseases of childhood; secondary attack rates of 80% occur among susceptible contacts. intestinalis, especially to the urinary tract, gallbladder, or respiratory tract, has been seen. Regardless of the identification system used, the clinical microbiologist may presumptively determine utilization of carbohydrates by observing the colony morphology of the isolate on differential or selective media such as MAC, SMAC, CIN, HE, or XLD. Fecal pathogens are generally nonlactose fermenters (NLFs). Some types of HPV will result in genital wart formation (condylomata acuminata), which can easily be identified. dumoffii. On BHI blood agar at 37° C, the mycelial phase rapidly converts to yeast phase. A better, albeit more expensive, method is to use a solid-state dehumidifier. Darting motility is a characteristic of: a. Most fungi are obligate aerobes that grow best at a neutral pH, although they tolerate a wide pH range. Roxstrom-Lindquist K, et al: Giardia immunity—an update, Trends Parasitol 22:26, 2006. agglomerans. After a few days, the infected host usually develops an asymptomatic viremia; however, some hosts become febrile, which is far less common. The capsular antigen K1 present in certain strains of E. World Health Organization: Cholera in Sierra Leone—update, 2012. The viruses are spread by aerosols, fomites, oral-fecal route, and personal contact. fragilis PEA or CNA S. triviale, and most rapidly growing Mycobacterium spp. These preparations incorporate dimethyl sulfoxide (DMSO) and a stain into the KOH solution. These are typically used in research or larger reference laboratories. submitted. Methylene blue stain demonstrating the typical morphology of Corynebacterium diphtheriae (arrows). Bordetella Christian Whelen CHAPTER OUTLINE ■ GENERAL CHARACTERISTICS ■ CLINICALLY SIGNIFICANT SPECIES Bordetella pertussis and Bordetella parapertussis Miscellaneous Species ■ LABORATORY DIAGNOSIS Specimen Collection and Handling Nucleic Acid Detection Microscopic Examination Isolation and Identification Serologic Testing ■ ANTIMICROBIAL SUSCEPTIBILITY OBJECTIVES After reading and studying this chapter, you should be able to: 1. 2H 2H 2H Electron transport and oxidative phosphorylation • Converts glucose-6-phosphate (rather than glucose) to pyruvate and glyceraldehyde phosphate, which can be funneled into other pathways • Generates one NADPH per molecule of glucose but uses one ATP • Aerobic process used by Pseudomonas, Alcaligenes, Enterococcus faecalis, and other bacteria lacking certain glycolytic enzymes • Butyric acid fermentation: Certain obligate anaerobes, including many Clostridium species, Fusobacterium, and Eubacterium, produce butyric acid as their primary end product along with acetic acid, carbon dioxide, and hydrogen. Determine the appropriate culture media required for isolation of Legionella spp. Carrier State. Individuals who recover from infection may harbor the organisms in the gallbladder, which becomes the site of chronic carriage. *Vancomycin, 5 µg. Most of these microorganisms are nonpathogenic. parapertussis is unnecessary because of predictable sensitivity to macrolides. Gram-positive cocci, yeasts, and Pseudomonas aeruginosa are also usually present in the large intestine. Third, direct examination might provide evidence of infection despite negative cultures. Reference Laboratory for Tuberculosis and Other Mycobacterial Diseases, Veterans Administration Medical Center, West Haven, CT, and Department of Laboratory Medicine, Yale University School of Medicine, New Haven, CT. Myroides odoratimimus (Flavobacterium odoratum) is an environmental, lowvirulence, nonfermenting, gram-negative bacillus that has been found to cause UTIs, endocarditis, ventriculitis, and cutaneous infections in immunocompromised patients. are noted for their bright salmon-pink color. Three major shifts occurred during the twentieth century. Madoff L: Infections from bites, scratches, and burns. In cases of severe diarrhea in children younger than 1 year, infection with EPEC should be suspected. Definitive Identification Isolates from stool specimens and rectal swabs can be presumptively identified as Campylobacter spp. The World Health Organization estimates that 1.3 million people are blind because of trachoma. List the stages of parasites found during microscopic examination of fecal material with direct wet mount, fecal concentration, and permanent stained smears. Hair A Wood’s lamp emits ultraviolet light >365 nm and can be useful in identifying infected hairs. The incubation period is 1 to 4 days. acnes. They are almost indistinguishable from eggs of F. trichiura rarely cause symptoms; heavy infections result in intestinal bleeding, weight tahir99 VRG 676 PART II Laboratory Identification of Significant Isolates FIGURE 28-58 Trichuris trichiura egg (unstained, ×400). (Nomarski optics, ×450.) FIGURE 27-34 Mucor sp. In 2012, the World Health Organization reported an outbreak of cholera in Sierra Leone; the national cumulative number for all 12 districts was about 20,700 cases, including 280 deaths. Shigella spp. However, the gram-negative control organism, E. (Figure 28-55). The eggs and rhabditiform larvae of the two species are indistinguishable; therefore, the laboratory can report only “hookworm” when a characteristic egg or larva is found in a stool specimen. Dold C, Holland CV: Ascaris and ascariasis, Microbes Infect 13:632, 2011. bronchiseptica infections in humans generally manifest with a nonspecific cough or bronchitis, and often patients have underlying conditions, such as immunosuppression or contact with animals. ozaenae pneumoniae subsp. have erect sporangiophores terminated by dark sporangia and sporangiospores (Figure 27-35). Like the biochemical-based multitest systems, these systems are primarily of value for identifying only the most commonly isolated anaerobes. bronchiseptica is a respiratory tract pathogen of numerous animals including dogs, in which it causes kennel cough. In a permanently stained preparation, the cytoplasm of the trophozoite may stain a dark purplish gray and contains vacuoles and ingested materials. It also exhibits a tertian life cycle. Key factors in the life cycle of this organism that contribute to its pathogenicity include the following: • The oocysts are infective when passed. In this case, the organ may contain bradyzoites, slowgrowing encysted forms of the parasite responsible for chronic infections that reactivate in the immunocompromised recipient. Although this method holds great promise, sequences in some reference databases are not accurate and procedures are not yet standardized. are also sexually transmitted. The recommended cutoff for positivity is 80 pg/mL. aeruginosa is innately resistant to many antimicrobial agents, including penicillin, ampicillin, first- and second-generation cephalosporins, trimethoprimsulfamethoxazole (SXT), chloramphenicol, and tetracyclines, along with other agents to which most gram-negative bacilli are resistant. Polioviruses, type B coxsackieviruses, and echoviruses grow readily in a number of cell lines, including PMK, continuous human and primate, and human fetal diploid fibroblast lines. Legionnaires’ disease is best diagnosed using a combination of culture and urine antigen detection. aegypti mosquitoes transmit the infection to people. 858. haemophilum, and M. Vibrio spp. The kidneys may fail, and about 50% of the patients in the toxic phase die. A problem with this method is that the agents change color at too high a level of oxygen concentration, which can make it difficult to react in time to maintain the viability of strict anaerobes. anisa No Color L. Chronic cough and chest pain might also accompany these infections. Serologic tests are being evaluated and may soon be available. Arizonae Escherichia coli Klebsiella spp. They are susceptible to the various effects of physical and chemical agents, such as disinfectants and high concentrations of acids and bile. Either of these media will inhibit facultative anaerobic gram-negative bacteria that can overgrow anaerobes present in the specimen. Some common agents that infect the nails are Trichophyton rubrum, T. Babesia microti Babesiosis is a zoonotic intraerythrocytic infection transmitted by the bite of a tick. The cytostome and nucleus are prominent in the anterior of the organism, and a spiral groove encircles the body of the organism. These initial cases were limited to patients who had undergone splenectomy, but since then, cases of babesiosis have been reported in patients who are not asplenic. Iron Uptake. Some mycobacteria can convert ferric ammonium citrate to an iron oxide. Clinical symptoms of HBoV include cough, rhinorrhea, fever, difficulty breathing, diarrhea, conjunctivitis, and rash. Because the peptidoglycan layer is the principal component of the gram-positive cell wall, many antibiotics effective against gram-positive organisms (e.g., penicillin) act by preventing synthesis of peptidoglycan. After all the specimens have been inoculated, the agar plate can be incubated anaerobically. botulinum as a critical result, requiring that the nursing unit or physician be notified immediately. 551 BIBLIOGRAPHY Beninati T, et al: First detection of spotted fever group rickettsiae in ricinus from Italy, Emerg Infect Dis 8:983, 2002. These mycoses are usually the result of the traumatic implantation of foreign objects into the deep layers of the skin, permitting the fungus to gain entry into the host. Biochemical Pathways from Glucose to Pyruvic Acid The starting carbohydrate for bacterial fermentations or oxidations is glucose. septicum. Campylobacter fetus subsp. immitis, H. aeruginosa is isolated from a sterile body site, such as blood, pleural fluid, joint fluids or tissues, or cerebrospinal fluid (CSF), it almost always constitutes a true infection. As shown in the life cycle illustrated in Figure 28-35, humans can acquire infection with T. Infections caused by common single species or by classic infectious agents include Streptococcus pneumoniae pneumonia, Staphylococcus aureus abscesses or pyodermas, H. The group booked a block of rooms together and spent time socializing over drinks and cigarettes in the cabins, saunas, and poolside. ITRA is especially useful for treating aspergilli and phaeoid fungi infections. Such specimens are more subject to contamination and may contain fewer viable tubercle bacilli. TABLE 27-10 Summary of Primary Fungal Culture Media Medium Expected Growth Results At 22° C SDA SDA with antibiotics* BHI BHI with antibiotics Inhibitory mold agar Cycloheximide Initial isolation of pathogens and saprobes Dimorphic fungi may exhibit their mycelial phase. and other yeasts. The type and quantity of mycolic acids are speciesspecific. Bern C, et al: Trypanosoma cruzi and Chagas’ disease in the United States, Clin Microbiol Rev 24:655, 2011. Compare the causative agents and arthropod vectors of relapsing fever and Lyme disease. Positive lactose fermentation 4. As the species name implies, it emits a dirty, musty odor resembling that of rotten potatoes. cysts. Routine vaccination against smallpox in the United States ended in 1972, and other countries have also stopped their vaccination programs. Reagent-impregnated strips have eliminated the need to handle and dispose of cyanogen bromide, which is caustic and toxic. Hermann C, et al: Comparison of quantitative and semiquantitative enzyme-linked immunosorbent assays for immunoglobulin G against Chlamydophila pneumoniae to a microimmunofluorescence test for use with patients with respiratory tract infections, J Clin Microbiol 42:2476, 2004. Postmortem examination revealed rickettsial-like inclusions in the monocytes of the dead animals. The color of Aspergillus spp. This chapter describes the interactions between the host and infectious agent in the pathogenesis of disease. Corneal involvement can result in destructive ulceration and perforation of the cornea, leading to blindness. conorii into the skin of a nonimmune person. fortuitum, and M. Veillonella spp. A positive reaction can occur within 15 minutes, but the tube must be held for up to 6 hours before a true negative reaction is determined. This is evidenced by the higher incidence of tinea versicolor among persons receiving corticosteroid therapy, tahir99 VRG CHAPTER 27 Medically Significant Fungi which decreases the rate of squamous epithelial cell turnover. tuberculosis M. • K antigen, or capsular antigen—this is a heat-labile polysaccharide found only in certain encapsulated species. The symptoms of the initial catarrhal phase are insidious and nonspecific and include sneezing, mild cough, runny nose, and perhaps conjunctivitis, although infants can develop apnea or respiratory distress or both. FIGURE 22-21 Gram-stained appearance of Fusobacterium nucleatum subsp. The isolate is sensitive to O/129 and is oxidase-positive. By MIF, C. For these reasons, the BACTEC 460 is gradually being replaced in clinical laboratories. No fever or evidence of inflammation is present. 3. Find additional cases. These tests are 80% to 85% effective. Some laboratories also use this test to distinguish penetration-capable Microsporum canis from M. Patients usually seek medical attention to address cosmetic rather than medical concerns caused by these fungi. Accessed November 14, 2013. coli S. tularensis colonies may not be visible before 48 hours of incubation at 37° C. The major disadvantage of any anaerobic jar system is that the plates have to be removed from the jar to be examined and processed. Initially, an infection control risk assessment should be conducted to determine whether the air is the likely source of infectious particles. duboisii, endemic in Central Africa, causes a clinically distinct form of disease involving primarily the skin and bones, whereas H. Patients develop DHF after they have already been exposed to one serotype of dengue virus and are then exposed to one of the other three serotypes. Mahon, MS, MT(ASCP), CLS Microbiologist and Senior Education Program Specialist Staff College, Center for Veterinary Medicine U.S. Food and Drug Administration Rockville, Maryland Adjunct Faculty Department of Clinical Laboratory Sciences School of Medicine and Health Sciences George Washington University Washington, DC Gail E. The organism lives in the large intestine, where it may cause mucosal lesions but not extraintestinal infections. Because many intestinal organisms are shed into the stool irregularly, a single stool specimen may be insufficient to detect an intestinal parasite. Describe the general procedures for performing the direct wet mount, fecal concentration, and permanent stained smears. The amastigote has also been reported extracellularly in cases of cutaneous leishmaniasis. Enlargement in the posterolateral triangle of the neck is known as Winterbottom’s sign. are also available for detecting Campylobacter antigen in fecal samples. FIGURE 27-11 Diagram of the typical so-called spaghetti and meatballs appearance (top arrow hypha and bottom arrow yeast cell) of Malassezia furfur in a potassium hydroxide preparation (×400). Is the final stage usually seen in developed countries? Probably a member of the Bacteroides fragilis group, but could be a Prevotella sp. Libman MD, et al: Detection of pathogenic protozoa in the diagnostic laboratory: result of reproducibility, specimen pooling, and competency assessment, J Clin Microbiol 46:2200, 2008. The adaptive immune system is able to enhance the protective mechanisms of the innate system, as previously mentioned. coli are DAEC and EAEC. trachomatis and Chlamydophila pneumoniae infections. Several EIAs for detecting NoV in stool samples are available. What is the test of choice for the laboratory diagnosis of relapsing fever borreliosis? atlantae. –Ketoglutarate CO_2 Succinate Entner-Doudoroff Pathway (see Figure 1-10) ATP, Adenosine triphosphate; EMP, Embden-Meyerhof-Parnas; $NADH_2$, nicotinamide adenine dinucleotide dehydrogenase; NADPH, nicotinamide adenine dinucleotide phosphate. EO-3 has been reported in a case of CAPD infection. (Courtesy Anaerobe Systems, Morgan Hill, CA.) presumptively identified as Peptoniphilus (Peptostreptococcus) asaccharolyticus. Unlike RMSF, the rash of rickettsialpox appears on the face, trunk, and extremities and does not involve the palms of the hands or soles of the feet. Eubacterium spp. Most laboratories use a wide variety of nonselective media, such as SBA and CHOC agar, as well as selective media, such as MAC, to recover enteric organisms from wounds, respiratory tract secretions, urine, and sterile body fluids. Infection with the adult tapeworm of either species usually causes few clinical symptoms, although vague abdominal pain, indigestion, and loss of appetite may be present. Cases of cutaneous leishmaniasis and visceral leishmaniasis have been seen in a number of military personnel serving in Middle East countries, such as Iraq and Afghanistan. The second section of the chapter discusses the virulence factors that contribute to the invasiveness of organisms, protective mechanisms the host employs, and how microbes are able to evade the host’s defenses. do not use acetate or mucate as a source of carbon. Such media may also have redox potentials above those required for anaerobes to initiate growth. odorifera contains two biogroups. longbeachae. psittaci infections because of transient bird exposure were actually infected with C. *Often only the virulent strains show the type of interference listed. Characteristics of Indigenous Microbial Flora Microorganisms that are commonly found on or in body sites of healthy persons are called normal, usual, or indigenous flora. Although several species, including Babesia microti and B. cholerae (serogroups O1 and non-O1), V. Susceptibility tests must be performed on each isolate to determine appropriate antimicrobial therapy. Humans acquire the infection by ingesting the organisms in contaminated animal food products or insufficiently cooked poultry, milk, eggs, and dairy products. Metronidazole is given to treat the infection. Available at: antibiotic-treatment.html. Chronic pulmonary histoplasmosis in patients with chronic obstructive pulmonary disease may also occur. Rapid assays useful for the detection of Legionella spp. Some serovars of L. The four genera include Sapporo virus, Norwalk virus, rabbit hemorrhagic disease virus, and feline calicivirus. The microscopic morphology is important because it differentiates Campylobacter from other bacteria, such as Aeromonas and Pseudomonas, which are oxidase-positive and can grow at 42° C in a microaerophilic environment. In 2012, several more outbreaks were reported. The cell lines are grown on coverslips in 1-dram shell vials or on the surface of multiwell cell culture dishes containing cell culture media with cycloheximide. Figure 22-5 FIGURE 22-4 Prereduced, anaerobically sterilized (PRAS) plated media. tropicalis. The greater sensitivity of the BACTEC system has resulted in a higher yield from smear-negative specimens. cepacia complex. influenzae tracheobronchitis or meningitis in infants, Clostridium perfringens gas gangrene, Nocardia spp. An iatrogenic infection is an infection that occurs as the result of medical treatment or procedures. Wet mounts are also made from the fecal specimen following a concentration procedure. Of these B FIGURE 29-1 A, Card format rapid immunochromatographic membrane assay, BinaxNOW (Scarborough, ME), for three common respiratory viruses—influenza A and B and respiratory syncytial viruses. trachomatis that vary in sensitivity, specificity, and positive predictive value. Figure 28-8, A, shows an MIF wet mount of a cyst of E. Each ringworm lesion is the result of a local inoculation of the skin with the causative agent. This pneumonia also can occur as a mixed infec tion with gonococcus, cytomegalovirus and other viruses, and TABLE 541 24-4 Inclusion Conjunctivitis in the Neonate Caused by Chlamydia trachomatis Characteristic Incubation period Signs Discharge Course Complications Comments 4-5 days Edematous eyelids Copious, yellow Untreated, weeks to months Corneal panus formation, conjunctival scarring Pneumocystis. In Versalovic J, et al, editors: Manual of clinical microbiology, ed 10, Washington, DC, 2011, ASM Press, p. It is found worldwide; in the temperate zones, epidemics occur during the winter and spring. Arcobacter spp. B, Same colony after 4 days of incubation. The disease progression is similar to that of RMSF, including involvement of the palms of the hands and soles of the feet with the rash. tertium will grow minimally on the aerotolerance plate and can be mistaken for a facultative anaerobe. Certain organisms that survive phagocytosis may be disseminated rapidly to many body sites, but the organisms themselves are not invasive. All members of the family: (1) are oxidase-negative except for Plesiomonas shigelloides (see Chapter 20), (2) ferment glucose, and (3) reduce nitrate to nitrite except Photorhabdus and Xenorhabdus (an environmental isolate). (Courtesy A.J. Horneman.) well as all A. Although half of all adenovirus infections are asymptomatic, the virus causes about 10% of all cases of pneumonia and 5% to 15% of all cases of gastroenteritis in children. The complex B. Because of the hazards to skin and eyes associated with excess UV light, the UV light should be turned on only when the safety cabinet is not in use. are phaeoid, rapidly growing fungi with colonies ranging from shades of gray to brown to black. Without treatment, mortality approaches 30%. aeruginosa from other fluorescent pseudomonads? Mycoplasma pneumoniae, Chlamydophila pneu moniae, and Legionella produce symptoms different from S. pylori. Saliva and tears contain enzymes such as lysozyme, and the sebaceous glands of the skin contain oils capable of inhibiting invasion by pathogenic organisms. produce rapidly growing, woolly colonies that cover the entire surface of the culture medium. Larger tissues of more than 1 cm^2 can maintain a reduced atmosphere as long as the transport time to the laboratory is minimized. In addition to farm and domestic animals, birds are natural reservoirs; turkeys, geese, pigeons, doves, and canaries have yielded positive cultures for this organism. Care should be taken to ensure the appropriate concentration of cephalexin in the medium. Some reasons for this significant rise in the clinical isolation of Vibrio include the following: • Increase in ocean water temperature because of climate change • Increased travel to coastal or cholera-endemic areas • Increased consumption of seafood (particularly raw or undercooked) • Increased use of recreational water facilities, which encourages aquatic exposure • Larger populations of immunocompromised individuals • Increased awareness of the existence and significance of these organisms in the clinical microbiology laboratory General Characteristics Clinical Manifestations Vibrio spp. Escherichia vulneris has been isolated from humans with infected wounds. Without treatment, the disease is often fatal; even with treatment, the fatality rate is 15% to 25%. Most bacteria are easily destroyed by the low pH found in the stomach. murliniae have also been isolated from human specimens. Issues to Consider After reading the patient’s case history, consider: ■ The clinical significance of anaerobes in human infections ■ Indications of an anaerobic infection ■ The importance of proper selection, collection, transport, and processing of anaerobic specimens ■ Various anaerobes that may be found in different specimen types ■ Extent of anaerobe identification ■ When and how to perform susceptibility testing of anaerobes Key Terms Actinomycosis Aerotolerance test Aerotolerant anaerobe Anaerobe Anaerobic chamber Bacterial vaginosis (BV) Botulism Capnophilic Catalase Endogenous anaerobe Enteritis necroticans TABLE Exogenous anaerobe Facultative anaerobe Hydroxyl radical Microaerophilic Myonecrosis Obligate anaerobe Pseudomembranous colitis Superoxide anion Superoxide dismutase Tetanus A naerobic bacteria are important in human and veterinary medicine because they play a role in serious, often fatal, infections and intoxications. Whether dealing with emerging diseases, such as SARS, or reemerging diseases, such as smallpox and anthrax, the laboratory must stay closely aligned with the infection control activities in the setting that the laboratory serves. trachomatis is unique in that it carries 10 stable plasmids whose function is currently unknown. In April 2013, a novel avian influenza A (H7N9) was reported in China. Figure 25-2 depicts electron micrographs of ciliated tracheal epithelial cells before and after M. Their genome consists of a single circular chromosome. For these reasons, cases of diarrheal illness resulting from EIEC might be underreported. TABLE 27-1 Various Forms of Dermatophytoses and the Respective Affective Sites Type of Ringworm Tinea Tinea Tinea Tinea Tinea Tinea Tinea Tinea Tinea Tinea capitis favosa barbae corporis manuum unguium cruris pedis imbricate Site Affected Head Head (distinctive pathology) Beard Body (glabrous skin) Hand Nails Groin Feet Body (distinctive lesion) Sporothrix schenckii commonly presents as a progressive lymphocutaneous infection, beginning with a single draining lesion and progressing along the limbs via the lymphatic system, forming multiple draining lesions. Other manifestations are reduced birth weight, CNS involvement, mental impairment, deafness, and even death. Primary infections are usually asymptomatic, but when apparent, they commonly manifest as mucosal vesicles inside the mouth, which are rarely seen, or ulcerations, which may be widespread and involve the buccal mucosa, posterior pharynx, and gingival and palatal mucosae. EO-2 exhibits a high level of homology to Paracoccus, and it has been named Paracoccus yeei. • Density measurement: The density (referred to as cloudiness or turbidity) of a bacterial broth culture in log phase can be correlated to CFU/mL of the culture. For instance, the terminal spore of Clostridium tetani, the etiologic (causative) agent of tetanus, gives the organism a characteristic tennis racquet–shaped or lollipop-shaped appearance. Box 2-2 provides a partial list of microorganisms found in the mouth. Personal communication for 2004 leptospirosis data. Transmission is primarily via aerosols, but contact with secretions and fomites can also cause infection. The environmental conditions described here may change with age, nutritional status, disease states, and drug or antibiotic effects. Splinter hemorrhages beneath the nails can be seen in many patients. Children younger than 5 years are at risk for infection, and G. pallidum transmission normally occurs during direct sexual contact with an individual who has an active primary or secondary syphilitic lesion. vesicularis septicemia, and three of the eight infants also had meningitis caused by the organism. Other antigenic polysaccharides may be present on the surface of the peptidoglycan layer. have been known to infect cattle and other animals, but the first cases reported in humans occurred in the 1950s. Often, these diseases are ruled out early in diagnosis, which leads to a presumptive identification of hMPV. Legionella colonies are the smallest ones (arrow) in the first quadrant. In 2011, the Foodborne Diseases Active Surveillance Network (FoodNet) estimated that 845,024 cases of Campylobacter infections occurred, ranking fourth in most common causes of foodborne gastrointestinal illness, behind norovirus, Salmonella, and Clostridium perfringens. Lemierre’s disease, a syndrome of thrombophlebitis of the jugular vein that occurs rarely following group A streptococcal pharyngitis, is caused by F. kansasii, M. Garcia-Hermoso D, et al: Agents of systemic and subcutaneous mucormycosis and entomophthoromycosis. Serosurveys also determined that 30% of the deer mice tested in the New Mexico area were seropositive for the same unknown virus. Therefore, laboratory diagnosis of EBV is often accomplished with serologic tests. The trophozoite is 30 to 60 µm, with broad pseudopods and a large single nucleus, with multiple nucleoli. Ziehl-Neelsen Stain 1. This organism has the most chronic course, and recrudescence occurs even decades after the initial infection. The mature oocyst has two sporocysts, walled bodies that produce sporozoites. The microbial flora plays an important role in both health and disease. The main adhesins in bacteria are the fimbriae (pili) and surface polysaccharides. reside in hair follicles and colonize the sebaceous glands because they are resistant to skin lipids and fatty acids as well as to superficial antiseptic agents commonly used to cleanse the skin. As more molecular biology methods become available commercially and increasingly automated, identification and detection of mycobacteria will become faster, less costly, and more specific. After 24 to 48 hours of incubation, test results are read, a code number is generated for each isolate, and identification is determined from a compendium codebook or electronic database. The bacterial cell then lyses (lytic phase), releasing new phage, which can infect other bacterial cells. Some microbiologists believe that it is better to batch-process specimens than to process each specimen as it arrives in the laboratory. tahir99 VRG 18 PART I Introduction to Clinical Microbiology Anatomy of a DNA and RNA Molecule DNA is a double helical chain of nucleotides. vesicularis isolates are positive, whereas B. Because PVA becomes cloudy when exposed to air, PVA-preserved specimens are not acceptable for wet mounts. The cross walls help determine whether the structures are true hyphae or pseudohyphae. Actinomyces spp. Culture Media The salt concentration (0.5%) in most commonly used laboratory media, such as nutrient agar or sheep blood agar (SBA), is sufficient to support the growth of many vibrios. enterocolitica grows better with cold enrichment, and motility is clearly noted at 25° C but not at 35° C. The laboratory diagnosis of Legionella infection typically depends on one or more of the following methods: • Isolation using special media • Urine antigen detection • Direct fluorescent antibody (DFA) • Serology Clinical Significance Infections caused by Legionella spp. TABLE Type 425 Enterotoxigenic Escherichia coli. ETEC strains are associated with diarrhea of adults and especially children in tropical and subtropical climates, especially in developing countries, where it is one of the major causes of infant bacterial diarrhea. • Viruses are obligate intracellular parasites that require host cells for replication (increase in number does not involve mitosis, meiosis, or binary fission) and metabolism. In the former case, it may be decided that definitive identification and susceptibility testing are required. In developing countries with malnutrition and poor hygiene, measles can have a high fatality rate. Even in well-immunized populations such as the United States, periodic outbreaks occur every 3 to 5 years, and isolated cases occur at all times. Additional characteristics can differentiate this group of nonfermenters from other gram-negative bacilli— motility, pigmentation, and their ability or lack of ability to grow on selective gram-negative media such as MacConkey (MAC) agar. nucleatum and Porphyromonas are recovered from the bloodstream or from abscesses located far from the oral cavity. Current options for treating fungal infection include drugs primarily from four classes—the polyenes, azoles, echinocandins, and allylamines. The presence and type of hemolysis among multiple aeromonad colony types in a single culture often are the only clues to an infection involving more than one species of Aeromonas. neoformans from C. If HSV-1 is present in the cell line, a viral protein will activate the promoter, resulting in β-galactosidase expression. Use of media with antimicrobial agents should not be needed because CSF is normally sterile. sibonii Morganella morganii biogroup 1 Obesumbacterium proteus biogroup 2 Pantoea agglomerans Pantoea dispersa Photorhabdus luminescens (all tests at 25° C) Photorhabdus DNA hybridization group 5 Pragia fontium Proteus mirabilis Proteus myxofaciens Proteus penneri Proteus vulgaris Providencia alcalifaciens Providencia heimbachae Providencia rettgeri Providencia rustigianii Providencia stuartii Rahnella aquatilis Salmonella bongori Salmonella enterica subsp. Food poisoning caused by C. Clinical Manifestations.

Trichosporon beigelii has been described as an extremely variable human pathogen. veronii biovar sobria, include hemolytic-uremic syndrome or kidney disease that might require kidney transplantation. In a wet preparation, the motility is sluggish. Batch processing is an acceptable alternative for aspirates and specimens received in proper transport containers—those kept moist under anaerobic conditions. Treatment for H. Antibodies to certain rickettsial species are known to cross-react with bacteria in the genus Proteus. The study showed that these EPEC strains caused distinct diarrhea. Lo Y-C, et al: Severe leptospirosis similar to pandemic (H1N1) 2009, Florida and Missouri, USA, Emerg Infect Dis 17:1145, 2011. As shown in Box 2-2, C. petrii, and B. Conway-Klaassen, PhD, MT(ASCP)SM Director, Clinical Laboratory Science Program University of Minnesota Minneapolis, Minnesota PowerPoint Writer Perry Scanlan, PhD, MT(ASCP) Associate Professor and Program Director Medical Laboratory Science Program Department of Allied Health Sciences Austin Peay State University Clarksville, Tennessee Laboratory Manual Writer Stephen D. Table 19-4 lists non-O157:H7 EHEC/STEC isolated from patients with bloody diarrhea, hemorrhagic colitis, or HUS. pneumoniae E. Species that produce carotene pigment on exposure to light are photochromogens. Susceptibility testing of leptospires is not normally performed in the clinical laboratory; leptospires have been shown to be susceptible in vitro to streptomycin, tetracycline, doxycycline, and the macrolide antimicrobials in vitro. Available at: preview/mmwrhtml/mm5936a1.htm. Coronaviruses may be responsible for 15% of coldlike infections in adults, but higher seroconversion rates have been seen in children. The texture of the yeast colonies also varies. bifermentans and C. One side of the paddle is covered with nonselective Middlebrook 7H11 agar. Several organisms are responsible for the disease, and certain organisms appear to reside in specific endemic areas throughout the world. The spores germinate into vegetative cells that produce tetanospasmin. The yeast cells measure 2 to 3 µm × 4 to 5 µm. Patients with hepatic abscesses may have symptoms such as fever, chills, and pain in the upper right quadrant or can be asymptomatic. Centers for Disease Control and Prevention: Public health then and now: celebrating 50 years of MMWR at CDC, MMWR 60(Suppl):1, 2011. novyi, and C. The vancomycin special potency antimicrobial disk set up at the same time as the aerotolerance test is used to determine the true Gram stain reaction of a pink-staining anaerobic bacillus. It is endemic in the humid, tropical belt, the tropical regions of Africa, parts of South America, India, and Indonesia, and many of the Pacific Islands. Diagnosis of Pontiac fever is usually limited to serology. Colony morphology varies with the extent of conidia production. Otherwise, aeromonads are generally susceptible to trimethoprim-sulfamethoxazole, aminoglycosides, and quinolones, although A. cholerae O139 V. Serologic assays are more valuable for epidemiology studies than for diagnostic purposes. For students, the Evolve website includes a laboratory manual. Adults, which may live as long as 15 years in humans, migrate through the subcutaneous tissue, causing temporary inflammatory reactions, called Calabar swellings. Other species of ticks, however, can be vectors. Bacteremia, renal failure, liver function abnormalities, watery diarrhea, nausea, vomiting, headache, confusion, lethargy, and other CNS abnormalities have been associated with these infections. It is important that the inoculated medium be fresh and moist and that the culture be incubated in a microaerophilic environment, with increased humidity. The ring forms of P. A summary of the published sensitivity, specificity, and negative and positive predictive values, as well as test specimens, is listed in Table 24-6. Once the organism has entered through the lungs or skin, it spreads hematogenously. The miracidium (first-stage larva) is ingested by a snail while within the egg or is released from eggs and penetrates the snail. In this cycle the rhabditiform larvae, rather than being passed in the stool, develop into the filariform larvae in the intestine. Centers for Disease Control and Prevention: Outbreaks of multidrugresistant Shigella sonnei gastroenteritis associated with day care centers—Kansas, Kentucky, and Missouri, 2005, MMWR 55:1068, 2006. coli strains on lactose-containing MAC or other routine enteric agar. This would be like taking the word “stops” and using the same letters to form the word “spots” or “posts,” which have different meanings but all the same letters. tuberculosis d. rustigianii, formerly identified as a strain of P. The causative agents responsible are organisms commonly found in soil or on decaying vegetation; therefore, agricultural workers are most often affected. The INNO-LiPA Rif TB (Innogenetics) is a reverse hybridization–based probe assay for the rapid detection of rifampin mutations leading to rifampin resistance in M. leprae fails to grow on artificial media. In addition, many people reported nausea, vomiting, and/or diarrhea. *Range—low to high prevalence as described in the text. Pritt BS, Clark CG: Amebiasis, Mayo Clin Proc 83:1154, 2008. Some of these kits are low-complexity and CLIAwaived. Discuss the sequence of events in the phagocytosis and killing of an infectious agent. can stain pink and thus appear to be gram-negative bacilli. Iolates of C. Adults may also exit through the mouth, tear duct, or nose and have been reported to enter and block catheters. If a culture is requested, organism identification should be limited to brief evaluation for S. However, not all strains produce a positive reaction after the culture is heated to 68° C for 20 minutes. The incubation period is usually 2 to 3 weeks, and early symptoms are nonspecific, consisting of headache, low-grade fever, malaise, and anorexia. Although grinding of tissue might be necessary for KOH and calcofluor white preparations, it should not be done unless sufficient tissue is present for mincing and grinding. IFA is the method of choice. sordellii C. In the downy form, conidia are less abundant. The viruses destroy their host cells. vulneris also produce yellow-pigmented colonies. Table 28-6 gives the general characteristics of trophozoites and schizonts of the human malarial species. Case in Point A 35-year-old infectious disease specialist complained of experiencing fever, chills, myalgia, and severe headache for the past several days. vulgaris ferments sucrose and gives an acid/acid reaction in triple sugar iron (TSI) agar. If more than one anamorph is present for the same teleomorph, the anamorphic strains are termed synanamorphs. Tubes are examined weekly for evidence of growth such as turbidity, haze, or a ring of growth. Most roundworm infections can be treated with oral albendazole or mebendazole. are susceptible to penicillin and other antimicrobial agents. tuberculosis may be detected as early as 7 to 8 days and M. In the United States, more than 90% of adults have antibody to VZV. putrefaciens is more frequently isolated from environmental sources. Although infrequently performed, environmental cultures may play a role in outbreak investigation. HBoV infection is highest during the winter months and has been detected worldwide in 5% to 10% of children 7 to 18 months of age with upper and lower respiratory tract infections. Preserved specimens that have been reported are reexamined as part of this program to see if the initial results (organism identification and quantification) are duplicated. Transcription is the synthesis of ssRNA (with the aid of the enzyme RNA Pyrimidines NH2 Purines O H3C N N H Cytosine C DNA and RNA O NH2 O N N H Thymine T N N O DNA only N H Uracil U RNA only O O N N H N N N H Adenine A N N Guanine G DNA and RNA FIGURE 1-12 Molecular structure of nucleic acid bases. A The taxonomy of B. Bacterial Vaginosis. Bacterial vaginosis (BV) is thought to arise because of a shift in the ecology of the endogenous microbiota of the vagina. It should be noted that a family-reactive monoclonal antibody can identify C. Onset of symptoms can occur weeks to months after infection, but once the brain is affected, the time to death is short. This commercially available medium contains Brucella agar base, 10% sheep red blood cells, and a combination of antimicrobials—vancomycin, trimethoprim, polymyxin B, amphotericin B, and cephalothin. The viral genome then directs the host cell to make viral proteins and replicate the viral genome. The domain Archaea (archaeobacteria) cell type appears to be more closely related to eukaryotic cells than to prokaryotic cells and is found in microorganisms that grow under extreme environmental conditions. pneumoniae must develop their own in-house protocols. The diagnosis of sparganosis is made by finding a small, white, ribbon-like organism with a rudimentary scolex. tahir99 VRG CHAPTER 22 Anaerobes of Clinical Importance 497 Why Some Organisms Are Anaerobes Where Anaerobes Are Found Molecular oxygen can be toxic to some anaerobes, but substances produced when oxygen becomes reduced through metabolic processes are even more toxic. Terminology for Direct Examinations FIGURE 7-5 Cytocentrifuge preparations deposit the concentrated sample within a limited area for viewing. A KOH wet mount is prepared with some of the scrapings; the KOH breaks down tissue, making it easier to view fungal hyphae. Lehman, George Manuselis.—Fifth edition. The wound caused by the tractor accident was likely contaminated by the soil containing the organism or its spores. 6. (Courtesy BD Diagnostic Systems, Sparks, MD.) combining with oxygen to form water. urethralis is a commensal of the genitourinary tract, but has been rarely associated with urosepsis, and there was a report of a case of infectious arthritis that was mistaken for gonococcal arthritis. In the latex agglutination assay, isolates must be tested with the negative control to detect nonspecific agglutination. The genus Hantavirus includes Hantaan virus, Seoul virus, Puumala virus, and Dobrava virus, which cause a disease called hemorrhagic fever with renal syndrome (HFRS). They are usually susceptible to quinolones, third-generation cephalosporins, piperacillin, and doxycycline, although not many isolates have been tested. Factors that have led to this greater awareness include the rising number of immunocompromised patients susceptible to infections caused by known pathogens and opportunistic organisms, the increasing number of people who travel to countries that have less than ideal sanitation and a large number of endemic parasites, and the growing population of immigrants from areas with endemic parasites. Microscopic examination of the culture shows fertile hyphae arising at right angles to the vegetative hyphae, producing alternating (separated by a disjunctor cell) hyaline arthroconidia (Figure 27-28). Description Establish a case definition. However, the persistence of hypnozoites that remain dormant in the liver can lead to relapses within 3 years of initial exposure. Finite cell lines, like primary cell lines, are diploid, meaning that they contain two copies of each chromosome. Armed with these test procedures, the laboratory scientist should be able to identify the most commonly encountered fungal isolates. B, Enlarged view of G. The balance between health and infectious disease is complex and mediated by humoral and cellular factors. Hafnia The genus Hafnia is composed of one species, H. Clinical Infections Acinetobacter spp. to red. • It acts as a sieve, allowing water-soluble molecules to enter through protein-lined channels called porins. Blader IJ, Saeu J: Communication between Toxoplasma gondii and its host: impact on parasite growth, development, immune evasion, and virulence, APMIS 117:458, 2009. lamblia are oval and measure approximately 8 to 12 µm × 7 to 10 µm. Some stools contain CharcotLeyden crystals, which are breakdown remnants of eosinophils; their presence in the stool is suggestive of an intestinal parasitic infection. The decision to identify many of the nonfermenters is based on the site from which they have been isolated—that is, whether they have been recoverd from a sterile site in pure culture or from a nonsterile site in which three or four other bacterial species are also present. This process or phenomenon is called opsonization. Develop an algorithm for the serologic diagnosis of viral hepatitis. Positive or equivocal results are confirmed with IgM and/or just IgG Western blot, depending on whether symptoms were present for longer than 30 days (IgG only). The three major biochemical pathways bacteria use to break down glucose to pyruvic acid are: (1) the Embden-Meyerhof-Parnas (EMP) glycolytic pathway (Figure 1-9), (2) the pentose phosphate pathway (Figure 1-10), and (3) the Entner-Doudoroff pathway (see Figure 1-10). In the CNS, the organism multiplies by binary fission and causes a necrotizing hemorrhagic infection similar to that of Acanthamoeba spp. Measles is easily diagnosed clinically, so few requests for laboratory identification are made. These outbreaks have been attributed to the plasmid transfer of antimicrobial resistance. In Versalovic J, et al, editors: Manual of clinical microbiology, ed 10, Washington, DC, 2011, American Society for Microbiology. Specimens that are acceptable for anaerobic culture are listed in Table 22-6. Klebsiella and Raoutella Members of the genera Klebsiella, Enterobacter, Serratia, Pantoea, Cronobacter, and Hafnia belong to the tribe Klebsielleae. Biochemical testing has limited value in the further identification of isolates to the species level. Skin testing previously demonstrated that about 80% of the population of longterm residents in endemic areas has been infected. The BACTEC 460TB system is an automated radiometric method using Middlebrook 7H12 (BACTEC 12B) containing a 14 C-labeled substrate (palmitic acid) that is metabolized by mycobacteria, liberating radioactive CO2 (14CO2) into the headspace of a glass vial. Photorhabdus The genus Photorhabdus includes three species: P. Lymphatic filariasis (caused by W. To enhance the red color of gram-negative anaerobes, the use of 0.1% basic fuchsin as the counterstain or extending the counterstaining with safranin for 3 to 5 minutes is recommended. These two key positive reactions help to distinguish it from the other three species. Once inside the cell, the virus loses its protein coat, releasing the genome. gladioli isolates are more susceptible to antimicrobials than B. histolytica, was named E. However, the clinical presentation is so similar to other conditions that misdiagnosis could occur, resulting in unnecessary surgical procedures, especially when confused with malignant melanoma. McAuley JB: Toxoplasmosis in children, Pediatr Infect Dis 27:161, 2008. Co-infections with another virus, including enterovirus, rhinovirus, influenza virus, and PIV; were detected in 8 (17%) of the infected children. Table 22-10 describes how the aerotolerance test is interpreted. *Polysciences, Warrington, PA. triviale have rapid arylsulfatase activity that can be detected in 3 days. It contains many important human and animal pathogens. What is the genus of this organism? Accessed June 14, 2013. coli to cause UTIs is the production of pili, which allow uropathogenic strains to adhere to epithelial cells and not be washed out with urine flow. There are more than 100 types of these small dsDNA viruses; more than 40 types are sexually transmitted and are known as the genital types. The rounding may be diffuse or focal, appearing like a cluster of grapes. Moraxella spp. Members of the usual microbial flora also may initiate an infection or make an infection more serious in patients with chronic illnesses, including diabetes or severe hepatic disease such as cirrhosis. caviae, has been showing increasing incidence of β-hemolysis on SBA. The cell demonstrates increases in glycolysis, the hexose monophosphate shunt pathway, oxygen use, and production of lactic acid and hydrogen peroxide. Gram stain characteristics may be atypical in antibiotic-treated and dead or degenerating organisms. In direct fluorescent antibody (DFA) tests, cells from a patient are fixed to a microscope slide and fluorescence-labeled antibodies are added. In addition, reports of antibodies directed against sporozoites and gametes (sexual reproductive cell that unites with another cell to form a zygote) indicate that these also may help reduce the rate of infection and the severity of illness. trematum). 740. lausannensis subsp. The malarial paroxysm, which begins 10 to 15 days after the bite of an infected mosquito, is associated with the growth of the parasites in RBCs. Symptoms are linked to the rupture of RBCs and release of merozoites (parasitic forms produced from multiple fission), malarial metabolites, and endotoxin-like substances into the bloodstream. This fifth edition discusses the continuing spread of infectious diseases and the emerging public health issues associated with them. The organism produces proteolytic enzymes such as collagenases and metalloproteases that facilitate tissue damage, and the host's inflammatory response helps increase the permeability of the barrier. coli cyst (trichrome stain). Saprobes are generally inhibited on this medium. tuberculosis to antimycobacterial agents: BACTEC 460TB, BACTEC MGIT 960, ESPII Culture System (Trek Diagnostic Systems), and MB/Bac T Alert 3D (bioMérieux). Clinical features of BV include a gray-white, homogenous, malodorous vaginal discharge, with little or no discomfort and no inflammation. mandrillaris, another freeliving ameba, is an emerging opportunistic pathogen that has been identified as a cause of skin lesions and GAE. During fission, two cells of equal size are formed. Two other gram-positive genera, Nocardia and Rhodococcus, may stain acid-fast by a modified method. mandrillaris is primarily associated with GAE and cutaneous lesions in humans. Describe the role of the usual microbial flora in the pathogenesis of infectious disease. This first line of defense has a limited capacity to distinguish one organism from another; however, previous exposure to a particular foreign substance is not required. Serotyping is accomplished by serum neutralization or hemagglutination inhibition. These include the following: • A foul odor on opening an anaerobic jar or bag. Organisms that can cause infection by penetrating the mucous membrane epithelium usually cannot penetrate unbroken skin. The ehrlichiae have a developmental cycle similar to that of the chlamydiae. mendocina produces nonwrinkled, flat colonies that may appear with a yellowish-brown pigment. nana is approximately 40 mm long and has a small scolex, with four suckers and a rostellum with spines. Which of the following Vibrio spp. Psychrobacter isolates of human origin, other than P. In the hemadsorption test, a suspension of RBCs is added to the infected cell monolayer. tahir99 VRG 614 PART II Laboratory Identification of Significant Isolates Life Cycle Pneumocystis is a nonfilamentous fungus. Numerous cases of Vibrio infections have been reported involving other serogroups, V. It should not be fixed with methanol before staining; fixing prevents lysis of the RBCs. Giemsa stain releases hemoglobin by lysing unfixed RBCs. Initial scanning of the stained smear at ×100 detects microfilariae. Hydrolysis of Tween 80. Some mycobacteria possess a lipase that can split the detergent Tween 80 into oleic acid and polyoxyethylated sorbitol. Complications may include respiratory, liver, or renal failure or disseminated intravascular coagulation. The scolex, if found, can also be used to distinguish the organisms. If an outbreak of smallpox did occur, there is sufficient smallpox vaccine to mount an effective vaccination program in the United States. The papule progresses to a pustule and then to an indurated eschar. It follows glycolysis and produces NAD. On microscopic examination, the organisms appear as small, fat bacilli or coccobacilli with intense peripheral yellowgreen fluorescence and darker centers. Because influenza viruses typically do not produce CPE, a hemagglutination or hemadsorption test is done to detect these viruses. The asaccharolytic members of the group, A. Discuss the development and transfer of antibiotic resistance in bacteria. Is highly contagious b. Dissemination via the circulatory system can lead to extrapulmonary infections with or without pneumonia. Occasionally short hyphal elements are also seen. (lactophenol cotton blue preparation, ×450). falciparum, erythrocyte membrane protein 1, is involved in mediating this attachment. Trypanosoma brucei rhodesiense and Trypanosoma brucei gambiense are the causative agents of human African trypanosomiasis, commonly called sleeping sickness, which is seen primarily in central Africa. They are not common agents of disease but have been known to cause opportunistic infection. veronii biovar sobria, A. For an additional example, see Corynebacterium diphtheriae in the section on transduction later in this chapter. These two chapters help students and practitioners who may have difficulty recognizing bacterial morphology on direct smear preparations, as well as colony morphology on primary culture plates, develop these skills through the use of color photomicrographs of stained direct smears and cultures from clinical samples. In a direct saline wet mount of a diarrheic stool, the trophozoite of E. The gastrointestinal tract is equipped with numerous defenses and effective antimicrobial factors. They are strictly aerobic and usually susceptible to penicillin, an unusual characteristic for a nonfermenter. In this case, antibiotic use altered the normal bacterial flora of the mouth and allowed yeast, probably Candida albicans, to cause an infection. The eggs are characteristically seen in packets of 15 to 25 eggs. parahaemolyticus are reported each year in the United States, although the actual number may be larger because many cases likely go unreported. The disease is identical to that which Ehrlichia equi causes in horses and Ehrlichia phagocytophilum causes in ruminants. In an iodine mount, the cytoplasm and central area of the vacuolar form stain brown. Ticks are the principal vector and reservoir for RMSF. Once the cysts are ingested, the gastric acid in the stomach triggers the beginning of excystation. Foreign proteins from these cercariae elicit a tissue reaction characterized by small papules 3 to 5 mm in diameter, edema, erythema, and intense itching. The recommended inoculum for the BACTEC 12B 4-mL vial is 0.5 mL of decontaminated concentrated specimen. paucimobilis does not grow on MAC agar and requires more than 48 hours for culture on SBA (Figure 21-16). Although Coccidioides spp. This species resembles the Neisseria by exhibiting gram-negative coccal morphology, and at one time was named Neisseria catarrhalis and also Branhamella catarrhalis. Geographic areas of tahir99 VRG 606 PART II Laboratory Identification of Significant Isolates highest incidence are typically humid, high-rainfall areas, with acidic soil conditions. This must include a written report that is kept on file and provided to all responsible individuals. The best time to collect eggs in urinary schistosomiasis is during peak excretion time in the early afternoon (noon to 2 PM). With the exception of the blood flukes, adult flukes are dorsoventrally flattened and have an oral sucker at the anterior end and a ventral sucker located midline, posterior to the anterior sucker. • Growth of large (>1 mm) gray-black colonies on BBE agar with growth on the KVLB agar after an overnight incubation is sufficient to identify an isolate as a member of the B. Vibrio parahaemolyticus 7. Define cytopathic effect and describe how it is used to presumptively identify viral agents. FIGURE 21-14 Elizabethkingia meningoseptica. Microbiota studies have found that anaerobes outnumber facultative anaerobes by a factor of 1000 : 1. Tularemia can also occur in pneumonic (contracted via the inhalation route), glandular, oro pharyngeal, oculoglandular, and typhoidal forms. On admission, physical examination showed that the patient was slightly febrile and in respiratory failure. Moellerella The genus Moellerella contains one species, M. Additionally, those who are severely immunosuppressed should avoid exposure of wounds to fresh, estuarine, and marine water sources. Isolates are weakly oxidase positive (some strains may be negative), motile at 18° C to 22° C but not at 37° C, indole negative, and oxidizers. Life Cycle. The sexual and asexual life cycles of Cryptosporidium occur in the same host. The cellophane tape preparation or commercially available sticky paddle is considered the diagnostic method of choice. Neither organism is associated with pica (eating dirt or other nonfood material). This process preserves the morphology and relationships of the microorganisms and cellular elements. Candida spp. Asexual reproduction results in the formation of conidia (singular, conidium) following mitosis. Because of their gram-positive nature, Mycobacterium and Nocardia spp. The egg, which is the most common stage found, is yellow-brown, round, and surrounded by a thick wall with radial striations; it measures 30 to 43 µm in diameter. However, PCR assay has been used to detect the virus in various specimens, including tissue, blood, bone marrow, saliva, and semen. Plesiomonas shigelloides phenotypically resembles the vibrios and aeromonads; however, this organism is the only oxidase-positive member of the family Enterobacteriaceae. Legionella spp. Serious clinical manifestations can develop if the mother acquires the primary infection during pregnancy. • Phenol, 5%, with a contact time of 10 to 30 minutes is adequate. gordonae, urease-negative (Figure 26-17). However, fungi have cell walls principally made of polysaccharides, such as chitin, mannan, and glucan. chelonae M. Representative organisms include Microsporum spp., Trichophyton spp., and Pseudallescheria boydii. Conventional Tubed Biochemical Identification Systems In general, conventional or traditional systems for the identification of anaerobic isolates use large test tubes containing various tahir99 VRG 518 PART II Laboratory Identification of Significant Isolates FIGURE 22-22 RapID ANA-II preformed enzyme system (Remel, Lenexa, KS). The insect vectors are sand flies of the genera Phlebotomus and Lutzomyia. Mature females produce microfilariae that are the infective stage for the insect during the insect's blood meal. At the base of the sporangiophores are brown rhizoids. Early infection includes two stages, the first of which is localized (stage 1). The initial symptoms, marked by high fever, chills, abdominal cramps, and pain accompanied by tenesmus, appear approximately 24 to 48 hours after ingestion of the organisms. It is used in determining the bacterial cell count in urine cultures. tahir99 VRG CHAPTER 19 Enterobacteriaceae TABLE 429 19-5 Differentiation of Common Species within the Genus Klebsiella K. lamblia has a worldwide distribution and has frequently been identified as the causative agent of outbreaks of gastroenteritis and traveler's diarrhea. Shifts result in novel strains of influenza virus, so the human population is likely to have little or no historic exposure or resistance to the new strain, which greatly increases the risk of pandemics. trematum are more recently described species and are agents of immunocompromised bacteremia (B. It must be used with caution because it is only tahir99 VRG 578 PART II Laboratory Identification of Significant Isolates slightly less harmful to the mycobacteria than to the contaminating organisms. The FDA has approved a microtiter method from Trek Diagnostic Systems (Cleveland, OH) and a diffusion method, Etest (bioMérieux). tropicalis (Figure 27-60). In general, the illness lasts about 1 week, and blood may appear in the stool. The time that the stool was passed and the time it was placed in the fixative should be noted on the laboratory requisition and container. Infections of these secondary hosts may give rise to strains that could potentially infect humans. B, Gram stain of Escherichia coli illustrating short gram-negative bacilli. Clinical Infection. A low number of cysts, as few as 10, can initiate infection with G. Sheep and other herbivores are the usual host of the larval stage (hydatid cyst). are rapidly growing phaeoid fungi, forming colonies ranging in color from brown to olivaceous to black. Microorganisms prevalent in the colon may produce a count between 108 and 1011 bacteria per 1 g of solid material. Sodium hypochlorite loses effectiveness in the presence of a large amount of protein material. B, Enlarged view of E. Knowledge of the composition of the microbiota at specific anatomic sites is useful for predicting the particular organisms most likely to be involved in infectious processes that arise at or adjacent to those sites. The genus Flavobacterium has undergone extensive taxonomic revision. Other characteristics include an erect conidiophore arising from a foot cell within the vegetative hyphae. In humans, plague can occur in three forms: the bubonic, or glandular, form; the septicemic form; and the pneumonic form. Media that are more complex and made of extracts of meat or soybeans are termed nutrient media (e.g., nutrient broth, trypticase soy broth). They are the result of an inflammatory and granulomatous reaction around the adult worms. During the second and third weeks of the disease, the patient generally experiences sustained fever with prolonged bacteremia. burgdorferi sensu lato is accomplished by a two-tiered serology. Subcutaneous layer FIGURE 27-10 Diagram of the layers of skin and tissues in which fungal infections can occur. Fader RC, et al: CACMLE self-study course 117: anaerobic bacteriology for today's clinical laboratory, Denver, 2007, Colorado Association for Continuing Medical Laboratory Education. The polysaccharide capsule of K. dermatitidis, and the endospores can be confused with the cells of C. cepacia, and B. tetani Peptostreptococci Propionibacterium acnes Colony Morphology on Blood Agar Cellular Morphology Spot Indole Large, flat colonies; barnyard odor, chartreuse fluorescence Large, irregular-shaped, double zone of β-hemolysis Smoothly swarming Very large, lobate, irregular, flat Smoothly swarming but slow growing Small, peaked, circular Small, opaque, enamel white, circular (catalase-positive) Thin rods, rare spores Boxcar, large, square rods Thin rods, subterminal spores Thin rods, subterminal spores Swollen terminal spores Cocci, pairs and chains Coryneform rods Negative Not done Negative Positive Positive Not done Positive *Additional information about presumptive identification of aerobes and facultative anaerobes can be found in Clinical and Laboratory Standards Institute: Abbreviated identification of bacteria and yeast: approved guidelines, ed 2, CLSI document M35-A2, Wayne, PA, 2008, CLSI. The factors that contribute to the ability of Legionella spp. Fluorescein isothiocyanate–labeled conjugates are available that detect all known serogroups of L. bronchiseptica + (3-5 days) + (2-3 days) + (1-2 days) − − + + − − − + − + − + (24 hours) − − + + + + + (4 hours) + + BOX 18-3 Summary of Laboratory Diagnosis of Pertussis by Culture FIGURE 18-31 B. Verotoxin II is biologically similar to, but immunologically different from, both Stx and verotoxin I. Adenoviruses are quite stable and can be isolated in human embryonic kidney, and many continuous epithelial cell lines. The condition is characterized by the presence of a crusted circular lesion on any exposed body surface, especially the face and extremities. This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein). FIGURE 29-4 Cytopathic effect of adenovirus on HeLa cells. Macroconidia are thin-walled, smooth, and cigar-shaped, with four to five cells separated by parallel cross walls. Microfilariae with no sheath and with nuclei that do not extend into the tip of the tail are characteristic of this organism. The family name is capitalized and has an “-aceae” ending (e.g., Micrococcaceae). psittaci strains that cause feline conjunctivitis, rhinitis, and respiratory infections among cats, guinea pig conjunctivitis, and abortion among ruminants have been replaced in three new species—Chlamydophila felis, Chlamydophila caviae, and Chlamydophila abortus, respectively. Brucella and Francisella spp. fowleri and Acanthamoeba spp. In immunocompetent hosts, the symptoms are self-limiting and persist for several weeks but may occur in a relapsing pattern for up to 2 months. Chronic mild diarrhea may be present. pseudomallei. organisms in water, soil, or sewage, and some are plant pathogens. When the chromatin split has been completed (mature schizont), each chromatin mass is surrounded by its own small amount of malarial cytoplasm (merozoite). haematobium, which is primarily found in the Nile Valley, the Middle East, and East Africa, lives in the veins surrounding the bladder. outbreak at the state fair discussed earlier, consider the number of fecal specimens that the health department laboratory processed, although patient cultures are only one component of the investigative activity. Single-Stranded DNA Viruses Parvoviridae FIGURE 29-11 Negatively stained transmission electron micrograph of the smallpox (variola) virus (×100,000). FIGURE 28-9 Endolimax nana trophozoite, identified by arrow (trichrome stain, ×1000). KOH, Potassium hydroxide; LPCB, Lactophenol cotton blue. A sheep blood agar (SBA) plate showed very moist large isolated colonies. Step nine, the final step and one of extreme and often overlooked importance, is to communicate the findings of the investigation. Once the larva has penetrated the skin, it enters the circulation, breaks out from capillaries in the lung, migrates up the bronchial tree and over the epiglottis, and enters the digestive tract, where it matures into the adult worm. Toxins, molecules that break down tissue, capsules that inhibit phagocytosis by macrophages, and adherence factors that aid in attachment to mucosal surfaces are thought to play a role in pathogenicity (Table 22-5). With the exception of the schistosomes, which infect humans by direct cercarial penetration, infection occurs when an individual ingests the metacercaria in raw or undercooked aquatic animals or on water vegetation. Define the atmospheric requirements of obligate aerobes, microaerophiles, facultative anaerobes, obligate anaerobes, and capnophilic bacteria. An anaerobic holding system should therefore be used in conjunction with any of the anaerobic bags. This characteristic occurs despite growth conditions and is best observed in Exophiala spp., in which the yeast phase is typically observed initially, followed by the mold phase as the colony ages. tahir99 VRG Connie R. On the other hand, if more than 5% of all specimens cultured are contaminated, the decontamination procedure may be inadequate. Define the following terms: parasitism, indigenous flora, commensal, symbiont, opportunist, resident flora, transient flora, and carrier. Studies have demonstrated that these media perform better than other commercially available media. They are currently placed in the genus Norovirus. One is the A. Direct antigen detection and serologic procedures for the diagnosis of histoplasmosis might be adjuncts to culture methods. in the clinical laboratory might be the first objective indication of suboptimal vaccination of children, colonization in adults, or waning immunity in adolescents—any of which may set the stage for an explosive outbreak because of the ease with which bacteria are transmitted person-to-person. pneumoniae does not occur as a normal commensal; therefore, its isolation is always significant and pathognomonic. This virus was named the Middle East respiratory syndromecoronavirus (MERS-CoV). Multicelled conidia have angular cross walls and taper toward the distal end. Their genome may be doublestranded DNA (dsDNA), single-stranded DNA (ssDNA), double-stranded RNA (dsRNA), or single-stranded RNA (ssRNA). Dark field microscopy requires considerable skill and experience; however, demonstration of motile treponemes in material from the chancre is diagnostic for primary syphilis. Phase contrast microscopy of direct wet mount preparations of corneal scrapings can reveal the trophozoite or cyst. tuberculosis from other mycobacteria, and the other side contains CHOC agar for the detection of contaminating bacteria. Specimen Collection, Handling, and Transport Collection of appropriate specimens is the primary criterion for accurate diagnosis of mycotic infections. Isolates of D. Growth (increase in size) does not occur in viruses. CL, colistin (10 µg); K, kanamycin (1000 µg); SPS, sodium polyanethol sulfonate; V, vancomycin (5 µg). SaVs are small (30 to 35 nm in diameter) diarrheagenic viruses distinguished by a cup-shaped morphology. sibonii. Garcia LS: Malaria, Clin Lab Med 30:93, 2010. Aspirates, or surface swabs, are usually appropriate for lesions. lamblia has caused outbreaks of diarrhea in nurseries and daycare centers as a result of person-to-person contact. The condition of these hosts is called the carrier state. Histologic stains such as Giemsa and Gomori methenamine silver are used. Compare primary amebic meningoencephalitis and granulomatous amebic encephalitis. The authors reported sensitivities for the NAATs of 88.5% to 96.2% and 42.3% and 61.5% for the non-NAATS. cholerae O139, and V. The presence of sclerotic bodies is diagnostic for this disease. Four genera of nonfermenters make up most isolates routinely seen in clinical laboratories—Pseudomonas aeruginosa, Acinetobacter spp., Burkholderia spp., and Stenotrophomonas maltophilia. Botulinum toxin attaches to the neuromuscular junction of nerves and prevents the release of acetylcholine (a neurotransmitter), which results in flaccid paralysis and death. Parasitic infections can be difficult to diagnose, however, because patients often have nonspecific clinical symptoms that can be attributed to a number of disease agents. The Firmicutes include many genera, but Lactobacillus is the only member encountered on a routine basis in the clinical microbiology laboratory. Measurement of any of these properties can be used as an indication of bacterial growth. Bacterial Genetics Carbohydrate Utilization and Lactose Fermentation The ability of microorganisms to use various sugars (carbohydrates) for growth is an integral part of most diagnostic identification schemes. The eight possible combinations of results are then used to group the nonfermenters, as shown in Figure 21-1. The collection systems are considered equivalent, although the Isolator system allows for quantitative analysis, which may be used to monitor therapy and evaluate prognosis. Prevotella sp. The streptococcal infection produces a peritonsillar abscess containing a number of bacterial species. It may, however, account for 5% to 15% of all nosocomial infections, especially pneumonia and bacteremia. Capsules act as virulence factors in helping the pathogen evade phagocytosis. express a capsule that produces the characteristic mucoid colony. Non-nutrient agar is seeded with an Escherichia coli overlay, and the spinal fluid sediment is inoculated onto the medium. The family Rickettsiaceae includes the genera Rickettsia and Orientia. Inside the cell, they become trophozoites and divide by asexual reproduction (budding) to form merozoites. Mutations A gene sequence must be read in the right “frame” for the correct protein to be produced. Direct Fluorescent Antibody Test. The DFA test is a rapid laboratory procedure for detection of the more common species of Legionella found in clinical samples from the lower respiratory tract. Microscopic examination of hyphae associated with the Zygomycetes often reveals occasional septations; therefore these hyphae are more correctly termed sparsely septate as opposed to aseptate. For the following three organisms, Toxoplasma gondii, C. Many fungi routinely recovered can be identified by either of these two methods. Tarnvik A, Berglund L: Tularaemia, Eur Respir J 21:361, 2003. histolytica (extraintestinal infections) are available for use in clinical laboratories. All aeromonads, in general, can typically grow from 4° C to 42° C. Researchers have developed polymerase chain reaction (PCR) assays, which increase the sensitivity of nucleic acid probes. cholerae, V. Arylsulfatase. Most members of the genus Mycobacterium possess the enzyme arylsulfatase. PIV-1 and PIV-2 cause the most serious illnesses in children between 2 and 4 years of age. The macular rash progresses into papules, then vesicles, and finally into pustules, which resemble chickenpox lesions. Slime layers are similar to capsules but are more diffuse layers surrounding the cell. Microsporum gypseum The fusiform, moderately thick-walled conidia (Figure 27-15) typical of Microsporum gypseum measure 8 to 15 µm × 25 to 60 µm and can have as many as six cells. Skin test reactivity to fungal antigens is sometimes used in the diagnosis of fungal allergies and infections. The organism is refractile, with the characteristic bull's eye nucleus, consisting of a small central karyosome. Fungi can cause mild infections, trigger allergic reactions, including asthma, and produce serious life-threatening disease. pestis may be isolated on routine culture medium. schenckii revealing hyaline conidia borne at the ends of conidiophore in “rosettes” as well as phaeoid conidia along the sides of the hyphae (Nomarski optics, ×625.) The morphology of the systemic dimorphic at 22° C is described in Table 27-4. Clostridium perfringens is an example of a highly invasive organism that may not disseminate. Because mycobacterial species may show only quantitative differences in the enzymes used in biochemical identification, no single biochemical test should be relied on for the identification of a species. A newer assay using real-time, reverse transcriptase–polymerase chain reaction (RT-PCR) is now the most commonly used diagnostic assay for detecting NoV. Vibrio vulnificus Vibrio vulnificus can be found in marine environments on the Atlantic, Gulf, and Pacific coasts of North America. This outbreak indicates a year-round risk of infection to institutionalized older patients. At a minimum, specimens for anaerobic culture should be plated onto a nonselective blood agar (e.g., Brucella or CDC blood agar), a biplate containing Bacteroides bile esculin (BBE), and kanamycin and vancomycin with laked sheep blood (KVLB) agars. Note the short buccal capsule and prominent genital primordium. The Etest (bioMérieux) is being used with increasing frequency for performing susceptibility testing on the rapid growers. Nucleic Acid–Based Detection An increasing interest in nucleic acid–based detection assays compared with traditional cell culture methods has shifted the focus of clinical virology. Points to Remember includes a bulleted list of important concepts that the reader should have learned from reading the chapter. (lactophenol cotton blue preparation, ×1250). However, Proteus spp. 3071. In vitro susceptibility testing of the nonfermenters is not always necessary. The inner peptidoglycan layer is much thinner than in gram-positive cell walls. Laked blood is blood lysed through a series of freeze-thaw cycles. Several dilutions should be used (undiluted, 1 : 10, and 1 : 100) and/or filtered (0.45 µm) to minimize the effects of inhibitory substances. Four pairs of flagella, midline axonemes, and two median bodies posterior to the nuclei are also present. A better presentation of granules is possible if granules or grains are “fished” from tahir99 VRG 128 PART I Introduction to Clinical Microbiology the surrounding materials and crushed on a separate slide using the technique shown in Figure 7-3. In the E. Available at: 0807.html. If specific rRNA is present, a stable DNA-RNA complex, or hybrid, is formed. IF, EIA, and nucleic acid amplification assays can also be used to detect and identify viruses in cell cultures. In situ hybridization can detect HHV-8–affected tissue. Trypanosoma cruzi: Chagas disease. stercoralis larval penetration of the skin does not cause a prominent papule, and migration through the lungs rarely elicits pneumonitis, but the patient may have wheezing and a mild cough. putrefaciens produces a tan to brown pigment. It was named the human bocavirus (HBoV) and causes a variety of upper and tahir99 VRG 704 PART II Laboratory Identification of Significant Isolates lower respiratory tract illnesses. vulgaris are easily identified in the clinical laboratory because of their characteristic colony morphology. Figure 28-66 shows a biopsy specimen of a muscle containing a larva of T. They are nonmotile, facultative anaerobes that ferment carbohydrates and are generally oxidase- and catalase-positive. Laboratory Diagnosis. The diagnostic stage in humans is the trypomastigote, which is usually seen in a Wright-stained blood smear, although wet films may be used to detect motile trypomastigotes. It has also been postulated that antigenic variation of cell surface proteins contributes to the organism's ability to evade host immune response and establish persistent infection. After the safety cabinet work area has been cleaned with disinfectant, the ultraviolet (UV) light, which kills microorganisms, inside the cabinet should be used to eliminate any further contamination of surfaces and airborne bacteria. Vinh DC, et al: Vibrio vulnificus septicemia after handling Tilapia species fish: a Canadian case report and review, Can J Infect Dis Med Microbiol 17:129, 2006. In other settings, physicians may expect periodic updates, including antibiograms and pathogen prevalence. Chako CZ, et al: Cryptosporidiosis in people: it's not just about the cows, J Vet Intern Med 24:37, 2010. This exposes the bacteria to oxygen, which is especially harmful to the anaerobes during their first 48 hours of growth. coli strains do decarboxylate lysine. loa, the eye worm, is limited to the African equatorial rain forest, where the fly vector (Chrysops) breeds. Common characteristics include the following: • Most grow on Simmons citrate and in potassium cyanide broth. Girginkardesler N, et al: Transmission of Dientamoeba fragilis: evaluation of the role of Enterobius vermicularis, Parasitol Int 57:72, 2008. The cells are then seeded onto a surface to form a monolayer, such as in a flask or test tube. First, it provides a rapid report to the physician, which may in turn result in the early initiation of treatment. putida, when it was found that some isolates had low-level nucleic acid relatedness to other Pseudomonas strains. tahir99 VRG CHAPTER 19 Enterobacteriaceae Aerobactin allows the bacterial cell to chelate iron; free iron is generally unavailable within the host for use by bacteria. immune defenses located at different body sites. Contact lens wear is a risk factor for corneal infection. The outbreak was likely caused by Campylobacter infection. The presence of large gram-positive bacilli with colonies exhibiting a double zone of β-hemolysis in the absence of organisms recovered on aerobic cultures of the specimen is sufficient to report a presumptive identification of C. which ruptures within the intestine and results in autoinfection, and the thick-walled oocyst, which is infective when passed in the stool. Ingestion of the infective oocyst initiates the asexual cycle (sporogony) with the release of sporozoites. Detection of immunoglobulin M (IgM) can be useful in identifying infection during the acute phase, but this class of antibody generally declines to nondetectable levels as the infection begins to resolve. Numerous studies have shown that the radiometric BACTEC isolation method significantly improves the isolation rate of mycobacteria and reduces the recovery time compared with conventional isolation media. Immunization programs began in the United States in 1963, and the reported number of cases dropped to fewer than 1500 by 1983. Depending on statutory requirements, the laboratory may also be responsible for the reporting of certain infectious diseases to public health jurisdictions. Permanent stains commonly used include iron hematoxylin and trichrome (Wheatley modification of the Gomori stain). Table 20-7 shows the composition of each of these selective media. Throat cultures have a much lower sensitivity and should be discouraged. Isolation and Identification Isolation Methods Since the original development of Bordet-Gengou potato infusion agar with glycerol and horse or sheep blood, few alternative formulations have been as successful. They are ribbon-like organisms whose method of growth involves the addition of segments, termed proglottids. sanguis, and Streptococcus salivarius. Herpes zoster is the clinical manifestation caused by reactivation of VZV; it usually occurs in adults. Centers for Disease Control and Prevention: Balamuthia mandrillaris transmitted through organ transplantation—Mississippi, 2009, MMWR 59:1165, 2010. Develop an epidemiologic curve. Optimally, several different cell lines will be used for a single specimen to recover different viruses that might be present, similar to the strategy used with media for the recovery of bacteria. Infected patients can present with a wide variety of conditions ranging from asymptomatic infection to life-threatening disease. tahir99 VRG 464 PART II Laboratory Identification of Significant Isolates The medical history of patients displaying diarrhea and harboring aeromonads often, but not always, involves aquatic exposure, such as an association with untreated groundwater or consumption of seafood, particularly raw oysters or clams. Chappuis F, et al: Options for field diagnosis of human African trypanosomiasis, Clin Microbiol Rev 18:133, 2005. Mims C, Nash A, Stephen J: Mims' pathogenesis of infectious disease, ed 5, San Diego, 2001, Academic Press. • None produce H2S. Although infection with the organisms is often linked to HIV infection, microsporidia have been identified in organ transplant patients, older adults, and patients with traveler's diarrhea. There tahir99 VRG 468 PART II Laboratory Identification of Significant Isolates have been reports of resistance to more than one aminoglycoside (e.g., gentamicin, tobramycin, amikacin), but the quinolones, cephalosporins, and carbapenems appear to be effective therapy. Leptospires are most likely to enter the human host through small breaks in the skin or intact mucosa. The organism has also been linked to several large foodborne outbreaks in North America. Ability to Resist Phagocytosis Phagocytic cells, such as macrophages and polymorphonuclear cells, play a major role in defending the host from microbial invasion. Daboul MW: Is the amastigote form of Leishmania the only form found in humans infected with cutaneous leishmaniasis? For example, E. Conversion of the mold form to the yeast form, using brain-heart infusion (BHI) agar incubated at 37° C, is confirmatory for H. Available at: . Some group members oxidize carbohydrates to derive energy for their metabolism; they are referred to as oxidizers. Most strains can grow on MAC agar. Colonies can be white, tan, or brown and may be fluffy to glabrous. are nonvacuolated, coccoid bacteria, forming pairs and short chains. For example, many patients who have indwelling urinary catheters develop a urinary tract infection. Mister, MS, MT(ASCP)SM Educational Coordinator, Medical Microbiology Johns Hopkins Hospital Baltimore, Maryland Linda S. Discuss the increasing incidence of infections caused by various nonfermenters in patients with cystic fibrosis. One of two courses may then be taken. Microorganisms that colonize an area for months or years represent resident microbial flora, whereas microorganisms that are present at a site temporarily represent transient flora. Flagella that extend from one end of the bacterium are polar. He had a history of periodontal disease and recently had two teeth extracted. A typical influenza season in the United States hospitalizes almost 200,000 and kills almost 36,000 people. In addition to persistent antigenuria following clinical disease mentioned earlier, prolonged secretion has been associated with immunosuppression, renal failure, and chronic alcoholism. The most common presentation is cellulitis, although there have been a few cases of myonecrosis and necrotizing fasciitis and even a rare case of ecthyma gangrenosum associated with sepsis. If the specimen has been preserved in 10% formalin, the drop of saline may be omitted from the unstained preparation. Photoreactivity. Mycobacterium spp. With congenital syphilis, comparing antibody responses in the mother's and baby's serum can aid diagnosis. Fungal elements fluoresce apple green or blue-white, depending on the combination of filters used on the microscope; therefore, any element with a polysaccharide skeleton fluoresces. In Isenberg HD, editor: Essential procedures for clinical microbiology, Washington, DC, 1998, American Society for Microbiology. entomophila, and S. has three stages—the trophozoite, which is 1 to 5 µm in size and is irregularly shaped; the precyst, 5 to 8 µm; and the cyst, which is a thick-walled sphere of about 8 µm containing up to eight intracystic bodies. The hemoglobin was 6.2 g/dL (reference range, 9 to 14 g/dL), and the reticulocyte count was 8% Issues to Consider After reading the patient's case history, consider: ■ Which features in the patient's history are significant ■ Which intestinal parasites should be considered as part of the patient's differential diagnosis ■ What additional laboratory tests could aid in the diagnosis tahir99 VRG 625 626 PART II Laboratory Identification of Significant Isolates Key Terms Amastigote Appliqué form Asexual reproduction Blackwater fever Bradyzoites Cercaria Chromatoidal bars Cyst Cysticercus Definitive host Erythrocytic phase Exoerythrocytic phase Filariform larvae Gamete Gametocyte Hexacanth embryo (oncosphere) Intermediate host Karyosome Kinetoplast Maurer's dots P Merozoite Metacercaria Microfilariae Peripheral chromatin Polyvinyl alcohol (PVA) Proglottid Rhabditiform larva Schizogony Schizont Schüffner's stippling Scolex Sexual reproduction Sporoblast Sporocyst Sporogony Sporozoite Tachyzoite Trophozoite Trypomastigote Vector Ziemann's dots arasites are an important cause of human morbidity and mortality. Multiplication of nuclei in the cyst stage also serves a reproductive function. africanum, M. acnes is considered an opportunistic pathogen and has been associated with cases of endocarditis, surgical wounds, and prosthetic joint infections. Aeromonas Clinical Manifestations The genus Aeromonas consists of ubiquitous, oxidase-positive, glucose-fermenting, gram-negative rods (Figure 20-5) that are widely distributed in freshwater, estuarine, and marine environments worldwide. Because of slow growth rates, F. Purines: adenine and guanine. xenopi, M. The organisms invade the cells lining the gut of the louse. Propionibacterium acnes is frequently isolated from blood cultures, but its presence often represents contamination from the patient's skin resulting from poor site preparation during the venipuncture. The CLSI recommends broth microdilution testing for S. Several different STEC strains have been identified; O157:H7 is only the first to have been widely reported. Dissemination of E. Anti-EA/R IgG antibody is elevated in patients with Burkitt lymphoma. Nakano, Centers for Disease Control and Prevention, Atlanta, GA.) of contention between scientists who advocate their destruction and those who wish to continue their study. Describe the microscopic and colony morphology of Bordetella spp. Like other vibrios, V. In general, toxoplasmosis in the immunocompetent host is not treated. houtenae (subspecies IV), and S. There also have been reports indicating simultaneous transmission of B. The lesion begins as a small, red papule at the site of the insect bite and progresses to a lesion with an elevated indurated margin that may reach 8 cm. cause keratitis, skin infections, or granulomatous amebic encephalitis, whereas Balamuthia is associated with GAE and skin infections. Their thick protein coat makes them highly resistant to chemical agents, temperature change, starvation, dehydration, ultraviolet and gamma radiation, and desiccation. Chryseobacterium indologenes is the most frequently isolated species in this genus found in human specimens, although it is often considered insignificant when isolated in rare numbers or from only one medium or one specimen. rhinoscleromatis were considered true species. coli population or as a small part of the E. Compare and contrast chromoblastomycosis and eumycotic mycetoma. More than 100 serotypes exist. hMPV infections usually occur in children. With what two diseases do you see this stage in the blood? tuberculosis complex organisms do not produce heat-stable catalase; exceptions are certain strains resistant to isoniazid. Only rarely will it invade nasally and spread along nerve fibers to the olfactory bulb. Transmission of the organism is known to occur through the respiratory route, with the cyst being the infective stage. In addition, to comply with mandatory infectious disease safety precautions, it is recommended that disposable gloves be worn if clinical specimens are being processed in gloveless chambers. The rash of boutonneuse fever, however, also involves the face. mallei is a nonmotile, gram-negative coccobacillus that produces nonpigmented colonies in 2 days. It was reported that 300 soldiers received treatment. The mollicutes, a common name used to describe members of the class Mollicutes, are characterized by permanently lacking a cell wall. Historically, they have been used to confirm positive nontreponemal test results, although some laboratories use reverse sequence syphilis screening. variicola has been isolated from primarily sterile sites. Stool decontamination is especially difficult and may require repeated attempts. marinum from M. The mortality rates for untreated or incorrectly treated patients can be as high as 20%, although correct antimicrobial therapy with tetracycline or chloramphenicol lowers the rates to 3% to 6%. may have fever, nonproductive cough, difficulty breathing, and a low-grade fever. maltophilia is rarely associated with lower respiratory tract infections, although it has been isolated from 6.4% to 10.2% of patients with CF. The microvilli are stunted and adsorption is reduced. Name two selective media for the detection of the mycoplasmas. Botulism. Foodborne botulism results from the ingestion of preformed botulinum toxin, produced in the food by C. Humans are the only host, and the presence of infection with the organism is associated with other sexually transmitted diseases, especially gonorrhea. The combined use of ampicillin SBA and a modified CIN II plate, with only 4 µg of cefsulodin instead of 15 µg, might yield the highest recovery of aeromonads. Mature sporozoites are released into the body cavity of the mosquito and migrate to the salivary glands. 453 ■ The use of an initial battery of selective agar media and key biochemical tests such as TSI, urea, and motility agars can usually result in a presumptive genus identification that can be confirmed with either conventional biochemical test or with the use of one of several multitest or rapid and automated identification systems. Similarly, P. Microbiology personnel must encourage immediate transport of clinical samples to the laboratory for processing, regardless of the source of the clinical specimen. werneckii include Phaeoannellomyces werneckii and Exophiala werneckii. This assay detects viral RNA and can be used to test stool, vomitus, and environmental samples. fetus and C. Ventilation Laboratory design and ventilation play important roles in mycobacteriology laboratory safety. The types of disease caused by these fungi are as varied as the species, sometimes more so, because a given fungus can have multiple clinical presentations. Specimens may be examined directly by IF and EIA methods. For refractile or late stages, prolonged treatment with ceftriaxone has been effective. The mortality rate of symptomatic patients is 3% to 20%. Because of the rapid dissemination of the tahir99 VRG CHAPTER 24 Chlamydia, Rickettsia, and Similar Organisms bacteria, a number of tissues can be infected, resulting in a chronic disease. The respiratory tract is also continuously exposed to microorganisms and is protected by nasal hairs, ciliary epithelium, and mucous membranes. ✓ 531 Isolation and Identification Isolation of leptospires is accomplished by direct inoculation of 1 to 2 drops of freshly drawn blood or CSF into laboratory media such as Fletcher, Stuart, or EMJH, and incubating the media in the dark at room temperature. These tests use nonisotopically labeled (e.g., an acridine ester–labeled nucleic acid) probes specific to mycobacterial ribosomal RNA (rRNA). DF, which is a relatively mild infection, occurs when patients are bitten by mosquitoes carrying the virus. falciparum and mature trophozoites and gametocytes that resemble those of P. In the clinical laboratory, most mold identifications are based on the structures formed as a result of asexual reproduction. Although infection with E. mallei and B. Pili (plural of pilus), also known as conjugation pili, are nonmotile, long, hollow protein tubes that connect two bacterial cells and mediate DNA exchange. Coronaviruses are extremely fragile and difficult to culture, but it is possible to test specimens directly by IF and EIA methods. The major disadvantage of this procedure is the disruption of conidia during the teasing process. Explain the use of the following stains in the diagnostic microbiology laboratory: Gram stain, acid-fast stains (Ziehl-Neelsen, Kinyoun, auramine-rhodamine), acridine orange, methylene blue, calcofluor white, lactophenol cotton blue, and India ink. Wash with running tap water, drain, and air dry. gambiense antigen to detect antibodies in the blood. albicans can be part of the normal biota, the infection was probably endogenous (originated from within). This cycle repeats itself at regular intervals, depending on the species of malarial organism present. cayetanensis is endemic in Nepal, Peru, Guatemala, and Haiti, but outbreaks have been reported in many countries, including those in Central and South America, parts of the Caribbean, India, and Europe. mimicus are nonhalophilic and grow well without additional salt, any salt tolerance test must be used in conjunction with the string test and O/129 disk to distinguish aeromonads from this major pandemic cholera species and the less common, sucrose-negative V. Individuals can have detectable antibodies but not an active infection. Strains of V. An anticodon is the triplet of bases on the tRNA that bind the triplet of bases on the mRNA. Patients with sickle cell disease and other hemolytic disorders, ulcerative colitis, and malignancy seem to be more susceptible to Salmonella spp. The cercaria then attaches to aquatic vegetation or invades the flesh of aquatic organisms. Left, Colonies of B. Isolates ferment glucose and, variably, sucrose; they grow on MAC agar and most enteric media, reduce nitrate, and grow at 42° C. List risk factors for acquiring legionellosis. Drinking unpasteurized milk 6. Tissue damage during migration through the liver can result in tahir99 VRG CHAPTER 28 Diagnostic Parasitology TABLE 665 28-7 Comparisons of Fluke Eggs Organism Average Size (µm) and Shape Other Identifying Features Fasciola hepatica 130 × 60-90, ellipsoidal Small, indistinct operculum Yellow-brown color Unembryonated when passed Fasciolopsis buski 130 × 80-85, ellipsoidal Cannot be distinguished from F. A commercially available two-vial system using polyvinyl alcohol (PVA), a resin polymer, in one vial and 10% formalin in TABLE 28-1 Preservatives Commonly Used for Fecal Samples Preservative Polyvinyl alcohol (PVA) 10% formalin Sodium acetate–acetic acid– formalin (SAF) Merthiolate-iodine-formalin (MIF) Single-vial systems PCR, Polymerase chain reaction. Lysosomes contain hydrolytic enzymes for degradation of macromolecules and microorganisms within the cell. tularensis growth. mosselii are nonpigmented and nonhemolytic, positive for oxidase, catalase, and ADH; optimal growth occurs at 30° C. Legionella Christian Whelen CHAPTER OUTLINE ■ GENERAL CHARACTERISTICS ■ CLINICAL SIGNIFICANCE Virulence Factors Infections Caused by Legionella Epidemiology ■ LABORATORY DIAGNOSIS Specimen Collection and Handling Microscopic Examination Isolation and Identification Serologic Testing ■ ANTIMICROBIAL SUSCEPTIBILITY tahir99 VRG CHAPTER 18 Haemophilus and Other Fastidious Gram-Negative Bacilli 409 OBJECTIVES After reading and studying this chapter, you should be able to: 1. Infections caused by β-lactamase–positive H. These jars have been used in clinical laboratories for many years, enabling even small laboratories to perform satisfactory anaerobic bacteriology. Anaerobic Incubation of Inoculated Media After specimens are processed and inoculated onto the appropriate media, the inoculated plates must be incubated anaerobically at 35° C to 37° C. Analysis of these end products is particularly useful for the identification of anaerobic bacteria. BV may result if the delicate balance between lactobacilli and other bacteria representing the normal vaginal biota is disrupted. The disease is primarily seen in sheep-raising areas of the world, including Australia, southern South America, and parts of the southwestern United States. Viruses cannot be seen under an ordinary light microscope, although their cytopathic effects on cell lines are visible. In addition, isolates have been linked to antibiotic-associated hemorrhagic colitis. Screen B. Bacterial genetics is increasingly important in the diagnostic microbiology laboratory. Histologic stains for tissue may also be useful. Aerotolerant anaerobes, previously referred to as facultative aerobes, can survive in the presence of oxygen but do not use oxygen in metabolism (e.g., certain Clostridium spp.). Lymphocytes originate in the bone marrow from stem and progenitor cells. bovis, M. Today, all donor blood and blood products are screened for HIV, which substantially reduces the risk. sordellii is the only Clostridium species that is urease-positive. hominis Oropharynx Oropharynx Oropharynx Oropharynx Oropharynx Oropharynx Urogenital tract Oropharynx M. Fungal media can be poured into Petri dishes or large test tubes. Sporadic cases in Missouri, Kentucky, and Washington State occurred with an organism that more closely resembles Babesia divergens, a parasite of cattle in Europe. The recognition of emerging pathogens d. Penicillinase or β-lactamase producers such as S. The lysis centrifugation system, the Isolator tube (Wampole, Cranbury, NJ), is the most sensitive method for the recovery of some fungi. It is an individual laboratory's decision whether such identification will suffice or whether definitive identifications on some isolates are necessary. Note the growth with yellow pigment on sheep blood agar (SBA) (left) and absence of growth on MacConkey plate (right). Urine 30 S. The number and arrangement of flagella are sometimes used for identification purposes. Most infections occur in children and adolescents, and 50% of adults are seropositive by the age of 50 years. In symptomatic patients fever, respiratory distress, cough, anorexia, headache, malaise, and myalgias can manifest for 6 weeks or longer. Clinically, the patient experiences flulike symptoms for approximately 1 week, which follows an incubation period of approximately 7 days. Centers for Disease Control and Prevention: A National strategic plan for public health preparedness and response; September 2011. The delicate nuclear membrane has no peripheral chromatin, and the karyosome consists of four to eight discrete granules, one of which is often larger than the others. Antibodies developed against one of the organisms can provide protection against infection by the same species, but antibodies developed against one species may provide only incomplete protection against another species. A physical examination demonstrated bilateral lower extremity weakness, and his reflexes were slowed throughout his body. The parasitic female threadworm is small (2.5 mm) and rarely seen in a stool specimen. Swabs must be made of Dacron or rayon. The serologic identification of the Vi antigen is important in identifying Salmonella serotype Typhi. This can be the result of tubercle burden at the collection site or of environmental effects, such as pH, that can affect recovery. The organism is round and has a ring of cytoplasm lining the inside of the plasma membrane, with as many as four nuclei present, which are usually pushed to the side. Disk diffusion susceptibility testing revealed the isolate to be susceptible to fluoroquinolones, carbapenems, third-generation cephalosporins, and piperacillintazobactam, but resistant to ampicillin, aminoglycosides, and colistin. Centers for Disease Control and Prevention: FDA approval of expanded age indication for a tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine, MMWR 58:374, 2009. The larvae are released from the tissue capsule in the intestine and mature into adults. In the field of biotechnology, phages are often used to insert cloned genes into bacteria for analysis. Coronaviruses infect a number of different animals; however, most individual strains of virus typically only infect a single animal species. The distinguishing characteristics of the microfilariae are the presence of a sheath and the arrangement of tail nuclei—the nuclei extend to the tip, but a space separates the two terminal nuclei. This organism should be considered especially when a nonfermentative wrinkled colony is isolated (Figure 21-11) that demonstrates bipolar staining on Gram-stained smears. stercoralis because treatment is different. A human becomes an intermediate host by accidentally ingesting the eggs of E. The morulae are found in granulocytes (Figure 24-11), and the sensitivity is about 60% because of a large number of infected white blood cells. haemophilum grow best at 30° C to 32° C and poorly, if at all, at 35° C to 37° C. The organisms colonizing different body sites play a significant role in providing host resistance to infections. Rickettsialpox has similarities to RMSF but is a milder infection. Cell Wall. The cell wall of prokaryotes is a rigid structure that maintains the shape of the cell and prevents bursting of the cell from the high osmotic pressure inside it. Figure 28-6 shows the cyst of E. See Table 26-1 for a summary of the identification characteristics of clinically important mycobacteria. Harrington, PhD, D(ABMM) Director, Microbiology Service Assistant Professor, Pathology University of Illinois at Chicago Chicago, Illinois Deborah Ann Josko, PhD Associate Professor Director, Medical Laboratory Science Program Rutgers, The State University of New Jersey School of Health Related Professions Newark, New Jersey Edward F. The life cycle of S. Cryptococcus gattii is an emerging pathogen, particularly in the Pacific Northwest of the United States. Dark-walled conidia are also produced along the sides of the hyphae and may be more readily viewable than the rosettes in mature cultures (see Figure 27-24, B). Other anaerobes found on the skin include gram-positive cocci (e.g., Peptostreptococcus). In some parts of the United States, as many as 20% of the Mus musculus mice carry the LCM virus. General Concepts in Parasitology Laboratory Methods Fecal Specimens Collection, Handling, and Transport The collection and handling of a stool specimen prior to laboratory examination may have an effect on whether or not organisms will be identified. No Peptococcus niger Peptostreptococcus spp. coli; EAEC, enteroaggregative E. During identification of certain bacteria by serologic typing, capsules sometimes must be removed to detect the somatic (cell wall) antigens present underneath them. luteola is usually sensitive to all β-lactams. Pyrazinamidase. Pyrazinamidase hydrolyzes pyrazinamide to pyrazinoic acid and ammonia in 4 days. In chronic giardiasis, there may be a malabsorption-like syndrome with up to 20% weight loss, fatigue, anorexia, and steatorrhea, with large amounts of gas. vermicularis is often asymptomatic, the patient may experience loss of appetite, abdominal pain, loss of sleep, and nausea and vomiting. nana cyst (trichrome stain). tahir99 VRG CHAPTER 28 Diagnostic Parasitology Linda A. The creation of a second genus was somewhat controversial and is still being debated. Although vancomycin is normally effective in treating infections caused by almost all gram-positive bacteria, many lactobacilli are resistant to the agent and consequently will be able to overgrow other inhibited gram-positive organisms during vancomycin therapy. This gave rise to the Weil-Felix agglutination test. General Characteristics Members of the genus Bordetella are small gram-negative bacilli or coccobacilli. Although this test is reliable, other auxiliary testing should accompany assimilation results before a final identification is made. Ehrlichia ewingii produces a disease indistinguishable from E. is shown in Table 28-4. Discuss the difference between hyaline and phaeoid fungi. The K antigen is heat labile and may be removed by boiling a cell suspension. At least two cases were reported in the United States in individuals who had recently traveled to Germany. Other organisms have been isolated from the skin but are found only occasionally or rarely and are not listed. cholerae non-O1. Retroviridae The family Retroviridae contains several subfamilies, including Oncovirinae and Lentivirinae. Gravid

proglottids at the distal end of the organism discharge eggs into the feces. Most strains of P. nonchromogenicum are usually “+” and M. The primary virulence factor associated with the ability of E. hominis as the cause of GI symptoms. Clinically Significant Species The genus Treponema comprises four microorganisms that are pathogenic for humans—T. • Viruses are becoming better known by their DNA or RNA makeup, host disease signs and symptoms, chemical makeup, geographic distribution, resistance to lipid solvents and detergents, resistance to changes in pH and temperature, and antigenicity (serologic methods). Bacteria Bacteria are unicellular organisms that lack a nuclear membrane and true nucleus. The DNA probe is allowed to react with the test solution. coli, and the enzyme HindIII was isolated from H. Note the fish eye appearance of the colonies. If the material is not too viscous, the specimens can be inoculated onto media with a sterile pipette. By using the guidelines in the CLSI document, an isolate can be identified with more than 95% accuracy. A, Bacterial transformation. The bacteria can infect fish, birds, rodents, livestock, and other mammals. Although it does not invade the gastric epithelium, the infection is recognized by the host immune system, which initiates an antibody response. Plasma Membrane (Cell Membrane). The plasma membrane (PM) is a phospholipid bilayer with embedded proteins that envelop the cytoplasm. Racquet hyphae contain enlarged, club-shaped areas. Describe the proper procedures for collection and transport of viral specimens. aureus, found in approximately 30% of healthy individuals, colonize the anterior nares. Adolescents and adults serve as reservoirs when their immunity from acellular pertussis (aP) vaccine, given in combination with the diphtheria-tetanus toxoids (DTaP), is either immature or waning. serve either to inhibit phagocytosis or, in some cases, to aid in adherence to host tissue or synthetic implants. Issues to Consider After reading the patient’s case history, consider: ■ Potential risks that the physician is taking in spreading germs to her patients ■ Importance of handwashing and use of an appropriate antiseptic ■ Quality control plan to minimize the risks to patients Key Terms Antisepsis Antiseptic Antiseptic drug Biofilms Destruction Disinfectants Disinfection Environmental Protection Agency (EPA) Fast-acting antiseptic Filtration Food and Drug Administration (FDA) Generally recognized as safe and effective (GRASE) Germ theory Health care antiseptic drug products Health care personnel handwash Lister Microbial load Moist heat Monograph New drug application (NDA) Over-the-counter (OTC) drug Pasteurization Patient preoperative skin preparation Persistent Prions Recognized as safe and effective (RASE) Resident flora Semmelweis Sporicidal Sterilization Surgical hand scrub Transient flora S afety in the laboratory cannot be overemphasized. 513 Color of Fluorescence Brick red Light orange to pink (coral) Brick red (some no fluorescence) Chartreuse Red but fades rapidly Red Chartreuse Red or no fluorescence The spot indole test is useful in identifying Propionibacterium acnes (indole-positive) from other Propionibacterium spp. Clinical Infections. In immunocompetent patients, the organisms cause a transient profuse, watery diarrhea along with mild to severe nausea and vomiting, headache, and cramps. 22-7 Characteristics to Note During the Macroscopic Examination of a Specimen Questions to Ask Is it an appropriate specimen? alvei biotype 1. HSV is the leading cause of fatal sporadic encephalitis in the United States. All unstained elements are subsequently counterstained red by safranin dye. Cytopathic Effect on Cell Cultures. Some viruses produce a very characteristic CPE, which can provide a presumptive identification of a virus isolated from a clinical specimen. The infection may be more severe in very young children, elderly adults, and patients with other underlying disease. These organisms are slowly to moderately growing phaeoid fungi, with granular velvety to fluffy colonies, ranging in color from olive to brown or black. (Courtesy BD Diagnostic Systems, Sparks, MD.) removed one by one for rapid microscopic examination of colonies, and then quickly returned to the holding system. Microfilariae of M. † Except for Clostridium difficile, Clostridium botulinum, and other specific causative agents. mentagrophytes, usually causes only surface erosion of hair shafts in this test, whereas T. Several species are currently placed in this genus; the two most frequently isolated species are S. Available at: global_report/en/index.html. The biphasic media system provides for rapid growth and identification and drug susceptibility testing, without the need for routine subculturing. Laboratory diagnosis of Taenia infection can be made by finding the egg, scolex, or proglottid in the feces. – Cocci Veillonella spp. In addition to the actual culture results, the laboratory may be asked to determine the serologic relationship of the isolates. Severe cases of shigellosis may become lifethreatening as extraintestinal complications develop. Identification and susceptibility testing systems requiring anaerobic incubation can be incubated in some bags or pouches. The microfilaria is sheathed, and nuclei extend to the tip of the tail. Infections are usually disseminated, with multiple organ involvement. These include: (1) physical and chemical barriers such as the skin and mucous membranes, (2) blood proteins that act as mediators of infection, and (3) a cellular mechanism capable of phagocytosis such as neutrophils and macrophages and other leukocytes such as natural killer cells. Mahon Donald C. *Coccidioides immitis can be converted to the spherule phase in modified Converse medium at 40° C in 5%-10% CO2. Examples of genotypic characteristics include base sequencing of DNA or RNA and DNA base composition ratio to measure the degree of relatedness of two organisms (see later in this chapter and Chapter 11). Cell cultures can be divided into three categories—primary, low passage (or finite), and continuous. It occurs in healthy, immunocompetent children and young adults with no predisposing condition. Two oval nuclei, each with a large central karyosome, are on each side of the midline. Obligate anaerobes reside in the gingival crevices where the anaerobic environment supports these organisms. burnetii allows it to survive harsh environmental conditions. Symptomatic clinical infection may appear as an acute or chronic form. While handling the Petri dish without gloves, he picked up the telephone to Issues to Consider After reading the patient’s case history, consider: ■ Potential risks to which laboratory workers are exposed while working in the microbiology laboratory ■ Exposure control plan to minimize the risks ■ Laboratory safety guidelines to protect laboratory personnel *This section was prepared by the author in her private capacity. dubliniensis does not is negative. Similarly, the foul odor usually associated with specimens containing anaerobes could be absent. A, Prokaryotic gram-positive and gram-negative bacteria. It is apparent from nucleic acid studies that P. Centers for Disease Control and Prevention: Summary of notifiable diseases—United States 2011, MMWR 60(53):1, 2013. In addition, other cases of bacteremia have been described. are extremely refractory to treatment and may be recovered from almost any source. pylori infections. asburiae is similar biochemically to E. Because of cross-reactivity among members of the same groups (spotted fever and typhus), generally only groupspecific antibody is available. Transposons often carry antibioticresistance genes and are usually located in plasmids. These fungi are found in the environment and have a predilection for cellulose products. The most likely identification is: a. false-positive results. (unstained, ×400). Tindall BJ, et al: Nomenclature and taxonomy of the genus Salmonella, Int J Syst Evol Microbiol 55:521, 2005. The relationship is an equilibrium that is in constant motion and subject to change. are also known for their resistance to a wide range of antimicrobials. hominis is a small organism not usually identified in the stool. nov., and emended description of Aggregatibacter aphrophilus to include V factordependent and V factor-independent isolates, Int J Syst Evol Micro biol 56:2135, 2006. • Large, flat colonies that produce a so-called barnyard or horse stable odor and fluoresce chartreuse under long-wave UV light can be identified as C. terrae are usually “−”. Enzyme-linked immunosorbent assay (ELISA) or latex agglutination can be used to detect the O157 antigen. pseudotuberculosis causes a disease characterized by caseous swellings called pseudotubercles. Despite the vast array of virulence factors, P. Specific antibodies in serum can be detected by enzymelinked immunosorbent assay or indirect immunofluorescent assay methods. The DNA relatedness between Y. Schools and businesses may request updated reports especially related to outbreaks that affect their operation. alcaligenes. Clinically, these organisms have been reported to cause bacteremia, peritonitis, synovitis, and skin ulcers, usually in immunocompromised hosts. Electron microscopy has a greater magnification and can be used to detect virions. If a constriction is present at the base of a germ tube, the yeast is not either species. PRAS media (Anaerobe Systems, Morgan Hill, CA) are prepared by boiling (to remove dissolved oxygen), autoclaving (to sterilize the mixture), and replacing any air with an oxygen-free gas mixture. An understanding of bacterial genetics is also necessary to understand the development and transfer of antimicrobial resistance by bacteria. Human hookworm infection may be caused by N. These eggs are identical to those of F. Borreliae General Characteristics The genus Borrelia comprises several species of spirochetes that are morphologically similar but have different pathogenic tahir99 VRG 532 PART II Laboratory Identification of Significant Isolates an adequate immune response. Obligate, or strict, aerobes require oxygen for metabolism, and they can grow well in an ambient air incubator. Virulence factors allow the pathogen to evade or overcome host defenses and cause disease and encompass functions such as inhibiting phagocytosis, facilitating adhesion to host cells or tissues, enhancing intracellular survival after phagocytosis, and damaging tissue through the production of toxins and extracellular enzymes. The ability to grow at room temperature (25° C) or at an elevated temperature (42° C) is used as diagnostic characteristics for some bacteria. Stuart broth and Amies and Cary-Blair transport media are common examples. With a dramatic rise in the incidence of West Nile fever in the United States, there has been an increased demand for WNV testing by PCR assay. Stool specimens should never be collected from bedpans or toilet bowls; such a practice might contaminate the specimen with urine or water, resulting in the destruction of trophozoites or introduction of free-living protozoa. It is the responsibility of practitioners, relying on their own experience and knowledge of their patients, to make diagnoses, to determine dosages and the best treatment for each individual patient, and to take all appropriate safety precautions. Serologic Grouping When an isolate is biochemically identified as Salmonella or Shigella, serologic grouping of the isolate for the O serogroups (somatic antigen) must be performed for confirmation. Included in this figure are some isolates of nonfermentative, gram-negative bacilli not always considered with the more typical nonfermenters. About 70% of cases of legionnaires’ disease are caused by L. Heavy infections induce diarrhea, upper right quadrant abdominal pain, hepatomegaly, cirrhosis, and liver obstruction, with resulting jaundice. A newer class of antivirals, termed neuraminidase inhibitors, is available. Borrelia burgdorferi Virulence Factors Bacterial spread may occur by the organism’s ability to bind plasminogen and urokinase-type plasminogen activator to its surface. However, it should be noted that the esculin hydrolysis test requires an agar-based medium, with or without bile, but never a standard broth or miniaturized cupule broth version. At least three species of B. pneumoniae infections are still largely unknown. These are certain components of complement, a number of bacterial products, products from damaged tissue cells, and products from responding immune cells. After centrifugation, the parasites can be viewed in a small area at the top of the erythrocyte column. Antibodies are produced in response to antigens, substances that are capable of inducing the immune response. Wright W, et al: (1-3)-β-D-glucan assay: a review of its laboratory and clinical application, Lab Med 42:679, 2011. Chromatography The cell walls of Mycobacterium spp. albicans is an important cause of hospital-acquired infections, especially in an immunocompromised patient, similar to the one in the Case in Point. Activity to SXT, fluoroquinolones, and piperacillintazobactam is often good. This species has a worldwide distribution. BCYE agar is available commercially as nonselective and semiselective media, and both should be used for optimal isolation. These worms live in the small intestine and attach to the mucosa by means of teeth (A. These tests can be used to detect RSV, influenza viruses A and B from respiratory specimens, rotavirus and enteric adenovirus from rectal swabs, and WNV from serum. Dermatophytes typically form two sizes of reproductive cells, macroconidium or microconidium. Testing colonies from highly selective media such as CIN can give a false-negative reaction, whereas MAC and EMB agars may give the appearance of a false-positive reaction from the pH indicators and dyes present in the media. Definitive Identification of Anaerobic Isolates For sterile site and some surgical isolates, it is important for the laboratory to provide full identification. Once established onto or into a particular body site of the host, microorganisms develop a particular relationship with that host. Most are composed of two subunits: the first is nontoxic and binds the toxin to the host cells; the second is toxic. Small numbers of organisms in samples from sterile sites must be seriously considered. Colonies are initially white but become gray to brown with age. Corticosteroids are used to reduce inflammation. psittaci is a problem, and 547 physicians need to know the tests that are most appropriate for differentiating these microorganisms. pallidum is an exclusively human pathogen under natural conditions. Fimbriae present on the outer surface of bacteria are used for: a. Serologic diagnosis of measles is accomplished by demonstrating measles-specific IgM in the specimens collected during the acute phase of the disease. Agents of Subcutaneous Mycoses Subcutaneous mycoses are fungal diseases that affect subcutaneous tissue. Kõnõnen E, et al: Bacteroides, Porphyromonas, Prevotella, Fusobacterium, and other anaerobic gram-negative rods. The unembryonated, operculated eggs are carried in the bile to the intestinal tract and are passed in the feces. spiritovorum has also been isolated from clinical specimens, primarily blood and urine, and from hospital environments; a case of necrotizing fasciitis and septic shock in an immunocompromised man was recently attributed to this species. Bacteria utilize two biochemical pathways, fermentation and respiration (oxidation), to catabolize carbohydrates to produce energy. In the Sudan outbreak, 284 people were infected and a mortality rate of 53% was reported. Distinguishing characteristics among E. Spot Indole Test. The spot indole test uses a small piece of filter paper saturated with p-dimethylamino-cinnamaldehyde. This leaves NAATs as the most frequently used method for direct detection of E. If the screening tests identify a group of organisms that are nonpathogens, the process is complete, and the culture is discarded (after 48 hours of incubation). Adenoviridae Adenovirus was first isolated from adenoid tissue and was thus named for the initial isolation location. Hanscheid T, et al: Screening of auramine-stained smears of all fecal samples is a rapid and inexpensive way to increase the detection of coccidial infections, Int J Infect Dis 12:47, 2008. perfringens lacks the ability to produce a number of essential amino acids, so meats and gravies are commonly implicated in outbreaks. Transmission is most commonly foodborne, although waterborne and person to person transmission can be significant. parahaemolyticus is generally self-limited. Parasitemia (percentage of erythrocytes parasitized) can be calculated form the thin blood smear. Petechiae and ecchymoses can form on mucosal surfaces and on skin. These products are incorporated into membrane-bound vesicles called lysosomes. capsulatum, like B. In addition, exposure to HHV-7 seems to occur later in life than with HHV-6. The virus is latent between active infections. After hydrolysis of Tween 80, neutral red can no longer bind, and it is released, causing a pink color to form. 10. The host shows few symptoms during this migration but may exhibit intermittent coughing and chest pain. During this process, adjacent bacterial genes may be excised with the phage DNA and packaged into the new phage. Blood culture collection is covered in more detail in Chapter 36. Symptoms of dermatophyte infections vary from slight to moderate and occasionally severe. Erect conidiophores bearing branched chains of one-celled, brown blastoconidia Conidium close to tip of conidiophore, termed shield cell Fragile chains Conidiophores erect, dark, bearing conidia only on upper portion near the tip Conidia elliptic, one-celled, produced sympodially B FIGURE 27-18 A, Conidia of Phialophora verrucosa at the tips of phialides with collarettes (Nomarski optics, ×1000.) B, Conidial arrangement of Cladophialophora carrionii. Metronidazole may provide some relief, but the symptoms recur once the drug is discontinued. In contrast, verotoxin II is not neutralized by antibody to Stx. catalase-positive after heating have a heat-stable catalase (Figure 26-12). felis. Short-chain volatile acids produced by anaerobes include formic, acetic, propionic, isobutyric, isovaleric, valeric, isocaproic, butyric, caproic, and heptanoic acids. Centers for Disease Control and Prevention: Investigation update: outbreak of Salmonella Typhimurium infections, 2008-2009. • They are negative for indole, the Voges-Proskauer test, phenylalanine deaminase, and urease. The vector is the chigger, Leptotrombidium deliensis, and the main reservoir is the rat. An example of nondermatophytic tinea is the disease tinea versicolor (pityriasis versicolor). The organism infects cells in the upper portion of the small intestine and causes frequent, watery, but nonbloody stools that may alternate with bouts of constipation. coli system, the donor strain (F+) possesses a fertility factor (F factor) on a plasmid that carries the genes for conjugative transfer. Other Precautions For sterilizing a wire-inoculating loop, an electric incinerator should be used within the biological safety cabinet. Other factors also contribute to the virulence of uropathogenic E. Suspicious colonies should be screened further for B. Infection begins with fever, myalgia, arthralgia, and photophobia. faecalis can further reduce nitrites to nitrogen gas. Person to person transmission has been reported, and some Campylobacter spp. enterica serotype Typhi); this is often more simply written as Salmonella Typhi (serotype is capitalized and not italicized). They often cause infections in animals and can be spread as agents of disease in humans. One reported case of C. The effectiveness of the various host defenses is evidenced by the relatively low incidence of infection in immunocompetent individuals by members of the usual or indigenous flora. Numerous outbreaks have also been linked to calves and poultry at petting zoos. They prefer moist environments, and in hospitals they can be isolated from nebulizers, dialysate fluids, saline, catheters and other devices. duodenale is seen in southern Europe and northern Africa along the Mediterranean, as well as in parts of Southeast Asia and South America. + + V. The disease usually is fatal within 1 week of the appearance of clinical symptoms. In the spring of 2009, a highly infectious novel form of influenza (H1N1), swine flu, emerged. Protozoan trophozoites do not survive the procedure. Some strains may produce a fruity odor and cause a green discoloration on SBA. Susceptibility to nalidixic acid 9. The ingestion of unpasteurized milk is also a recognized risk factor. hollisae, whereas Vibrio damsela has been reclassified as Photobacterium damselae. Mahon* CHAPTER OUTLINE ■ SIGNIFICANCE ■ OVERVIEW OF THE MICROBIAL WORLD Bacteria Parasites Fungi Viruses ■ CLASSIFICATION/TAXONOMY Nomenclature Classification by Phenotypic and Genotypic Characteristics Classification by Cellular Type: Prokaryotes, Eukaryotes, and Archaeobacteria ■ COMPARISON OF PROKARYOTIC AND EUKARYOTIC CELL STRUCTURE Prokaryotic Cell Structure Eukaryotic Cell Structure ■ BACTERIAL MORPHOLOGY Microscopic Shapes Common Stains Used for Microscopic Visualization ■ MICROBIAL GROWTH AND NUTRITION Nutritional Requirements for Growth Environmental Factors Influencing Growth Bacterial Growth ■ BACTERIAL BIOCHEMISTRY AND METABOLISM Metabolism Fermentation and Respiration Biochemical Pathways from Glucose to Pyruvic Acid Anaerobic Utilization of Pyruvic Acid (Fermentation) Aerobic Utilization of Pyruvate (Oxidation) Carbohydrate Utilization and Lactose Fermentation ■ BACTERIAL GENETICS Anatomy of a DNA and RNA Molecule Terminology Genetic Elements and Alterations Mechanisms of Gene Transfer OBJECTIVES After reading and studying this chapter, you should be able to: 1. Spores appear as highly refractile bodies in the cell. The metacercaria excysts in the small intestine and burrows through the duodenal wall into the peritoneal cavity. It is weaker than the fluorescence produced by pigmented species of TABLE FIGURE 22-11 Anaerobic blood agar plate from an intrauterine device culture as seen through a dissection microscope. Often, antibacterial and antifungal agents are added to transport systems to inhibit contaminating microorganisms. sakazakii. Discuss the indications and limitations of serologic assays in the diagnosis of viral infections. Resistance to aztreonam and the aminoglycosides is common. This class targets cell wall synthesis, which makes it an attractive option for treating fungi that have developed resistance to other agents. KOH (10%), or calcofluor white, may be used to enhance the detection of the yeast cells. Bacillus anthracis, Staphylococcus aureus, West Nile virus b. tahir99 VRG 15-50 4-12 5-12 6-20 Entamoeba hartmanni Endolimax nana Iodamoeba bütschlii 15-25 Entamoeba histolytica Entamoeba coli Size (μm) tahir99 VRG Nondirectional Nondirectional Nondirectional Nondirectional Progressive, directional Motility Vacuolated May contain ingested bacteria Vacuolated May contain ingested bacteria Finely granular Vacuolated Ingested bacteria Finely granular May contain ingested red blood cells Cytoplasm Trophozoite 28-3 Comparisons of Amebae Organism TABLE Large karyosome surrounded by achromatic granules No peripheral chromatin Large, irregularly shaped karyosome No peripheral chromatin Small, central karyosomes Fine, evenly distributed peripheral chromatin Large, eccentric karyosomes Coarse, uneven peripheral chromatin Small, central karyosome Fine, evenly distributed peripheral chromatin Trophozoite and Cyst Nuclear Structure 6-15, oval or irregular 5-12, oval 5-10, round 15-25, round 10-20, round Size (μm) and Shape One Four Four Eight Four No. of Nuclei in Mature Cyst Not present Not present Rounded ends Elongated Not always present Elongated Splintered ends Not always seen Rounded Elongated Usually seen Chromatoidal Bars Cyst Single, defined Not seen Not seen Not seen Not usually seen Diffuse in young cyst Glycogen Vacuole CHAPTER 28 Diagnostic Parasitology 635 636 PART II Laboratory Identification of Significant Isolates abscess may yield motile trophozoites and necrotic material composed of lysed cells. The oval trophozoite (Figure 28-15, A) demonstrates two size ranges, 45 to 60 μm × 30 to 40 μm and 90 to 120 μm × 60 to 80 μm. Some isolates form chlamydoconidiumlike swellings terminally on hyphae. In endemic areas with a high risk of reinfection, treatment may not be indicated. The use of MALDI-TOF for rapid confirmation of B. Evaluate the laboratory methods for the diagnosis of Clostridium difficile infection. This would cause the heavier than air, oxygen-free gas mixture to be displaced by room air, thus defeating the primary purpose of using such a transport medium. Clinical Manifestations Pneumocystis spp. Leclercia The name Leclercia was proposed in 1986 for 58 isolates from human clinical specimens, including blood, urine, sputum, and feces and 27 isolates from nonhuman sources. Behera B, et al: Parasites in patients with malabsorption syndrome: a clinical study in children and adults, Dig Dis Sci 53:672, 2008. However, they must be distinguished from other oxidase-positive organisms, such as Aeromonas and Pseudomonas, which will also grow on CIN. fowleri has greater than 95% mortality. Proteus mirabilis is the most common clinical isolate. Symptoms usually disappear within a few days, with few or no complications. Blood concentration methods are often needed because of the small number of organisms present. Patients with babesiosis may have malaria-like symptoms, such as malaise, fever, chills, sweating, and myalgia; however, many cases are asymptomatic. krusei and C. mimicus, are halophilic, or saltloving, and require the addition of Na+ for growth. Pseudomonas Nonfluorescent Group Pseudomonas stutzeri Pseudomonas stutzeri, although a rare isolate and even rarer pathogen in the clinical laboratory, is usually easily recognizable because of its characteristic macroscopic appearance of wrinkled, leathery, adherent colonies that may produce a light-yellow or brown pigment (Figure 21-6). Coxiella burnetii is the causative agent of Q fever. Proper installation, maintenance, and testing are essential to their performance. have a microscopic morphology similar to that of Campylobacter spp. amalonaticus is frequently found in feces, but no evidence has been found that it is a causative agent of diarrhea. After an incubation period of at least 5 to 6 hours, symptoms begin as an acute onset of severe abdominal pain and diarrhea, which is often bloody, and may be accompanied by vomiting. [DNLM: 1. Microbiological Techniques. 2. Bacterial Infections--diagnosis. 3. Communicable Diseases—diagnosis. 4. Mycoses—diagnosis. 5. Virus Diseases—diagnosis. QW 25] QR67 616.9'041—dc23 2013045846 Vice President and Publisher: Andrew Allen Managing Editor: Ellen Wurm-Cutter Content Development Specialist: Amy Whittier Publishing Services Manager: Julie Eddy Project Managers: Celeste Clingan/Nisha Selvaraj/Devendran Kannan Design Direction: Karen Pauls Printed in China Last digit is the print number: 9 8 7 6 5 4 3 2 1 tahir99 VRG To my husband, Dan, for his love and continued support and understanding, my son, Sean, who inspires me, my daughter, Kathleen, for showing me courage, and my granddaughters, Kelly Amelia and Natalie Page, who have given us so much pleasure. harveyi From Abbott SL et al: Vibrio and related organisms. pylori infections consists of a macrolide, amoxicillin, and proton pump inhibitor. The cyst wall does not pick up the stain, but the nuclei of all forms stain pink, and the intracystic bodies can be demonstrated as a circular arrangement within the cyst. PROCEDURE 1. All diseases respond well to penicillin or tetracycline. This selective medium has been modified, and manufacturers such as BD Diagnostic Systems (Sparks, MD) have added a differential property (mannitol) to the medium, named Yersinia-selective agar (YSA) base. The presence of sterols is also a trait of eukaryotic cell membranes. • What was the epidemiologic profile of the serogroup? This represents an area of chronic lysis and infiltration with neutrophils, lymphocytes, and eosinophils. Currently, four commercial methods are approved by the FDA to determine the susceptibility of M. HIV causes acquired immune deficiency syndrome (AIDS). The pigment is violacein, an ethanol-soluble, waterinsoluble pigment. Facultative anaerobes (aerobes that can grow anaerobically) are routinely cultured in an aerobic atmosphere because aerobic culture is easier and less expensive than anaerobic culture; an example is E. Between 1 and 2 million deaths worldwide occur annually from malaria, primarily caused by infection with P. Hunting c. Investigators have estimated this method as almost 95% sensitive, but it is technically demanding and influenced by the quality of the specimen and expertise of the laboratory scientist. An organism was recovered by the laboratory. Identification is very important in providing proper therapy for the patient and in limiting any possible complications that might develop. Adaptive, or Specific, Immunity Adaptive immunity enhances the protective capability of innate immunity. histolytic and E. The Pastorex Candida (Bio-Rad, Hercules, CA) and Cand-Tec (Ramco Laboratories, Houston) tests are latex agglutination assays that detect Candidaspecific mannan. tahir99 VRG CHAPTER 28 Diagnostic Parasitology FIGURE 28-53 Hymenolepis nana egg (iodine wet mount, ×400). The incubation period for HGA is 5 to 11 days. gonorrhoeae are protective by preventing the organism from attaching to the epithelial cells. Implement intervention activities to control the outbreak. A large number of rapid test kits, generally based on EIA or latex agglutination, are commercially available for detecting heterophile antibodies. FIGURE 22-16 Positive lecithinase reaction on egg yolk agar. The filariform larva also must be distinguished from that of S. Typical morphotypes should be sought. The high-numbered enteroviruses (68 to 71) require special handling. Dipylidium caninum. Humans serve as accidental hosts for Dipylidium caninum, the dog tapeworm. boydii; hence, the species name shigelloides. ‡ Species that require salt should have salt added to each biochemical tested. pestis, is a pathogen primarily of rodents, particularly guinea pigs. Bacilli with tapered, pointed ends are termed fusiform. Adenovirus has 49 distinct serotypes (six subgenera, A through F), and the different serotypes are associated with numerous common clinical manifestations. IgM antibodies are usually elevated during the first 2 weeks and then decline. A good quality control procedure to ensure that the agar is maintained under proper conditions is to inoculate an agar plate with F. The organ system approach in Part III has been the foundation of the Textbook of Diagnostic Microbiology and provides an tahir99 VRG xiii xiv PREFACE opportunity for students and other readers to “pull things together.” Chapters begin with the anatomic considerations of the organ system to be discussed and the role of the usual microbiota found at the particular site in the pathogenesis of a disease. htm?s_cid=mm6131a3_w. The household cat and other members of the family Felidae serve as definitive hosts for the organism. Phase 1 antigens agglutinate only with homologous H Ag Vi Ag O Ag FIGURE 19-8 Antigenic structures of salmonellae used in serologic typing. pertussis? Negative glutamate dehydrogenase and EIA for toxins A and/or B can be used as rapid screening to rule out C. Once the organism reaches the blood-brain barrier, it is able to bind to microvascular endothelial cells through receptor molecules. McLellan E, Partridge D: Prosthetic valve endocarditis caused by Pseudomonas mosselii, J Med Microbiol 58:144, 2009. † Oxidase-positive. A radiograph revealed pockets of gas in the tissue. Acid treatment of specimens contaminated with other bacteria before inoculation also enhances isolation of Legionella spp. The disease manifests in acute cases as a severe gastroenteritis accompanied by vomiting and followed by diarrhea. Therefore, they are identified on the basis of characteristic asexual reproductive structures. The system consists of a bottle containing Middlebrook 7H9 broth with an atmosphere of 5% to 8% CO2, an enrichment consisting of growth-enhancing factors and antimicrobial agents, and a paddle with agar media. Members of the genus Moraxella that are commonly encountered include M. testosteroni (formerly Pseudomonas testosterni) and C. An infection is often seen in the eye after a poultice has been applied to relieve an infection. ovale, P. Some organisms prevent fusion of phagosomes and lysosomes, others have a resistance to the effects of the lysosomal contents, and still others escape from the phagosome into the cytoplasm. Stool is the best sample to use to detect NoV; it should be collected when a person has acute illness (within 48 to 72 hours after onset of symptoms). The Case in Point is followed by “Issues to Consider.” These are points in a bulleted format that the learners are asked to think about as they read the chapter. Note discoloration of media, especially on the left. freundii has been associated with infectious diseases acquired in hospital settings; UTIs, pneumonias, and intraabdominal abscesses have been reported. Malvy D, Chappula F: Sleeping sickness, Clin Microbiol Infect 17:986, 2011. Although aeromonads grow on almost all enteric media, they often are overlooked on MAC agar, especially in stool cultures, because a number of aeromonads ferment lactose. Dogs, rats, and other rodents are the principal animal reservoirs. Genes that are expressed only under certain conditions are inducible. Serologic grouping of Shigella is also based on the somatic O antigen. In contrast, the heat-stable toxin (ST) stimulates guanylate cyclase, causing increased production of cyclic guanosine monophosphate, accumulation of which also causes hypersecretion. The colony morphology of many of the species resembles Klebsiella when growing on MAC agar. Cross-reactivity among related fungi has been reported. Table 19-3 lists some diseases associated with members of the family Enterobacteriaceae. Differentiation can be accomplished by using a minimal number of biochemical tests, such as urea hydrolysis and lysine decarboxylase. Third, the antibody level does not necessarily correlate with the acuteness or activity level of the infection, because this is also host-dependent. Name the appropriate specimen for maximum recovery of the suspected viral agent. A modification of this procedure is to use flat-bottomed microtiter plates. Compare the results of the urease test and the hair perforation test for T. 16S Ribosomal RNA Gene Sequencing Many of the techniques mentioned have been replaced largely in research and reference laboratories by 16S ribosomal RNA gene sequencing. However, ribosomal RNA (rRNA) analysis indicates it is related to the stramenopiles (brown algae and water molds). The remainder of the amebae is considered nonpathogens. Branched conidium-bearing cells may dislodge, and the three scars on each of these cells give them the appearance of a shield. burgdorferi sensu stricto occurs in North America. ■ Surveillance is important to establish baseline data and recognize the need to investigate potential outbreaks. Clinical Significance Members of the family Enterobacteriaceae are ubiquitous in nature. Some patients who remain symptomatic but show no evidence of the organism after repeated stool tests may require a duodenal aspirate or use of the Enterotest to demonstrate the trophozoite in the small intestine. This organism differs in several ways from many members of the families Rickettsiaceae and Anaplasmataceae. CHROMagar Candida presumptively identifies C. Discuss the habitat in which the organisms discussed in this chapter are found. Flagella are longer projections (>150 μm) used for locomotion by cells such as spermatozoa. A chronic diarrhea usually lasting more than 10 days 4. FIGURE 28-42 Fasciola (unstained, ×400). *All would contain endogenous anaerobic microbiota. Bacterial exotoxins and endotoxins are compared in Table 2-4. Kampfer P, et al: Description of Wautersiella falsenii gen.nov., sp. It is believed that individuals have a genetic predisposition to developing the disease because not everyone encountering infected skin scales becomes infected. As a result of nucleic acid studies, the nonfermentative, gramnegative bacilli continually undergo taxonomic changes. As shown in Table 3-7, diseases designated as class A1 are considered public health concerns and are to be reported as soon as they are suspected or identified. One drop of the concentrate is used for India ink preparation or latex agglutination for Cryptococcus, and the remainder is inoculated onto media. Methanol fixation preserves the morphology of leukocytes and bacteria better than heat fixation. It begins as a red macule and expands to form large annular erythema with partial central clearing, sometimes described as having a target appearance. This process involves lysis of the yeast cell wall so that a blastoconidium can form. Aerobic diphtheroids are usually found in moist areas such as the axillae and between the toes. Viruses can penetrate the cell by several different mechanisms and is tahir99 VRG CHAPTER 29 Clinical Virology virus-dependent. • Are unexpected structures present? Initial replication takes place in the mucosal cells of the respiratory tract; measles virus then replicates in the local lymph nodes and spreads systemically. Immunoassays. The most commonly used rapid antigen assay for the detection of C. africanum M. Roxby AC, et al: Strongyloidiasis in transplant patients, Clin Infect Dis 49:1411, 2009. Azithromycin has fewer and milder side effects, has a longer half-life, and requires fewer daily doses resulting in better patient compliance. Lactobacillus. Lactobacillus spp. Mosquitoes of the genera Mansonia, Anopheles, and Aedes have been shown to transmit the organism. (Giemsa stain, ×1000.) A B FIGURE 28-31 A, Plasmodium ovale trophozoite. The following are indications for serologic testing: • Diagnosis of infections with nonculturable agents, such as hepatitis viruses • Determination of immune status in regard to rubella, measles, VZV, HAV, and HBV • Monitoring of patients who are immunosuppressed or have had transplants • Epidemiologic or prevalence studies Double-Stranded DNA Viruses Viruses are discussed in this chapter in groups based on nucleic acid types: double-stranded DNA (dsDNA), single-stranded DNA (ssDNA), double-stranded RNA (dsRNA), and singlestranded RNA (ssRNA) viruses. Toxins are poisonous substances produced by organisms that interact with host cells, disrupting normal metabolism and causing harm. Fothergill CHAPTER OUTLINE Agents of Subcutaneous Mycoses Agents of Systemic Mycoses Agents of Opportunistic Mycoses Agents of Yeast Infections Pneumocystis Infection ■ LABORATORY DIAGNOSIS OF FUNGI Safety Issues Specimen Collection, Handling, and Transport Direct Microscopic Examination of Specimens Isolation Methods Identification of Fungi (1-3)-β-D-Glucan Detection ■ IMMUNODIAGNOSIS OF FUNGAL INFECTIONS ■ ANTIFUNGAL SUSCEPTIBILITY Antifungal Agents Antifungal Susceptibility Testing ■ GENERAL CHARACTERISTICS Yeasts Versus Molds Hyaline Versus Phaeoid Dimorphism and Polymorphism Reproduction ■ TAXONOMY Mucorales Ascomycota Basidiomycota Fungi Imperfecti ■ MYCOSES Superficial Mycoses Cutaneous Mycoses Subcutaneous Mycoses Systemic Mycoses ■ CLINICALLY SIGNIFICANT SPECIES Agents of Superficial Mycoses Agents of Cutaneous Mycoses OBJECTIVES After reading and studying this chapter, you should be able to: 1. Finely granular nuclear chromatin, which is evenly distributed on the nuclear membrane, and the small central karyosome stain a dark purple-red. • What antimicrobial agents can successfully treat them? Figure 28-3 shows trichrome-stained trophozoites of E. Serous transudate is placed onto a slide, diluting with nonbacteriocidal saline if the preparation is too thick. 2-3 Examples of Exotoxins of Pathogenic Bacteria Bacterium Disease Caused in Humans Toxins Bacillus anthracis Bordetella pertussis Clostridium botulinum Clostridium difficile Anthrax Whooping cough Botulism Antibiotic-associated diarrhea, pseudomembranous colitis Clostridium novyi Gas gangrene Clostridium perfringens Gas gangrene, food poisoning, enteritis necroticans Clostridium septicum Clostridium sordellii Gas gangrene Gas gangrene Clostridium tetani Tetanus Corynebacterium diphtheriae Escherichia coli Diphtheria Diarrhea Pseudomonas aeruginosa Staphylococcus aureus Pyogenic infections Pyogenic infections, enterotoxemia Streptococcus pyogenes Pyogenic infections, scarlet fever, rheumatic fever Vibrio cholerae Salmonella typhimurium Shigella spp. Filipinos and blacks run the highest risk of dissemination, with meningeal involvement being a common result of disseminated disease. More information on smallpox as an agent of bioterrorism can be found in Chapter 30. Patients infected with Pneumocystis sp. dublinensis subsp. Several of these patients have been identified as intravenous drug abusers. Although it does not invade the mucosal surface, it does attach to the surface of columnar epithelial cells. paucimobilis except that isolates of S. Rinse very lightly with water. The usual result is that a phagocytosed organism is quickly engulfed, killed, and digested. fragilis Pigmented species of Prevotella SBA CHOC FIGURE 22-6 Glove box type of anaerobic chamber (Coy Laboratory Products, Grand Lake, MI). (From Thibodeau GA, Patton KT: Anatomy and physiology, ed 6, St Louis, 2007, Mosby.) traditionally have been categorized according to their staining characteristics. Infections of the kidneys, liver, heart, CNS, lymph nodes, spleen, and bone marrow and cutaneous abscesses have been described. During childbearing years, high estrogen levels promote the deposition of glycogen in vaginal epithelial cells. IFA serologic kits are available for the detection of antibodies to A. coli has been detected. However, many isolates of E. † Kanamycin, 1000 μg. Reid, MD Assistant Professor, Medicine Division of Infectious Diseases Department of Medicine Loyola University Medical Center Maywood, Illinois George Manuselis, MA, MT(ASCP) Emeritus Medical Technology Division Ohio State University Columbus, Ohio Adjunct Faculty Department of Natural Sciences and Forensic Science Central Ohio Technical College Newark, Ohio Kevin McNabb, PhD, MT(ASCP) Director, Microbiology and Immunology New Hanover Regional Medical Center Wilmington, North Carolina Frederic J. Hobbs MM, et al: Methods for detection of Trichomonas vaginalis in the male partners of infected women: implications for control of trichomoniasis, J Clin Microbiol 44:3994, 2006. The DMSO facilitates more rapid breakdown of cellular debris without requiring heat while the stain is taken up by fungal elements, making them readily visible on microscopic examination of the slide preparation. stercoralis. stuartii P. trichiura may be seen in fecal specimens. nov., to accommodate clinical isolates phenotypically resembling members of the genera Chryseobacterium and Empedobacter, Int J Syst Evol Microbiol 56:2323, 2006. Fader CHAPTER OUTLINE ■ IMPORTANT CONCEPTS IN ANAEROBIC BACTERIOLOGY Anaerobes Defined Why

88 95 95 92 0 100 0 17 0 58 0 75 0 75 0 50 80 40 15 40 50 4 20 100 100 77 100 92 0 0 100 85 85 100 98 98 98 75 82 83 0 100 8 100 0 100 0 93 0 95 0 75 0 0 0 0 0 0 0 40 5 100 60 35 100 100 0 100 0 100 100 96 0 50 0 100 65 65 100 100 0 100 0 100 93 99 15 0 55 25 25 0 0 0 85 0 0 1 0 0 0 0 50 0 100 20 0 70 50 0 50 10 0 75 10 0 30 15 10 90 7 0 0 0 100 50 0 0 60 55 80 0 0 8 0 0 88 0 70 100 100 99 99 100 0 0 100 0 0 99 0 0 99 0 0 100 0 0 98 0 0 100 0 20 20 0 70 0 100 0 10 0 30 0 1 0 90 0 0 0 100 0 0 0 0 0 0 0 0 0 0 60 100 100 100 100 100 100 100 80 100 100 100 100 100 100 100 0 0 0 0 100 0 0 0 0 0 55 100 100 70 30 100 100 100 60 95 98 85 75 99 0 2 30 92 0 0 0 100 40 100 0 35 100 100 100 100 76 97 85 100 6 15 98 100 100 5 0 96 7 99 5 0 70 0 100 4 0 8 91 5 0 0 0 60 40 100 98 100 75 0 0 0 0 0 100 100 100 100 95 100 100 100 100 0 0 95 100 100 100 100 94 100 100 100 100 0 7 40 81 94 0 0 0 0 0 40 0 0 100 0 100 0 100 0 97 1 95 0 96 D-Mannose ONPG Test 0 Yellow Pigment Oxidase, Kovacs' 0 DNase (25° C) Nitrate Nitrite 97 0 100 100 100 100 100 99 100 50 65 100 20 1 100 0 43 94 0 30 45 0 29 60 0 2 95 0 0 0 0 100 100 0 0 0 94 0 94 94 99 1 99 99 0 1 20 11 0 30 15 4 40 5 30 2 3 75 90 2 11 0 0 9 0 100 100 100 0 0 0 0 100 88 100 94 85 0 90 0 65 0 100 5 93 0 100 100 0 0 70 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 30 20 15 15 0 0 0 0 0 0 40 0 0 100 100 5 1 75 70 30 99 100 100 45 100 100 100 0 0 100 85 0 0 60 60 0 1 80 90 15 70 95 100 80 100 98 100 100 100 100 100 100 100 75 100 96 100 100 0 0 0 0 0 0 77 0 0 0 0 0 0 0 0 0 0 0 20 0 25 0 85 0 100 0 0 0 0 0 0 50 0 95 0 0 0 0 1 40 0 100 80 45 0 45 0 0 20 0 70 0 0 0 90 85 60 70 20 50 50 0 100 100 0 85 5 55 6 88 0 40 0 100 0 0 0 50 0 0 15 15 18 8 0 0 0 0 0 55 55 12 0 0 0 0 0 0 5 0 0 0 0 0 0 100 100 98 100 94 100 100 85 95 0 0 0 80 0 95 0 100 0 90 0 70 0 20 0 50 0 70 0 0 0 0 0 0 0 0 0 62 0 0 38 100 25 5 0 95 0 95 5 25 100 100 100 100 0 0 0 0 0 0 50 0 92 0 0 0 38 30 0 0 100 0 30 0 0 0 0 25 0 30 0 0 88 0 75 0 100 0 50 0 50 0 100 0 100 0 100 0 0 0 0 0 100 0 0 0 0 0 0 0 0 0 0 0 100 100 100 100 100 100 50 0 60 100 98 100 100 77 100 100 50 0 100 100 0 50 5 0 0 100 0 100 100 100 100 0 0 100 0 100 100 100 0 0 0 25 0 75 0 0 0 0 0 0 0 50 0 0 100 100 100 100 100 100 100 100 55 100 100 10 100 0 0 100 0 0 100 100 100 0 0 67 30 0 10 60 75 0 50 0 0 100 60 50 75 0 0 tahir99 VRG 45 50 0 0 25 0 0 100 0 0 100 0 0 100 0 100 100 0 0 100 0 0 0 0 100 100 100 0 100 0 25 0 0 0 100 100 100 100 D-Galactose Glycerol Fermentation 0 Tyrosine Utilization D-Arabitol 5 Fermentation Melibiose Fermentation 90 Fermentation Esculin Hydrolysis 90 Fermentation 5 82 100 7 0 Erythritol Fermentation 0 2 α-Methyl-D-glucoside Fermentation 99 Cellobiose Fermentation 0 Trehalose Fermentation 1 100 D-Xylose 10 100 Maltose Fermentation Fermentation L-Rhamnose Raffinose Fermentation Fermentation L-Arabinose Fermentation D-Sorbitol 0 Citrate, Christensen's 451 CHAPTER 19 Enterobacteriaceae myo-Inositol Fermentation 452 PART II Laboratory Identification of Significant Isolates TSI agar K/A H2S+ A/A H2S+ Proteus mirabilis Salmonella spp. ozzardi, M. difficult to use with large numbers of specimens, it does offer rapidity in selected cases, particularly in detecting ocular infection in newborns. For the fluorescent antibody test from a plate isolate, slides should be carefully prepared so that organisms are well dispersed on the slide; this ensures that individual cells show characteristic peripheral staining. Initiation of Special Handling for Unsuspected or Special Pathogens The direct microscopic examination of infected materials, along with specimen site and historical information, may suggest modifications in routine culture techniques to allow isolation of a suspected pathogen. Other bacteria are decolorized by acid-alcohol, whereas mycobacteria and nocardiae retain the stain. Although antimicrobial resistance has been increasing within the family Enterobacteriaceae, it is probably most severe with K. Members of the family Enterobacteriaceae are routinely isolated from stool cultures; complete identification should be directed only toward true intestinal pathogens. Cell Envelope Structures Plasma Membrane. The PM (see Figure 1-2) is a phospholipid bilayer with embedded proteins that envelops the cytoplasm and regulates transport of macromolecules into and out of the cell. falciparum often demonstrates an tahir99 VRG CHAPTER 28 Diagnostic Parasitology A 655 B FIGURE 28-30 A, Plasmodium malariae band form trophozoite. In 2012, only 542 cases were reported, and dracunculiasis was limited to four African countries. Whereas tease and tape mounts typically disturb conidia, preventing viewing of how they are formed, slide cultures provide a more intact specimen. Some humans were infected during these poultry outbreaks. solium, the pork tapeworm, thus becoming an intermediate host. Other tests are packaged in single-test platforms, with positive specimens detected by colorimetric or optical density changes on membrane or silicon surfaces (Figure 29-1). agglomerans HG XIII, which may produce a yellow pigment, is primarily a plant pathogen. Vandamme P, et al: Genus Campylobacter Sebald and Véron 1963, 907AL emend. The capsid protein subunits aggregate to form capsomers, and the capsomers combine to form the capsid. Matrix-assisted, laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry is a method for the identification of bacteria and fungi from colonies based on analysis of the unique spectra of their peptides when separated in the mass spectrophotometer. biflexa sensu lato are pathogenic for a wide range of wild and domestic animals and humans, but mechanisms of pathogenicity are not well understood. PIV-1 is the primary cause of croup (laryngotracheobronchitis) in children. 421 BOX 19-1 Key Characteristics of the Family Enterobacteriaceae • They are gram-negative bacilli and coccobacilli. Sexual reproduction by trophozoites also occurs, first producing a precyst and then the cyst containing spores or intracystic bodies. In addition, more than one set of smears should be made within a 36-hour period because only one set is inadequate to completely rule out the presence of malarial organisms. A 65-year-old Chinese man, in obvious shock, was examined in the emergency department for a painful swelling of the left hand. Pressure from the increasing size of a cyst may cause necrosis of surrounding tissue. For a symptomatic patient, several blood smears from samples collected at approximate 6-hour intervals over 36 to 48 hours should be examined before a final negative diagnosis is made. Once isolated, monoclonal antibodies can be used to type the virus. Metabolism can be anaerobic, facultatively anaerobic, or aerobic, depending on the species. jejuni present with a diarrheal disease that begins with mild abdominal pain within 2 to 10 days after ingestion of the organisms. Hall, PhD, D(ABMM), F(AAM) Retired as Medical Director in Clinical Microbiology Forestville, New York Maximo O. In immunocompromised hosts, such as transplant recipients and HIVinfected patients, a CMV infection can become a significant, life-threatening, systemic disease involving almost any organ, including the lungs, liver, intestinal tract, retina, and CNS. This stain may be useful when morphology is ambiguous and the nonspecific staining of other techniques such as Grocott-Gomori methenamine–silver nitrate gives confusing results. tuberculosis is reduced from 6 to 3 weeks. pneumoniae inclusions but cannot differentiate this organism from the other chlamydiae. EBV-specific serologic tests (Table 29-4; Figure 29-9) measure the presence or absence of the following: • Anti-VCA (antibodies against the viral capsid antigen): IgM to the VCA occurs early in the infection and disappears in about 4 weeks, so its presence indicates current infection. Resistance to all these antimicrobial agents has been increasing, however, especially in the immunocompromised patient in whom A. nov., Cronobacter malonaticus sp. • They do not grow in medium containing potassium cyanide. It has also been shown to play a role in the development of primary effusion lymphomas and multicentric Castleman disease. Sharp S: Commensal and pathogenic microorganisms of humans. ozaenae and K. Systemic e. Septate and Hyaline Saprophytes Aspergillus. Aspergillus spp. Media for Aerobic Incubation. In most cases, a request for anaerobic culture on a specimen is also accompanied by a request for aerobic culture. Laboratory diagnosis of Lyme borreliosis caused by B. Describe the antigenic structures of the family Enterobacteriaceae, and explain how these antigens are used for identification. A virus that infects and possibly destroys bacterial cells is known as a bacteriophage (Greek phage: to eat). They often have a history of travel abroad and most frequently of consuming untreated water. It is not seen in the United States. The average size of the trophozoites of E. All of the above 10. In Sherris J, editor: Medical microbiology: an introduction to infectious diseases, ed 4, New York, 2004, McGraw-Hill, p. A549, Human lung carcinoma cell line; CPE, cytopathic effect; HDF, human diploid fibroblasts; HEp2, human laryngeal carcinoma cell line; PMK, primary monkey kidney; RK, rabbit kidney; −, negative; +, acceptable; ++good viral recovery, ; +++ recommended, ; ± positive or negative. The CPE and resistance to detergent, acid, and solvents constitute a presumptive diagnosis of enterovirus infection. Trachoma is associated with serotypes A, B, Ba, and C. Attached ticks should be removed immediately because pathogen transmission is associated with the length of attachment. Confirming an outbreak exists, calculating an infection rate, and performing serology and culture tests 9. The presence of cells representing a response to injury supports the probability of infection and directs attention toward specific types of pathogens. Their motion is rapid and rotational. It may be necessary to increase the safranin counterstaining time to 2 minutes to see typical morphology. The results can be obtained in approximately 5 hours. Patients suffering from Guillain-Barré syndrome (GBS) often test positive for Campylobacter antibodies. Proteus spp. At least three major clinical types of gastroenteritis are caused by Plesiomonas: • The more common watery or secretory diarrhea • A subacute or chronic disease that lasts from 14 days to 2 to 3 months • A more invasive, dysenteric form that resembles colitis On average, 25% to 40% of all patients present with fever, vomiting, or both, and the single most common clinical symptom for all such patients is abdominal pain. boydii are the most common isolates. The immunofluorescent antibody (IFA) test is considered the gold standard method for antibody detection. ✓ Case Check 7-2 The foundation in the diagnostic microbiology laboratory, as in the Case in Point, is the ability to combine the rapid response of direct specimen examination with culture isolation and antibiotic susceptibility testing to achieve the following: • Confirm that the material submitted for study is representative. They digest the tissue plug and pierce capillaries. The second or third specimen after the purge is more likely to contain trophozoites that inhabit the cecum. They are rodent-borne viruses. Except in certain patient populations, dissemination through the blood to major organs does not occur. belli are usually clinically indistinguishable from those with Cryptosporidium spp. The organism gains access to host cells by emergence of a polar tube through the spore wall and its insertion through the cell membrane. What initial clues indicate that an isolate is a nonfermenter? Lee SM, et al: Experience of Comamonas acidovorans keratitis with delayed onset and treatment response in immunocompromised cornea, Korean J Ophthalmol 22:49, 2008. Because Vegetative mycelia FIGURE 27-2 Aerial mycelia give mold the "woolly" appearance. thetaiotaomicron, and B. At this point, the patient was given a broad-spectrum antimicrobial agent, with the plan to give her antifungal agents if the fever persisted. Plasmodium vivax. Plasmodium vivax has a tertian life cycle pattern—that is, it takes approximately 48 hours for the life cycle to be completed. piechaudii may be susceptible to amoxicillin. The urban cycle occurs in larger towns and cities when infected A. tuberculosis is used as the antigen (i.e., purified protein derivative; PPD). Antibody titer Clinical illness Anti-VCA IgG Anti-EBNA Anti-EA Anti-VCA IgM Heterophil (PB) antibody Weeks 1 2 3 4 5 6 12 24 FIGURE 29-9 Serologic evaluation of Epstein-Barr virus infection (infectious mononucleosis) showing the rise and fall of detectable antibodies. If the decolorization is too vigorous or prolonged, the gram-positive complex will be removed, and the normally gram-positive organisms will not stain. Adapted from Mims CA: The pathogenesis of infectious disease, ed 5, New York, 2001, Academic Press. The first step requires an enzyme, β-galactoside permease, for the transport of lactose across the cell wall into the bacterial cytoplasm. Note the FIGURE 28-69 Cross section of tissue infected with Oncho- faintly staining sheath extending from both ends of organism. FIGURE 28-12 Iodamoeba bütschlii cyst with prominent glycogen vacuole (iron hematoxylin stain, ×1000). An alternative is to use commercial media, such as PRAS media, that have been prepared, packaged, shipped, and stored under anaerobic conditions. Glucose ATP ADP Glucose–6–PO4 NAD NADH2 6–Phosphogluconic acid 2–Keto–3–deoxy–6–phosphogluconic acid NAD NADH2 Pentose PO4 + Glyceraldehyde–3–PO4 CO2 Acetyl PO4 Pyruvic acid Glyceraldehyde–3–PO4 Acetaldehyde + CO2 (Via EMP pathway) NADH2 2 ATP Lactate NADH2 NAD NAD Acetaldehyde Ethanol 2 ADP 2 ATP 2 NAD 2 NADH2 Pyruvic acid NADH2 (Via EMP pathway) NAD Ethanol Acetaldehyde + CO2 NADH2 NAD Ethanol FIGURE 1-10 Alternative microbial pathways to the Embden-Meyerhof-Parnas (EMP) pathway for glucose fermentation. EAEC may be an important cause of diarrhea in infants in the United States and should be considered a cause of diarrhea in patients with human immunodeficiency virus infection. McKenzie SB: Clinical laboratory hematology, Upper Saddle River, NJ, 2009, Pearson Prentice Hall. Tularemia, infection with bacteria from the genus Francisella, is a zoonotic disease and has many other names, including rabbit fever, deerfly fever, lemming fever, and water rat trappers' disease. Rarely, larvae are seen in the sputum in heavy infections. Viruses transmitted by arthropods, such as ticks and mosquitoes, are referred to as arboviruses. The clostridial cell wall structure is similar to that of other gram-positive bacteria. Yokenella strains have been isolated from human specimens, but further study is required to determine their significance in human disease. The two gases have a function similar to that in the anaerobic chamber with H2 509 FIGURE 22-8 Anaerobic jars. Occasionally, fever or other disease conditions cause the adults to migrate from the intestine and invade other organs, resulting in peritonitis, liver abscess, or secondary infection in the lungs. Describe EPA regulations on chemical surface disinfectants and FDA regulations on chemical skin antiseptics. A portion of the formed stool should be placed in formalin for concentration procedures and another portion placed in PVA for permanently stained smears. This photograph depicts the GasPak Pouch, one of the commercially available anaerobic bag or pouch systems. Crusty, cup-shaped flakes called scutula are formed. It is thought to be one of the most common diseases transmitted by ticks in the United States. Examination using a dissecting microscope may help to characterize the nature of hard granules. The disease caused by this virus became known as hantavirus pulmonary syndrome (HPS). 1215. cincinnatiensis, V. possess peritrichous flagella (Figure 21-12) and are obligately aerobic gram-negative bacilli. Table 24-2 also lists additional properties of the Chlamydiaceae species that have helped further differentiate the three human species on the basis of natural host, major diseases, and number of antigenic variants (i.e., serovars). Antifungal Susceptibility Testing Until the last several years, many laboratories used methods that had been developed in house to determine susceptibility patterns for the fungi. tahir99 VRG NH2 CHAPTER 1 Bacterial Cell Structure, Physiology, Metabolism, and Genetics 3′ hydroxyl 5′ phosphate -O Base-pair Base P=O H H HO H H 19 Nucleotide Deoxyribose 3′ sugar H O O H2C5′ A T H2C O 1′ H O -O -O H 3′ H H P=O O O H2C H H H O H H 5′ H O H H Phosphodiester bond H2C O -O P=O A H T C G O H H O P=O A H T C O O H2C A H2C T G T A O H H O -O H H H - O P=O O O H2C C P=O H O H H H H2C G O H H H O -O H H H - O P=O O A O H - H P=O H C G 3′ O 3′ O H H 3′ H 5′ O H2C P=O H T H2C5′ A B O H H OH - O P=O 5′ phosphate H 3′ hydroxyl FIGURE 1-13 A, Molecular structure of DNA showing nucleotide structure, phosphodiester bond connecting nucleotides, and complementary pairing of bases (A, adenine; T, thymine; G, guanine; C, cytosine) between antiparallel nucleic acid strands. C, Same colony after 5 days of incubation. Of the following, which would most likely cause an opportunistic infection in the genitourinary tract of a woman of childbearing age? are responsible for 3% of all hospital-acquired infections in the United States, particularly UTIs. They ascend the urinary tract, causing infections in both the lower and the upper urinary tract. Although reasons for overgrowth by M. ■ The usual microbial flora benefits the normal host by priming the immune system, outcompeting potential pathogens for nutrients, and creating a hostile environment for other microbes. rickettsii. Similarly, 50% of the bacteria in cervical and vaginal secretions are anaerobes. Always check the quality of the stain before moving to interpretation. Bile Disk. This disk can be used to determine an organism's ability to grow in the presence of relatively high concentrations (20%) of bile; however, it is not necessary if BBE agar is used as a primary plating medium. This particular species is generally overlooked as normal biota E. The egg of S. • They do not hydrolyze urea. Great care must be exercised to avoid transfer of culture medium with the inoculum because even minuscule amounts of vitamin carried over can adequately supply the requirement and thereby result in a false growth reaction. A study in Finland surveyed 1338 children younger than 13 years and found that 47 (3.5%) with respiratory illness were positive for hMPV. The Wright-Giemsa stain is available in a modification that requires only 1 to 3 minutes. Mucosal penetration and invasion do not appear to be part of ETEC disease. gondii organisms as antigen are routinely used for diagnosis. Because most strains ferment lactose, albeit as a delayed positive reaction, the easiest screening procedure is an oxidase test performed on colonies from nonselective media, such as SBA or CHOC agar. A 411 Extending the safranin counterstaining time to at least 10 minutes can enhance the staining intensity of the organisms. This suggests that the food may have been cross-contaminated by raw shellfish after cooking. Flood with safranin or dilute carbolfuchsin or neutral red for 30 seconds to 1 minute (anaerobes). Following this diagram, it becomes easier to classify infections of the skin, depending on where the infection occurs. FIGURE 27-56 Bone marrow stained with Giemsa stain showing the yeast phase of Histoplasma capsulatum var. Microorganisms usually isolated from the genitourinary tract are listed in Box 2-6. It is recommended that the test be done on egg agar cultures 3 to 4 weeks old and with at least 50 colonies. Pathogenic staphylococci release leukocidins that cause lysosomal discharge into cell cytoplasm. The trachea is lined with ciliary epithelium. Exceptional reactions occur. The rickettsiae are spread throughout the host hematogenously and induce vasculitis in internal organs, including the brain, heart, lungs, and kidneys. An early-morning specimen should be collected on three consecutive days, although recent studies have suggested that the addition of a third specimen does not significantly increase the sensitivity of detecting mycobacteria. Ingredients in media that allow visualization of metabolic differences between groups or species of bacteria are called differential media. rectus, and C. General Characteristics FIGURE 20-5 Gram stain of Aeromonas (×1000). Results are available tahir99 VRG 588 PART II Laboratory Identification of Significant Isolates in 2 to 3 days. glabrata. Infections caused by Bordetella spp. Eggs subsequently find their way into the lumen of the intestines or bladder. method as the reference method for comparison, of 173 species level identified strains from 187 clinical samples, 75% were identified correctly by API 20NE (bioMérieux, Durham, NC) strip, 83% by Vitek-2 (bioMérieux), and 89% by MALDI-TOF. Points to Remember ■ ■ ■ ■ ■ ■ ■ ■ ■ ■ Chlamydiae and rickettsiae are obligate intracellular organisms. Candida albicans cells or in which chemotactic activity is impaired also may have a reduced immune response. A long-term resident species of bacteria in the gastrointestinal tract produces vitamin K, which is required for blood clotting in mammals. Young cultures are off-white to pink, but with age they become black, with the production of darkly pigmented arthroconidia. This is because of a lack of sufficient discriminatory markers to detect interspecies differences and poor correlation between conventional test results and rapid or miniaturized versions, specifically esculin hydrolysis, decarboxylase reactions, and sugar fermentation. Other Escherichia Species There are currently six recognized species within the genus Escherichia. In smear-negative samples, M. An attenuated vaccine to prevent chickenpox was approved for use in the United States in 1995. These conidia measure about 7 µm × 20 to 50 µm and are produced singly on undifferentiated hyphae. cholerae, also exhibit a positive string test observed as a mucoid "stringing" reaction after emulsification of colonies in 0.5% sodium desoxycholate. It usually appears as a lactose-positive (pink) colony with a surrounding area of precipitated bile salts on MAC agar (Figure 19-1). Given the signs and manifestations of an anaerobic infection, name the most probable causative agent of the following: • Wound botulism • Tetanus • Gas gangrene • Actinomycosis • Pseudomembranous colitis • Bacterial vaginosis 8. Liver hemorrhaging, myocarditis, kidney damage, and mental status changes occur, often followed by death. Although this method is tahir99 VRG 542 TABLE PART II Laboratory Identification of Significant Isolates 24-5 Appropriate Chlamydia trachomatis Assays for Selected Patient Population Patient Population Prenatal Assay Culture Newborn Clinics* Low Risk High Risk Eye Throat Low Risk High Risk Legal Applicability (Rape or Child Abuse?) Test of Cure A/B A B A A/B B Yes Yes A A — A A — A A — B A/B B A A B No No No No No No A A IUO IUO A A IUO IUO B, LGV B NA B, LGV B B NA A A NA A A(IgM) B, LGV B B B, LGV B B No NA No No NA No Nonculture, Nonamplified DFA EIA OIA B A/B — Nonculture, Amplified PCR, SDA, TMA Serology CF EIA MIF A, Most useful, stands alone; B, probable, but needs verification or complementary assay recognizing different Chlamydia trachomatis macromolecules, i.e., LPS (EIA) vs. Later, Rosalind Franklin discovered the helical structure by x-ray crystallography. For example, human immunodeficiency virus (HIV) infects T helper lymphocytes, not muscle cells, in humans; other viruses, such as the rabies virus, can infect dogs, skunks, bats, and humans. Juranek, Centers for Disease Control and Prevention, Atlanta, GA.) Louse-Borne Typhus. Louse-borne (epidemic) typhus is caused by R. Niacin Accumulation. Most mycobacteria possess the enzyme that converts free niacin to niacin ribonucleotide. serotype Choleraesuis S. Borrelia recurrentis and Similar Borreliae Virulence Factors As the disease name suggests, relapsing fever is characterized by acute febrile episodes that subside spontaneously but tend to recur over a period of weeks. The incubation period is approximately 1 week. Other selective media that have been successful in recovering Campylobacter spp. bestiarum, and certain motile strains of A. Rotaviruses have a worldwide distribution and cause an estimated 611,000 deaths annually. The modified acid-fast stain demonstrates variably staining organisms, from dark pink to almost colorless, with no visible internal structures. Wash in running tap water. Humans acquire infection with these organisms by ingesting the metacercaria in undercooked or raw fish. This organism resides in soil with a high nitrogen content, particularly in areas heavily contaminated with bat and bird guano. The viruses attack the ciliated epithelial cells lining the respiratory tract, causing necrosis and sloughing of the cells. Weeksella and Bergeyella spp. Isolation of other yeasts from clinical samples is also increasing. There have been a number of cases of Babesia duncani and B. Safety, specimen collection, agent identification, and agent control are components of the response plan to which the laboratory can contribute its input. Members of the former genus Arizona, now subspecies IIIa of S. aeruginosa, S. During the past 35 years, an average of 193 cases has been reported annually in the United States. jordanis. This disease is diagnosed by the presence of characteristic lesions accompanied by microscopic sclerotic bodies, often referred to as copper pennies because of their shape and staining properties in tissue sections. P. A few can cross the placenta and cause congenital infection. On solid media, some species (e.g., M. Levett PN: Leptospira. When an infection occurs, massive numbers of PMNs accumulate at the site. Cutaneous acanthamebiasis has been associated primarily with patients with AIDS whose CD4 count is less than 250/mm3. Calcofluor white was the original "blueing" used in high-volume laundries to whiten yellow-appearing white cotton and other fabrics. Some states require periodic reporting of these rates. Although we usually think of the facultative anaerobes as being the predominant organisms, obligate anaerobes far outnumber the facultative gram-negative rods, making up more than 90% of the microbial flora of the large intestine. Inoculated media are incubated at 35° C to 37° C in air for at least 7 days. It is now recognized that organisms fall into three distinct groups based on type of cell organization and function: prokaryotes, eukaryotes, and archaeobacteria. EIEC 6. Patients with erythema infectiosum experience a prodrome of fever, headache, malaise, and myalgia, with respiratory and GI symptoms (nausea and vomiting). These heavily colonized surfaces are the usual portals of entry into the tissues and bloodstream for endogenous anaerobes. Both natural and recombinant interferons have been shown to be effective in preventing infection and illness when given intranasally over short periods. Because of subjectivity in reading the samples and the use of expensive fluorescent microscopy, the FTA-ABS test has become less frequently used in favor of the EIAs. Antimicrobial Susceptibility Penicillin is the drug of choice for treating patients with syphilis. The shell vial is inoculated with the clinical sample and then centrifuged to promote viral absorption. Contaminated tap water has been implicated as a cause of positive blood cultures in a patient receiving irrigations who had recently undergone bone marrow transplantation; a leukemic patient who had undergone stem cell transplantation was found to have bacteremia with Methylobacterium fujisawaense. werneckii produces shiny, moist, yeastlike colonies that start with a brownish coloration that eventually turns olive to greenish black. PCR methods and antigen detection assays are also available commercially. jirovecii (Giemsa stain). of the organism. Serum also contains low-molecular-weight cationic proteins, termed β-lysins. Right, ESwab with prereduced liquid Amies (Copan Diagnostics, Corona, CA). MOMP (DFA) or competition assay for DNA probes; CFI, complement fixation; DFA, direct fluorescent antibody; EIA, enzyme immunoassay; IUO, investigational use only; LGV, lymphogranuloma venereum; MIF, microimmunofluorescence; MOMP, major outer membrane protein; NA, not available; OIA, optical immunoassay; PCR, polymerase chain reaction; SDA, strand displacement amplification; TMA, transcription-mediated amplification. In Brenner DJ, et al, editors: Bergey's manual of systematic bacteriology, ed 2, vol 2, the proteobacteria, part C, New York, 2005, Springer, p. rectus) and Mobiluncus spp., among many others. The MicroSeq Microbial Identification System (Applied Biosystems, Foster City, CA) has kits and an electronic database that can be used for bacterial or fungal identification. Other notable species of Candida are C. Coughing spells may occur many times a day and are sometimes followed by vomiting. No PCR kit is commercially available at the present time. Automated blood cultures are often insensitive, although lysis centrifugation using the Isolator (Wampole Laboratories, Cranbury, NJ) system is a good alternative, albeit labor-intensive. saginata show 15 to 20 per side. Smears from Thick, Granular, or Mucoid Materials Opaque material must be thinly spread so that a monolayer of material is deposited in some areas. Centers for Disease Control and Prevention: Multi-state outbreaks of Salmonella infections associated with raw tomatoes eaten in restaurants—United States, 2005-2006, MMWR 56:909, 2007. Both IgG and IgM antibodies are commonly assayed in a variety of serologic tests. tahir99 VRG Rather than repeat cultures for similar respiratory pathogens (i.e., Mycoplasma pneumoniae), establish cause and impact on diagnosis-related group reimbursement CHAPTER 24 Chlamydia, Rickettsia, and Similar Organisms FIGURE 24-8 Chlamydophila pneumoniae detection from direct sputum smear using fluorescent-labeled monoclonal antibody, highlighting cytoplasmic inclusion (×400). This increase is due to expansion of reporting into adolescents and adults as well as additional diagnostic tools such as PCR. Serodiagnosis can be useful to diagnose infection in patients from nonendemic countries who develop symptoms after visiting endemic areas. For instance, inflammation, a nonspecific response of the innate system, provides a signal that triggers an adaptive type of immune response. All herpesviruses share the property of producing latency and lifelong persistence in their hosts. After cholera, the second most serious types of Vibrio-associated infections are those caused by V. These introductory case studies represent an important pathogen, infectious disease, concept, or principle that is discussed in the chapter text and is used to introduce the learner to the main context discussed in the chapter. Acetone is a more rapid decolorizer and may give better results, but the reaction must be stopped with water as soon as the purple color disappears. Flood slides with 70% ethanol, and rinse with tap water. The cyst stage is small (3 to 5 µm), oval to round, and possesses one to four nuclei and multiple vacuoles. Centers for Disease Control and Prevention: Progress toward eliminating Haemophilus influenzae type b disease among infants and children— United States, 1998-2000, MMWR 51:2234, 2002. Based on this evidence, the noninvasive organism, formerly referred to as nonpathogenic E. Available at: . Merozoites are released to infect other RBCs. Some will differentiate into gametocytes, but this is not an easily identifiable stage from the asexual form. vulnificus strains from the nonsucrose-fermenting (green) species—V. vulneris. Part I presents basic principles and concepts of diagnostic microbiology, including quality assurance, which provide students with a firm theoretic foundation. Most infections in women and men can remain asymptomatic, which facilitates the spread of the bacteria by unprotected sexual contact. maltophilia may be inherently resistant. In the 1990s, several health agencies, including the WHO and United Nations Children's Fund, launched an eradication program. ozaenae Klebsiella pneumoniae subsp. The capsule can be detected surrounding the budding yeast in spinal fluid with the aid of India ink or Nigrosin (Figure 27-53). Spore formation by C. The other members of the tribe Escherichiae, the shigellae, are discussed in the section on enteric pathogens of this chapter. Other Genera of the Family Enterobacteriaceae Budivicia Based on DNA hybridization, Budivicia aquatica is a group of closely related organisms. RSV can be a significant cause of morbidity and mortality in older patients as well. von König CHW, et al: Bordetella and related genera. Amebic ulcers of the liver often are detected by ultrasonographic or radiographic tests. Knowledge of common pathogens and nonpathogens that exist in specific geographic regions and for a given body site is necessary to ensure proper identification and, if necessary, therapy. TABLE 24-6 Detection Capabilities of Various Methods for Chlamydia trachomatis Culture Specimen Site SENS* (%) SPEC* (%) PPV* (%) NPV* (%) 50-85 100 73-98 90-100 + + + + False − None False ± Staphylococci Nonculture, nonamplified Reported Cervical-Urethral Rectal Urine Eye False ± Cross-Reactivity DFA 70-95 92-98 73-98 95-99 + + − + EIA 72-95 90-99 45-92 95-99 + − LA LA 85-95 100 + − + Nonculture, Amplified PCR, SDA, TMA 85-95 99-100 Comments Labor-intensive gold standard for specificity Screen only; experience in FA needed False ± Streptococci, GC, Verify with Acinetobacter complementary assay False − None reported No verification necessary DFA, Direct fluorescent antibody; EIA, enzyme immunoassay; FA fluorescent antibody; LA limited availability; NPV, negative predictive value; PCR, polymerase chain reaction; PPV, positive predictive value; SDA, strand displacement amplification; SENS, sensitivity; SPEC, specificity; TMA, transcription-mediated amplification. In P. Dogs and pigs serve as reservoir hosts. pneumoniae Klebsiella pneumoniae subsp. nov., a new genus to accommodate the biogroups of Enterobacter sakazakii, and proposal of Cronobacter sakazakii gen. such as E. FIGURE 22-15 Typical special potency antimicrobial disk results for Clostridium ramosum, susceptible to vancomycin (left) and kanamycin (right) and resistant to colistin (center disk). cholerae O1 organisms are divided into the following serotypes: Ogawa (A, B), Inaba (A, C), and Hikojima (A, B, C). The formalin–ethyl acetate sedimentation (FES) method was the standard sedimentation method. The organism ferments glucose and is superoxol- and catalase-negative. The infection is seen primarily in children 1 to 4 years old. The organism multiplies in brain tissue and within 2 to 4 days, the patient can suffer drowsiness, confusion, and seizures, and progress into a coma. This is because of possible serious discrepancies in β-lactamase detection by rapid minimal inhibitory concentration methods that would directly affect the proper treatment of aeromonad infections. The organism is considered by government agencies to be a potential bioterrorism agent. In the United States, there is a racial distribution, with infections more prevalent in whites than in African Americans. Differentiation of each genus and definitive identification of species are based on biochemical characteristics and DNA homology. A B FIGURE 28-39 Cyclospora cayetanensis oocyst. The Amplicor MTB PCR assay (Roche Molecular Systems, Branchburg, NJ) consists of PCR amplification of the 584-bp region of the 16S rRNA gene sequence. Pithomyces spp. These bacteria are classified as either lactose fermenters or lactose nonfermenters. Medium V, a modification of the original Butzler medium, contains cefoperazone, rifampin, colistin, and amphotericin B; it seems to inhibit normal colon microbiota better than the original formulation. Peroxisomes contain protective enzymes that break down hydrogen peroxide and other peroxides generated within the cell. Avoid getting reagents in eyes or on skin or clothing; if this does occur, flush with copious quantities of water. The isolate is subcultured to a sheep blood agar plate with an O/129 disk. mirabilis Lysine decarboxylase + – Salmonella spp. Based on analysis of 16S and 23S rRNA gene sequences, a revised taxonomic classification has been accepted. The larva is released in the intestine, penetrates the intestinal wall, and migrates to the body cavity, in which males and females mature. histolytica–E. Most often these bacterial species are isolated with other organisms, including more fastidious pathogens. They are the site of protein biosynthesis. difficile), diarrhea (C. It is FIGURE 29-10 Electron micrograph of a varicella virus (×100,000). Antigenic heterogeneity Masking of antigen Destruction of antibody Antigenic variation Failure to activate alternative pathway Inactivation of complement components Resistance to bacteriolysis Formation of abscess Fimbriae, capsules, LPS, M protein Capsules, Ig-binding proteins Borrelia Fibrinolysis Collagenase, elastase Nonspecific B-cell activation Inhibition of delayed hypersensitivity Rapidly fatal (toxin) Inhibition of chemotaxis Streptococcus spp. The traditional starting place is to decide whether the isolate is a yeast or a mold. tahir99 VRG 628 PART II Laboratory Identification of Significant Isolates Relative numbers Cysts Trophozoites Liquid Soft Formed Consistency of stool specimen FIGURE 28-1 Relationship of stool consistency to protozoan stage. Chronic heavy infection with hookworm can lead to microcytic hypochromic anemia, especially in children whose diet is inadequate in iron and protein. Bosshard PP, et al: 16 S rRNA gene sequencing versus the API 20NE system and the VITEK 2 ID-GNB card for identification of nonfermenting gram-negative bacteria in the clinical laboratory, J Clin Microbiol 44:1359, 2006. Note the pink to red colonies. After incubation, the metabolic end products are extracted with chloroform and ether, injected into the chromatograph, volatilized, and carried as a gas through a packed column. Granules within the material must be crushed so that their makeup can be assessed. For example, Cryptococcus spp. Approximately 43 species of dermatophytes and dermatophyte-like fungi have been described, and approximately 24 of these have been documented to cause human infection. Machado CM, Levi JE: Transplant-associated and blood transfusion– associated tropical and parasitic infections, Infect Dis Clin N Am 26: 225, 2012. Fresh or formalinpreserved stool specimens may be used. mansoni and S. One of the most serious complications is ileus, an obstruction of the intestines, with marked abdominal dilation, possibly leading to toxic megacolon. They range in size for coccoid forms from approximately 0.2 to 0.3 µm in diameter to tapered rods of approximately 1 to 2 µm in length and 0.2 to 0.3 µm in diameter. The fever can last 12 to 22 days. Single-titer evaluations, although not diagnostic, may be suggestive. pneumophila serogroup 1, which is responsible for 85% of cases of legionellosis. Hantaviruses typically affect the peritoneal cavity, kidneys, thoracic cavity, or lungs. Plesiomonas can be serotyped by somatic O antigens and their flagellar H antigen. fonticola, Serratia spp. Blood Flukes. The blood flukes, Schistosoma spp., differ from other flukes in the following manner: • There is both a male and female form; the female lives in an involuted chamber, the gynecophoral canal, which extends the length of the male. Most newborns are infected by mothers who are asymptomatically shedding the virus during a primary infection. Biochemical tests to identify the isolate were performed with the following results: triple sugar iron, acid over acid with gas production; indole, methyl red, VogesProskauer, citrate (IMViC) reactions were − − + +; urea was hydrolyzed; lysine was decarboxylated; malonate was utilized; the organism was nonmotile. One type of program might assess the reproducibility of results in the examination of fecal specimens. In the insect's stomach, exflagellation by the male and subsequent fertilization of the female result in formation of an ookinete that migrates through the gut wall and forms an oocyst on the exterior gut wall. Most cases were linked to red blood cell components. The antigen can be detected by day 3 of the infection and can persist for 1 year; consequently, the test is of limited value in persons with a recent history of Legionella infection. fluorescens and P. Patients in the tertiary stage are usually not infectious. Yeast isolates are suspended in an agar

Embryonated when passed Schistosoma haematobium 110-170 × 40-70, oval Rounded anterior Terminal spine Embryonated when passed Schistosoma japonicum 60-95 × 40-60, round to slightly oval Small, inconspicuous, hooked lateral spine Embryonated when passed *These two species and C. Examine. R. It is caused by the hemoflagellate T. There is evidence that the organism may also be associated with urinary tract infections in young girls. Eggs of the lung fluke Paragonimus westermani can also be identified in a sputum wet mount. freundii has been associated with endocarditis in intravenous drug abusers. diversus) amalonaticus youngae braakii farmeri pneumoniae subsp. chelonae, and M. Visceral leishmaniasis has also been reported to be transmitted in solid organ transplants. Serologic tests, including immunoblot techniques, have been developed to detect antibodies against specific cysticercal antigens. Case reports show that treatment with a drug such as metronidazole results in the clearance of the organism and alleviation of symptoms. At a later time, the phage may be “induced,” and a lytic cycle then ensues. histolytica, Trypanosoma cruzi, or T. bifermentans have all been associated with myonecrosis. An acute, dysenteric form of diarrhea (similar to shigellosis), with blood and mucus 3. are curved, non–spore-forming, gram-negative rods that measure approximately 0.2 to 0.9 µm × 0.5 to 5.0 µm (Figure 20-9). The WHO announced in April of 2010 that the H1N1 influenza virus moved into the postpandemic period. Heggie TW: Swimming with death: Naegleria fowleri infections in recreational waters, Travel Med Infect Dis 8:201, 2010. Almost half of all infants are infected with RSV during their first year of life, and by the age of 2 years, almost all have been exposed to RSV. The causative agent of syphilis was not discovered until 1905. tahir99 VRG CHAPTER 24 Chlamydia, Rickettsia, and Similar Organisms Other Urogenital Diseases. C. Transmission to humans involves ingestion of a larval stage, called the cysticercus (larva consisting of a fluid-filled sac containing an invaginated scolex), cysticercoid, or plerocercoid larva (depending on the genus) in raw or undercooked meat or fish or of insects harboring the larval stage. The organisms are found in water and muddy soils in Southeast Asia (including Vietnam and Thailand), Northern Australia, and Mexico. aeruginosa infections usually requires combination therapy, often ceftazidime or cefepime, piperacillin, or a carbapenem (imipenem or meropenem) with an aminoglycoside (tobramycin or amikacin). tahir99 VRG 15 95 100 97 92 0 100 100 100 100 0 0 99 97 99 0 0 100 0 100 0 100 0 100 30 75 0 100 97 93 13 74 0 0 0 0 0 99 0 100 0 99 0 100 0 99 0 100 0 97 0 95 0 0 0 0 D-Xylose 100 99 100 99 85 100 100 100 100 100 100 99 100 99 2 100 96 100 100 83 0 30 90 0 100 100 0 97 97 0 0 0 0 100 100 100 100 100 100 100 100 0 100 100 0 100 100 0 100 100 0 100 100 0 0 0 100 0 100 0 100 0 100 0 20 0 0 0 35 0 5 0 0 0 100 0 100 5 75 5 65 0 0 50 95 30 0 100 50 0 95 90 85 40 0 0 0 0 0 0 0 0 0 0 100 100 100 100 98 0 100 0 100 0 0 0 0 95 0 45 0 100 0 100 0 100 0 98 0 97 0 97 78 0 0 0 96 35 2 35 70 30 96 78 30 10 15 0 0 0 0 0 0 0 0 0 0 0 0 0 100 100 100 97 100 100 0 83 0 98 0 100 0 85 0 90 0 30 93 98 96 100 90 95 0 0 0 100 0 100 0 100 0 100 1 100 0 100 92 100 100 100 100 100 100 100 100 100 100 65 98 90 55 95 95 98 92 70 0 100 100 100 100 100 0 80 97 95 65 25 50 62 2 0 0 0 100 0 80 0 100 0 80 1 100 0 100 99 0 100 0 0 1 1 0 100 0 100 0 100 94 99 75 85 0 0 0 0 0 0 0 98 0 100 1 100 0 0 0 95 0 0 0 100 100 100 100 100 0 100 100 0 100 100 0 0 60 0 60 0 0 100 100 100 75 0 50 80 95 95 98 2 15 65 80 70 90 2 0 40 99 0 2 0 92 96 96 96 97 100 100 100 97 93 100 100 100 100 23 16 13 99 10 97 100 98 95 15 0 0 0 70 0 0 0 0 0 0 0 0 25 0 0 0 98 99 98 100 100 100 100 100 100 98 95 100 100 100 100 100 100 100 100 100 55 95 2 100 99 98 99 0 100 100 100 98 90 0 99 98 97 90 95 75 0 0 99 0 99 95 100 100 90 96 100 100 100 0 0 30 100 100 50 0 50 0 0 0 100 0 0 100 100 100 100 100 100 99 100 100 98 100 91 100 100 95 100 100 100 100 100 100 100 100 0 0 0 0 0 0 100 100 100 100 100 99 0 40 90 35 100 0 5 81 19 100 0 33 83 50 100 96 3 93 83 20 50 86 83 28 0 0 0 0 0 0 0 0 0 0 100 100 100 100 100 0 0 0 0 100 100 100 100 100 0 0 0 0 100 100 100 0 10 0 0 0 0 100 0 100 0 90 0 0 0 0 90 0 0 0 0 83 0 100 30 0 0 0 0 0 0 0 0 0 0 0 0 100 99 100 100 100 99 0 99 0 100 0 0 100 0 0 0 100 99 20 3 25 24 95 0 0 99 0 0 0 99 46 0 100 40 0 8 20 100 0 50 0 99 7 0 0 0 0 0 0 100 99 80 100 100 100 0 40 100 98 100 0 45 100 100 0 0 100 100 83 0 0 100 66 100 98 0 0 0 0 0 0 0 0 0 75 40 15 40 75 0 0 100 97 100 2 0 4 96 0 100 0 0 0 0 100 0 0 75 17 100 100 0 50 100 10 0 30 0 98 50 0 0 0 D-Galactose 0 Tyrosine Utilization Lipase (Corn Oil) 35 Fermentation Acetate Utilization 0 D-Mannose Tartrate, Jordan’s 75 Yellow Pigment Mucate Fermentation 1 ONPG Test Glycerol Fermentation 0 Oxidase, Kovacs’ D-Arabitol 0 Nitrate Nitrite Melibiose Fermentation 90 DNase (25° C) Esculin Hydrolysis 0 Fermentation Erythritol Fermentation 1 Trehalose Fermentation 99 100 100 100 Fermentation α-Methyl-D-glucoside Fermentation 0 100 Maltose Fermentation Fermentation L-Rhamnose Raffinose Fermentation Fermentation L-Arabinose Fermentation D-Sorbitol 1 100 Cellobiose Fermentation 0 Citrate, Christensen’s 447 CHAPTER 19 Enterobacteriaceae myo-Inositol Fermentation 100 100 100 100 100 100 100 99 100 100 99 0 100 0 0 0 0 37 100 100 100 100 100 0 0 0 0 100 Continued tahir99 VRG 448 Arginine Dihydrolase Ornithine Decarboxylase Motility Gelatin Hydrolysis (22° C) Growth in KCN Malonate Utilization D-Glucose, Acid D-Glucose, Lactose Fermentation Sucrose Fermentation D-Mannitol Dulcitol Fermentation Salicin Fermentation Adonitol Fermentation 0 95 95 0 98 1 99 90 1 0 0 0 0 0 7 100 93 29 0 64 79 0 79 0 100 86 0 7 0 0 0 0 15 100 100 100 0 80 0 0 90 5 100 93 0 0 0 0 0 0 0 0 100 0 0 0 0 100 0 0 0 0 0 0 0 0 0 0 85 0 100 0 100 2 0 50 35 82 0 65 100 9 100 0 75 20 0 0 40 0 0 75 100 1 100 0 0 15 0 0 65 0 0 7 0 0 0 0 0 100 80 20 0 100 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 50 86 0 0 100 95 0 0 0 15 0 0 0 0 20 0 50 50 82 0 70 50 64 100 0 50 0 0 0 20 0 25 20 9 0 0 0 0 0 0 0 60 0 89 0 22 98 98 98 0 100 100 30 100 99 95 95 99 0 0 98 0 20 0 100 0 2 97 50 0 100 100 0 100 0 98 95 0 99 99 0 89 65 50 0 15 98 0 85 0 99 93 0 98 65 0 98 100 0 0 88 100 0 100 0 1 100 0 0 H2S (TSI) 95 20 0 0 100 0 100 0 100 0 0 99 95 90 98 0 100 100 100 0 85 50 99 0 95 91 99 1 96 0 100 0 46 0 8 0 2 0 0 0 0 100 0 100 96 100 100 100 45 100 85 100 85 0 100 0 Fermentation Lysine Decarboxylase 1 95 Gas Phenylalanine Deaminase 95 Citrate (Simmons) 95 Voges-Proskauer Morganella morganii subsp. The sporozoites enter the human circulation and take approximately 60 minutes to reach the liver, where they begin the exoerythrocytic phase by penetrating parenchymal cells. Sexual and asexual reproduction occur in the human intestinal tract. Severe systemic disease (Weil disease) includes renal failure, hepatic failure, and intravascular disease and can result in death. Genes that are always expressed are constitutive. Instead of using two teasing needles, a portion of the colony may be removed by one teasing needle or by a sterile applicator stick. perfringens. Bacterial species vary in their possession of flagella from none (nonmotile) to many (Figure 1-4). In contrast, Neisseria meningitidis may be found in the nasopharynx of asymptomatic individuals during an outbreak of meningitis. Conversely, isolates from immunocompromised hosts consist of a wide variety of strains. Specimens fixed in 10% formalin, SAF, or Ecofix can be concentrated. It also uses other receptors and has a broad range of host cells. tarda Indole E. Adult worms live in the large intestine (cecum), although they have occasionally been found in the appendix or vagina. difficile produce toxins A and B, assays that detect both toxins are generally more sensitive. xylosoxidans Alcaligenes faecalis Pseudomonas luteola Brucella sp. This isolate is readily recovered from the environment worldwide. Of interest is that some of these O139 strains share cross-reacting antigens with Aeromonas trota, a somewhat uncommon cause of diarrheal disease (see later). Freeze specimens at −70° C if processing will be delayed for several days. Ellis J, et al: Tularemia, Clin Microbiol Rev 15:631, 2002. conorii, occurs in France, Spain, and Italy. Sigmoid biopsies may be used to demonstrate the characteristic morphology of the A intestinal ulcers or to identify trophozoites in tissue when none can be isolated from the stool specimen. Nørskov-Lauritsen N, Kilian M: Reclassification of Actinobacillus actinomycetemcomitans, Haemophilus aphrophilus, Haemophilus paraphrophilus and Haemophilus segnis as Aggregatibacter actino mycetemcomitans gen. parapertussis can be adequately identified and separated with these serologic reagents alone. The latent phase can last 10 to 40 years after infection, but only about 30% to 40% of patients develop the chronic form of Chagas disease. Picornaviridae The Picornaviridae is one of the largest families of viruses, with more than 230 members. salmonicida, which is a fish pathogen with several subspecies. *Antibiotics generally used are cycloheximide and chloramphenicol. For instance, the enzyme EcoRI was isolated from E. Compare the difference(s) between treponemal and nontreponemal tests for syphilis. An oxidase-positive, indole-positive, β-hemolytic, gram-negative bacillus resistant to O/129, cannot grow in 6% NaCl broth, and is a non–lactose fermenter on MacConkey agar is isolated from an adult stool culture. The patient had a history of poorly controlled diabetes, chronic obstructive pulmonary disease, and Kaposi’s sarcoma of the right leg. Moraxella catarrhalis d. Although a safe vaccine has been available for decades, about 200,000 cases of yellow fever and 30,000 resulting deaths are reported annually worldwide. Brill-Zinsser disease is a milder disease than louse-borne typhus, and death is rare. KOH Preparation A 10% to 20% solution of KOH is useful for detecting fungal elements embedded within skin, hair, nails, and tissue. In most diagnostic microbiology laboratories, this procedure consists of visual inspection at the time of smear and culture preparation and microscopic examination of a Gram-stained preparation for structures too small to be seen with the unaided eye. Rapid antigen detection kits are also available for RSV. The smallest of the DNA viruses are the Parvoviridae, which are naked ssDNA viruses that measure about 22 to 26 nm in diameter. Once inside the cell, the active A1 subunit stimulates the production of adenylate cyclase through the inactivation of G protein. sparsely septate • Rhizoids FIGURE 27-55 Guideline for the identification of fungal isolates. Lee Y-M, et al: Serologic diagnosis of human Taenia solium cysticercosis by using recombinant and synthetic antigens in QuickELISA, Am J Trop Med Hyg, 84:587, 2011. Explosive, foul-smelling, nonbloody diarrhea is present. Biphasic illness—prolonged sore throat, crouplike hoarseness, followed by lower respiratory (flulike) symptoms Pneumonia and bronchitis, rarely accompanied by sinusitis Fever relatively uncommon Chest radiograph shows isolated pneumonitis One in nine infections results in pneumonia. Acremonium falciforme. Acremonium falciforme produces mucoid clusters of single- or two-celled, slightly curved conidia borne from phialides at the tips of long, unbranched, multiseptate conidiophores. This process is referred to as splitting or passaging. It was formerly called Enteric Group 90. Generally, enteric opportunistic organisms isolated from sites that are normally sterile are highly significant. 2. Confirm that an outbreak exists. This microscopic appearance has gained M. answer a page. This stainless steel and Plexiglas chamber is manufactured by Anaerobe Systems. The onset is insidious, with fever, headache, stiff neck, vomiting, and photophobia, and progresses to personality changes and seizures. It is imperative for any laboratory performing bacteriology to be able to identify serologically Salmonella serotype Typhi particularly and other members of Salmonella in O groups tahir99 VRG CHAPTER 19 Enterobacteriaceae A through G. They circulate as monocytes for 1 to 2 days and then migrate through the blood vessel walls into the tissues and reside in specific tissues as part of the mononuclear phagocyte system. Previously, it was thought that Scedosporium apiospermum was TABLE 27-2 Microscopic Morphology of Fungi Causing Chromoblastomycosis Organism Phialophora verrucosa Fonsecaea pedrosoi Fonsecaea compactum Cladophialophora carrionii Rhinocladiella aquaspersa Microscopic Morphology Conidiogenous cells, phaeoid, flask-shaped phialides, with collarettes Conidia oval, one-celled, occur in balls at tips of phialides Primary one-celled conidia formed on sympodial conidiophores Primary conidia function as conidiogenous cells to form secondary one-celled conidia. Superficial b. and Trypanosoma cruzi) Blood/cerebrospinal fluid (Trypanosoma sp.) Nucleus Kinetoplast Amastigote Trypomastigote • Free flagellum • Undulating membrane attached posterior to nucleus • Retracted flagellum INSECT Sand fly (vector for Leishmania sp.) Tsetse fly Reduviid bugs Epimastigote • Free flagellum • Undulating membrane attached anterior to nucleus Promastigote • Free flagellum FIGURE 28-21 Life cycle stages of the blood and tissue flagellates. The occurrence of all biochemical reactions in the cell depends on the presence and activity of specific enzymes. Testing for toxins or colonizing factors is performed by research and reference laboratories; its use is not justified in the clinical laboratory for diagnostic purposes. tahir99 VRG CHAPTER 18 Haemophilus and Other Fastidious Gram-Negative Bacilli 407 Learning Assessment Questions FIGURE 18-25 Francisella tularensis colonies grown on chocolate (CHOC) agar. Grampositive archaea have a thick wall and stain purple. Consequently, treponemal tests are also not useful in following therapy or detecting reinfection. Because of the special nutritional requirements of this organism, routine fungal cultures are negative for growth. aeruginosa will also produce the blue, water-soluble pigment pyocyanin. Because of the low infective dose required to produce the disease, shigellosis is highly communicable. In 2002, the CDC documented 4156 human cases of WNV in 44 states. Cutaneous c. This diversity is also found in the microorganisms that inhabit the human body as normal biota (normal flora), as opportunistic pathogens, or as true pathogens. Laboratory Diagnosis Microscopic Examination. Diagnosis of borreliosis is readily made by observing Giemsa- or Wright-stained blood smears of blood taken during the febrile period. Bacterial genomes contain many IS elements. The opportunistic pathogens are often a part of the usual intestinal microbiota of both humans and animals. Three specimens should be collected within 3 days. Botulinum toxin is a neurotoxin that blocks nerve impulse transmission, causing flaccid paralysis, especially in infants. epidermidis are called opportunistic tahir99 VRG 30 TABLE PART I Introduction to Clinical Microbiology 2-1 Abbreviated List of Opportunistic Microorganisms Conditions Compromising Host Defenses Foreign bodies (catheters, shunts, prosthetic heart valves) Alcoholism Burns Hematoproliferative disorders Cystic fibrosis Immunosuppression (drugs, congenital disease) Organism(s) Staphylococcus epidermidis Propionibacterium acnes Aspergillus spp. Treponema pallidum Subsp. oryzihabitans isolates are resistant to first- and second-generation cephalosporins but are usually susceptible to penicillin. tend to be mucoid and can flow across the plate, a trait shared by some bacterial isolates, such as Klebsiella Candida Candida spp. Transport systems should be selected based on anticipated 417 transport time and test desired. enterocolitica and most Aeromonas spp., but one must remember that Yersinia is oxidasenegative, Aeromonas is oxidase-positive, and testing for this trait must be done on SBA, not on selective or differential media. +, Feature present; −, feature absent. There is speculation that long-term H. Approximately 80% of individuals infected with WNV are asymptomatic. Microbial Growth and Nutrition All bacteria have three major nutritional needs for growth: • A source of carbon (for making cellular constituents). Isolation Methods Culture Media In general, fungi do not share the broad range of nutritional and environmental needs that characterize bacteria, so relatively few types of standard media are needed for primary isolation. Although pockets of individuals, mostly in West Africa, are infected with HIV-2, it is the impact of HIV-1 that continues to be felt around the world. are opportunistic pathogens associated with outbreaks in health care settings. First-void urine and vaginal swab specimens are excellent for detecting infection. The herpesviruses have a genome of linear dsDNA, icosahedral capsid, amorphous integument surrounding the capsid, and outer envelope. Varicella-Zoster Virus Varicella-zoster virus, in the genus Varicellovirus, spreads by droplet inhalation or direct contact with infectious lesions. The Clinical and Laboratory Standards Institute (CLSI) offers a document (M35-A2, Abbreviated Identification of Bacteria and Yeast) that provides guidelines for the rapid presumptive identification of many commonly encountered anaerobic bacteria. Opportunistic pathogens such as S. Human Metapneumovirus The human metapneumovirus (hMPV) was first described in children with previously virus-negative cultures. dubliniensis is differentiated from C. An effective attenuated vaccine is available and recommended for all children. Available at: index.html. In the fifth step, a tentative hypothesis is established as a best guess about the likely reservoir, source, and means of transmission. falciparum infection. In most patients, the illness is self-limited and usually resolves in 2 to 6 days. The Fontana-Masson method stains melanin in the cell wall and identifies the presence of phaeoid fungi. dubliniensis are identified by germ tube production (Figure 27-59). CDC Groups EO-3, EO-4, and Paracoccus The taxonomy of CDC groups EO-2, EO-3, and EO-4 is unclear; EO refers to eugonic oxidizer. Ubiquitous in soil and water, they are rarely isolated from clinical specimens but have been found in hospital equipment and fluids. The truncate-based conidia tend to remain in chains on the annellides. Three methods are used for the isolation of viruses in diagnostic virology: cell culture, animal inoculation, and embryonated eggs. This is necessary because lactose-positive colonies from selective differential media, such as MAC or cefsulodin-Irgasin-novobiocin (CIN) agar, can give false-positive oxidase reactions. Specimens are collected in sterile, leakproof containers and should be stored at 2° C to 8° C or frozen at −20° C if testing is delayed. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary. The suspension is used to inoculate media, as described earlier for an aspirate. The swab is immersed in the specimen for several seconds and used to prepare a thin spread of material on the glass slide for staining and viewing. It is recovered worldwide primarily in wet conditions, such as shower tiles and water lines. PIV type 2, and to a lesser extent PIV type 3, can also produce syncytia. An F+ cell connects with an F− cell via sex pili. The identification of protozoan trophozoites and cysts is confirmed on a permanently stained smear. Smith, Baltimore, MD.) grow on most simple laboratory media. medinensis Dracunculus medinensis. Dracunculus (guinea worm, fiery serpent of the Israelites) causes serious infections in the Middle East, parts of Africa, and India. The Case in Point describes these circumstances and host factors such as smoking, alcohol consumption, and inadequate rest, which also contribute to disease acquisition and severity. When stained with methylene blue or Wayson stain, it shows intense staining at each end of the bacillus, referred to as bipolar staining, which gives it a “safety-pin” appearance. To recover H. Differentiate the cell walls of gram-positive from gram-negative bacteria. Under harsh conditions, each vegetative cell (active, capable of growing and dividing) produces internally one endospore (inactive), which germinates under favorable environmental conditions into one vegetative cell (active). Issues to Consider After reading the patient’s case history, consider: ■ The cause of meningeal infections in the given patient population *My comments are my own and do not represent the view of the Health Resources and Services Administration of the Department of Health and Human Services.” 552 7. It was originally thought to be a Cyanobacterium-like body, blue-green algae, or large C. 420 5. schenckii at 22° C can be variable. Specimen Selection, Collection, and Transport A number of different clinical specimens are suitable for the diagnosis of viral diseases. Wells remain clear when the sugar is not assimilated. Direct Nucleic Acid Amplification Tests. Nucleic acid amplification assays designed to detect M. Also in contrast with RMSF, this disease is characterized by the presence of taches noires (black spots) at the primary site of infection. After uptake, the DNA may take one of three courses: (1) it is integrated into existing bacterial genetic material; (2) it is degraded; or (3) if it is a compatible plasmid, it may replicate in the cytoplasm. About 12 hours after the first blood cultures were drawn, both blood cultures tested positive for a gram-negative bacillus. Most cases in organ transplant patients are caused by reactivation of latent or chronic infection, although some cases may result from primary infection. Siegel JD, Rhinehart E, Jackson M, Chiarello L; the Healthcare Infection Control Practices Advisory Committee: 2007 guideline for isolation precautions: preventing transmission of infectious agents in health care settings. Salmonella spp. However, those incubated in anaerobic jars, bags, or pouches will be exposed to the potentially damaging effects of oxygen whenever the containers are opened. It has changed over the years from viral diagnostic testing performed in only a very few, highly specialized laboratories to the modern, high-complexity laboratory of today. Specimen Collection and Handling Nasopharyngeal aspirates or swabs (calcium alginate or Dacron polyester with a flexible wire shaft) constitute the specimen of choice for culture, DFA, and PCR testing for Bordetella. Speciation is based on the number of coils in the polar tubule, septations in the spore, and size. One clue that a mold is a basidiomycete is the presence of clamp connections. 677. Burkholderia gladioli A plant pathogen, Burkholderia (Pseudomonas) gladioli, resembles B. However, many individuals have subtle infections from which fewer organisms will be shed. Adults, which are reddish brown and approximately 1 cm long, live within capsules in the bronchioles. Epidermophyton is distributed worldwide. They have also been implicated in cases of peritonitis and possibly meningitis, although often in association with each other or with other bacteria. vesicularis strains will grow on MAC agar. are unable to oxidize methanol or assimilate acetamide. To eliminate confusion, the first two letters or the first syllable are used when two or more genera names begin with the same first letter (e.g., Staph. Clinical and Laboratory Standards Institute: Abbreviated identification of bacteria and yeast: approved guideline, ed 2, CLSI Document M35-A2, Wayne, PA, 2008, CLSI. Microaerophilic bacteria require a reduced level of oxygen to grow. albicans is a yeast that may be part of the normal oral flora in people. It requires a skillful microscopist, however, to discern amebic motility in a field of neu trophils. can tolerate chlorine concentrations of 3 mg/L, they resist water treatment and subsequently gain entry into and colonize human-made water supplies and distribution systems. Some characteristic clues can indicate the presence of a nonfermenter in the clinical laboratory: • Thin, gram-negative bacilli or coccobacilli on Gram stain • Oxidase-positive reaction, although reaction can be weak and variable • Nonreactivity in 24 hours in commercial multitest kit systems used primarily for the identification of Enterobacteriaceae • No acid production in the slant or butt of TSIA or KIA BOX 21-1 Risk Factors for Diseases Caused by Nonfermentative, Gram-Negative Bacilli Immunosuppression Diabetes mellitus Cancer Steroids Transplantation Trauma Gunshot, knife wounds, punctures Surgery Burns Foreign Body Implantation Catheters, urinary or bloodstream Prosthetic devices—joints, valves Corneal implants or contact lenses Infused Fluids Dialysate Saline irrigations tahir99 VRG 476 PART II Laboratory Identification of Significant Isolates • Resistance to a variety of classes of antimicrobial agents, such as aminoglycosides, third-generation cephalosporins, penicillins, and fluoroquinolones Many classification systems have been devised for grouping the nonfermenters. Ingestion The next step of phagocytosis is ingestion. Individuals with extensive disease shed large numbers of organisms. Surveillance is defined as: a. tahir99 VRG CHAPTER 19 Enterobacteriaceae Kimberly E. Sometimes that education needs to be provided, even when it is not sought. Shigella K antigens, when present, interfere with the detection of the O antigen during serologic grouping. yeei have been recovered from blood cultures and a cutaneous bulla in a 67-year-old man. In addition, the strong association between long-term H. In vitro, the organism demonstrates resistance to ampicillin, cefoxitin, and nalidixic acid; intrinsic resistance to fluoroquinolones has been detected. Well water and food may have been cultured in addition to specimens from the patients with diarrhea and food handlers. For each organism presented describe the morphology; life cycle, including the infective and diagnostic stages; and usual procedure for identification. It also grows in PMK and human diploid fetal cells. The information contained in DNA is determined primarily by the sequence of letters along the “staircase” or “zipper.” The sequence ACGCT represents different information than the sequence AGTCC. infection in these patients has been reported. An individual should never open the jar in the vicinity of an open flame. Laboratory Diagnosis Initially, identification methods were limited to finding the small spores in Giemsa-stained tissue sections or in electron microscopic examination of biopsy specimens. Leptospires can survive in neutral or slightly alkaline waters for months. albicans cannot be differentiated from C. Based on the growth pattern, identification is made. Damage to the mucosal surface results in atrophy of the microvilli. shigelloides is found in soil and aquatic environments, but because of intolerance to increased NaCl and a minimum growth temperature of 8° C, isolates are generally found only in the fresh and estuarine waters of tropical and subtropical climates. Because infections of the skin, hair, and nails were at one time believed to be the result of burrowing worms that formed ring-shaped patterns in the skin, the term tinea (Latin meaning worm) was applied to each disease, along with a Latin term for the body site. tahir99 VRG CHAPTER 28 Diagnostic Parasitology Stark D, et al: Comparison of stool antigen detection kits to PCR for diagnosis of amebiasis, J Clin Microbiol 46:1678, 2008. Figure 28-7, B, is an enlargement of the organism to demonstrate the characteristic nuclear morphology. What is the role of the capsule in the pathogenesis of infectious diseases? The absolute concentration method determines the MIC. Special filters are required for optimal use. Rotate the two glass surfaces against each other so that the shear forces break up the material. C 45 FIGURE 26-13 Semiquantitative catalase test. For example, because Shigella spp. FIGURE 27-36 Syncephalastrum sp. A film that is too thick peels from the slide; thickness is optimal when newsprint is barely visible through the drop of blood before it dries. caviae, A. The diagnosis is based primarily on history and clinical symptoms. One difference in this life cycle from that of malaria is the lack of an exoerythrocytic cycle in humans. septicum is present in the bloodstream, it is often a marker organism for a malignancy in the GI tract. cholerae serogroup O139 in 1992 led to a widespread occurrence of cholera cases throughout India and Bangladesh. The lesions regress in about 1 week. This can be accomplished by a variety of mechanisms (see later “Processing Clinical Samples for Recovery of Anaerobic Pathogens”). In Sudan, 34 cases occurred in 1979, and 65% of the 34 patients died. Bacteria are placed in domains Bacteria and Archaea, separate from the animals; plants and protists are placed in domain Eukarya. Other Culture Media for Recovery of Mycobacteria A CHOC agar plate should be included in the primary isolation media for skin and other body surface specimens for the recovery of M. The toxin gene is commonly encoded by phages, plasmids, or transposons. Nucleic acid testing using PCR assay, although considered very sensitive, has been shown to be less sensitive than some immunologic assays. The classes differ from one another Light chain Disulfide bonds s s Heavy chains s s Nature of the Immune Response to Infectious Agents Although both humoral immunity and cell-mediated immunity are important in protecting the human from a wide variety of infectious agents, each contributes differently, in terms of overall importance, according to the type of pathogen and virulence mechanisms. (From the CDC Public Health Image Library. For example, although C. NAP selectively inhibits the M. In RNA, the nitrogenous base thymine is replaced by uracil, another pyrimidine. Figure 19-15 shows an example of a schematic diagram for the identification of commonly isolated enterics using conventional biochemical tests. These patients may have watery diarrhea and concurrent weight loss or develop a chronic infection. A skin test (Mantoux) with PPD should be administered the first day of employment and thereafter regularly to persons previously skin test–negative. Vibrio cholerae c. Definitive identification of less common species is usually performed at reference or public health laboratories, frequently using 16S ribosomal RNA sequencing methodology. Contamination of specimens not collected from sterile sites diminishes the value of culture studies. Case in Point vaginal delivery in the parking lot of a local hospital. Helicobacter pylori H. flavescens, M. Proceed as for Kinyoun method, beginning with Step 2. 7. Another selective and differential medium is SS (Salmonella-Shigella) agar, a light straw-colored medium where Salmonella colonies appear colorless with dark black centers from the production of H2S and Shigella colonies 19-11 Stool Culture Screening for Enteric Pathogens Using Triple Sugar Iron and Lysine-Iron Agar in Combination TSI Reactions LIA Reactions K/A H2S K/AG H2S K/AG K/A A/A H2S A/AG A/A K/K M. Vibrio parahaemolyticus b. This edition maintains the building block approach to learning, critical thinking, and problem solving, features that clinical laboratory science and clinical laboratory technician students, entry-level clinical laboratory practitioners, and others have found valuable and effective. Certain species can grow at low tahir99 VRG 442 PART II Laboratory Identification of Significant Isolates temperatures (1° C to 5° C, such as Serratia and Yersinia) or tolerate high temperatures (45° C to 50° C, such as E. In contrast to the lecithinase reaction, the lipase reaction is essentially a surface phenomenon (Figure 22-17). The mature schizont contains 12 to 24 merozoites, with an average of 16. enterocolitica infections manifest in several forms: an acute enteritis, an appendicitis-like syndrome, arthritis, and erythema nodosum. Define the following genetic terms: genotype, phenotype, constitutive, inducible, replication, transcription, translation, genome, chromosome, plasmids, insertion sequence (IS) element, transposon, point mutations, frame-shift mutations, and recombination. Tularemia can be contracted through ingestion, inhalation, arthropod bite (e.g., ticks, biting flies), or contact with infected tissues. Other anaerobes can survive some oxygen exposure but will not be able to perform metabolic processes unless placed into an anaerobic environment. Budding involves maturation of the bud to an independent blastoconidium (daughter cell), as shown in Figure 27-1. Artemisinin-based drugs have been used in combination with classic drugs in areas in which highly resistant strains of P. Furthermore, ulcers caused by syphilis may contribute to the efficiency of HIV transmission in populations with high rates of both infections. In a review covering 10 years, 17 of 20 patients from whom Roseomonas spp. The API 20NE is specifically for the identification of nonfermenters and the API 20E for enterics. The mortality rate for legionnaires’ disease is 15% to 30% and may approach 50% in patients with nosocomial pneumonia if the correct diagnosis is not made early. They are considered aerotolerant anaerobes, growing better under anaerobic conditions. *Formerly Moraxella phenylpyruvica. Liver Flukes. Fasciola hepatica, the sheep liver fluke, is seen in the major sheep-raising areas of the world, including parts of the southwestern United States. Rotaviruses are the most common cause of viral gastroenteritis in infants and children. tahir99 VRG 490 PART II Laboratory Identification of Significant Isolates C. The differences in size and configuration of IgG and IgM molecules result in differences in functional activity of the molecules in serologic tests (Table 2-8). A blood agar plate can also be, in a nonstrict sense, differential because it distinguishes between hemolytic and nonhemolytic organisms. Surfaces The culturing of environmental surfaces should be performed only under the combined direction of the laboratory and infection control program. EIEC infection is rare in the United States and seen less commonly in developing countries than ETEC or EPEC. For the infant described in the Case in Point, what other clinical conditions could be due to the causative organisms? Reports of P. About 30% of the patients die. They are not inhibited by the presence of lactoferrin and are able to use it for growth. Graczk Z, et al: Novel and promising compounds to treat Cryptosporidium parvum infections, Parasitol Res 109:591, 2011. Because eggs are laid outside the body in the perianal area and are rarely present in the stool, a fecal specimen is unsatisfactory for diagnosis. perstans are filarial worms not usually associated with serious infections. With the zinc sulfate method, the operculum might open and release the contents or cause the egg to sink. Society for Healthcare Epidemiology of America/Association for Professionals in Infection Control/Infectious Diseases Society of America, MMWR Recomm Rep 51(RR-16):1, 2002. Dark field microscopy is not useful because of normal oral spirochetal biota. The thick smear is used to detect malarial parasites; however, distortions of parasite morphology and the lack of intact RBCs require that a thin smear be examined to identify the species. A radiometric application was the first assay developed to detect the selective inhibition. Conidia, in many cases, are used to identify different fungal genera. parahaemolyticus V. Stools from patients with parasitic infections caused by Giardia lamblia are usually characterized as having a pale, frothy, gassy, and greasy appearance. These fibrils, termed the periplasmic flagella (also known as axial fibrils, axial filaments, endoflagella, and periplasmic fibrils), are responsible for motility. The onset of clinical illness is usually abrupt, with nonspecific, influenza-like constitutional symptoms such as fever, chills, headache, severe myalgia, and malaise. Infections range from asymptomatic to potentially fatal. Urease. Detection of urease activity can be used to distinguish M. However, one of the most effective cleansing mechanisms humans have is the desquamation of the skin surface. ureolytica has been reported to cause bacteremia in a patient with AIDS and in an 18-month-old child with pneumonia. haemophilum, which requires ferric ammonium citrate or hemin for growth. The human mycoplasmas are susceptible to adverse environmental conditions, such as heat and drying. Comparison of Prokaryotic and Eukaryotic Cell Structure Prokaryotic Cell Structure The bacterial cell is smaller and less compartmentalized than the eukaryotic cell. Mucorales Cunninghamella. Cunninghamella spp. of MAC (Figure 26-16) and thus is useful in distinguishing MAC from other nonchromogenic species. Urine is the most common specimen submitted for viral detection in these patients. Antimicrobial Susceptibility Antimicrobial susceptibility testing for Campylobacter spp. cenocepacia and B. List the media of choice for isolation of each organism. histolytica, E. Viruses causing HFRS target the kidney. Transmission can occur by direct person-to-person contact, and spread can take place via the fecal-oral route, with carriers as the source. There are two purines in the molecule: adenine (A) and guanine (G). Smith CHAPTER OUTLINE ■ GENERAL CONCEPTS IN PARASITOLOGY LABORATORY METHODS Fecal Specimens Other Specimens Examined for Intestinal and Urogenital Parasites Examination of Specimens for Blood and Tissue Parasites Immunologic Diagnosis Quality Assurance in the Parasitology Laboratory ■ MEDICALLY IMPORTANT PARASITIC AGENTS Protozoa Apicomplexa Microsporidia Helminths OBJECTIVES After reading and studying this chapter, you should be able to: 1. Clinical Infections. Infections may be symptomatic or asymptomatic. Sterile, nonfortified rice is inoculated lightly with a portion of a colony. Compared with other dermatophytes, T. cholerae O139 are associated with epidemic cholera. Most isolates of B. tahir99 VRG 438 PART II Laboratory Identification of Significant Isolates Yersinia The genus Yersinia currently consists of 14 named species; most are considered environmental species. Plates for the recovery of Bordetella spp. If the transit time is less than 2 hours or DFA is the desired test, the swab can be expressed into a solution of 1% casein hydrolysate (casamino acids) broth. This group of organisms is important because some of them, the Vibrio spp. Patients who suffer from superficial fungal infections do not show any overt symptoms because the fungal agents do not activate any tissue response or inflammatory reaction. In 1998, an outbreak of influenza A (H5N1), the avian flu, appeared in poultry in Hong Kong. Laboratory Diagnosis. Examination of tissue or purulent material in cutaneous skin lesions may reveal large, spherical, refractile yeast cells, 8 to 15 µm in diameter, with a doublecontoured wall and buds connected by a broad base (Figure 27-25). Schaeffer-Fulton is the most commonly used endospore stain. hMPV will grow slowly in standard cell culture lines, such as monkey kidney and A549 cell lines. This chapter contains several color photographs to help students understand the principles and interpretations of these important tests. Dacron, cotton, and calcium alginate swabs can be used, but it should be noted that toxicity has been associated with different lots of each, which is a concern if culture is attempted. Outside the United States, areas of high endemicity are found in northern Mexico, Guatemala, Honduras, Venezuela, Paraguay, Argentina, and Columbia. The anterior flagellum is not visible in this organism. It accounts for approximately 10% to 15% of community-acquired cases of pneumonia. Bern C, et al: Chagas disease and the U.S. blood supply, Curr Opin Infect Dis 21:476, 2008. parapaucimobilis has been isolated from sputum, urine, and vaginal specimens. Information for protein synthesis is encoded in the bacterial DNA and transmitted in the chromosome to each generation. Bloody stools containing mucus and numerous leukocytes follow the watery diarrhea, as the organisms invade the colonic tissues and cause an inflammatory reaction. Frequently, the initial site of infection is the lung, from which the organism disseminates hematogenously to other organs or the skin. The major function of the Golgi apparatus or complex is to modify and package proteins sent to it by the rough ER, depending on the protein’s final destination. tahir99 VRG Human ingestion of undercooked meat Dissolution of cysticercus in intestine 672 PART II Laboratory Identification of Significant Isolates FIGURE 28-51 Taenia sp. The organisms also secrete substances that interfere with the action of digestive enzymes and inhibit host absorption of nutrients. Bu

asymptomatic pose a great risk to others because they are cyst passers and therefore infective. morganii, with two subspecies: M. tuberculosis in an average of 7.8 days but with diminished sensitivity. Initial symptoms include severe bifrontal headache, fever (38° C to 41° C), stiff neck, and nausea and vomiting. Identification is accomplished by PCR amplification of DNA followed by sequencing of the amplicons (amplified products). Healthy skin is an effective barrier against infection. was based on selected characteristics of the growth cycle, susceptibility to sulfa drugs, accumulation of glycogen in inclusions, and DNA relatedness. Individuals with lymphoma, leukemia, or other blood disorders in which there is a functional defect in phagocytic activity or a decrease in the number of functioning 27 BOX 2-6 Microorganisms Found in the Genitourinary Tract Common Less Common Lactobacillus spp. Staining for Acid-Fast Bacilli When Gram-stained, Mycobacterium spp. Direct Microscopic Examination. Direct specimen examination by cytologic methods primarily involves trachoma and inclusion conjunctivitis (Figure 24-5). In addition, the parasitology laboratory should have the following: • A reference book collection, including texts and atlases • A set of digital images of common parasites • A set of clinical reference specimens, including permanently stained smears and formalin-preserved feces The department should also be enrolled in an external proficiency testing program. This extractable LPS, with ketodeoxyoctonate, is shared by most members of the family and is the primary antigen detectable in genus-specific tests and serologic assays for the chlamydiae. The chromatin may be surrounded by a clear halo. Aeromonas hydrophila b. When this occurs, the asexual form is termed the anamorph. bütschlii. Once trophozoites have matured, the development of meronts with merozoites begins. pseudomallei should be ceftazidine, imipenem, doxycycline, and tetracycline. tahir99 VRG 540 PART II Laboratory Identification of Significant Isolates TABLE 24-3 Human Diseases Caused by Chlamydiaceae Species Species Chlamydia trachomatis Serovars* Disease Host A, B, Ba, C D, Da, E, F, G, H, I, Ia, J, Ja, K Hyperendemic trachoma Inclusion conjunctivitis (adult and newborn) Nongonococcal urethritis Cervicitis Salpingitis Pelvic inflammatory disease Endometritis Acute urethral syndrome Proctitis Epididymitis Pneumonia of newborns Perihepatitis (Fitz-Hugh-Curtis syndrome) Lymphogranuloma venereum Pneumonia, bronchitis Pharyngitis Influenza-like febrile illness Psittacosis Endocarditis Abortion Humans Humans Chlamydophila pneumoniae L1, L2, L2a, L2b, L3 1 Chlamydophila psittaci 10 serotypes Humans Birds *Predominant serovars associated with disease. coli in the colon. Depending on the amount of melanin present, the hyphae will appear pale to dark brown or almost black. The reaction proceeds without the generation of hydrogen, so it eliminates the need for a catalyst. Exotoxins Many of the bacterial exotoxins are highly characterized. Solaymani-Mohammadi S, Singer SM: Giardia duodenalis: the doubleedged sword of immune responses in giardiasis, Exp Parasitol 126: 292, 2010. Recent reports include cases of continuous ambulatory, peritoneal dialysisassociated peritonitis. Discuss the natural habitat of the nonfermentative, gram-negative bacilli. do not belong with the vibrios. Evaluate the typical antimicrobial susceptibility pattern of the most commonly encountered nonfermentative organisms. coli: enterotoxigenic Escherichia coli (ETEC), enteroinvasive Escherichia coli (EIEC), enteropathogenic Escherichia coli (EPEC), enterohemorrhagic Escherichia coli (EHEC), and enteroadherent, which includes diffusely adherent Escherichia coli (DAEC) and enteroaggregative Escherichia coli (EAEC). maltophilia infections was associated with inappropriate antimicrobial treatment because many were initially placed on a carbapenem. Mandell LA, et al: Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults, Clin Infect Dis 44(S2):S27, 2007. plymuthica often produce a characteristic pink-to-red pigment, prodigiosin, especially when the cultures are incubated at room temperature. However, when found in secretions, tahir99 VRG CHAPTER 2 Host-Parasite Interaction Disulfide bonds J chain Secondary IgG Antibody response IgG-like subunit Primary 41 IgM Time First exposure to antigen Antigen binding sites FIGURE 2-8 Primary versus secondary response. The average size of the trophozoite is 15 to 50 µm, with most measuring 25 µm (Figure 28-7, A). Within hours, septicemia can develop, with a mortality rate of 40% to 60%. cholerae b. General Characteristics Plesiomonads are straight (0.8 to 1 µm by 3 µm), gram-negative bacilli that occur singly, in pairs, or in short chains or filamentous forms. capsulatum grows as a white to brownish mold. All are obligate aerobic bacteria, grow best at 35° C to 37° C, do not ferment carbohydrates, oxidize amino acids, are relatively inactive in biochemical test systems, and produce catalase, although this is variable in B. gonorrhoeae b. For tick-borne relapsing fever, exposure control includes wearing protective clothing, rodent control, and the use of repellents. Agents of Yeast Infections The escalating incidence of yeast and yeastlike fungi isolated from patient specimens has emphasized the importance of identifying yeast isolates to the species level. pickettii is oxidase and catalase positive (there are reports of rare catalase negative strains), grows on MAC agar, reduces nitrate, oxidizes glucose and xylose, and is motile by means of a single polar flagellum. What cells do the Ehrlichia and Anaplasma species typically infect in humans? vulnificus be reported to local and state public health departments. A summary of the clinically significant viruses is shown in Table 29-1. Some kits require a large number of organisms to be present for a positive result. Other patients have a history that involves handling animals. The remaining material is inoculated directly onto the agar. The most prominent component of this membrane is the major outer membrane protein (MOMP). Human monkeypox infection occurs primarily in central and western Africa. Trichosporon sp. HHV-7 can be isolated in culture in peripheral blood lymphocytes or in cord blood lymphocytes. Systemic human infections are rare and are associated with the patient's endogenous organisms. Outbreaks have been reported from focally contaminated swimming pools. This is particularly useful when the slide culture from a known isolate is stored in a collection for future comparison against isolates awaiting identification. Antifungal therapy is frequently ineffective when diagnosis is delayed. hepatica Unembryonated when passed Paragonimus westermani 80-118 × 48-60, oval Brown, thick shell Slightly flattened operculum Shoulders at operculum Unembryonated when passed Clonorchis sinensis 29-35 × 12-19, vase-shaped Domed operculum Prominent shoulders Knob at end opposite operculum Embryonated when passed Heterophyes heterophyes and Metagonimus yokogawai* 28-30 × 15-17, vase-shaped Schistosoma mansoni 115-175 × 45-75, oval Operculated Shoulders not distinct Small knob Embryonated Similar to C. Peters, Baltimore, MD; and H, courtesy of Dr. Andrew G. The cyst form can be found in stool and in culture. Proteus mirabilis is a commensal species in the gastrointestinal tract of humans. The organism lacks a cyst stage, and the life span of the trophozoite outside the body is very short, so direct transmission is unlikely. The biochemical tests BOX 26-4 Photoreactivity Mycobacteria of Clinically Important Nonchromogens Slow Growers M. The GenoType Mycobacteria Direct test (Hain Lifescience GmbH, Nehrin, Germany) uses a similar format and has additional probes for M. tularensis is a CDC category A select biological agent. Because of their high density, these eggs sink to the bottom of the tube. The concentrate sediment may then be examined unstained or stained with iodine. Since 2000, the incidence of C. Marsik, PhD, ABMM Microbiology Consultant New Freedom, Pennsylvania Sarojini R. pseudotuberculosis Y. One such case involved a fatal episode of multiple organ dysfunction, only 7 days after a 58-year-old Canadian developed a septicemia with V. A, Peritrichous flagella (e.g., Alcaligenes faecalis and Achromobacter spp.) (×1000). Molecular methods show that there are at least two genetic types, but their significance is unknown. Place the smear in an upright position in a staining rack, allowing the excess water to drain off and the smear to dry. Patients experience extremely painful bowel movements, which contain predominantly mucus and blood. In an iron hematoxylin stain, the parasites stain gray-black, nuclear material stains black, and background material is light blue-gray. These cultures are kept under strict security measures and their very existence remains a point 29-5 Human Papillomaviruses and Their Clinical Significance Human Papillomavirus Type Clinical Manifestation Association with Malignancy 1 2-4 5, 8, 9, 12, 14, 15, 17, 19-25, 36-38 Plantar warts Common warts Flat and macular warts 26-29, 34 Common and flat warts None None >30% of patients with epidermodysplasia verruciformis (a rare autosomal disease) with types 5, 8, 14, 17, and 20 develop malignancy Frequent, especially in immunosuppressed patients Cutaneous Mucosal 6, 11 42, 43, 44 31, 33, 35, 51, 52 16, 18, 45, 56, 58, 59, 68 Papillomatosis, primarily laryngeal, also upper respiratory tract and condylomata acuminata (genital warts) Condylomata acuminata Condylomata acuminata Condylomata acuminata Condylomata acuminata Low risk Low risk Intermediate risk High risk Adapted from Gravitt PE, Ginocchio CC: Human papillomaviruses, In Versalovic J, et al, editors: Manual of clinical microbiology, ed 10, Washington, DC, 2011, American Society for Microbiology, p. In 2011, 106 patients with C. It has been recommended that permanent stained smears be made and examined for each specimen individually. The egg spines cause trauma to the tissues and walls of the vessels during the early stage of acute infections and can result in hematuria (S. Given the results of key biochemical laboratory results, identify the most likely anaerobe. Delftia tsuruhatensis, a newly named species, has been associated with catheterrelated bacteremia. Schüffner's dots are less commonly seen than with P. Unlike C. Two additional species pathogenic for humans are S. A gram-negative, oxidase-negative coccobacillus was isolated from the cerebrospinal fluid of an infant in the newborn nursery. The capsule inhibits phagocytosis primarily by masking the cell surface structures that are recognized by receptors on the surface of the phagocytic cell and in the same manner inhibits the tahir99 VRG CHAPTER 2 Host-Parasite Interaction TABLE 2-2 Microbial Interference with Phagocytic Activities Microorganisms* Mechanism (or Responsible Factor) Type of Interference† Streptococci Kill phagocyte Staphylococci Inhibit chemotaxis Resist phagocytosis Resist digestion Kill phagocyte Streptolysin induces lysosomal discharge into cell cytoplasm Streptolysin M substance Leukocidin induces lysosomal discharge into cell cytoplasm Protein A blocks Fc portion of Ab Cell wall mucopeptide Toxic complex Capsular polyglutamic acid Polysaccharide capsule Inhibit opsonization Bacillus anthracis Haemophilus influenzae Streptococcus pneumoniae Klebsiella pneumoniae Pseudomonas aeruginosa Escherichia coli Salmonella typhi Cryptococcus neoformans Treponema pallidum Yersinia pestis Mycobacteria Brucella abortus Toxoplasma gondii Resist killing Kill phagocyte Resist killing Resist phagocytosis (unless Ab present) Resist digestion Resist phagocytosis (unless Ab present) Resist digestion Resist phagocytosis (unless Ab present) Resist killing Resist phagocytosis (unless Ab present) Resist killing Resist phagocytosis Resist phagocytosis Resist killing Resist killing and digestion Inhibit lysosomal fusion Resist killing Inhibit attachment to PMN Inhibit lysosomal fusion "Surface slime" (polysaccharide) O antigen (smooth strains) K antigen (acid polysaccharide) K antigen Vi antigen Polysaccharide capsule Cell wall Protein-carbohydrate cell wall Cell wall structure ? The lesion shows a pinpoint ulceration on the mucosal surface and a gradual widening in the submucosal areas as the parasites invades the tissue. Brevundimonas Brevundimonas spp. Bacteria are incubated in 2 mL of sodium nitrate at 37° C for 2 hours. Overall, however, the permanently stained smear is the best preparation for identification of the amebae. influenzae would be _____ because the organism _____ biosynthesize heme. The thick smear is examined at ×1000 for the presence of malarial organisms. indicates that the patient was infected. histolytica antigens without excluding E. dispar; currently, only one differentiates E. Four hours later, the other two sets were also positive for a gram-negative bacillus. A viral culture for EBV requires human B lymphocytes, which is beyond the capabilities of most clinical virology laboratories. diarizonae (subspecies IIIb), S. (Courtesy Dr. Dennis D. Reporting to Public Health There is the requirement to report the identification or suspicion of certain infectious diseases to the local, state, or federal public health entities. Microsporum audouinii A slow-growing anthropomorphic dermatophyte, Microsporum audouinii was responsible for most of the gray patch tinea capitis of children until a few decades ago, when T. Influenza-infected culture cells adsorb RBCs, a feature that can be used to detect positive cell cultures. The incidence of WNV in the United States increased during the mid-1990s, prompting the development of a national surveillance system in 1999. Uniseriate species are those whose phialides attach directly to the vesicle at the end of the conidiophore. serotype Paratyphi S. Younger cultures are primarily composed of budding blastoconidia, whereas the older mycelial portion of the colony shows hyphae with blastoconidia in clusters. flavescens Smooth Rough Smooth 25-40 M. Cilia may be seen retracted within the cyst wall. Ascaris lumbricoides. More than 1 billion people worldwide are infected with A. Enteric Campylobacter and Helicobacter spp. However, it is important to distinguish the two species because the clinical course and treatment outcomes can be different. Therefore, a fecal specimen is not the optimal specimen for diagnosis of infection with this organism. The last mesophilic complex is the A. All rights reserved. Pneumocystis Infection FIGURE 27-53 India ink preparation is used primarily to examine cerebrospinal fluid for the presence of the encapsulated yeast Cryptococcus neoformans. Generally, the typhus rickettsiae differ from the other rickettsial groups in that they replicate in the cytoplasm of the host cell and cause cell lysis, thereby releasing the rickettsiae. tahir99 VRG 604 PART II Laboratory Identification of Significant Isolates Coccidioides immitis Clinical Manifestations. Coccidioides spp. coli is associated with the following properties: • Fermentation of glucose, lactose, trehalose, and xylose • Production of indole from tryptophan • Glucose fermentation by the mixed acid pathway: methyl redpositive and Voges-Proskauer-negative • Does not produce H2S, DNase, urease, or phenylalanine deaminase • Cannot use citrate as a sole carbon source Uropathogenic Escherichia coli E. Multicelled conidia are produced on sympodial conidiophores (Figure 27-49). TABLE 653 At this point, two outcomes are possible. Infections have also been linked to fresh produce and dairy products. Trypomastigotes enter the blood and lymphatics, and the patient experiences generalized symptoms, including fever, headache, joint and muscle pain, weakness, and lymphadenopathy. parapertussis B. The first three letters in the restriction endonuclease name indicate the bacterial source of the enzyme. In the Case in Point, this patient with AIDS would become susceptible to opportunistic organisms as his immune system continued to deteriorate. cheonae subsp. cruzi is based on patient history, clinical symptoms, and presence of IgG antibodies to the organism. xenopi Species (Subspecies) Sodium Citrate Inositol Carbon Sources Mannitol Arylsulfatase SemiQuant >45 26-1 Identification of Clinically Important Mycobacteria—cont'd Heat Stable (68° C) TABLE Descriptive term Nonphotochromogens Photochromes tahir99 VRG Scotochromogens Niacin −(99) −(99) −(99) −(99) −(99) −(80) −(99) +(85) − −(99) −(97) −(99) −(99) − −(99) −(99) Nitrate Reduction +(96) −(95) +(99) −(85) −(99) −(96) +(99) −(85) − −(99) +(99) +(72) −(99) − −(94) −(92) Growth on 5% NaCI Pyrazinamidase† +(63) −(99) 7d +(86) −(94) 7d − −(99) + −(99) −(97) −(67) +(99) +(71) −(77) +(56) −(57) −(70) −(79) −(84) +(85) − +(71) −(99) −(75) −(74) −(59) +(82) Tellurite Reduction −(67) +(78) −(99) 7d 4d +(60) +(99) −(99) 7d + −(99) 4d +(99) +(69) −(99) 4d 7d − + +(60) +(90) +(99) 4d 7d + −(99) +(99) 4d +(63) 4d + +(86) +(99) −(99) 4d 7d +(51) −(99) Growth on T2H +(99) +(98) +(99) +(99) +(99) +(99) +(99) +(99) +(99) +(99) +(99) +(99) + +(99) +(99) Tween Hydrolysis +10 Day +(97) +(99) ±(50) −(98) +(91) +(99) +(99) −(85) − +(97) +(99) +(99) +(99) + −(99) −(98) Tween Opacity (1 Week) +(99) +(71) −(99) −(85) −(99) +(94) −(99) +(99) −(99) +(99) −(64) +(97) +(64) −(67) −(86) −(93) −(91) −(99) +(79) − −(99) −(99) +(99) Urease‡ 574 PART II Laboratory Identification of Significant Isolates CHAPTER 26 Mycobacterium tuberculosis and Nontuberculous Mycobacteria identification of culture isolates. A slide with wells is easy to use for the agglutination test. This organism may establish itself in the bile duct and be excreted in the stool over years. Patients often experience high fever, headache, malaise, and myalgia. vulnificus strains. 941. In Versalovic J et al, editors: Manual of clinical microbiology, ed 10, Washington, DC, 2011, ASM Press, p. apiospermum are not related. 500 PART II Laboratory Identification of Significant Isolates relationship between facultative anaerobes and obligate anaerobes frequently exists in polymicrobial infections, which can contribute to an infectious disease. Genetic Elements and Alterations Bacterial Genome The bacterial chromosome (also called the genome) consists of a single, closed, circular piece of dsDNA that is supercoiled to fit inside the cell. The lack of association between antibody levels and immunity to pertussis makes results of serologic testing difficult to interpret, so tests for routine purposes are not approved for diagnostic use. However, an unusual sequence of events occurred in 1989, when monkeys imported to Reston, Virginia, from the Philippines experienced an epidemic of what eventually became the third type of Ebola virus, EBO-R. Other sources include cold water systems, ornamental fountains, whirlpool spas, humidifiers, respiratory therapy equipment, and industrial process waters. • The Gram stain often reveals the presence of leukocytes, indicating an inflammatory response at the site of the infection. However, these assays tend to have low sensitivity, possibly from transient mannan antigenemia. The life cycle of N. Several Candida spp. Almost all clinically encountered Candida spp. planticola, and K. Outbreaks of public health concern (e.g., scabies) d. Describe the pathogenesis of the clinically relevant members of the family Enterobacteriaceae. EIEC strains can be nonmotile and generally do not ferment lactose; cross-reaction between shigellae and EIEC O antigens has been reported. As mentioned previously, the cell wall structure determines the Gram-staining characteristics of a species. Proglottids of T. pneumoniae carbapenemase, found in Klebsiella spp., Enterobacter spp., Serratia marcescens, and other genera, is the most common. Information that should be recorded about an isolate includes the number of days until first visible growth and the number of days required to see fruiting structures, whether mold or yeast forms are recovered, media on which the fungus is isolated, temperature at which growth occurs, and the morphology of the colonies. Blood and wound cultures subsequently grew an oxidase-positive, Issues to Consider After reading the patient's case history, consider: ■ Various pathogenic organisms associated with aquatic life that cause human infections tahir99 VRG 455 456 ■ ■ ■ ■ PART II Laboratory Identification of Significant Isolates Disease spectrum and states associated with these organisms Presentation of clinical signs and symptoms Media of choice to aid in a rapid diagnosis Key biochemical reactions to presumptively and definitively identify the organism responsible for this infection Treatment of choice Key Terms Cholera Cholera toxin Choleragen "Darting" motility El Tor Halophilic Kanagawa phenomenon Microaerophilic Thiosulfate citrate bile salt sucrose (TCBS) agar Type B gastritis Urea breath test T his chapter discusses agents of diarrheal diseases and other infections caused by species of Vibrio, Aeromonas, Plesiomonas, Campylobacter, and Helicobacter. This form of infection, which often affects infants and young children between the ages of 1 and 5 years, is usually mild and self-limiting. In addition, invasion through the oral mucosa should be suspected whenever oral anaerobes such as F. Newer tests use fluorescent or enzyme immunoassay (EIA) techniques. Anaerobes may stain better at 1 minute or with dilute carbolfuchsin. A codon is a group of three nucleotides in an mRNA molecule that signifies a specific amino acid. Reported cases decreased to 740 in 2010. Small amounts of oxygen in these gases can generally be removed by the anaerobic chamber. anaerobius MAC E. Table 27-9 lists the characteristic fungal reactions seen with selected stains. The lessons of the chapter must be carefully learned and implemented. The role of pigs as a natural reservoir has been greatly emphasized in Europe. The incubation time should be extended to 72 hours to isolate enteric Campylobacter spp. Travelers from industrialized countries often become infected with ETEC when they visit developing nations. A positive hippurate hydrolysis is an important characteristic for the identification of C. The virus circulates in the T and B cells and monocytes, until eventually the lungs, gut, bile duct, bladder, skin, and lymphatic organs are involved. (Courtesy Janice Carr, Centers for Disease Control and Prevention, Atlanta, GA.) infectious nature of the disease, when they infected laboratory animals with the blood of infected patients. Heavy smokers have a significant reduction in ciliated epithelial cells and therefore are more susceptible to respiratory infections. B, Polar, monotrichous (e.g., Pseudomonas aeruginosa) (×1000). A large variety of clinical diseases have been documented as being caused by P. The invasion of a new group of RBCs begins on the third day. Available at: http:// www.cdc.gov/salmonella/typhimurium-laboratory/011712. The absence of motility distinguishes Klebsiella spp. fragilis group, such as B. Patients can experience severe complications, including toxic shock–like syndrome, central nervous system involvement, and adult respiratory distress syndrome. The presumptive identification of suspected colonies as belonging to the genus Legionella can be accomplished by demonstrating requirements for L-cysteine, Gram stain, and DFA tests (Figure 18-30). B, Structure of the generalized eukaryotic cell. aeruginosa, however, do not produce pyocyanin. The genera most commonly encountered in clinical specimens include the B. The oocyst requires 2 to 5 days in the environment to mature and become infective. ✓ Case Check 22-1 In the Case in Point, the infecting organism was likely Clostridium perfringens, which can be found as exogenous microbiota in soil. Some tests detect both types of protein to provide a more complete picture of the infective agent. Kanis MJ, et al: Corneal graft rejected complicated by Paracoccus yeei infection in a patient who had undergone a penetrating keratoplasty, J Clin Microbiol 48:323, 2010. Plates should be examined daily because older colonies lose these characteristic features and may be mistaken for other bacteria. Blunt pseudopods and characteristic spinelike projections of the cytoplasm (acanthopodia) may also be seen on a wet mount. The relevance of isolation of nonfermentative bacillus from the sputum of an immunocompetent, healthy individual with a cough might differ from that in a patient who is on heavy doses of steroids because they have just received a lung transplant and they have cystic fibrosis (CF). FIGURE 26-14 Iron uptake. Isolates of EO-3 and EO-4 have been obtained from urine, eye discharge, blood, pleural fluid, and CSF and from lung, throat, and genitourinary tract specimens, in which their clinical significance remains unclear. Significance Microbial inhabitants have evolved to survive in various ecologic niches (way in which an organism uses its resources) and habitats (organism's location and where its resources may be found). vivax and P. ■ E. trachomatis is the enzyme immunoassay (EIA). clostridioforme; however, if the kanamycin result is resistant, it could be a Porphyromonas sp. Animals such as civets may acquire infections with SARS-like CoV from contact with infected bats. Laboratory identification of malarial species involves examination of the morphology of infected RBCs and characteristic morphology of the parasite. Immunity may be short-lived, leading to the potential for multiple infections throughout life. Ascomycota Fungi associated with the class Ascomycetes are characterized by the production of sexual spores known as ascospores. vulgatus, B. By adulthood, about 80% of Americans have been infected with HSV-1. The skin also has substantial numbers of microbial flora that are usually not pathogens, organisms that contribute to a low pH, compete for nutrients, and produce bactericidal substances. Is serology a good method to identify and respond to pertussis outbreaks in real time? neoformans but is easily differentiated on the basis of physiologic and morphologic characteristics. putida is negative and P. As with the other herpes group viruses, infection is very common and results in latency, and most adults demonstrate antibody against the virus. It eventually penetrates the diaphragm and enters the lung. penneri has been isolated from patients with diarrhea, although the organism's role in disease has not been proven. Sputum cultures from hospitalized patients may contain enteric organisms that may require complete identification. Longstanding lesions have a cauliflower-like surface. Isolates resemble Moraxella but are usually not penicillin-susceptible. In contrast to shock caused by fluid loss, such as shock seen in severe bleeding, septic shock is unaffected by fluid administration. Readers are referred to standard parasitology references for detailed procedures, reagent preparation, and a comprehensive description of parasites that have been implicated in human disease. Cytopathic changes may not be evident for 3 to 7 days. Anaerobic gas is one of the biggest expenditures in operating an anaerobic chamber. Secondary bacterial infections, which occur as a result of massive larval migration, may be seen in up to 40% of patients with disseminated tahir99 VRG CHAPTER 28 Diagnostic Parasitology A 679 B FIGURE 28-63 A, Hookworm rhabditiform larva. tuberculosis complex bacilli directly from patient specimens can be performed in as little as 6 to 8 hours on processed specimens and offer the promise of same-day reporting of results for detection and identification of M. coli is widely recognized as the most common cause of UTIs in humans. Recently, the use of azithromycin and atovaquone has been recommended. For example, Staphylococcus (genus) aureus (species epithet) belongs to the family Micrococcaceae. Improper disposal of sewage, poor sanitation, and lack of a modern potable water system have caused outbreaks of typhoid fever when the organisms reach a water source. Murray PR, et al: Manual of clinical microbiology, ed 8, vol 1, Washington, DC, 2003, ASM. Staphylococcus aureus Staphylococcus epidermidis Enterococcus spp. The most severe infection, visceral leishmaniasis or kala-azar, is endemic in parts of South America, Africa, Southern Europe, and Asia. The PMNs and macrophages (monocytes in the peripheral blood) are the body's first line of defense. The sachet is activated on exposure to air once the foil pouch is removed. In atypical cases, such as in immunosuppressed patients, the diagnosis may be more difficult or questionable. HHV-7 causes latent infections in T lymphocytes. What is the difference between resident and transient flora? Discuss the problems with serologic cross-reactivity among the rickettsial species. Pathogenic Intestinal and Urogenital Flagellates The flagellates constitute another major group of parasites that can inhabit the intestinal tract. Influenza viruses are enveloped, and types A and B have eight segments of ssRNA. Species that produce hydrogen sulfide (H2S) may be readily distinguished when placed on HE or XLD agar. falciparum is blackwater fever, a condition characterized by hemoglobinuria (hence, the term blackwater). A clinical isolate in an immunocompromised patient, however, was recently considered to be a pathogen and the isolate carried a VIM-2-metallo-β-lactamase, contributing to its multidrug resistance. Hafnia has been linked to gastroenteritis and is occasionally isolated from stool cultures. Laboratory Diagnosis. Diagnosis is made by finding embryonated eggs in the feces (S. The three major metabolic pathways and their key characteristics are described in Box 1-1. It is always warranted in wound infections and septicemia. Many of these microbes tahir99 VRG 4 PART I Introduction to Clinical Microbiology do not cause disease. In the United States, the organism is most frequently seen in rural parts of the Southeast. The bacterial cell is lysed, releasing mature phage. fortuitum and MAC. Because the worms feed on liquid intestinal contents, chronic infection with A. A nonpigmented mycobacterium is isolated that reduces nitrate to nitrite and is niacin-positive. Second, arthropods directly inoculate new hosts with rickettsiae during feeding. Trichophyton rubrum Although Trichophyton rubrum is known to produce three- to eight-celled cylindric macroconidia measuring somewhat smaller than those of T. A presumptive identification can be made by a positive test result in any one of the following assays: direct fluorescent antibody (DFA), immunohistologic staining with monoclonal antibody, PCR, slide agglutination, or single serology test. The disease is endemic in tropical areas of Africa, Asia, and Latin America. Some species also produce capsules and slime layers. Screening of blood donors for antibodies to T. Edema in the legs and arms and around the eyes is possible. The cell culture cytotoxicity assay that detects toxin B in fecal samples by tissue culture is generally considered the gold standard. It is beyond the scope of this chapter to list all the current names of rarely encountered anaerobes. From there, it is free to disseminate throughout the CNS. Aldous, PhD(ABMM) Chief, Microbiology Department of Clinical Support Services U.S. Army Medical Department Center and School Fort Sam Houston, Texas Carl Brinkley, PhD Department of Chemistry and Life Science Colonel United States Military Academy West Point, New York Gerri S. Microconidia are found primarily in clusters described as grapelike. Although the original source of infection has not been determined, it is surmised that it is the gastrointestinal, biliary or, even more rarely, respiratory tract. Starnes CT, et al: Brucellosis in two hunt club members in South Carolina, J SC Med Assoc 100:113, 2004. However, various structures are unique to prokaryotic cells (see Figure 1-1). Candida albicans is the most commonly isolated yeast. Media relations represent an educational opportunity that might be investigated before the laboratory technologist speaks to media personnel. Systemic Dermatophyte Infections Immunocompromised persons may suffer systemic dermatophyte infections. Screwcapped tubes also tend to show less media dehydration than Petri dishes and are more easily handled and stored. When mature and gravid, the female migrates through the subcutaneous tissues to the arm or leg to release liveborn larvae into the water. The SabinFeldman dye test was the first antibody test developed but is no longer used in clinical laboratories because it requires live organisms. Laboratory Diagnosis. The primary diagnostic stage in the blood is the trypomastigote. Campy-CVA (cefoperazone-vancomycin-amphotericin B) medium has been reported to provide better suppression of fecal biota, even when this medium is incubated at 37° C. canis, H. Special potency disks to determine the true Gram stain of an organism can play a major role in the use of the preformed enzyme-based identification systems. Microscopes are divided into two basic types: compound light microscopes, with common resolving limits of 1 to 10 µm and enlargements up to ×2000, and electron microscopes, with enlargements greater than ×1,000,000 (Table 7-3). Trichosporon ovoides, T. doylei Campylobacter coli Campylobacter lari Campylobacter fetus subsp. D, Acid-fast stain, fluorochrome-based. longbeachae is notable because human infections are associated with exposure to gardening materials, such as compost and potting soil, and constitutes a significant source of infection in New Zealand and Australia. If this DNA probe is proven successful, it may be applicable for laboratory diagnostic testing. Establish a "best guess" hypothesis about the outbreak. In addition, Campylobacter spp. 35 Antimicrobial Substances Various substances produced in the human host have antimicrobial activity. Differentiate the various types of anaerobes with regard to atmospheric requirements (i.e. obligate anaerobes, facultative anaerobes, and aerotolerant anaerobes). ducreyi is one of the most fastidious species of Haemophilus. luteola and P. Mutations are changes that occur in the DNA code and often (not always; "silent mutations" do not make a change in the protein) result in a change in the coded protein or in the prevention of its synthesis. Laboratory technologists must not only keep themselves educated in their contribution to the infection control team, but also keep the infection control personnel educated regarding the laboratory's contribution to the team. Clinical Infections Trachoma. C. If the decolorization is insufficient, organisms may be falsely gram-positive, and tahir99 VRG CHAPTER 7 Microscopic Examination of Materials from Infected Sites 131 PROCEDURE 7-1 Gram Stain—cont'd organisms in the thicker areas of the sample may be obscured. produce a moderately rapid growing gray to brown colony. The H antigen is used to bind to host cells, and N cleaves budding viruses from infected cells. Currently, no commercial kits are FDA-approved. Rapid antigen detection tests are based on immunochromatographic EIA procedures using monoclonal antibodies. As an alternative, the specimen could be collected on a clean piece of waxed paper or newspaper and transferred to the container. Agglutination indicates the presence of anti-treponemal antibodies. Rapid urea broths are also available from various manufacturers. Does the specimen have a foul odor? Although Bacteroides fragilis is the most common species of anaerobic bacteria isolated from soft tissue infections and bacteremia, it accounts for less than 1% TABLE 22-4 Endogenous Anaerobes of Various Anatomic Sites Site Skin Upper respiratory tract Oral cavity Intestine Genitourinary tract Anaerobes Propionibacterium, peptostreptococci Peptostreptococci, Actinomyces, Propionibacterium, Campylobacter, Fusobacterium, Prevotella, Veillonella Actinomyces, Eubacterium/Eggerthella, peptostreptococci, Campylobacter, Fusobacterium, Prevotella, Bifidobacterium, Porphyromonas, Veillonella Bifidobacterium, Eubacterium/Eggerthella, Clostridium, peptostreptococci, Bacteroides fragilis group, Bilophila, Campylobacter, Fusobacterium, Porphyromonas, Prevotella, Sutterella, Veillonella Peptostreptococci, Bifidobacterium, Fusobacterium, Lactobacillus, Mobiluncus, Prevotella, Veillonella From Jouseimies HR et al: Wadsworth-KTL anaerobic bacteriology manual, ed 6, Belmont, CA, 2002, Star. Characterize the following different types of mycoses, defining the tissues they affect: a. falciparum infection can lead to anemia and deposits of malarial pigment in organs such as the spleen. The coating of the bacterium with antibody or complement components results in enhanced phagocytosis by the PMN. The second group of bacteria, the heterotrophs, requires more complex substances for growth. Enteric FIGURE 20-9 Campylobacter Gram stain (×1000). This generalization is misleading because the severity of disease caused by different organisms varies from one to another. Candida albicans is the fourth most common cause of bloodborne infection in the United States, accounting for 10% to 15% of all hospital-acquired septicemias. Currently, however, fluorescent antibody microscopy or the modified acidfast stain has replaced this method of detection. The Flavobacterium spp. As noted, most anaerobic infections are caused by members of the endogenous microbiota. Serovars D through K are associated with these clinical infections, which can be persistent and subclinical, as well as acute and demonstrable. rubrum and T. The organism shares the general life cycle characteristics of other intestinal Apicomplexa. aureus, streptococci or viridans streptococci, Lactobacillus, diphtheroids, and β-hemolytic streptococci. BHI, Brain-heart infusion agar; SDA, Sabouraud dextrose agar. The sediment is fixed and decontaminated in 70% alcohol for 5 minutes. Specimen Selection, Collection, Transport, and Processing Specimen Quality Specimen Transport and Processing Regardless of the type of specimen submitted for anaerobic bacteriology, it must be transported and processed as rapidly as possible and with minimum exposure to oxygen. 1793. Edwardsiella spp. Using a combination of nonselective and semiselective BCYE agar is the best strategy for isolation of Legionella organisms. These antigens include the following: • O antigen, or somatic antigen—this is a heat-stable antigen located on the cell wall. PIV-3 causes bronchiolitis and pneumonia in infants and is second in importance only to RSV. They are transmitted by midges belonging to the genus Culicoides. Using a wooden applicator stick, a small portion of a colony should be touched to a drop of saline on a microscope slide. However, some bacteria are able to survive and pass into the small intestine. The solution should be made fresh daily, and contact time should be 10 to 30 minutes. in several ways, including chemical structure, serum concentration, half-life, and functional activity. The organism has also been identified as a sexually transmitted agent among men who have sex with men. It is the most commonly isolated species of Yersinia. In particular, their inoculating suspensions should contain at least 0.85% NaCl, such as that found with the API-20E identification strips (bioMérieux Vitek, Hazelwood, MO). HHV-6 has also been proposed as having some involvement in the development of progressive multifocal leukoencephalopathy and multiple sclerosis. Available at: http:// www.cdc.gov/abcs/reports-findings/survreports/hib10.html. Heineman's method emphasizes the ratio between SECs and PMNs. Many diagnostic microbiology laboratories use the documented observations related to SECs and PMNs but attempt to coordinate observations related to background materials, which consist of local materials, contaminating materials, and purulence, and to describe the relationship of microorganisms to these background materials. In general, nonfermentative, gram-negative bacilli or coccobacilli are ubiquitous and found in most environments, in soil and water, on plants and decaying vegetation, and in many foodstuffs. In the United States, PAM is most Clinical course Population at risk Immunocompromised CNS, Central nervous system. High risk for contracting the virus includes having unprotected sex with multiple sex partners, IV drug abuse, receiving blood and blood products, and infected mothers passing the virus to their children during pregnancy. Bacterial isolates are presented based on a taxonomic approach. Finding site-specific organisms at a distant and/or unusual site can serve as a clue to the underlying origin of an infectious process. The trophozoites of G. multivorans, P. Brandt ME, et al: Histoplasma, Blastomyces, Coccidioides, and other dimorphic fungi causing systemic mycoses. The presence of the pigment can hamper proper oxidase reactions. Transmission between humans has not been demonstrated. The size of the trophozoite ranges from 5 to 12 µm, and the cytoplasm contains many food vacuoles and bacteria (Figure 28-19). The time frames listed are not exact and vary with the organism; rinsing with water between each step is important. Are adults immune to Bordetella. Most of the systems generate code numbers, which are referenced in a manufacturer-supplied codebook. Carbohydrate assimilations Blastoconidia True hyphae Blastoconidia Pseudohyphae True hyphae Arthroconidia Candida sp. Some strains of A. Over the last decade, diagnostic advances have transformed the field of virology by developing newer methods that are many times faster, so that the results are clinically useful. Syphilis can also be acquired by nongenital contact with a lesion (e.g., on the lip) or transplacental transmission to a fetus, resulting in congenital syphilis. This morning, she arrived late to work because she had to take her sick son to her mother's house and drop off her dog to the vet. The genera Helicobacter and Wolinella are members of the family Helicobactereaceae. Both species, particularly P. They are differentiated from other members of the tribe by their ability to produce extracellular DNase. Lecithinase-positive clostridia include C. The Gram stain consists of gentle heat fixing (methyl alcohol tahir99 VRG CHAPTER 1 Bacterial Cell Structure, Physiology, Metabolism, and Genetics Microscopic Morphology of Bacteria ✓ COCCI 11 Case Check 1-3 Review of quality control slides is important in the detection of errors in the performance of the Gram stain procedure and in interpretation of results. Table 28-9 compares the diagnostic characteristics of the eggs and larvae of intestinal roundworms. For example, the gametocytes (cells capable of dividing to form gametes) of Plasmodium falciparum may lose their characteristic banana shape and round up. suis are ≥95% positive; B. Opportunistic Members of the Family Enterobacteriaceae and Associated Infections TABLE 19-3 Bacterial Species and Infections They Commonly Produce Bacterial Species Escherichia coli Shigella spp. Rinse with running tap water, and air dry. The group prokaryotes ("before nucleus") includes the domains Archaea and Bacteria (Eubacteria), whereas fungi, algae, protozoa, animals, and plants

mmwr/preview/mmwrhtml/mm5240a2.htm. grisea, only sterile hyphae are observed. Describe the general characteristics of the genera of spirochetes. Cell Envelope Structures The cell envelope consists of the membrane and structures surrounding the cytoplasm. histolytica with other amebae. are noted for producing blastoconidia only, without producing true hyphae or pseudohyphae on cornmeal agar. influenzae? N. Psychrobacter immobilis is psychrotropic, and the optimal temperature for growth is 20° C. The status of the host's immune system and ability of the host to defend itself from microbial invasion, combined with microbial factors inherent to the invading organism, often determine whether disease occurs. Many times we can see their effects on cell lines, such as inclusions, rounding up of cells, and syncytium (cell fusion of host cells into multinucleated infected forms), where these characteristics become diagnostic for many viral diseases. americanus and A. Aspergilli may be uniseriate or biseriate. It is also important to note whether or not FIGURE 27-37 Aspergillus sp. Scarring of the veins, development of ascites, pain, anemia, hypertension, hepatomegaly, and splenomegaly are also seen. Some are designed to detect antigen in the stool and others to detect antibodies in the serum. Cyanogen bromide must be alkalinized with NaOH before disposal. Complications are rare, and recovery usually occurs without incident. Humans acquire the infection by ingesting metacercaria on raw water vegetation, especially watercress. pneumoniae, and Klebsiella oxytoca clinical strains produce plasmid-mediated extendedspectrum β-lactamases (ESBLs) including carbapenemases, cephalosporinases, or metallo-β-lactamases, which can inactivate extended-spectrum cephalosporins (e.g., cefotaxime), penicillins, and aztreonam. Infection arises from infected skin scales coming into contact with exposed skin via a carpet, shower, or other environment. The seventh step in the investigation may actually occur at any point along the investigation time line. (Courtesy DAKO Reagents, Carpinteria, CA.) Two antibody response patterns have been identified for C. infections do not have detectable levels of antibody. Develop protocols for sample collection, handling, and transport of specimens for blood and tissue parasites. cholerae O1, spread quickly throughout Asia and reached the African continent in 1970. For each of the following dimorphic fungi, describe the characteristic microscopic appearance at 37° C or when grown at 22° C: a. Isolation of Bordetella spp. This is in large part because of its unique, positive trio profile of positive ornithine and lysine decarboxylases and arginine dihydrolase reactions, combined with the fermentation of inositol. verrucosa and C. An increase in body temperature occurs within 1 hour after exposure. posadasii Paracoccidioides brasiliensis Central and South America In soil Small, oval yeast (2-5 µm) in histiocytes, phagocytes Spherules (30-60 µm) containing endospores Thick-walled yeasts (15-30 µm) Multiple buds, "Mariner's wheel" *Histoplasma capsulatum var. Numerous gas-generating systems are available and can be divided into newer systems that are waterless and older systems, which require water. Other disadvantages of the BACTEC 460TB system include opportunity for crosscontamination of specimens, radioisotope disposal, and safety concerns from the use of syringes. They are also negative for nitrate reduction. Rales, dyspnea, and shaking chills are clinical manifestations of progressing disease. No previous laboratory reports indicated infectious agents in the cerebrospinal fluid (CSF). The disease is endemic in India, Africa, and South America. veronii biovar veronii is 100% susceptible to cefoxitin. Relapsing fever is the only spirochetal disease in which the organisms are visible in blood with bright-field microscopy. Discuss appropriate respiratory and nonrespiratory specimens for the diagnosis of legionellosis. Thus, successful isolation of mycobacteria from clinical specimens begins with properly collected and handled specimens. Catalase. Catalase is an enzyme that can split hydrogen peroxide into water and oxygen. The size of the organism ranges from 10 to 50 µm, averaging 15 to 25 µm. It fluoresces when exposed to long-wavelength ultraviolet and shortwavelength visible light. The fermentation of the sugar is usually detected by acid production and a concomitant change of color resulting from a pH indicator present in the culture medium. Figure 24-6 shows inclusion bodies demonstrated by direct examination of cytologic stains of endocervical smears. These microorganisms have been associated with various opportunistic and hospital-acquired infections, particularly pneumonia, wound infections, and UTIs. Members of these genera demonstrate variable biochemical reactions. It appears that the species can be traced to specific geographic locations. However, from January to October 2006, a large multistate outbreak of almost 6000 cases of mumps was documented. Secretions from the larva create a severe allergic reaction, with intensely itchy skin lesions that are vesicular and erythematous. Laboratory Diagnosis of Rickettsial Diseases Because of their infectious nature, isolation of the rickettsiae is not recommended and should only be attempted by biosafety level 3 laboratories. As is the case with RMSF, the clinical course of endemic typhus includes fever, headache, and rash. Cladosporium. An infrequent cause of disease, Cladosporium spp. Other bacteria with thinner walls containing lipopolysaccharides do not retain the dye complex; they are gram-negative bacteria. For example, Aspergillus spp. The genera Plesiomonas and Shigella share biochemical and antigenic features, and plesiomonads often cross-agglutinate with Shigella sonnei, S. They have been known to devastate printed literature and library holdings and have been associated with indoor air quality problems. (From Joklik WK et al: Zinsser microbiology, ed 20, Norwalk, CT, 1992, Appleton & Lange.) An important step in classifying members of the Enterobacteriaceae family is the determination of the microorganism's ability to ferment lactose. The virus attaches to specific receptors on the surface of a susceptible cell by means of specialized structures on its surface, called adhesion molecules. A disadvantage of the BACTEC system is that no colony morphology or pigmentation is available to suggest that the growth is of a mycobacterial species and not that of a contaminant or commensal organism. • Prepare smears from colonies that require L-cysteine and test with polyvalent and monovalent conjugates to determine specific species and serogroup. coli O157:H7 strains produce high levels of cytotoxins, and STEC strains may be detected using cell culture assays with Vero cells. Blood and CSF were collected. Figure 19-2 compares the colony morphology of E. The cytocentrifugation process deposits cellular elements and microorganisms from the specimen onto the surface of a glass slide as a monolayer. Cellular Fatty Acid Analysis by High-Resolution Gas-Liquid Chromatography Cellular fatty acid analysis is another method of identifying anaerobes. Albendazole or ivermectin can be used to treat intestinal and disseminated Strongyloides infection. eucrenophila. In LGV, patients have inguinal and anorectal symptoms (Figure 24-4). He had a number of small erythematous vesicles on his feet. enterocolitica Y. Anaerobic Chambers. The ideal anaerobic incubation system is an anaerobic chamber, which provides an oxygen-free environment for inoculating media and incubating cultures. The inoculum should be sufficiently small to produce isolated colonies. The organisms multiply intracellularly; later they are released into the bloodstream for the second time. taiwensis, all formerly Ralstonia spp. The small size of the spores, however, makes them easy to overlook in clinical specimens. The active portion (A1) of the A subunit catalyzes the adenosine diphosphate (ADP) ribosylation of the Gs (stimulatory) regulatory protein, "locking" it in the active state. In addition, the most commonly isolated species, A. Following DNA homology and 485 sequencing analysis, it was classified as a member of the genus Stenotrophomonas, where it remains today. When noninvasive techniques have failed to provide a diagnosis, surgical procedures may need to be considered. A combination of antibody, complement, lysozyme, and β-lysin is significantly more effective in killing bacteria than each alone or than any combination in which one or more are missing. Carriers, particularly food handlers, are important sources of infection anywhere in the world. Other important isolates are B. Sodium Chloride Tolerance. High salt concentration (5% NaCl) in egg-based media (e.g., LJ) inhibits the growth of most mycobacteria. Commercial kits FIGURE 20-10 Urease reactions. Although several vitamin deficiencies are recognized in fungi, the test for thiamine requirement is perhaps the single most useful nutritional test for dermatophytes. However, yellow fever is still epidemic in parts of Africa and tahir99 VRG CHAPTER 29 Clinical Virology South America, where about 80% of the population must be vaccinated to reduce the impact of the disease. Brain abscesses are frequently caused by anaerobic organisms such as Prevotella, Porphyromonas, and Fusobacterium found as endogenous microbiota of the oral cavity. These proteins are produced by a variety of gram-positive and gram-negative bacteria and appear to give the secreting bacterium an advantage because they can eliminate other bacteria that would compete for nutrients and space. schenckii is dimorphic, cultures are examined at 22° C and 37° C. Overall, sedimentation methods concentrate a greater diversity of organisms, including cysts, larvae, and eggs. Plesiomonas d. Campylobacter and CampylobacterLike Species Campylobacter and Campylobacter-like species, which include Helicobacter and Wolinella, have undergone changes in taxonomy. They all oxidize glucose and xylose, but differ in oxidation of lactose and mannitol. intracellulare, M. pallidum Other Treponemal Diseases ■ LEPTOSPIRES General Characteristics Virulence Factors and Pathogenicity Infections Caused by Leptospires Epidemiology Laboratory Diagnosis Antimicrobial Susceptibility ■ BORRELIAE General Characteristics Clinically Significant Species OBJECTIVES After reading and studying this chapter, you should be able to: 1. These nail and nail bed infections may be among the most difficult dermatomycoses to treat. enteritidis, S. In bacteria, these are the cell membrane and the cell wall. They are unembryonated when passed (Figure 28-42). ducreyi causes the STD chancroid. Perform microscopy. The childhood vaccination series consists of five doses from age 6 weeks to 6 years, and decreases in immunization rates lead to increased attack rates or major epidemics. BIBLIOGRAPHY Aguero-Rosenfeld ME, et al: Diagnosis of Lyme borreliosis, Clin Microbiol Rev 18:484, 2005. Colonies of MAC have a variable appearance, with glossy whitish colonies often occurring with smaller translucent colonies. histolytica trophozoites and cysts and compares E. Undoubtedly, interventions of some type (e.g., increased hand hygiene) are introduced early in the investigation. • Anti-EA/R (antibody to early antigen, restricted): Antibodies to EA/R appear in the acute phase and disappear soon after anti-EA/D, but can persist for up to 2 years and may be lifelong in some patients. Monoclonal antibodies directed against the spotted fever or typhus group have been used, but no antibody is commercially available. As with all organisms, sniffing of plates should be discouraged. The febrile period lasts about 3 to 7 days and ends abruptly with the development of Epidemiology Relapsing fever can be tick-borne (endemic relapsing fever) or louse-borne (epidemic relapsing fever). tuberculosis infection may also produce induration smaller than 10 mm. The test is often referred to as the proportion method because it allows one to predict the probability that 1% of the cells are resistant or not. The cell membrane of the phagocytic cell invaginates and surrounds the attached particle. The genus Sphingomonas contains at least 12 species, but only two are believed to be clinically significant; S. tuberculosis complex. Streptococcus pneumoniae, Streptococcus pyogenes (rarely), Stomatococcus mucilaginosus Streptococcus pneumoniae Pathogenic Neisseria spp., Moraxella catarrhalis Bacilli Gram-positive bacilli Small Medium Large Diphtheroid Pleomorphic, gram-variable Beaded Filamentous Filamentous, beaded, branched Bifid or V forms Gram-negative coccobacilli Masses Chains Gram-negative bacilli Small Bipolar Medium Large Curved Spiral Fusiform Filaments Listeria monocytogenes, Corynebacterium spp. Describe the primary, secondary, and tertiary clinical manifestations of syphilis. Sigmoidoscopy Specimens Scrapings or aspirates obtained by sigmoidoscopy may be used to diagnose amebiasis or cryptosporidiosis. Centers for Disease Control and Prevention: Investigation update: outbreak of Shiga toxin-producing E. The LPS is contained in the outer membrane along with proteins and phospholipids (see Figure 2-1). Many other liquid media (e.g., BHI, trypticase soy broth, nutrient broth) have been used successfully as an alternative. Negative vials should be retested every 3 to 4 days for the first 2 weeks and then once weekly for the remaining 6 weeks. The host has innate and acquired immune responses that come into play in an attempt to prevent colonization. were recorded in flood water samples in New Orleans following Hurricane Katrina in 2005. Enteroviruses have no group antigen, so they must be identified individually by a serum neutralization test. Most serotypes of the enteroviruses are distributed worldwide. The antibodies produced are not protective, however. A clostridia are capable of producing spores, some species do so readily, whereas others require extremely harsh conditions. Describe diseases caused by fungi. Providencia spp. For infection of a cell to occur, virions must absorb or attach to the cell surface. Antibody titers may be unreliable in immunocompromised patients because these patients lack the ability to produce sufficient antibody to cause a significant rise in titer. Two treponemes are shown adjacent to an erythrocyte (Nichols strain, ×2500). Adherence to the intestinal mucosa is achieved by the ventral sucking disk. Various metals and ions for enzymatic activity must also be present. maltophilia is an oxidase-negative, nonfermentative, gramnegative bacillus. pneumoniae, C. At this temperature, colonies are often initially white, glabrous, and yeastlike, turning darker and more mycelial as they mature. The chromatoidal bars are cigar-shaped, with rounded ends. ✓ Case Check 1-2 The most common mechanism for evading phagocytosis that is used by many different microorganisms is having a polysaccharide capsule on the surface. The patient becomes febrile, and oral lesions can appear. It has been a disease of major public health significance for centuries. It is not known what triggers the change into other forms. Their unique growth cycle involves two distinct forms, an elementary body (EB), which is infectious, and a reticulate body (RB), which is noninfectious. The clinical infection is characterized by fever, severe abdominal cramps, malaise, and watery diarrhea. Tiny, round to oval, gram-negative cocci with a tendency to stain gram-variable are suggestive of Veillonella, whereas gramnegative coccobacilli may be Bacteroides, Porphyromonas, or Prevotella. Those laboratories that used conventional mycobacteriologic methods for culturing and identification were not able to report results as quickly as those that had already incorporated newer methodologies; the amount of time needed was 43 versus 22 days, respectively. Chapters 7 (Microscopic Examination of Infected Materials) and 8 (Use of Colony Morphology for the Presumptive Identification of Microorganisms) still play vital roles in this text. See Appendix D for the NALC-NaOH digestion and decontamination procedure. Most infections are caused by MAC. Phaeoid fungi produce dark pigments. The endospores appear green within pink-appearing or red-appearing bacterial cells. pneumoniae. None of the above 4. Aeromonads have a seasonal pattern of increased isolation from May through October, reflecting their increased numbers in aquatic environments in the warmer months in the United States. Granules that are too hard to crush between two glass slides probably do not represent infectious materials. mucoides also causes systemic disease, recovered frequently from the cerebrospinal fluid (CSF) as a cause of meningitis. As with the anaerobic jar, plates must be removed from the bags to work with the colonies at the bench. You are examining a blood smear and find an extracellular structure that is approximately 18 µm long. The egg, which is rarely seen except in cases of severe diarrhea, resembles that of a hookworm. solium may reach only 7 m. H1N1 was the predominant strain until a shift to influenza A (H2N2) occurred in 1957 to 1958. Compare asexual and sexual reproduction of fungi. (Iodine wet mount, ×200.) B, S. A granular texture is seen in species with abundant conidial formation. canadensis, H. These fragments are anticoagulants and can cause serious bleeding. Individuals undergoing transplantation who have a history of travel to or residence in endemic areas may need to be screened for S. The patient suffered from posthepatic cirrhosis, a predisposing factor. This was the first influenza pandemic in 40 years. • Examine more than one area of the smear. Ciprofloxacin and other quinolones can also be used. The resulting infections are generally asymptomatic. aeruginosa, including bacteremia, often presenting with ecthyma gangrenosum of the skin, wound infections, pulmonary disease, especially among individuals with CF, nosocomial urinary tract infections (UTIs), endocarditis, infections following burns or trauma and, in rare cases, central nervous system infections, including meningitis. histolytica normally subsists on intestinal bacteria and partially digested food of the host. When basidiomycetous molds are recovered in the laboratory, they typically remain sterile, complicating the identification process. Histoplasma capsulatum var. Sporangiophores are erect, branching into several vesicles that bear sporangioles (Figure 27-32), and can be covered with long, fine spines. Other defense mechanisms must play a major role in immunity to these intracellular organisms. This may manifest as myocarditis or meningoencephalitis. Short rods may have clubbed ends and may be seen in diphtheroid arrangements, short chains, or small clusters. In the laboratory, verotoxin-producing E. Collect as much information as possible about the cases, including people, place, and time elements. A specific therapeutic regimen has not been established because most infections have been diagnosed at autopsy. This part of the county had only recently been connected to the local city's sanitation system. It generally resembles E. The FIGURE 29-12 Transmission electron micrograph revealing the ultrastructure morphology of norovirus virions (×100,000). In clinical trials, both vaccines were shown to be safe and effective. In the chain of nucleotides, bonds form between the phosphate group of one nucleotide and the 3′ sugar of the next nucleotide. parapertussis cause pertussis, or whooping cough, and are primary human pathogens of the respiratory tract. Historically, DNA was first discovered by Frederick Miescher in 1869. One end of the string is taped to the side of the patient's mouth; the weighted end is carried into the upper small intestine. The usually mild, self-limiting disease caused by ETEC is characterized by watery diarrhea, abdominal cramps, and sometimes nausea, usually with no vomiting or fever. Commercially available engineered cell lines improve the detection of HSV. Available at: .cdc.gov/std/stats10/chlamydia.htm. Three periplasmic flagella are inserted into each end of the cell. Morulae are round to oval clusters of bacteria 1 to 3 µm in diameter. O2 + e− → O2− (superoxide anion) O2 + e− + 2H+ → H2O2 (hydrogen peroxide) Furthermore, one hypothesis of oxygen toxicity proposes that the superoxide anion reacts with hydrogen peroxide in the presence of iron (Fe3+/Fe2+) to generate the hydroxyl radical (OH−). The treponemal tests retain a very high sensitivity in the tertiary stage as well. In the intestines, damage is temporary because the cells lining the gut are rapidly replaced. hepatica and, when seen, should be reported as Fasciolopsis buski–Fasciola hepatica eggs. This process depletes clotting factors and activates fibrinolysis so that fibrin-split products accumulate in the blood. Life Cycle. The life cycle of Plasmodium involves sexual reproduction (sporogony) and asexual reproduction (schizogony), as shown in Figure 28-27. Citrobacter freundii tahir99 VRG 454 PART II Laboratory Identification of Significant Isolates 9. Key Terms Case in Point A 2-month-old girl with a 6-day history of persistent and worsening cough was taken to the pediatric clinic. psittaci. Some are produced as part of the phagocytic defense and are discussed later. Given the key reactions for identification, place an unknown organism in its proper tribe, genus, and species. tahir99 VRG 580 PART II Laboratory Identification of Significant Isolates Egg-Based Media The basic ingredients in an inspissated egg medium, such as LJ, Petragnani, and American Thoracic Society (ATS) media, are fresh whole eggs, potato flour, and glycerol, with slight variations in defined salts, milk, and potato flour. Most molds have a fuzzy or woolly appearance because of the formation of mycelia (Figure 27-2). The use of an enclosed electric incinerator is recommended to eliminate the hazards of open gas flames and to contain particles emitted when loops or needles are incinerated. Although these kits offer easier disposability and a cleaner preparation because of the filtration method used, studies indicate that their use is more expensive than the traditional FES method. tahir99 VRG CHAPTER 20 Vibrio, Aeromonas, Plesiomonas, and Campylobacter Species Deborah Ann Josko CHAPTER OUTLINE ■ PLESIOMONAS Epidemiology Clinical Manifestations General Characteristics Laboratory Diagnosis Antimicrobial Susceptibility ■ CAMPYLOBACTER AND CAMPYLOBACTER-LIKE SPECIES Epidemiology Clinical Manifestations Laboratory Diagnosis Antimicrobial Susceptibility ■ VIBRIO General Characteristics Vibrio cholerae Vibrio parahaemolyticus Vibrio vulnificus Vibrio alginolyticus Laboratory Diagnosis Antimicrobial Susceptibility ■ AEROMONAS General Characteristics Clinical Manifestations Laboratory Diagnosis Antimicrobial Susceptibility OBJECTIVES After reading and studying this chapter, you should be able to: 1. The infective dose is not known, but it is thought to be relatively low, between 10 and 100 oocysts. The ELISAs have major advantages, namely being less time-consuming, and the method does not rely on the quality of the fluorescent microscope used or the experience of the laboratory scientist. tahir99 VRG CHAPTER 28 Diagnostic Parasitology Clinical Infections. Although malaria is usually transmitted through the bite of an anopheline mosquito, transmission through blood transfusion and infected needles has been reported. Urine For examination of urine, a first morning midstream specimen is preferred. The asci contained within the perithecia are evanescent, so at maturity the pigmented, lemon-shaped ascospores are released within the perithecium. Either end of the nail is first infected, with spread continuing to the nail plate. You should suspect: a. 12. In the example of 565 suspected cases, only 47 cultures were processed. The only exception is the hepatitis viruses, which will be discussed as one group, despite the fact that they do not all have the same type of nucleic acid. The following standard rules for denoting bacterial names are used. Place the second slide face down over the material. Although they are biologically nonspecific and known to react with organisms of other diseases and conditions (causing false-positive reactions), the nontreponemal tests are excellent screening tests. Because the H and N antigens of the influenza A virus continually change, the CDC and WHO make recommendations regarding the composition of the trivalent influenza vaccine several months before the influenza season begins. Connie R. The specimen is centrifuged onto the cell monolayer and incubated for 72 hours. Most 2-year-olds are seronegative for HHV-7, but most children are seropositive by the age of 6 years. It is an elongated structure 15 to 20 µm long that often appears in a C or U shape. In areas in which the organism appears endemic, natural disease occurs in dogs and horses, with the disease process mimicking that seen in human infections. In some instances, the phage DNA instead becomes incorporated into the bacterial genome, where it is replicated along with the bacterial chromosomal DNA; this state is known as lysogeny, and the phage is referred to as being temperate. All specimens for mycology should be transported and processed as soon as possible. Capnophilic organisms grow best when the atmosphere is enriched with extra carbon dioxide (5% to 10%). Alternatively, iodine or Giemsa stain can be used, but these methods are less sensitive and specific (Figure 24-7). The disease is commonly found in children and usually develops in the summer, frequently referred to as the summer flu or summer cold. Gaydos C, Essig A: Chlamydiaceae. novyi type A. This last species has, to date, been isolated almost exclusively from aquatic wound and bloodstream infections. There are a total of 9 N antigens (N1 through N9); human infections usually occur with N1 and N2. These include pyruvic, lactic, oxaloacetic, oxalic, malonic, fumaric, succinic, benzoic, phenylacetic, and hydrocinnamic acids. With liquefaction of the specimen, the surviving mycobacteria can be concentrated with centrifugation. They have been described typically as curved gram-negative rods, but this morphology is often seen only in the initial Gram stain of the clinical specimen (Figure 20-1). pecorum, C. stain a faint blue (gram-positive) color. General Characteristics Mycoplasmas are pleomorphic organisms that do not possess a cell wall, a characteristic that makes them resistant to cell wall– active antibiotics such as the penicillins and cephalosporins. The mediators cause erythema as a result of greater blood flow, edema from an increase in vascular permeability, and continued phagocyte accumulation, resulting in pus. Lichtheimia. Lichtheimia spp. diarizonae Salmonella enterica subsp. As of August 2013, MERS-CoV had been associated with 94 infections and 46 deaths, primarily in Saudi Arabia. Travelers in areas with poor sanitation are often exposed to conditions in which waterborne or foodborne (rare) cysts are ingested. Intestinal roundworms are the most common of all the helminths that infect humans in the United States. The case studies included in the chapters in Part III enhance problem solving and critical-thinking skills, and help students apply knowledge acquired in Parts I and II. A, Wet mount. In the United States, S. Lesions are usually confined to the extremities, often the feet and lower legs, and are a result of trauma to these areas. tahir99 VRG CHAPTER 21 Nonfermenting and Miscellaneous Gram-Negative Bacilli 493 BIBLIOGRAPHY FIGURE 21-16 Sphingomonas paucimobilis on sheep blood agar (SBA). salamae (subspecies II), S. influenzae in vaccinated children in the United States by 99%, but serotype b remains prevalent in developing countries. Septic or endotoxic shock is a serious and potentially life-threatening problem. A volume of 5 to 10 mL of sputum produced by a deep cough of expectorated sputum or induced by inhalation of an aerosol of hypertonic saline should be used. This isolate is a hyaline, septate, filamentous mold. Cysts may remain viable for several months in cold water. In temperate zones, enterovirus epidemics occur in the summer and early fall. Vibrio parahaemolyticus Epidemiology Vibrio parahaemolyticus is the second most common Vibrio species implicated in gastroenteritis after V. The thermally dimorphic fungi Blastomyces dermatitidis, Coccidioides immitis, Histoplasma capsulatum capsulatum, Paracoccidioides brasiliensis, Sporothrix schenckii, and Penicillium marneffei, are often associated with systemic mycoses. coli, C. In young adults, a primary herpes simplex infection can involve the posterior pharynx and look like acute pharyngitis. cruzi, the agent of American trypanosomiasis or Chagas disease, is discussed in the next section. (Courtesy Erskine Palmer and B.G. Partin, Centers for Disease Control and Prevention, Atlanta, GA.) tahir99 VRG CHAPTER 29 Clinical Virology thought that the virus remains latent in the dorsal root or cranial nerve ganglia after primary infection. Identify staphylococcal species b. albicans infection is thrush, an infection of the oral mucosa. Treating symptoms and reducing the spread in the household is the typical response. These enzymes are used in the field of biotechnology to create sites for insertion of new genes. 1% casein hydrolysate (1- to 2-hour holding time) b. EIAs, however, are not recommended for testing urine or vaginal swab specimens. Sterile scissors are used to cut complete nails into small thin strips, which are used to inoculate media. bancrofti infections. The microscopist must have a consistent vocabulary for the description of materials seen in samples. There appears to be higher risk for infection by these organisms in the presence of foreign materials (e.g., catheters), corticosteroid use, and immunocompromised states. Other species, such as M. However, they rarely cause disease in humans, with the exception of M. Animals born and raised in a germ-free environment have a poorly functioning immune system. Identifying Characteristics All the Acinetobacter spp. monteilii, produce pyoverdin, a yellow-green or yellow-brown pigment. The lesion is teeming with treponemes and is extremely infectious. bronchiseptica is considered normal oral biota in: a. algae is more frequently isolated from clinical specimens than S. histolytica infections; treatment for nonpathogens is not indicated. The use of this differential medium facilitates the primary screening of E. Lichtheimia hyphae are broad and ribbon-like, with few septations (Figure 27-33). The millions of lymphocytes found in the body have been preengineered during embryogenesis and throughout life in the primary lymphoid tissues to recognize a vast array of substances as foreign while learning which substances constitute self. Isolation of the virus is most sensitive with lymphocyte cell culture, which is not practical for routine diagnosis. Klebsiella pneumoniae d. Along with schistosomiasis and amebiasis, malaria is a major cause of mortality in people in underdeveloped countries. HSV is one of the most frequently isolated viruses in the clinical virology laboratory. TABLE 20-7 Selective Media for the Cultivation of Campylobacter Species Medium Base Antimicrobial Agent Campy blood agar plate Brucella agar 10% sheep red blood cells Skirrow's Oxoid blood agar base Lysed, defibrinated horse red blood cells Thioglycolate fluid with agar added 10% sheep red blood cells Vancomycin Trimethoprim Polymyxin B Amphotericin B Cephalothin Vancomycin Trimethoprim Polymyxin B Bacitracin Novobiocin Actidione Colistin Cefazolin Cefoperazone Amphotericin B Butzler CCDA Nutrient agar Charcoal Sodium deoxycholate CCDA, Charcoal cefoperazone deoxycholate agar (Oxiod, Cambridge, England). The MIDI Sherlock Microbial Identification System (MIDI, Newark, DE) is a fully automated gas chromatography system that can be used to identify bacteria (including anaerobes) and yeasts. Isolator Lysis-Centrifugation System Isolator is a blood collection system that contains saponin to liberate intracellular organisms. Dientamoeba fragilis. D. Plasmodium malariae. Plasmodium malariae usually invades older RBCs, perhaps accounting for the occasional darker appearance of the invaded RBC. tahir99 VRG CHAPTER 18 Haemophilus and Other Fastidious Gram-Negative Bacilli Optimal diagnostic sensitivity requires paired sera and testing for multiple immunoglobulin class–antigen combinations (e.g., IgG to FHA and PT, along with IgA to FHA). The presence of the migrating larvae and adults causes little inflammatory damage because they acquire host human leukocyte antigens and ABO blood group antigens on their surface that diminish the host's immune response. This genus is among the easiest to identify because of the frequently seen crescent-shaped conidia with three to five cells of unequal size and an enlarged central cell. japonicum, which is commonly found in the Far East, including Japan, China, and the Philippines, lives in venules of the small intestine. tahir99 VRG CHAPTER 22 Anaerobes of Clinical Importance of the human intestinal biota. tropica or L. aegyptius with H. All people who come through the laboratory must also observe the safety guidelines to ensure proper protection and reduce risk to exposure to potential hazardous biological agents. Isolate, identify, and sometimes perform susceptibility tests for M. Theoretically, the plates can be examined for growth without removing the plates from the bags, so the colonies are not exposed to oxygen. that have been isolated from clinical samples include Enterobacter cloacae, Enterobacter aerogenes, Enterobacter gergoviae, and Enterobacter hormaechei. Later, as pulmonary infiltrates develop, sputum may be bloody or purulent. It is a common inhabitant of marine environments and is a strict halophile, requiring at least 1% NaCl; it is able to tolerate up to 10% NaCl. Almost all isolates originate from extraintestinal sources, such as eye and ear infections or wound and burn infections. abscessus M. arizonae (subspecies IIIa), S. cruzi in some countries of Central and South America has decreased the rate of transfusion transmission. from an environmental source to a human host, including important bacterial factors involved. See Chapter 30 for a discussion of C. trachomatis serovars L1, L2, L2a, L2b, and L3 cause LGV, a sexually transmitted disease (STD); these serovars are more invasive than the others. Chacin-Bonilla L: Epidemiology of Cyclospora cayetanensis: a review focusing in endemic areas, Acta Tropica 115:181, 2010. Centers for Disease Control and Prevention: Investigation update: outbreak of Salmonella Litchfield infections associated with a hotel— Atlantic City, New Jersey, 2007. ovale, however, may persist in the liver in a dormant stage known as hypnozoites, which accounts for the relapse (recurrence) of the disease within 1 to 3 years after the primary infection. The niacin test may be negative when performed on young cultures with few colonies. Clinical Manifestations Treponema pallidum subsp. However, as technology becomes more widespread, they will be moving into the clinical laboratory. Disposable gloves should be worn and a biosafety cabinet used when handling clinical specimens containing potentially infectious agents. Prolonged exposure to gastric acid kills mycobacteria and diminishes culture yield. knowlesi has early life cycle stages that resemble those of P. In the thick film, the RBCs are destroyed so that only white cells, platelets, and parasites are visible. The most successful of tahir99 VRG 418 PART II Laboratory Identification of Significant Isolates these is charcoal agar supplemented with 10% horse blood and 40 mg/L cephalexin. tahir99 VRG CHAPTER 21 Nonfermenting and Miscellaneous Gram-Negative Bacilli Gerri S. micdadei are most frequently implicated in human infections. FIGURE 28-49 Diphyllobothrium latum egg (unstained, ×400). This diarrheal illness is very similar to that produced by Shigella spp. For louseborne relapsing fever, control is best achieved by good personal and public hygiene, especially improvements in overcrowding and delousing. For example, patients who undergo surgery become susceptible to infections caused by organisms that colonize the particular surgical site (e.g., abdominal cavity); leakage following perforation of the colon spills the contents of the colon into the peritoneal cavity, leading to an overwhelming infection by the colon flora. Another feature of patients with endotoxic shock is severe neutropenia, which can occur within minutes after exposure. Phenotypic identification will not identify all nonfermenters; MALDI-TOF or 16 rRNA DNA sequencing will be needed for confirmation, if needed. Enteropathogenic Escherichia coli. Although the EPEC strain has been known to cause infantile diarrhea since the 1940s, its pathogenic role has remained controversial over the last few decades. aeruginosa and certain other contaminants are present. falciparum being the causative agent in more than 50% of cases. coli O157:H7 appears colorless on SMAC agar, whereas most other strains produce pink colonies for sorbitol fermentation. What risk factors contribute to the more severe form of legionellosis? False-positives have been attributed to patients undergoing hemodialysis with cellulose membranes and bacterial infections, and cross-reactions with β-lactam antimicrobials and antitumor polysaccharides have been noted. The nuclear structure is characterized by a large eccentric karyosome and coarse, uneven peripheral chromatin on the nuclear membrane. (Nomarski optics, ×650.) Phoma. Disease caused by the Phoma spp. The sensitivity of viral culture can decrease rapidly 3 days after the acute onset of symptoms, so care needs to be taken to appropriately collect specimens to maximize detection and identification. Transplacental infection also has been documented. Antigenic drift is a minor change in antigenic structure as mutations accumulate. Because many anaerobic infections consist of a mixture of aerobic and tahir99 VRG CHAPTER 22 Anaerobes of Clinical Importance TABLE 511 22-9 Options Available for Identifying Anaerobic Bacteria Identification Technique Presumptive identification—based on colony morphology, Gram stain observations, and results of simple tests (e.g., disks, catalase, spot indole) Definitive identification—commercially available, preexisting, enzyme-based identification systems (e.g., ANIDENT, MicroScan, RapID ANA-II, BBL Crystal, Vitek ANI card) Commercially available biochemical-based identification systems (e.g., API 20A, Minitek, Sceptor) Cellular fatty acid analysis by high-resolution GLC (e.g., MIDI system) Conventional tubed biochemical tests and metabolic endproduct analysis by GLC 16S rDNA sequencing Time to Obtain Results Extent of Identification Same day that PC-SC plate is available Limited capability; many clinically encountered anaerobes cannot be identified Same day that PC-SC plate is available (4-h incubation) Most commonly encountered, clinically significant anaerobes can be identified 24-48 h after PC-SC plate is available Most commonly encountered saccharolytic anaerobes can be identified, but many asaccharolytic anaerobes cannot be identified Most clinically encountered anaerobes can be identified 24-48 h after PC-SC plate is available 24-72 h after PC-SC plate is available 5 h after PC-SC plate is available Most clinically encountered anaerobes can be identified Most clinically encountered anaerobes can be identified GLC, Gas-liquid chromatography; PC-SC, pure culture-subculture. Moraxella, Oligella, and Psychrobacter Moraxella and Olligella, along with Acinetobacter and Psychrobacter, are members of the family Moraxellaceae. A nonfermenter (an oxidizer or nonoxidizer) produces no change in the butt and slant or may produce an alkaline (red) slant. Thus, organisms that have a positive VP reaction usually have a negative reaction on the methyl red test, and vice versa. trachomatis, although its viability is relatively stable at 4° C. freundii hydrolyze urea, but all fail to decarboxylate lysine, whereas Salmonella fails to hydrolyze urea, and most isolates decarboxylate lysine. VORI has shown improved activity against the aspergilli, in addition to several emerging pathogens. The tapering, sometimes elongated, spiny distal ends of the macroconidia are key features that distinguish this species. 639. Several commercial assays are produced, but only one, the Fungitell test (Associates of Cape Cod, Falmouth, MA), is available in the United States. Asexual reproduction is carried out by specialized fruiting structures known as conidiogenous cells. Broth-based susceptibility results are usually available in 5 to 7 days because it is not necessary to wait to visually detect growth. After 10 days of incubation at room temperature, the medium is observed for growth. The incubation period is approximately 10 to 17 days. Most disturbing are the reports of multidrugresistant S. trachomatis is the most common sexually transmitted bacterial pathogen in the United States. Modifications of the KOH test can provide easier and more reliable results. bozemanii, L. Lassa virus is present in throat secretions, can be transmitted from person to person, and can also be transmitted through sexual contact and nosocomially. Resistant strains have not developed. Two types of oocysts may be formed, the thin-walled oocyst, tahir99 VRG 660 PART II Laboratory Identification of Significant Isolates Sporozoite release Entry into intestinal cell to form type I meront Release outside body Autoinfection Rupture in lumen of intestine Merozoite release OR Human ingestion of thick-walled oocyst in fecally contaminated food or water Maturation into thin-walled oocyst Maturation into thick-walled oocyst Type II meront OR Merozoite release Zygote Macrogamont Microgamont Microgamete release FIGURE 28-36 Life cycle of Cryptosporidium sp. Most infections are seen in people from East Asia, Africa, South America, and some areas of the Caribbean. nov. The laboratory scientist places one drop of antisera on the appropriately labeled slide. Local Materials Criteria Less than 25 PMN leukocytes (white blood cells [WBCs]) per LPF and less than 10 contaminating epithelial cells per LPF are seen, along with cellular and fluid elements local to the area sampled. The virus has also been increasingly recognized as an important infectious agent in FIGURE 29-8 Negatively stained transmission electron micrograph revealing the presence of numerous Epstein-Barr virus virions (×40,000). She then changed planes in New York for When an outbreak is suspected, steps are taken to investigate the event. Pantoea (Enterobacter) agglomerans gained notoriety with a nationwide outbreak of septicemia resulting from contaminated intravenous fluids. Yersinia pestis, Staphylococcus aureus, hepatitis C c. These detection times are an obvious improvement in the recovery time from conventional media. Colonies of P. Microorganisms can be described in such a way that, based on prevalence, the description implies the identification of the organism. The IgM antibody molecules are large; the whole molecule has a molecular weight of about 900,000 D and consists of five basic subunits—each composed of two heavy chains and two light chains (similar to an IgG molecule) and linked to another polypeptide chain (J chain) by disulfide bonds (Figure 2-7). Because the gravid female can migrate into the vagina, eggs can also be seen in vaginal specimens. Identification of nonfermenters by 16 S rRNA sequencing was compared with that accomplished with the API 20NE and Vitek-2 ID-GNB cards; 92% of the isolates could be assigned to a species and 8% to a genus level with sequencing as compared with 54% species and 7% genus with API 20NE, and 53% and 1% with Vitek-2 identification; 15% and 43%, respectively, were not found in the API 20NE and Vitek-2 databases. Over the last several years, patient populations have changed. Centers for Disease Control and Prevention: Epidemiology and pre vention of vaccine-preventable diseases, ed 12, 2012. The vaccine usually contains two different strains of influenza A virus and a single strain of influenza B virus. Hypochromic anemia may occur in children with inadequate iron and protein intake in the presence of constant, low-level bleeding with chronic infection. coli strains from strains of normal biota. Most other stains are directed toward specific organism groups and should be available where needed. their macroscopic and microscopic morphologies are similar, identification of yeasts is based primarily on biochemical testing. Padgett JJ, Jacobsen KH: Loiasis: African eye worm, Trans R So

enrichment broth generally is not considered necessary. Miscellaneous Tests for the Identification of Molds Hair Perforation Test. In the hair perforation tests, sterile 5- to 10-mm hair fragments are floated on sterile water supplemented with a few drops of sterile 10% yeast extract. Some researchers use species-specific monoclonal antibodies that bind to the MOMP, whereas others prefer the family-specific antibody, which binds to an LPS component. The lysis of white blood cells and red blood cells releases microorganisms, which are then concentrated into sediment during centrifugation. The stomach acidity greatly reduces the number of organisms that reach the small intestine. pestis is a class A bioterrorism agent; further information relating to this classification can be found in Chapter 30. The sequential steps provide for crystal violet (hexamethyl-p-rosanaline chloride) to color all cells and background material a deep blue and for Gram's iodine to provide the larger iodine element to replace the smaller chloride in the stain molecule. In addition, they may show splenomegaly and hepatomegaly with elevated liver enzyme levels, hypogam maglobulinemia, and hypoproteinemia. nucleatum illustrating the fusiform morphology of this organism (×1000). There is a single nucleus that shows even peripheral chromatin and a small central karyosome. The main activity of PT is modification of host proteins by adenosine diphosphate–ribosyl transferase, which interferes with signal transduction. Vibrios usually appear as small, straight, gramnegative rods, but they can be highly pleomorphic, especially under suboptimal growth conditions. Yeasts Versus Molds Yeasts are single vegetative cells that typically form a smooth, creamy, bacterial-like colony without aerial hyphae. It was first isolated in 1987 during an outbreak of pneumonia and meningitis linked to individuals attending a hot springs spa. The patient develops tachycardia, hypoxia, and hypotension. With the exception of S. Gram-negative anaerobic bacilli are all non–spore-forming and are often found as members of the endogenous microbiota. The disease can be severe; complications include viremia, thrombocytopenia, granulocytopenia, pancytopenia, flulike symptoms, and congestive heart failure. enterica subsp. Kim K, Weiss LM: Toxoplasma: the next 100 years, Microbes Infect 10:978, 2008. Smears from Thin Fluids FIGURE 7-2 Smears from opaque thick liquids or semisolids, such as stool, can be made using a swab to sample and smear the material. Herpes Simplex Viruses HSV-1 and HSV-2 belong to the genus Simplexvirus. RESULTS Gram-positive bacteria stain dark blue to blue-black. Which organism is an opportunistic pathogen that causes wound and urinary tract infections and may cause the production of kidney stones? Recently, some partially automated enzymelinked immunosorbent assays (ELISAs) have become commercially available, but they too are not yet FDA-approved. The virus may be carried in the nares of asymptomatic adults. enterica serotype Typhi. Inflammation Inflammation is the body's response to injury or foreign body. Modified from Engelkirk PG et al: Principles and practice of clinical anaerobic bacteriology, Belmont, CA, 1992, Star. H2S, Hydrogen sulfide; TSI, triple sugar iron agar; −, ≤9% of strains positive; +, ≥90% of strains positive; V, 10% to 89% of strains positive. Sinha AS, et al: Early onset Morganella morganii sepsis in a newborn infant with emergence of cephalosporin resistance caused by depression of AMPC beta-lactamase production, Pediatr Infect Dis J 25: 376, 2006. However, none of them equals the sensitivity of culture, and most are significantly less sensitive. Microaerophilic organisms, such as Campylobacter, require the oxygen concentration to be reduced to 5% or less. Like HHV-6, HHV-7 is extremely common and is shed in the saliva of 75% of adults. As noted, the life cycles of the two Taenia spp. Adherence can be improved by fixation in 70% to 95% alcohol or by gently warming the slide to remove all water from the material. Although patients with primary infection may exhibit flulike symptoms, most are asymptomatic and cannot accurately define the time of onset. However, most strains of Porphyromonas will not grow on KVLB because of their sensitivity to vancomycin. Pseudomonas spp. hydrolyze urea slowly and ferment lactose, producing colonies on MAC agar that resemble those of E. The absence of the preceding bacterial cell structures differentiates them from eukaryotes. It is important to ensure adequate moisture during this period to prevent plates from drying out. Cell morphology is good, and the concentrating effect shortens viewing time and increases the volume of cellular material reviewed. Available at: index.html. Ziehl-Neelsen d. Most laboratories use more than one method to maximize their diagnostic capabilities. The adaptive immune response adds a high degree of specialization to the tahir99 VRG PART I Introduction to Clinical Microbiology Classification and Characteristics of Antibodies Antibody molecules, found in serum and other body fluids and secretions, may be classified into one of five distinct immunoglobulin groups or classes. The bacteria are initially stained purple by the crystal violet, which is bound to the cell wall with the aid of iodine. The eggs are found in the feces of dogs or other definitive hosts and resemble those of Taenia spp. The E. Oxidation of carbohydrates is weak. Since 2007, adenovirus has been associated with outbreaks of acute respiratory illness in U.S. military recruits and the general public. Exogenous anaerobic infections are usually caused by grampositive, spore-forming bacilli belonging to the genus Clostridium. There is no thin-walled oocyst to cause continued autoinfection. The micrometer should be calibrated for each objective on the microscope. Yersinia pestis Cholera Enteritis Dysentery Plague Complex, lethal, edema-producing toxin Lethal, dermonecrotizing toxin 6 type-specific lethal neurotoxins* Toxin A, enterotoxin* Toxin B, cytotoxin* Alpha, lethal, dermonecrotizing Beta, lethal, dermonecrotizing, hemolytic Gamma, lethal, dermonecrotizing, hemolytic Delta, hemolytic Epsilon, lethal, hemolytic Zeta, hemolytic Alpha, lethal, dermonecrotizing, hemolytic* Beta, lethal Gamma, lethal Delta, lethal Epsilon, lethal, dermonecrotizing Eta, lethal (?) Iota, lethal, dermonecrotizing Theta, lethal, cardiotoxic, hemolytic Kappa, lethal, proteolytic Enterotoxin* Alpha, lethal, hemolytic, necrotizing Lethal toxin* Hemorrhagic toxin* Tetanospasmin, lethal, neurotoxic* Neurotoxin, nonspasmogenic Tetanolysin, lethal, cardiotoxic, hemolytic Diphtheria toxin, lethal, dermonecrotizing* Heat-labile enterotoxin* Heat-stable enterotoxin Shiga toxin Exotoxin A Alpha, lethal, dermonecrotizing, hemolytic Beta, lethal, hemolytic Gamma, lethal, hemolytic Delta, hemolytic Exfoliating toxin* Enterotoxin* Dick toxin, erythrogenic, nonlethal Streptolysin O, lethal, hemolytic, cardiotoxic Streptolysin S, lethal, hemolytic Cholera toxin, lethal, enterotoxic* Enterotoxin?* Enterotoxin* Murine toxin From Braude AI, Davis CE, Fierer J, editors: Infectious diseases and medical microbiology, Philadelphia, 1986, Saunders. As the organisms enter the blood, the acute phase begins. Detection of acetoin is the basis for the positive VP reaction characteristic of these microorganisms. Because of this association of IgA with mucosal surfaces, it provides protection against microorganisms invading at those sites. As discussed in Chapter 4, safety in the microbiology laboratory encompasses the infection control program. Acute infections are generally mild and can resemble infectious mononucleosis. Although this method saves time, there is a risk that if organisms are present in small numbers, they may be missed on microscopic examination of wet mounts because of a dilution effect (i.e., reduced sensitivity). Staphylococcal leukocidin, called PantonValentine, is lethal to leukocytes and contributes to the invasiveness of the organism. • They do not produce cytochrome oxidase except for Plesiomonas (see Chapter 20). psittaci, and C. The fungus colonizes primarily the outer portion of hair shafts, so-called ectothrix hair involvement. braakii. pseudoalcaligenes is ADHpositive and will weakly ferment fructose, two characteristics that differentiate it from P. botulinum, which germinate; the vegetative cells multiply and produce toxins. Fungi can be isolated from almost any type of clinical specimen. curvus can be isolated using routine culture media. This algorithm resulted in higher numbers of false-positives in five laboratories studied from 2006 to 2010, so the CDC continues to recommend the original approach. All species except one have been recovered from human specimens. The normal biota of all of these sites adds another dimension to the host's ability to resist invading pathogens. Based on the type of clinical specimen and suspected viruses, a variety of fluorescent-labeled antibodies can be used. The Chinese liver fluke, Clonorchis (Opisthorchis) sinensis, is geographically limited to the Far East, where dogs and cats serve as reservoir hosts. Centers for Disease Control and Prevention: Extensively drug-resistant tuberculosis—United States, 1993-2006, MMWR 56:250, 2007. aeruginosa is a common cause of ventilatorassociated pneumonia, especially in the immunocompromised or otherwise severely ill patient. Larger particles have higher S values. The nodules in which adults live may measure up to 25 mm and can be found on most parts of the body. Calcofluor White Calcofluor white is a fluorochrome that binds to chitin in fungal cell walls. Use of multiplex polymerase chain reaction (PCR) assays for the identification of many of the Burkholderia spp., including B. The immature oocyst usually shows a single sporoblast (an early stage in the development of a sporocyst, prior to differentiation of the sporozoites). Plasmodium Plasmodium spp., which cause malaria, remain endemic throughout the world in tropical and subtropical countries, with an estimated 300 million to 500 million individuals infected. The auramine or auramine-rhodamine fluorochrome stains are more sensitive than the carbolfuchsin stains. Although any of these indicators should alert the physician to the possible involvement of anaerobes, most are not specific for anaerobes. Thrush is also recognized as an indicator of immunosuppression. Mycobacteria are generally categorized as rapid growers, having visible growth in Acid-fast bacillus Growth rate >7 days on LJ at 37° C Pigmentation in absence of light Buff Orange Pigmentation after exposure to light Yellow Tween hydrolysis Buff Nitrate reduction – + Tween hydrolysis + SQ catalase + Mycobacterium kansasii + Niacin Nitrate reduction + – + 68° C catalase – SQ catalase – Nitrate reduction + Mycobacterium tuberculosis SQ catalase + + – Mycobacterium asiaticum Mycobacterium gordonae – Mycobacterium flavescens Tween – Tween hydrolysis Mycobacterium scrofulaceum – Photochromogen at 22° C Mycobacterium szulgai + – + – Mycobacterium Nitrate marinum reduction (optimum growth + at 30° C) – 68° C catalase + Mycobacterium simiae Mycobacterium terrae-triviale complex SQ catalase + 68° C catalase weak + Nitrate reduction – SQ catalase – Mycobacterium avium complex Mycobacterium xenopi + – 68° C catalase + Mycobacterium nonchromogenicum 68° C catalase + – Mycobacterium malmoense Mycobacterium gastri FIGURE 26-8 Schematic diagram for the identification of slowly growing Mycobacterium spp. During the next 8 hours, they organize into larger, less dense RBs, which divert the host cell's synthesizing functions to their own metabolic needs and begin to multiply by binary fission. Many commercial kits are available, all having similar advantages. There is a direct association between dermatophytic infections of the feet or hands and infections of the nails. baumannii, the glucose-oxidizing nonhemolytic strain, and A. parahaemolyticus infection associated with the consumption of raw shellfish in three different states (New York, Oregon, and Washington) between May 20 and July 31, 2006. Certainly, as more of these assays become commercially available and approved for use in laboratories, their value will become even more recognized. A KOH preparation should also be made. trachomatis–specific IgG and IgM are not detected by MIF and a fourfold rise in chlamydiae antibodies is detected by CF, then C. are commonly isolated from the environment worldwide. Epidermophyton floccosum Despite the fact that E. Using a sterile Pasteur pipette, one drop of purulent material or two to three drops of nonpurulent material should be added to each plate and streaked so as to obtain well-isolated colonies. | Several strains in this complex have varied in carbon sources, as follows: "+" sodium citrate; "+" mannitol; "−" inositol. belli is oval, 20 to 33 µm by 10 to 19 µm, with a hyaline cell wall. The first step is to confirm that the isolate recovered in broth or on solid media is an acid-fast organism by performing an acid-fast stain. Campylobacter c. pestis, one of the most virulent species in the family Enterobacteriaceae, causes the extraintestinal infection plague. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility. Stock Solution PROCEDURE 1. Protein A in the cell wall of Staphylococcus aureus helps the organism avoid phagocytosis by 31 interfering with the binding of the host's antibodies to the surface of the organism. In immunocompetent individuals, most infections are mild or asymptomatic. melitensis, and B. Although extinct in nature, at least two cultures are known to be maintained. The promastigote is transmitted to humans through the salivary glands of the insect when it takes a blood meal. Table 27-6 summarizes systemic mycoses, their agents, and their characteristics. Several different immunoassays, collectively referred to as RDTs, use malarial antibody–impregnated dipsticks to detect P. xylosoxidans and A. (Courtesy Bartley SL, Howard JD, Simon R: Centers for Disease Control and Prevention, Atlanta, GA.) 517 specimens. base of the germ tube, called a pseudo–germ tube. Some lipase-positive clostridia are C. It has been shown that approximately twice as many parasites are detected when a single laboratory scientist staffs the parasitology department compared with departments that rotate personnel through the department. The populations that most often manifest the disease include infants and young adults, although all age groups are at risk. The primary hosts usually have minimal or no disease from their rickettsial infection. Skin and nail bed infections as well as severe respiratory infections can be caused by a variety of fungi in patients with AIDS. The specificity of the DFA test has been reported to be 94% to 99%, but tahir99 VRG 414 PART II Laboratory Identification of Significant Isolates some species of Pseudomonas, Bacteroides, Corynebacterium, and other bacteria can cross-react with the polyvalent conjugates. Tiny colonies may appear on CHOC agar that contains L-cysteine; however, BCYE agar with L-cysteine is best for Legionella isolation. Available at: syphilis.htm. Mumps virus is spread by droplets of infected saliva and has a global distribution. Organisms such as Staphylococcus epidermidis under usual conditions do not cause disease but can induce an infectious process in patients with prosthetic devices. This may inhibit the growth of strict anaerobes because of the toxic effect of oxygen. The transmission of campylobacterioses has been attributed to direct contact with animals and handling infected pets, such as dogs, cats, and birds, and indirectly by the consumption of contaminated water and dairy products and improperly cooked poultry. These isolates are fastidious, slow-growing organisms at 37° C and have not been found in human specimens. Mycobacteria and nocardiae are best stained with an acid-fast stain, in which the bacteria are stained with carbolfuchsin, followed by acid-alcohol as a decolorizer. Include size, appearance on routine wet mount or trichrome, and appearance with special stains. The nucleus is bounded by a bilayered lipoprotein nuclear membrane. On a permanently tahir99 VRG Falling leaf 9-21 × 5-15 Giardia lamblia Nondirectional Jerky, nondirectional 6-14 5-12 7-23 × 5-12; average, 15-18 Trichomonas hominis Dientamoeba fragilis Trichomonas vaginalis Jerky 10-20 × 3-10 Chilomastix mesnili Rotary Motility Size (µm) tahir99 VRG One Two (20% have one) One One Two No. of Nuclei Trophozoite 28-5 Comparison of Intestinal and Urogenital Flagellates Organism TABLE Undulating membrane half the length of the body Found in urine Nucleus made of four to eight clustered granules Resembles ameba Undulating membrane the entire length of the organism Axostyle through body Spiral groove Cytostome Sucking disk, ventral surface Parabasal bodies and axonemes Other Features 6-10, lemon-shaped 8-12, oval Size (µm) and Shape One Four No. of Nuclei Cyst No cyst stage No cyst stage No cyst stage Anterior of cyst has nipple-like protrusion Cytoplasm retracted from cyst wall Fibrils and flagella inside cyst Other Features CHAPTER 28 Diagnostic Parasitology 643 644 PART II Laboratory Identification of Significant Isolates A B FIGURE 28-17 A, Giardia lamblia trophozoite, identified by arrow (trichrome stain). Pandemics (worldwide epidemics) of cholera, a devastating diarrheal disease caused by V. Other species commonly inhabiting the GI tract include Bifidobacterium, Clostridium, Eubacterium, and the anaerobic gram-positive cocci. Wound infection invariably involves a recent traumatic aquatic exposure, such as a boating or fishing accident, and generally occurs on the extremities. The trypomastigote, an extracellular nonreplicating form, is readily visible because of the motion of the flagellum and undulating membrane. The preservative used is determined by the procedure to be performed on the fecal sample. Tetanus. The clinical manifestations of tetanus are attributed to the neurotoxin tetanospasmin produced by Clostridium tetani. Nevertheless, these organisms may cause significant, often serious, infections or may exacerbate existing infections in individuals lacking a fully responsive immune system. Colonies rapidly develop abundant aerial mycelium, and the colony appears to enlarge in a circular bloom. Rhinoviruses infect the nasal epithelial cells and activate inflammatory mediators. Prepare smear or imprint. Table 24-5 identifies the situations in which the tests may be most applicable and identifies the population groups at greatest risk. Dimorphic fungi include a mold phase and a yeast or spherule phase. Inoculated plates should be held under near-anaerobic conditions until placed into an anaerobic jar or bag. Lipuma J, et al: Burkholderia, Stenotrophomonas, Ralstonia, Cupriavidus, Pandoraea, Brevundimonas, Comamonas, Delftia, and Acidovorax. Adults of the two species can be differentiated by the morphology of the buccal capsule or, in the male, the copulatory bursa. Patients begin bleeding from the nose, gums, and GI tract during the latter part of the first week. They are nitrate positive and can grow on modified Thayer-Martin medium. Gram-negative isolates, especially NLFs, should be tested for cytochrome oxidase production. In this procedure, a drop of the KOH preparation is added to a slide. Epidemiology Organisms are transmitted via the bite of infected Ixodes ticks, so most cases occur during June through September, when more people are involved in outdoor activities and ticks are more active. posadasii is found in the desert southwest of the United States, Mexico, and South America. There are rare cases of endocarditis described in the literature. Transient flora comes to "visit" but does not usually live or stay. H, India ink. Values for each ocular unit can be calculated by using the stage micrometer according to the procedure found in Appendix D. microti; Figure 28-33, B, demonstrates the tetrad formation characteristic of the organism. Most flagellates have both cyst and trophozoite stages. that cause gastrointestinal disease (enteric campylobacters) are C. spiritovorum. Similarly, testing should be repeated if the patient's cultures for M. In addition, many secretions provide a chemical barrier, such as the acidic pH of the stomach and vagina. At first he noticed only a local reaction in his finger, but after 12 hours the left hand began to swell, accompanied by nausea, vomiting, and diarrhea. In addition, smears can be screened at a lower magnification (×250 to ×400), thus allowing for more fields to be examined in a shorter time. The skin contains a wide variety of microorganisms, most of which are found on the 25 Case Check 2-2 In the Case in Point at the beginning of this section, antibiotic use led to an alteration of the patient's normal oral flora. Fermentation of mannitol results in a decrease in pH around the colony, causing the pH indicator, neutral red, to turn red at the center of the colony and the bile to precipitate. pneumoniae is among the most commonly recovered isolates from respiratory tract samples of hospitalized patients. They must be carefully distinguished from leukocytes. When the cell ruptures, amastigotes are released and invade other macrophages. An example of such a growth curve is shown in Figure 1-7. Unlike hookworms, S. Items that should be included in such a checklist appear here and on following pages in bold italic type preceded by a bullet (•). Issues to Consider After reading the patient's case history, consider: ■ The risk factors in this patient for infection with a nonfermentative gram-negative bacillus ■ The significance of the number of positive blood cultures and the time until the cultures were positive ■ How you might rapidly identify this organism for better clinical care tahir99 VRG CHAPTER 21 Nonfermenting and Miscellaneous Gram-Negative Bacilli Key Terms Saccharolytic Eugonic oxidizer (EO) Fermentative Nonfermentative Nonoxidizers 475 Clinical Infections Oxidizers Pseudomonad Pyocyanin Pyoverdin A erobic gram-negative bacilli can be divided into at least two large groups, those that ferment carbohydrates, called fermentative or fermenters, and those that do not ferment carbohydrates, called nonfermentative or nonfermenters. Inserted into the viral envelope are viral glycoprotein (gp) trimers or spikes. • Colonies present on the anaerobically incubated blood agar plates but not on the CO2-incubated blood or chocolate agar plates • Good growth (>1 mm in diameter) of gray colonies on a BBE plate, characteristic of members of the B. parapsilosis has become a major cause of outbreaks of nosocomial infections. duttonii acquire complement regulators C4b-binding protein and factor H, Infect Immun 74:4157, 2006. All of the above 8. These types of systems will help epidemiologists, infectious disease physicians, and others in the public health community by rapidly identifying viral pathogens during an outbreak. sonnei, which has only one serotype. Current technology has allowed the analysis of genetic relatedness (DNA and RNA structure and homology) for taxonomic purposes. Colorado Tick Fever Virus The genus Coltivirus contains the Colorado tick fever virus, which causes a dengue-like infection in the western United States and Canada. houtenae Salmonella enterica subsp. An ongoing internal proficiency testing program should be used to enhance the identification skills of the laboratory scientists, especially if a full-time parasitolo gist is not employed. Specimens submitted for influenza virus cultures are usually inoculated onto PMK, LLC-MK2 (a continuous line derived from rhesus monkey kidney), or MDCK (canine kidney) cells. These colonies are a buff (tan) color and are nonphotoreactive; exposure to light does not induce pigment formation. The most common presentation is tracheobronchitis; however, about one third of infected patients demonstrate clinically apparent pneumonia (Figure 25-4). Cocci Anaerococcus spp. lamblia has been identified as a sexually transmitted pathogen among those who practice oral-anal sex. Many clinical virologists use these terms interchangeably; however, cell culture is technically more correct. Edward Jenner demonstrated the efficacy of vaccination to smallpox in 1796, which ultimately led to control of the disease. They are found in certain protozoa and in ciliated epithelial cells of the respiratory tract. Many individuals with antibodies to HSV-2 have not been diagnosed with genital herpes. Preservation Several methods are available for stool preservation if the specimen will not be delivered immediately to the laboratory. The key to the persistence of the influenza virus is its antigenic variation. Primary cell lines can only be passaged a few times before new cells must be obtained. nov., Cronobacter muytjensii sp. Sphingobacterium are oxidase, catalase, and esculin positive but generally indole negative. influenzae biogroup aegyptius. Archaeal cell walls lack peptidoglycan, a major reason they are placed in a domain separate from bacteria. Although the virus can be isolated from the saliva of healthy individuals, it is rarely isolated from peripheral blood mononuclear cells. After 48 hours of incubation at 37° C, growth on SBA was described as mucoid colonies exhibiting a greenish brown halo. Colonies often show β-hemolysis at 48 hours. No acceptable identification could be made with a rapid identification system for gram-negative bacilli, and final identification was made with conventional biochemicals supplemented with 1% NaCl. The patient suffered a cardiac arrest and died 11 hours after admission. mucosa was the usual isolate, and catheter-related bloodstream infection was the most common infection seen. Sometimes electron microscopy of clinical samples will yield the characteristic virions. cryocrescens ferments glucose at this temperature. Smear positivity and culture yield vary with the extent of the disease (i.e., whether there is cavitary or noncavitary pulmonary disease or endobronchial or laryngeal disease). Small aliquots of fluid (0.1 to 0.2 mL) are placed in the cytocentrifuge holders. In the case of the blood flukes, individuals should wear clothing and shoes to prevent cercarial penetration. Organisms that are "intracellular" (e.g., Mycobacterium tuberculosis, Listeria monocytogenes, Brucella spp.) are able to survive phagocytosis and may multiply within the phagocyte. (A, From Murray PR, Rosenthal KS, Pfaller MA: Medical microbiology, ed 6, Philadelphia, 2009, Mosby; B, from Thibodeau GA, Patton KT: Anatomy and physiology, ed 6, St Louis, 2007, Mosby.) temperature. More likely, they are small stones or foreign bodies. In the pandemic of 1918 and 1919, influenza killed an estimated 20 to 50 million people, with more than 500,000 of them from the United States. The word "syphilis" comes from a poem written in 1530 that described a mythical shepherd named Syphilus who was afflicted with the disease as punishment for cursing the gods. What are the stages of a Treponema pallidum subsp. Similar results can occur in the presence of tissue necrosis and when the redox potential in tissue is decreased. (Hematoxylin and eosin, ×200.) number of parasites, tissues invaded, and person's general health. Fihman V, et al: Stenotrophomonas maltophilia—the most worrisome threat among unusual non-fermentative gram-negative bacilli from hospitalized patients: a prospective multicenter study, J Infect 64:391, 2012. None of the members of this tribe ferment lactose. EBO-R was isolated from the bloodstream of one of the workers. fragilis and B. In most cases, the cause of the illness is not disclosed in the case study; rather, it is presented elsewhere in the chapter to give students the opportunity to think the case through. The organisms found in the gastrointestinal tract are summarized in Box 2-5. However, the instrumentation required may not be available for use in all areas of the world. Some normal flora species produce bacteriocins, substances that inhibit the growth of closely related bacteria. Larger laboratories usually maintain antisera to serogroup salmonellae for all the somatic types. G, Lactophenol cotton blue stain. Only B. The speed and magnitude of this response are easily visualized by recalling how quickly a splinter or similar injury becomes infected and how much pus is produced. The trophozoite is uncommonly seen in CSF, and diagnosis is typically made by brain biopsy demonstrating cysts, trophozoites, or both. parahaemolyticus has also been isolated in Europe, the Baltic area, Australia, Africa, Canada, and almost every coastal state in the United States. Lipase-positive organisms produce a colony covered with an iridescent, multicolored sheen, sometimes described as resembling the appearance of gasoline on water or mother of pearl. Mucorales, such as Mucor and Rhizopus spp., grow rapidly and may fill the tube with aerial mycelium within a few days, whereas more slowly growing organisms such as Fonsecaea or Phialophora spp. For example, the female genital tract flora depends on the pH of that environment, which in women of childbearing age is approximately 4.0 to 5.0. Most bacteria do not survive at these extreme pH ranges. Natural hosts of the organism include dogs and deer, as well as humans, with the lone star tick (Amblyomma americanum) being the primary vector. The tape is placed onto a drop of LPCB on a slide and examined. Box 22-1 lists examples of conditions that may predispose a patient to anaerobic infections. Dengue virus has four serotypes, 1 through 4. pestis by its motility at 18° C to 22° C, production of urease, and ability to ferment rhamnose. FIGURE 29-3 Cytomegalovirus from cerebrospinal fluid forming a cytopathic effect on diploid fibroblast cells. BOX 2-4 Microorganisms Common α-Hemolytic and nonhemolytic streptococci Diphtheroids (Corynebacterium spp.) Staphylococcus aureus Staphylococcus epidermidis Streptococcus pneumoniae Streptococcus mutans Streptococcus milleri Streptococcus mitis Streptococcus sanguis Streptococcus salivarius Found in the Oropharynx Moraxella catarrhalis Haemophilus parainfluenzae Anaerobic streptococci Bacteroides spp. Sexual Phase. Sporogony, which takes place in the mosquito, results in the production of sporozoites infective for humans. Both plasmids and chromosomal genes can be transferred by this method. Patients develop a sore throat, abdominal pain, diarrhea, and vomiting and also start to bleed from injection sites and GI tract. volvulus is referred to as onchocercosis, or river blindness. avium are respiratory tract pathogens of wild and domestic birds and mammals and are generally nonfastidious and recoverable on routine microbiological culture media. histolytica, however, can differentiate between E. Gryseels B: Schistosomiasis, Infect Dis Clin N Am 26:383, 2012. Organisms causing chromoblastomycosis are darkly pigmented or phaeoid molds. Adults often have a milder form of the disease, and the most severe form of acute infection usually occurs in children. Cornmeal Agar. Yeast morphology on cornmeal agar is second in importance to sugar assimilation in determining yeast identification (see Appendix D). Table 19-8 shows the characteristic features of Salmonella serotype Typhi, Salmonella serotype Choleraesuis, and Salmonella serotype Paratyphi. Some tests use monoclonal antibody directed against an antigen specific to P. Microorganisms have been categorized according to their optimal temperature for growth. The three different transmission cycles for yellow fever virus are the sylvatic, urban, and intermediate cycles. spiralis. bongori is a rarely isolated species that is named after the town of Bongor in Chad, Africa, and was initially isolated in 1966 from a lizard. This cycle results in the production of acid and the evolution of carbon dioxide (Figure 1-11). In a direct wet preparation, the motility of the trophozoite may aid in presumptive identification. The trauma to the legs following the accident allowed the organism to penetrate the skin surface. Direct smears prepared from samples, such as sputum, that contain endogenous microbiota do not provide valuable information because their significance cannot be fully assessed unless the gram-negative bacteria are prevalent and endogenous inhabitants are absent. The egg and occasionally the adult of T. with pyoverdin, the green color characteristic of P. HBoV was detected less frequently than common respiratory agents (e.g., influenza, parainfluenza, adenovirus, RSV) in South African children. grow rapidly on primary fungal media and produce arthroconidia, hyphae, and blastoconidia (Figure 27-12). Physicians have far fewer options for treating fungal infection than for their bacterial counterparts. Along with Giardia, these organisms account for most outbreaks caused by waterborne parasites. coli isolates. PIVs are a primary cause of respiratory disease in young children. The use of India ink preparation is being replaced by the latex agglutination test for cryptococcal antigen because of the former's low sensitivity. Infections are thought to be related to inhalation of a large number of organisms or forceful entry of water into the nose (microtrauma) and to the virulence of the strain. An average of 500,000 cases of measles was reported annually in the 1950s, with an average mortality of 500. If a fungal infection such as aspergillosis occurs during or immediately after construction, an outbreak investigation may be initiated and control measures implemented. Wauters G, Vaneechoute M: Approaches to the identification of aerobic gram-negative bacteria. Positive; cannot; positive c. The reaction between the hydroxyl radical and superoxide anion forms singlet oxygen, which is also damaging to cells. pseudomallei in septicemic melioidosis, Am J Trop Med Hyg 86:1039, 2012. In some cases, the background quality was poor, and in others a less sharp morphology of the organism was observed. avium complex, M. Endospores are not a means of reproduction. Until other drugs are studied and breakpoints are established, the usefulness of antifungal susceptibility testing will most likely remain limited. Environmental Culturing ✓ 3-6 Pathogens Related to Waterborne Infections Viruses Index Case Case Check 3-3 As part of an effective infection control program, the microbiology laboratory may be called on to perform cultures of various environmental sites. The patient involved in the suspected index case flew from Russia to London, where she changed planes for a flight to New York. Pneumocystis spp. A gram-negative bacillus isolated from a stool specimen produces clear colonies on MacConkey agar and yellow colonies on thiosulfate citrate bile salt sucrose medium. Table 20-8 lists the biochemical tests most useful for definitively identifying the most commonly encountered Campylobacter, Helicobacter, and Arcobacter species. coli Citrobacter Enterobacter Serratia — — Salmonella* Plesiomonas† Hafnia Serratia Shigella* Yersinia Aeromonas† E. The most important species in this genus is T. When delays of more than 2 hours between collection and processing are unavoidable, specimens should be refrigerated to avoid overgrowth of contaminating microbiota. Species-specific borreliae often bear the same epithet as their vectors (e.g., Ornithodoros hermsii transmits Borrelia hermsii). The scolex of T. In the center are two identical copies of ssRNA and reverse transcriptase surrounded by an icosahedral capsid. cholerae non-O1 8. This is one of the reasons Escherichia and Shigella spp. stuartii has been implicated in outbreaks in burn units and has been isolated from urine cultures. Because most humans are now susceptible to variola virus, it remains a threat as a bioterrorist weapon. They are susceptible to polymyxin B, which differentiates isolates from members of the genus Sphingobacterium, which it resembles. All laboratory procedures to recover and identify these agents must be performed in a biological safety cabinet. The CSF will usually exhibit increased A 641 protein, normal or decreased glucose, and increased lymphocytes. The antiviral drugs amantadine and rimantadine can prevent infection or reduce the severity of symptoms if administered within 48 hours of onset. rubrum is the 5-day urease test. Whether in everyday activities or activities in response to an outbreak or emerging diseases, the microbiology laboratory and laboratory technologist constitute a critical component of an efficient and successful infection control program. The primary human pathogen, L. Composition of Microbial Flora at Different Body Sites most superficial layers of cells and the upper parts of hair follicles. The flexible, clear vinyl chambers are available in three lengths—36, 59, and 78 inches—including one fitted with two pairs of gloves so that two microbiologists can use the chamber simultaneously. The reported rate of chlamydial infections in women increased from 496.5 cases/100,000 women in 2005 to 610.6 cases/100,000 women in 2010, an increase of about 23%. However, since 2000 the disease has increased, peaking at 46,290 cases in 2008. Envelop culture methods for T. A basic saponification followed by acidification and chloroform extraction allows accurate identification of most mycobacterial species using chromatograms and colony characteristics. Nasopharyngeal aspirates or swab specimens should be plated directly onto culture media or transferred to an appropriate transport system (1% casein hydrolysate, Amies, or Regan-Lowe transport medium) at the bedside. Sources include contaminated potable water distribution systems, respiratory therapy equipment, and recreational waters. Monoclonal antibodies against the MOMP are reported to offer the brightest fluorescence, with consistent bacterial morphology and less nonspecific staining than monoclonal antibodies against the LPS. Lack of pigment, presence of extraerythrocytic organisms, and absence of other life cycle stages serve as keys to differentiate Babesia from P. catarrhalis is the most frequent isolate in the genus Moraxella from clinical specimens, especially from respiratory and ear specimens. Infections resulting from lower infective doses have been reported. Establishing a case definition and culturing air and water b. If therapy begins within the first 6 days of exposure, Lassa virus can be effectively treated with the antiviral drug ribavirin. Schweiger M, et al: Case of Paracoccus yeei infection documented in a transplanted heart, Transpl Infect Dis 13:200, 2011. It begins with the breakdown of pyruvic acid. (Courtesy Anaerobe Systems, Morgan Hill, CA.) Case Check 22-3 In the Case in Point, a Gram stain of the patient's leg wound revealed boxcar-shaped, gram-positive rods suggestive of clostridia. sordellii is the only member of the clostridia to exhibit urease activity. The occurrence of surgical site infections (SSIs) is generally calculated as: a. avium complex M. Abscess Fluid, Wound Exudates, and Tissue Using a dissecting microscope, one can examine abscess fluid and exudate from wounds for the presence of granules. The growth cycle (Figure 24-1) begins when the small EB infects the host cell by inducing energy-requiring active phagocytosis. However, D. Yersinia enterocolitica b. marcescens, S. • Phenol-soap mixtures containing orthophenol or other phenolic derivatives are effective with contact periods of 10 to 30 minutes. Examination of Specimens for Blood and Tissue Parasites Blood Smears Examination of a blood smear stained with Giemsa or Wright stain is the most common method of detecting malaria, Babesia, Trypanosoma, and some species of microfilaria. It is transmitted to humans by bites of fleas, which are its most common and effective vectors. The ultimate aim of a cell is to produce the proteins that are responsible for cellular structure and function and to transmit the information for accomplishing this to the next generation of cells. Health departments frequently require the reporting by the laboratory of: a. Petry F, et al: Host immune response to Cryptosporidium parvum infection, Exp Parasitol 126:304, 2010. Shared by numerous serotypes, phase 2 antigens react with heterologous antisera. How would you differentiate P. alginolyticus 75 99 9 98 95 25 0 70 95 99 100 99 8 1 0 99 0 0 5 0 0 0 100 97 30 0 99 100 3 4 V. aureus. aureus can be distinguished from MRSA in an outbreak of skin infections in prisoners. This autoinfective life cycle is most common, although an insect vector may serve as intermediate host in an alternate form of the life cycle. aeruginosa, but can produce a nonfluorescing yellow or green pigment that may diffuse into the media. pylori can be presumptively identified in a gastric biopsy specimen by testing for the presence of a rapid urease reaction (Figure 20-10). The arthropod transmits the virus when feeding on the blood of vertebrate hosts, which includes humans. L– or D–Lactic acid + 2H Acetaldehyde H2 + CO2 + 2H + 4H PYRUVIC ACID Oxaloacetic acid Formic acid Acetyl CoA Acetolactic acid Acetoacetyl CoA + 4H + 4H Ethanol Acetoin Acetic acid Acetone Butyryl CoA + 4H + 2H + 2H 2,3–Butanediol 15 Isopropanol Butyric acid Butanol FIGURE 1-8 The fate of pyruvate in major fermentation pathways by microorganisms. These eggs can appear similar to those of Diphyllobothrium latum and must be carefully examined when seen in the feces. Notice darkly staining, ingested red blood cell near the nucleus (trichrome stain). Dip slide in fixative solution five times for 1 second each time. vaginalis, such as the InPouch TV (BioMed Diagnostics, San Jose, CA), are also available. EIA kits are simple to perform, commercially available, and comparable to other treponemal tests. FIGURE 27-51 Pithomyces sp. If needed, pyocyanin typing can be used to epidemiologically link strains of P. • The eggs are unperculated. This reaction occurs in about 4 days and may be useful in distinguishing M. In smear-positive samples, M. Infections are generally spread by contact with contaminated secretions. Treatment and management of cholera are best accomplished by the administration of copious amounts of intravenous or oral fluids to replace fluids lost from the severe diarrhea. • Humans are infected by direct cercarial penetration of the skin. Unlike the leptospires and treponemes, the borreliae stain easily and can be visualized by bright field microscopy. Stage 2 is early disseminated and produces widely variable symptoms that include secondary skin lesions, migratory joint and bone pain, alarming neurologic and cardiac pathology, splenomegaly, and severe malaise and fatigue. Fungi Fungi are heterotrophic eukaryotes that obtain nutrients through absorption. Symptoms resolve in several weeks, although infections can last up to 1 month. Infertile Ascaris lumbricoides eggs and Schistosoma spp. Microbiologists use these metabolic differences as phenotypic markers in the identification of bacteria. intracellular forms. Clinical Infection. E. fragilis group • Colonies on KVLB or anaerobic blood agar plates that fluoresce brick red under ultraviolet (UV) light or are brown to black in ordinary light, characteristics of pigmented Porphyromonas and Prevotella. Aeromonads are straight rods (1.0 to 3.5 µm long by 0.3 to 1.0 µm wide). West African nations suffer from more than 200,000 cases and approximately 3000 deaths annually. The amount of fluids recommended for culture varies—2 mL for CSF, 3 to 5 mL for exudates and pericardial and synovial fluids, and 10 to 15 mL for abdominal and chest fluids. Intravenous fluids are often needed in children this young. Most infections occur in the summer months. Thick-walled rhomboid cells containing ascospores are seen. The genera Leishmania and Trypanosoma are blood flagellates of humans transmit

Fastidious Gram-Negative Bacilli 413 Legionellosis suspected Urine antigen test LRT specimen DFA Culture (BCYE) LRT specimen No growth Growth on BCYE only Reported negative Gram-negative bacillus* Positive Negative Reported and confirmed with culture Result reported; culture pending Identification of Legionella and its species with DFA FIGURE 18-29 Schema for identification of Legionella organisms. FIGURE 24-11 Anaplasma morula (arrow) in an infected white blood cell (×1000). Like the vibrios and aeromonads, these organisms are oxidase-positive, glucosefermenting, facultatively anaerobic, gram-negative bacilli that are motile by polar flagella. By this method, results can be obtained in 2 to 3 weeks, depending on the growth rate of the organism. When decolorizer is applied to bacteria with a gram-negative type of cell wall structure, the crystal violet washes out of the cells, which take up the pink counterstain, safranin. tahir99 VRG 586 PART II Laboratory Identification of Significant Isolates Performance of the test with isolated colonies or with broth from a BACTEC medium with a growth index of 50 to 100 or more is recommended. Weeksella isolates tahir99 VRG CHAPTER 21 Nonfermenting and Miscellaneous Gram-Negative Bacilli are asaccharolytic, indole and oxidase positive, and fail to grow on MAC agar. pylori has been identified in as many as 80% of gastric ulcer patients. Blood b. There is a wrinkled outer wall and smooth inner wall. The ordered tests provide a guide to the examination, but a routine approach to specimen management should include an examination procedure that would discover unexpected pathogens. It is optimum to process viral specimens for culture immediately. Microscopic Examination Several diagnostic methods can be used in the microscopic examination of a fecal specimen: • Direct wet mount examination (stained and unstained) of fresh stool specimens • Concentration procedures with wet mount examination of the concentrate • Preparation of permanently stained smears In general, concentration and permanent staining procedures should be performed on all specimens. Bacterial species, such as Chlamydia, Mycobacterium, Brucella, and Listeria species, are easily engulfed by macrophages and phagocytes; however, these species not only are able to survive inside the macrophages—protected from the host's other immune defenses—but also are able to multiply intracellularly. How did the patient in the Case in Point at the beginning of this section develop the yeast infection in her mouth? Figure 28-1 shows the relationship between stool consistency and protozoan stage. Adults may also experience arthralgia, arthritis, or both. Two types of infection are important to distinguish: infections caused by a single species, or monomicrobial, and infections 133 caused by multiple species, or polymicrobial. pertussis DNA by PCR amplification was added to case inclusion criteria in 1995, and use of PCR has gained considerable use, but a lack of standardization and single target testing has also led to false-positive results and outbreaks that were mistakenly attributed to pertussis in several states. World Health Organization: Global epidemics and impact of cholera, 2013. Are morphotypes present that characterize the source of the organisms? gondii DNA may be used for CSF or amniotic fluid and are useful in detecting congenital infection in utero. The parasite induces an inflammatory response in the lung characterized by the presence of neutrophils and eosinophils. Cytoplasmic Structures Bacteria do not contain a membrane-bound nucleus. pylori infection can also be diagnosed by fecal antigen detection, microscopic examination of stained gastric tissue, and DNA amplification tests (e.g., polymerase chain reaction assay). Discuss the criteria used to differentiate the bacterial species that cause gastrointestinal illnesses from other diarrheal agents. Cells infected with influenza virus express a viral hemagglutinin (H) protein on their surface that binds red blood cells (RBCs). It stimulates proliferation of B lymphocytes in some animal species, activates macrophages, activates complement, and has an adjuvant effect with protein antigens. No surgery was needed to replace the valve, which was unusual compared with endocarditis caused by P. may also be used to fix) of the smear and the addition of four sequential components: crystal violet (the primary stain, 1 minute), iodine (the mordant or fixative, 1 minute), alcohol or an alcohol acetone solution (the decolorizer, quick on and rinse), and safranin (the counterstain, 30 seconds). The second step is to confirm that an outbreak exists. Three other azoles, ITRA, VORI, and POSA, are more frequently prescribed for mold infections. When inserting swabs or small pieces of tissue into an anaerobic transport container, care must be taken not to tip the container. Note the prominent karyosome (iron hematoxylin stain, ×1000). Colonies of B. These containers are described in more detail later (see "Anaerobic Incubation of Inoculated Media"). Most isolates are DNase, oxidase, gelatin hydrolysis, and weakly indole positive (the more sensitive Ehrlich indole test is recommended over the Kovacs test). • Butanediol fermentation: Members of the genera Klebsiella, Enterobacter, and Serratia within the Enterobacteriaceae use this pathway for sugar fermentation. (A, original magnification ×1000; B, ×1000.) cells. Discrepancies in sensitivity could be based on differences in sample size, disease prevalence, population characteristics, collection sampling techniques, and laboratory standards. carrionii are shown in Figure 27-18. The less commonly seen male is 2 to 5 mm long, with a curved posterior. fragilis group, Actinomyces spp. Diagnosis of trichinosis is made through biopsy of tissue to identify the coiled larval stage. gingivalis Catalyze the degradation of collagen Toxic to specific types of cells Destroy DNA Toxic to cells of the intestinal mucosa Lyse red blood cells liberating hemoglobin Catalyzes the hydrolysis of hyaluronic acid, the cement substance of tissues Catalyze the hydrolysis of ester linkages between fatty acids and glycerol of triglycerides and phospholipids Destroy or disrupt nerve tissue Certain Clostridium C. These features have been used initially to differentiate and characterize certain genera. Obligate anaerobes cannot grow in the presence of oxygen. Laboratory Diagnosis Traditionally, diagnosis was made by finding the cyst or trophozoite in tissue obtained through open lung biopsy. When he was admitted to the hospital, a stool culture grew an organism identified as Escherichia coli. Table 2-2 lists some of the ways microorganisms interfere with phagocytic activities. B, Gram-stained appearance of subterminal spores of Clostridium sordellii (×1000). The primary diagnostic stage in humans is the rhabditiform larva. Tubed media have the advantage of being safer to handle and less susceptible to drying. The smear is examined under a mercury vapor lamp with a strong blue-filtered light. One option is the GasPak jar (BD Diagnostic Systems; Figure 22-8). Common symptoms include periorbital edema, fever, muscular pain or tenderness, headache, and general weakness. Chiang E, Haller N: Babesiosis: An emerging infectious disease that can affect those who travel to the northeastern United States, Travel Med Infect Dis 9:238, 2011. It is a relatively rare infection; however, many studies have shown the presence of antibodies directed against N. It is the only proven therapy that has been widely used for patients with neurosyphilis, congenital syphilis, and syphilis during pregnancy. Occasionally, these fungi will also reproduce asexually. FIGURE 21-9 Acinetobacter baumannii on MacConkey (MAC) agar. In addition to C. Cephalothin and carbenicillin disk susceptibility tests separate the first two species; K. The remaining 20% display symptoms of what is termed West Nile fever, which includes fever, headache, fatigue, occasional skin rash on the trunk, swollen lymph glands, and/or eye pain. In addition to vancomycin, lactobacilli are frequently resistant to the cephalosporins. rectus. Trends Microbiol 11:118, 2003. LJ, Löwenstein-Jensen. Define the following terms: true pathogen, opportunistic pathogen, and virulence. Antimicrobial Susceptibility Case Check 23-1 Leptospires are present in water and mud contaminated by the urine of reservoir animals. Infection with H. lung abscesses, and gonococcal urethritis. Based on the clinical infections that they produce, members of the family Enterobacteriaceae may be divided into two broad categories: (1) opportunistic pathogens and (2) primary pathogens. Where are most cases of leptospiroses contracted within the United States, and why is this important when considering the typical incubation period of the infection? perfringens When anaerobes are suspected, the following steps must be performed and recorded for each colony morphotype present on the anaerobic blood agar plate to initiate presumptive identification of the isolates: • Describe the colony morphology and note whether growth occurred on each of the selective (e.g., BBE, KVLB) and nonselective anaerobic blood agar plates. pneu mophila serogroups 4 and 6, L. Sealing the preparation prevents rapid drying and allows longer examination. Hentschel U, Steinert M, Hacker J: Common molecular mechanisms of symbiosis and pathogenesis, Trends Microbiol 8:226, 2000. vivax has the widest geographic distribution and is the one most likely to be found in temperate climates. Information on the container should include the patient's name and the date and time the stool was collected. • They do not decarboxylate lysine. Figures 26-8 and 26-9 show schematic diagrams for the identification of slowly growing and rapidly growing Mycobacterium spp., respectively. Single-Stranded RNA Viruses Arenaviridae The arenaviruses get their name from the Latin arena, meaning "sand." Under an electron microscope, arenaviruses appear sandy and granular. These organisms are usually found in water; however, they occasionally occur in clinical specimens. Because archaea are not encountered in clinical microbiology, they are not discussed further in this chapter. Sediments for mycobacteria (concentrated smears, auramine, and rhodamine) Preferred acid-fast stain Acid-fast stain modification of Ziehl-Neelsen method for Cryptosporidium and Cyclospora parasites in stool specimens Bronchoalveolar fungi and some parasitic cysts Differentiates them from background materials of similar morphology Routine stain for diagnostic area Yeast differentiated from all other organisms Provides same differential staining but enhances red-negative organisms by staining the background material green to gray-green Genus (Species)-Specific Stains Antibody or DNA probe stains Used for specific identification of selected pathogens, such as Chlamydia trachomatis, Bordetella pertussis, Legionella pneumophila, herpes simplex virus, varicella-zoster virus, cytomegalovirus, adenovirus, and respiratory viruses PROCEDURE 7-1 Gram Stain PRINCIPLES The following Gram staining method was developed empirically by the Danish bacteriologist Christian Gram in 1884. Elephantiasis, a debilitating and deforming complication, occurs in less than 10% of infections, usually after many years of continual filarial infection. For example, the strains of Escherichia coli that cause traveler's diarrhea use their fimbriae to adhere to cells of the small intestine, where they secrete a toxin that causes the disease symptoms. A study of 81 patients with symptoms suggestive of CDAD compared three NAATs and two non-NAATS for the detection of CDAD. Brook I: Antimicrobial treatment of anaerobic infections, Expert Opin Pharmacother 12:1691, 2011. The aerotolerance test needs to be one of the first tests performed. terrae complex M. Other factors, such as excessively thick smears, which tend to obscure microorganisms, and the technical skill of the observer, may influence the sensitivity of the test. Epidemiologists constantly seek means of further subdividing bacterial species to follow the spread of bacterial infections. Outside the peptidoglycan layer is an additional outer membrane unique to the gram-negative cell wall. Arthroconidia begin as true hyphae but break apart at the cross walls with maturity. lilacinus, and in about 6% of Candida lusitaniae isolates. Enveloped viruses are typically more susceptible to inactivation by high temperature, extreme pH, and chemicals than nonenveloped (naked) viruses. • Mixed acid fermentation: Members of the genera Escherichia, Salmonella, and Shigella within the Enterobacteriaceae use this pathway for sugar fermentation and produce a number of acids as end products—lactic, acetic, succinic, and formic acids. In the United States, as many as 20% of normal adults have antibodies to HHV-8, as do 27% of patients with HIV-1 who do not have Kaposi sarcoma and 60% of patients with HIV and Kaposi sarcoma. What are the clinical samples of choice for the diagnosis of B. In the last several years, another organism, Entamoeba moshkovkii, has been identified as belonging to this complex of organisms that can infect humans. Compare the epidemiology and pathogenesis of the serovars of Chlamydia trachomatis. respiraculi, and C. xenopi, and M. Between 20% and 25% of neonates born to Chlamydia culture–positive mothers develop conjunctivitis, 15% to 20% develop nasopharyngeal infection, and 3% to 18% develop pneumonia. Generalized symptoms include fever and fatigue. Figure 28-5, A, shows the trichrome-stained trophozoite, and Figure 28-5, B, is an enlarged view of E. The egg, which must be distinguished from that of H. K/K, Alkaline-alkaline; Rhizobium radiobacter Achromobacter xylosoxidans Acinetobacter baumannii Achromobacter xylosoxidans Achromobacter denitrificans Brucella canis var. Bacilli may occur as single rods or in chains or may align themselves side by side (palisading). Trimethoprim-sulfamethoxazole is used to treat the infection; once treatment is started, symptoms usually abate within several days. CMV is the most common congenital infection in the United States. Lichtheimia spp. FIGURE 27-41 Geotrichum sp. from most other members of the family Enterobacteriaceae. The remaining two immunoglobulin classes, immunoglobulin D (IgD) and immunoglobulin E (IgE), are found in very low concentrations in serum (500) were reported but not cultured. Luminal amebicides such as metronidazole are given to carriers in nonendemic areas to prevent the invasive phase and reduce the risk of transmission. The transitional group contains R. Many pathogens encountered in the clinical microbiology laboratory fall into this group (e.g., Bacteroides fragilis). Four of the stains—Gram, acid-fast, calcofluor white, and rapid modified Wright-Giemsa—should be available in all diagnostic microbiology laboratories (Procedures 7-1 to 7-4). Infection in young children is almost always asymptomatic. Some cancers also have been associated with EBV, including Burkitt lymphoma, Hodgkin disease, and nasopharyngeal carcinoma (NPC). As a result, the incidence of infection caused by opportunistic organisms increases. When used in conjunction with the additional tests shown in Table 20-5, the clinical microbiologist should be able to identify almost all Aeromonas isolates to the species level. Therefore, laboratories that isolate few M. Examinations of wet mounts and Gram-stained preparations of pus from draining sinuses are useful diagnostic procedures for demonstrating the non–sporeforming, thin, gram-positive bacilli that frequently exhibit branching in clinical specimens (Figure 22-2). Control organisms that may be used are Cryptococcus albidus (positive) and C. Helminths Helminth infections in humans are caused by flukes, tapeworms, or roundworms. Skin Skin samples are scraped from the outer edge of a surface lesion. The biological safety cabinet is the single most important piece of equipment in a mycobacteriology laboratory. To induce mycelia conversion to yeast, the fungus is inoculated onto brain heart infusion agar supplemented with sheep red blood cells and incubated at 37° C in a CO2 incubator. A B FIGURE 18-30 A, Legionella pneumophila (arrow) in specimen smear stained by direct fluorescent antibody (DFA) technique (×450). A 47-year-old patient who had just returned from Mexico was admitted to the hospital with a 3-day history of vomiting and diarrhea, without fever, and no fecal leukocytes were found in the stool. They cause outbreaks of acute gastroenteritis in schools, colleges, nursing homes, and families, as well as on cruise ships and in resort areas. The antiviral compound ribavirin is approved as a treatment for patients with RSV. It is 200 to 250 µm long, with a short buccal capsule (Figure 28-65, A), large bulb in the esophagus, and prominent genital primordium located in its posterior half to posterior third (see Figure 28-65, B). maltophilia, may be all that is needed, especially when cultures are mixed with many other bacterial species. The WHO distributes pools of enterovirus antisera that allow identification by neutralization patterns in the antisera. Similarly, the mycobacteria isolated may be other than M. In individuals with an intact immune system, infections are localized and limited. The appropriate collection container for feces is clean, dry, sealed tightly, and waterproof (e.g., a plastic container with lid). Air Usually, infections traced to air quality occur during construction activities in a health care setting. Recovery of the organism from blood is associated with clinical disease. Foster G, et al: A review of Brucella sp. Other Campylobacter species produce colonies similar to those of C. Rapid surgical débridement is necessary for patient management. rhinoscleromatis varicola ornitholytica V. Klebsielleae, cont'd Genus Species Enterobacter aerogenes cloacae gergoviae cancerogenus (taylorae) hormaechei agglomerans sakazakii alvei marcescens liquefaciens rubidaea fonticola odorifera plymuthica mirabilis vulgaris penneri hauseri myxofaciens morganii alcalifaciens rettgeri stuartii pseudotuberculosis pestis enterocolitica frederiksenii kristensenii intermedia ruckeri Pantoea Cronobacter Hafnia Serratia VI. Proteeae Proteus Morganella Providencia VII. Yersinieae Yersinia Modified and revised from Ewing WH: Edwards and Ewing's identification of Enterobacteriaceae, ed 4, East Norwalk, CT, 1986, Appleton & Lange. coli, it is negative for lysine and ornithine decarboxylase and arginine dihydrolase (i.e., triple decarboxylase-negative). Fever, sepsis, skin lesions, and abdominal pain were the most common presenting symptoms and liver was the most common site of localized abscesses in the series. Amebic keratitis, associated with Acanthamoeba spp., has been identified since the 1980s. ✓ Case Check 18-5 Children require five vaccinations by the age of 6 years, beginning at 2 months of age. Candida albicans Viridans streptococci Serratia marcescens Pseudomonas aeruginosa Streptococcus pneumoniae Klebsiella pneumoniae Pseudomonas aeruginosa Cryptococcus neoformans Varicella-zoster virus Pseudomonas aeruginosa Burkholderia cepacia Candida albicans Pneumocystis jirovecii (carinii) Herpes simplex virus Aspergillus spp. Naegleria fowleri. PAM has been reported from countries worldwide. Iodamoeba bütschlii. Iodamoeba bütschlii is less commonly encountered than E. CO2 Fumarate ATP FIGURE 1-11 Krebs tricarboxylic acid cycle allowing complete oxidation of a substrate. The studies showed that most strains in asymptomatic cyst passers and in most men who have sex with men were nonpathogenic, whereas several strains from areas with high rates of endemic disease were pathogenic. histolyticum, C. Epidemiology Campylobacter spp. Because multiple blind passes are not necessary to maximize the isolation rate in a 1-dram vial, the shell vial technique (see Chapter 29) has been found to be more sensitive than the microwell method. It has also been linked to chronic illnesses such as atherosclerosis, coronary heart disease, and stroke. prowazekii, R. are commonly recovered from the blood. As demonstrated in the Case in Point, care must be taken for proper diagnosis because colonies may initially appear yeastlike on subculture but rapidly become wooly as hyphae are formed. (Giemsa stain, ×1000.) proposed as another method for diagnosing malaria. Most symptoms occur during the migration and encapsulation periods; the severity of symptoms depends on the tahir99 VRG 680 PART II Laboratory Identification of Significant Isolates Adults in small intestine Eggs in small intestine Rhabditiform larvae in feces Hatching of eggs 1 Feces deposition in soil Autoinfection 2 Complete development in intestine 3 Direct route Free-living male and female adult development Penetration of intestinal wall Entry into circulation Migration up trachea Free-living cycle Filariform larvae development in intestine Egg production Filariform larvae development in soil (infective for humans) Hatching of rhabditiform larvae Entering circulation Breaking through to alveoli Penetration of human skin Swallowed FIGURE 28-64 Life cycle of Strongyloides stercoralis. EHEC causes about 80% of the cases of HUS in the United States. birminghamensis cincinnatiensis hackeliae jordanis longbeachae maceachernii micdadei oakridgensis pneumophila† sainthelensi wadworthii L. A congenital infection is best confirmed by isolation of CMV from the infant within the first 2 weeks of life. botulinum is isolated or if a case of botulism is suspected. H1N1 infection causes a wide range of flulike symptoms, including fever, cough, sore throat, body aches, headache, chills and fatigue. Mycoplasmas are the smallest self-replicating organisms in nature. Box 18-3 summarizes the laboratory diagnosis of pertussis by culture. NoVs have been found in drinking water, swimming areas, and contaminated food. Transmission is probably through aerosol droplet spray produced while coughing. Each safety cabinet should be tested and recertified at least yearly by trained personnel with special monitoring equipment. Within 24 hours, anaerobic cultures grew grampositive rods, with a double zone of β-hemolysis surrounding the colonies on sheep blood agar (SBA) incubated anaerobically. Trichophyton Agars. Seven different Trichophyton agars, numbered 1 through 7, are used to determine the nutritional requirements of the Trichophyton spp. pylori in the stomach releases 13CO2 or 14CO2, which is absorbed into the bloodstream and detected in the exhaled breath by a scintillation counter. The phage may then take a lytic pathway, in which the bacteriophage DNA directs the bacterial cell to synthesize phage DNA and phage protein and package it into new phage particles. Omer MK, et al: Prevalence of antibodies to Brucella spp. MAC and EMB agars do not support F. Cases of cholera are not commonly reported in the United States, and most cases are considered imported cases. Failure to achieve anaerobic conditions could be the result of a "poisoned catalyst" or a crack in the jar, lid, or O-ring. Define teleomorph, anamorph, and synanamorph. A trichrome-stained smear of a patient's fecal specimen shows the presence of cysts that are oval, approximately 11 µm in size and have four nuclei containing large karyosomes with no peripheral chromatin and a cluttered appearance in the cytoplasm. stutzeri from most other Pseudomonas spp. For the following DNA, write the complementary sequence. The entire volume of voided urine, or a minimum of 15 mL, is collected in a sterile container. Yaws Yaws is a spirochetal disease caused by T. The borreliae are highly flexible organisms varying in thickness from 0.2 to 0.5 µm and in length from 3 to 20 µm. However, faster laboratory-directed diagnostics must be followed with appropriate medical interventions or patients will receive poor service. In chronic stages, dysuria, urine retention, and urinary tract infections occur. A cover slip is not needed if the cellophane tape technique is used because the tape serves as a cover slip. alginolyticus, V. Proteolysis is best observed by holding the plate up to a strong light source. Virulence Factors and Pathogenicity Leptospiral disease in the United States is caused by more than 20 different serovars, the most common of which are Icterohaem orrhagiae, Australis, and Canicola. Egg production and egg migration through the tissues are responsible for most of the acute damage because the eggs are highly immunogenic. DNA hybridization identification can be applied to growth on conventional agar and to growth in liquid media. Excess mucus will compound this phenomenon. The arthritic form resembles the arthritis seen in other bacterial infections—that is, in infections with Shigella spp., Salmonella spp., and Neisseria gonorrhoeae and in acute rheumatic fever. However, staining of formalin-preserved feces using the Weber modification of the trichrome stain and the Ryan trichrome blue stain can be used to detect microsporidial spores. Small, empty spherules may resemble the yeast cells of B. The infections are commonly chronic and usually incite the development of lesions at the site of trauma. Specimens should not be frozen unless a significant delay, more than 4 days, in processing is anticipated. The virus infected at least 8000 people and had a mortality rate of approximately 10%. (Hematoxylin and eosin, ×1000.) tahir99 VRG 658 PART II Laboratory Identification of Significant Isolates Human ingestion of raw meat containing cyst with bradyzoites Human ingestion of infective oocyst from cat feces Release of sporozoite, which penetrates intestinal cell Congenital toxoplasmosis Formation of "cyst" containing bradyzoites in various organs Infection of fetus Tachyzoite formation Tachyzoites crossing placental barrier Immune system response Asexual reproduction in cells Invasion of other tissue cells by tachyzoites Pregnant woman Rupture of cells Hematogenous spread FIGURE 28-35 Life cycle of Toxoplasma gondii. It has been isolated from respiratory specimens of patients with CF and in non-CF patients as well; the significance is not always clear, although it appears that colonization increases in patients who are mechanically ventilated compared with patients who are not. Young C, et al: Transfusion-acquired Trypanosoma cruzi infection, Transfusion 47:540, 2007. In California residents, 59% of leptospirosis cases were acquired during freshwater recreation from 1982 to 2001; in the last 5 of those years, the rate was 85%. The uniqueness of these cells lies in the presence of specific cell surface receptor molecules that recognize and bind a unique antigen, activating the cell to divide, differentiate, and secrete numerous effector substances. Tapeworms Tapeworms (cestodes) are another group of human parasites in the phylum Platyhelminthes. In the United States, cutaneous larva migrans occurs primarily in the Southwest, Mid-Atlantic, and Gulf Coast areas and is most commonly caused by the filariform larva of the dog or cat hookworm (Ancylostoma braziliense). The difference between the newer tests and those of the previous generation is the antigens used. A biphasic system (broth and agar), such as the Septi-Chek (Becton-Dickinson Diagnostic Systems, Sparks, MD), can also be used. Treponemal tests are also helpful in the detection of late-stage infections because the titers remain high and usually do not drop in response to therapy, as do the nontreponemal test 535 results. Virusinfected cells are usually found at the edge and in the base of lesions; however, the virus can be transmitted from older lesions, as well as from asymptomatic patients. These organisms are considered mesophiles because they grow well at 37° C. Mycobacteria are catalasepositive. Brown, round sclerotic bodies, which are nonbudding structures occurring singly or in clusters, are seen in tissues. Most people with this infection are asymptomatic, but the organism can cause a self-limiting diarrhea with nausea, vomiting, and abdominal tenderness. (pleomorphic) Veillonella spp. tahir99 VRG CHAPTER 27 Medically Significant Fungi FIGURE 27-47 Chaetomium sp. Because the goal of the Textbook of Diagnostic Microbiology is to provide a strong foundation for clinical laboratory science students, entry-level practitioners, and other health care professionals, discussions of organisms are limited to those that are medically important and commonly encountered, as well as new and re-emerging pathogens. harveyi isolates are sucrose-positive. The mature schizont contains merozoites, which are individual chromatin masses, each surrounded by cytoplasm. 487 microbiologists for rapid and accurate laboratory diagnosis. With viscous materials such as a thick tracheal aspirate, a Dacron swab may be used to inoculate the material onto the media, or preferably the specimen can be digested with the mucolytic agent N-acetyl-L-cysteine and concentrated before inoculation. Available at: .htm. flexneri infection have changed during recent years, from a disease affecting mostly young children to one producing infections in young adults (approximately 25 years old). In summary, the digestion-decontamination agent used, its concentration, length of exposure of the agent to the specimen, and centrifugation speed and temperature all affect the recovery of Mycobacterium spp. holmesii) and wound or ear infection (B. Both species typically produce wrinkled or rough colonies at 48 hours (Figure 21-7). The B portion binds to the GM1 ganglioside of the intestinal mucosa, providing entry for the A portion. are strongly oxidase-positive, nonmotile, and gram-negative coccobacillary to bacillary. They have been implicated in tomato rot and are readily recovered from the environment in air-settling plates. hominis to colitis and irritable bowel syndrome, B FIGURE 28-7 A, Entamoeba coli trophozoite. Jacquier H, et al: Revisited distribution of nonfermenting gram-negative bacilli in clinical isolates, Eur J Clin Microbiol Infect Dis 30:1579, 2011. If these tests yield equivocal results, suspicious organisms should be subcultured to charcoal– horse blood agar, SBA, and CHOC agar (the last to assess for Haemophilus spp. filariform larvae are about 500 µm long, with a pointed tail and an esophageal-intestinal ratio of 1 : 4. Discuss the differences between bacteria and fungi. Table 28-7 compares the characteristics of the fluke eggs. kingae c. B, Acridine orange stain of Vibrio cholerae (×1000). The infective form of the organism is the EB, which replicates in the phagosome and prevents phagolysosome formation. The adult worm is approximately 5 mm long and contains only three proglottids. Care should be taken in the interpretation of very low numbers of bacteria, especially in the absence of inflammation or necrosis and in specimens from nonsterile sites. parapertussis generally causes a similar disease with milder symptoms. trota + + V + − + − + + + − + − + V − V+ − + − − − + − − + + − + + − + + − + + + + V + + − − − − − − + − − + + V R V V R V V R R V R V V R V V R V V S S V + − + + + + + − + + + + + + + + + + − − V+ − − V + − − − − + + − + + + + + − + + + V From Horneman AJ et al: Aeromonas. Glove boxes also use less gas and are therefore cheaper to operate. Does the number of cases diminish or disappear? The arthropod host allows rickettsiae to persist in nature in two ways. moshkovkii also be included. fetus Campylobacter hyointestinalis Campylobacter upsaliensis Campylobacter concisus Campylobacter curvus Campylobacter rectus Arcobacter butzleri Helicobacter pylori Helicobacter fennelliae Helicobacter cinaedi Susceptibility to Catalase Nitrate Reduction Urease H2S Production (TSI) Hippurate Hydrolysis Indoxyl Acetate Hydrolysis 15° C 25° C 42° C Nalidixic Acid (30 µg) Cephalothin (30 µg) + + − − + + − − + + − V − − − V + − − − + + + + + + + + − − V − − − − − + − − − − − − + + + + − + − − − − + + − − − − − + + − + − − − V −V + + + + + + + V − + − − − − − − − − − V V − − − − − − − V − − − − − + − V + + − + V − − − − + − − − − − − − + − − − + + + W V V − − + − + + V − + + + − ND ND − + + + Growth at From Fitzgerald C, Nachamkin I: Campylobacter and Arcobacter; and Fox JG, Megraud F: Helicobacter. When asked to perform environmental cultures of water, the microbiology laboratory must determine which specific pathogens are sought, if that information is known. coli; EPEC, enteropathogenic E. These infections are rarely transmitted by sexual contact, and congenital infections do not occur. Plates never should remain in room air on the open bench. Patients experience photophobia, blurred vision, inflammation, ring infiltrates, and pain. oryzihabitans by the ortho-nitrophenyl-galactopyranoside (ONPG) test and esculin hydrolysis. borne at the tips of annellides (Nomarski optics, ×1250.) 601 S. Colonization of gram-negative bacilli in the respiratory tracts of hospitalized patients, particularly by K. Gram stain + Bacilli Spore Yes Clostridium spp. Infection control programs rely on microbiology laboratory support in: a. belli are asymptomatic, but symptoms such as low-grade fever, headache, diarrhea, and colicky tahir99 VRG CHAPTER 28 Diagnostic Parasitology 661 FIGURE 28-37 Cryptosporidium oocysts (modified acid-fast FIGURE 28-38 Cystoisospora belli oocysts (modified acid-fast abdominal pain may be present. Otherwise, the initial clinical impression might resemble influenza, especially if presentation occurs during periods of high influenza activity. A less useful method involves cutting the hairs close to the scalp with sterile scissors. coli Enterobacter Citrobacter — Aeromonas*† Yersinia Citrobacter Enterobacter — — R/A K/K H2S — Citrobacter Data from Microbiology Laboratory, The Ohio State University Hospitals and Maureta Ott, Columbus, OH. What three bacterial agents are the main causes of communityacquired, atypical pneumonia? Tetracycline, erythromycin, and chloramphenicol can be substituted for gentamicin. Another growing area of EIA application testing is the field diagnosis of malaria. (Nomarski optics, ×450.) tahir99 VRG CHAPTER 27 Medically Significant Fungi rate that with age, can develop light shades of pink, gray, or tan pigment. fragilis is now recognized as a pathogen. Table 24-4 shows selected features associated with neonatal inclusion conjunctivitis. Energy for the new constructions is generated during the metabolic breakdown of the substrate. Found in the Mouth Prevotella/Porphyromonas Bacteroides oralis Treponema denticola Treponema refringens Less Common Staphylococcus aureus Enterococcus spp. Within the host cell, the sporoplasm divides and develops into meronts. However, this method provides the least ideal environmental condition. Gram-positive diplococci, encapsulated, extracellular. Some newer nucleic acid assay panels now include this virus, which helps with treatment and epidemiology efforts. The organism is susceptible to rapid drying in the environment, but a few cases of nonsexual transmission have been reported. In the hemagglutination assay, supernatant from the infected monolayer containing influenza virus is mixed with a suspension of RBCs. Influenza viruses also have the H protein on their surface; therefore, the RBCs will visibly agglutinate. Some anaerobes, especially C. TCBS agar is the medium of choice to isolate and differentiate the Vibrio spp. Accessed November 1, 2013. "Ground-glass" appearance Legionnaires' disease Pontiac fever Approximately 20 species of Legionella have been isolated from humans (Box 18-1). It has been isolated from sites of extraintestinal infections, such as blood and wounds. For each drug tested, a standardized inoculum is added to the control (drug-free) media and media containing several dilutions of the drug. These organisms, however, have also been identified as opportunistic pathogens in immunocompromised hosts, particularly in those with AIDS. Certain genera, such as Bacillus and Clostridium, produce endospores in response to harsh environmental conditions. The patient begins to sweat profusely and is left exhausted and sleepy. Serologic Assays Viral serology detects circulating antibodies to viruses after exposure. The Probe-Tec ET Legionella assay (BD Diagnostic Systems) is approved by the FDA. Cases of liver abscess and acute infective endocarditis caused by Y. Merozoites may be arranged in a characteristic "loose daisy petal" arrangement around the clumped pigment; however, they may also be randomly arranged. Lactose is a disaccharide consisting of one molecule of glucose and one molecule of galactose linked together by a glucoside bond. This method provides a count of viable cells only. (99) Smooth Temp. TABLE 25-2 Pathogens in the Class Mollicutes Feature Cell wall–deficient Gram stain Penicillin-susceptible Urease activity Lack of cell wall induced in hypertonic solution and penicillin, lysozyme, or salts Exists in nature as free-living organism Pleomorphic shape Other shared characteristics Mycoplasma Ureaplasma Acholeplasma + − − − + − + − + − − − − − + Smaller than other bacteria; close in size to myxoviruses Smaller genome than other bacteria Lower guanidine-to-cytosine (G/C) ratio than most bacteria Limited metabolic activity (i.e., fastidious) Many mollicutes contain DNase. Pustules are deeply embedded into tissues. BCYE, Buffered charcoal yeast extract; LRT, lower respiratory tract; DFA, direct fluorescent antibody. About 2 to 3 days later, a maculopapular rash appears on the head and trunk. FIGURE 20-4 Vibrio cholerae on thiosulfate citrate bile salt sucrose agar. Organisms should be evaluated for shape, size, and Gram reaction. Molecular methods are used to identify strains. Other various manifestations of the disease are mediastinitis, pericarditis, and mucocutaneous lesions. MATERIALS TB auramine-rhodamine T stain Carbolfuchsin stain (prepared by laboratory) 0.5% acid alcohol (0.5% HCl in 70% ethanol) 2% acid alcohol (prepared by laboratory) 1% sulfuric acid (partial acid-fast) 0.5% aqueous potassium permanganate solution 0.3% aqueous methylene blue solution (prepared by laboratory) Microscope slides 1 × 3 inches Sterile water Fluorescent Stain 1. Infections within a family are common. A thin smear of feces must be used so that debris does not obscure the small, faintly staining spores. 503. Most importantly, the specific response is able to remember each time it encounters a particular foreign antigen, called immunologic memory. Both excess heat and exposure of the prepared media to light can result in the release of formaldehyde, which is toxic to mycobacterial growth. With this negative stain, budding yeast surrounded by a large clear area against a black background is presumptive evidence of C. The laboratory is integral in several of the steps. cruzi infection. What organisms would be expected to be potential contaminants of improperly collected blood cultures? 452 444 0 0 0 0 0 0 0 0 0 0 50 0 80 0 100 0 100 95 95 0 80 0 100 0 100 0 86 91 100 70 0 100 99 100 100 0 65 100 100 100 0 100 0 100 100 100 0 100 50 100 0 0 0 0 50 50 100 0 100 0 100 100 1 100 D-Glucose, Gas 0 0 0 0 D-Glucose, Acid 0 0 0 0 0 0 27 0 100 100 0 33 100 0 80 60 20 0 80 0 100 100 67 0 100 0 0 100 Growth in KCN 0 0 0 0 Motility 50 95 80 99 50 100 50 100 Urea Hydrolysis 0 100 0 40 0 100 0 100 H2S (TSI) 80 0 0 0 0 0 0 0 Voges-Proskauer 0 0 0 100 0 0 0 0 0 20 0 0 0 33 0 33 0 99 0 100 0 100 0 100 0 0 0 0 0 100 100 0 100 0 9 100 1 30 0 80 7 100 15 100 100 78 89 100 67 33 100 50 40 99 33 2 11 0 40 0 9 0 0 15 0 0 0 0 99 100 0 100 100 67 33 100 100 33 0 100 100 100 100 0 100 100 17 100 100 100 100 17 0 100 0 0 95 5 100 75 25 20 100 85 10 99 18 100 98 99 100 100 5 99 100 0 100 0 100 100 0 100 0 0 0 0 0 0 0 0 95 5 85 0 0 85 95 95 0 33 100 33 0 99 100 100 100 100 100 0 0 0 0 0 87 10 78 33 99 60 0 78 67 0 47 59 44 0 75 0 0 0 0 0 0 0 0 0 0 67 93 85 100 67 0 33 0 80 99 87 97 89 67 95 0 100 0 100 93 0 93 0 100 96 0 89 11 100 89 0 100 100 100 100 0 0 95 100 98 100 0 83 0 15 11 100 100 0 100 67 67 100 0 0 0 100 100 0 83 0 100 100 0 100 100 100 100 100 0 75 65 80 5 100 99 0 1 100 0 0 0 70 0 0 0 0 0 50 0 0 67 0 0 0 100 0 100 100 0 100 0 0 50 5 0 99 91 0 20 100 100 0 100 100 95 96 100 0 0 0 0 0 0 0 0 95 100 0 65 0 0 100 98 0 100 100 0 0 0 0 0 0 0 0 0 0 0 1 100 0 0 0 0 0 0 0 0 0 0 100 100 100 100 0 0 100 0 100 67 100 0 100 100 100 0 100 0 100 19 100 100 60 0 100 35 100 100 0 50 100 100 100 50 100 0 0 99 100 100 100 Adonitol Fermentation 60 100 100 100 100 100 100 100 93 100 100 100 100 100 100 Salicin Fermentation Dulcitol Fermentation 0 0 0 0 0 0 0 0 0 0 0 0 0 0 33 0 33 0 0 0 0 0 0 0 0 0 0 100 0 0 0 100 0 0 100 0 Fermentation D-Mannitol 0 0 0 100 53 87 0 80 60 100 100 100 100 100 100 100 67 0 40 0 100 100 0 0 60 100 100 40 60 0 67 100 100 100 100 0 100 100 100 100 0 0 33 100 100 100 0 Malonate Utilization Sucrose Fermentation Gelatin Hydrolysis (22° C) Ornithine Decarboxylase Arginine Dihydrolase Lysine Decarboxylase Phenylalanine Deaminase Lactose Fermentation Budvicia aquatic Buttiauxella agrestis Buttiauxella brennerae Buttiauxella ferragutiae Buttiauxella gaviniae Buttiauxella izardii Buttiauxella noackiae Buttiauxella warmboldiae Cedecea davisae Cedecea lapagei Cedecea neteri Cedecea species 3 Cedecea species 5 Citrobacter amalonaticus Citrobacter braakii Citrobacter farmeri Citrobacter freundii Citrobacter gillenii Citrobacter koseri (C. Endospores are small, dormant (inactive), asexual spores that develop inside the bacterial cell (active vegetative cell) as a means of survival, although they do become vegetative when the harsh conditions are removed. The formal steps of the intervention process may not be developed until after the hypothesis is developed and tested. Inoculation of Appropriate Plated and Tubed Media The choice of media for use in the anaerobic bacteriology laboratory is an extremely important aspect of

grown on a round coverslip in a shell vial. By early 2006, influenza A (H5N1) had been isolated from birds in Turkey, Greece, Italy, Germany, Iran, Iraq, Nigeria, and many other countries. environmental fungi typically considered contaminants. Salmonellae are gram-negative, facultatively anaerobic bacilli that morphologically resemble other enteric bacteria. The primary group at risk for developing this condition is individuals who wear contact lenses, especially the soft and extended-wear types. Trichomoniasis has been associated with increased adverse pregnancy outcomes, including transmission of the organism to the newborn, in addition to cervical neoplasia and pelvic inflammatory disease. The organism is commonly found in soil contaminated with pigeon droppings and is most likely acquired by inhalation. The recommended detection methods for Cryptosporidium infection are the modified acid-fast stain and a direct fluorescent antibody test using a monoclonal antibody directed against Cryptosporidium spp. A, acid; IMV:C, indole, methyl red, Voges-Proskauer, citrate; K, alkaline; NLF, nonlactose fermenter; PAD, phenylalanine deaminase. Approximately one third of untreated patients exhibit a biologic cure, losing serologic reactivity. During this maturation, the cells synthesize myeloperoxidase, proteases, cathepsin, lactoferrin, lysozyme, and elastase. Often the genus name is abbreviated by using the first letter (capitalized) of the genus followed by a period and the species epithet (e.g., S. APPLICATION The Gram stain is used routinely and as requested in the clinical microbiology laboratory for the primary microscopic examination of specimens submitted for smear and culture. The specimens of choice are from the nasopharynx and urine, but the virus can only be recovered from these sources in the early stages of infection. Gram-negative anaerobic bacilli (Prevotella, Porphyromonas, and Fusobacterium) and anaerobic cocci are the anaerobes occurring in the largest numbers. An example of a pathogenic microaerophile is Campylobacter spp., which requires 5% to 6% oxygen. stuartii and P. Reports describe hospitalacquired outbreaks of Klebsiella resistant to multiple antimicrobial agents in newborn nurseries. Because of low sensitivity and specificity, antigen detections methods are generally not used. capsule and are urease-positive. trota, and A. It is not known why so few individuals are infected with such common exposure. Rhinoviruses Rhinoviruses, found in the genus Rhinovirus, are small naked viruses closely related to the enteroviruses. Patients infected with C. The newest species added to this genus, Escherichia albertii, is associated with diarrheal disease in children. On Gram-stained smears, these organisms stain poorly. These include PC (Pseudomonas cepacia), OFPBL (oxidative-fermentative base, polymyxin B, bacitracin, lactose), and BCSA (B. The virus is shed in saliva, tears, urine, stool, and breast milk. ‡ Young cultures may be nonchromogenic. dermatitidis or the microconidia of H. A Y. In the lysogenic cycle, the phage DNA is incorporated into the bacterial genetic material, and genes encoded on the phage DNA may be expressed from this site. The pandemic strain, V. The stain of choice in most laboratories is the trichrome stain, because results are somewhat less dependent on technique and the procedure is less time-consuming. Humans are infected when the filariform larvae penetrate their skin. Helicobacter infections usually are identified by nonculture methods. 557. Early growth of the mycelial culture produces round to pyriform microconidia measuring 2 to 5 µm. grow on Simmons citrate medium and in potassium cyanide broth; the methyl red test is negative, and the Voges-Proskauer test is positive. koseri is a pathogen documented as the cause of nursery outbreaks of neonatal meningitis and brain abscesses. Arthritis is a common extraintestinal form of Y. Current data indicate that bats, primarily the horseshoe bat, are the most likely reservoir for the SARS-CoV, although the bat coronaviruses are species-specific. The alcohol-acetone decolorizer damages these thin lipid walls and allows the stain complex to wash out. Key biochemical tests to differentiate various Vibrio spp, include reaction on TCBS, agent O/129 susceptibility or resistance, string test, and growth in different concentrations of NaCl. The Case in Point uses the results obtained from these reactions to identify the responsible organism. donovani). RSV causes croup, bronchitis, bronchiolitis, and interstitial pneumonia. In the event of a positive agglutination in the Vi antisera with no agglutination in the other groups, the suspension should be heated to 100° C for 10 minutes to inactivate the capsular Vi antigen. Fluconazole is the leading agent for treating yeast infections but has limited to no activity against molds. Neonatal Herpes. Transmission of HSV from infected mothers to neonates is less common than might be expected but the risk of mother to infant transmission is 10 times higher in mothers with an unrecognized primary infection during labor and delivery. A diagnosis of septic shock was made, and antimicrobial therapy was initiated. Enteroviruses have also been implicated in early-onset diabetes, cardiomyopathy, and fetal malformations. Compare and contrast legionnaires' disease and Pontiac fever. Humans are not the usual hosts when they enter jungle areas in which the sylvatic cycle exists. Culture Identification Guidelines The designation "usual flora" or a brief presumptive description of "colony-type growth" may be used (see section on contaminating materials). Ferrous ammonium sulfate combines with pyrazinoic acid, producing a red pigment (Figure 26-15). boydii but molecular characterization has revealed that P. The micronucleus is rarely visible in routinely stained smears. The following procedures should be performed on clinical specimens for the recovery of anaerobic bacteria: • Macroscopic examination of the specimen • Preparation of Gram-stained smears for microscopic examination • Inoculation of appropriately plated and tubed media, including media specifically designed for culturing anaerobes • Anaerobic incubation of inoculated media Macroscopic Examination of Specimens Each specimen received in the anaerobic bacteriology section should be examined macroscopically and pertinent observations recorded. Available at: http:// www.cdc.gov/salmonella/agona. Direct transmission from person to person has been reported in institutions. The heat-stable O antigen of salmonellae, as is the case with other enteric bacteria, is the lipopolysaccharide located in the outer membrane of the cell wall. The microbiologist inserts his or her hands into the gloves and manipulates specimens, plates, and tubes inside the chamber. Enterobius vermicularis. Enterobius vermicularis, often called the pinworm, is a worldwide parasite commonly detected in children, especially those 5 to 10 years old. Examination of these blood smears remains the classic method of diagnosing malaria. The lack of phagocytic cells leaves the host susceptible to overwhelming infection. These usually resolve within a few weeks, although malaise can be prolonged in some cases. Available at: . tahir99 VRG spp. The smooth ER does not have ribosomes on the outer surface of its membrane—hence the smooth appearance. Absence of Cell Wall. Prokaryotes that belong to the Mycoplasma and Ureaplasma genera are unique in that they lack a cell wall and contain sterols in their cell membranes. Previous editions copyrighted 2011, 2007, 2000, 1995 Library of Congress Cataloging-in-Publication Data Textbook of diagnostic microbiology / [edited by] Connie R. Relapsing fever is best prevented by control of exposure to the arthropod vectors. Determine the appropriate transport and culture media required for isolation of Bordetella spp. spiritovorum and S. pertussis and B. albicans or C. Guarro J, de Hoog GS: Bipolaris, Exophiala, Scedosporium, Sporothrix, and other melanized fungi. C, Non–lactose-fermenting E. Decolorize the slide with acetone or absolute alcohol or a mixture of the two decolorizers, and wash immediately with water. are a problem to those with sensitivities to molds. Eggs that are deposited in warm, moist soil become infective within about 2 weeks. The Rickettsia spp. Most strains of Trichosporon spp. This specimen is allowed to air-dry thoroughly before staining. Table 2-7 summarizes the defenses used by the human host against infection. odorifera biogroup 2 is negative for sucrose, raffinose, and ornithine and has been isolated from blood and CSF. dispar, and E. conorii, and R. The following types of disinfectants are suitable for cleaning work areas, but some have limitations on their use. perfringens enterotoxin linked to sporulation. Microsporum canis Macroconidia from Microsporum canis are spindle-shaped, with echinulate, thick walls; they measure 12 to 25 µm × 35 to 110 µm and have 3 to 15 cells (Figure 27-14). Calibration requires two parts: the stage micrometer, a 0.1-mm line that is ruled in 0.01-mm tahir99 VRG 632 PART II Laboratory Identification of Significant Isolates units, and the ocular micrometer, which is ruled in 100 units but has no value assigned to the units. It is an uncommon part of the normal bacterial microbiota and is isolated from fewer than 12% of normal stool specimens. It is not unusual for an outbreak to end before all data are collected and analyzed. Both species can grow at 4 °C and have been linked to transfusion-associated septicemia. In this strategy, automated treponemal test–positive sera is tested with nontreponemal and a second treponemal assay. dysenteriae type 1 and S. They actively divide and eventually lyse the host cells, spilling the organisms into the lumen of the gut. brucei rhodesiense. The paired adult flukes use the portal system to reach veins of the intestine or bladder. (Courtesy Fred Murphy, Centers for Disease Control and Prevention, Atlanta, GA.) transplant recipients. Other groups may expect reports as well, such as committees, persons managing specific programs, and the news media. Viral agents are not routinely cultured, nor would we expect viral causes to extend for this long of a time period. The family Leptospiraceae contains the genus Leptospira, and the family Spirochaetaceae contains Borrelia and Treponema. Hair, skin, or nails submitted for dermatophyte culture are generally contaminated with bacteria, rapidly growing fungi, or both. Unstained cells are not visible by bright field microscopy but are visible by dark field, phase contrast, and immunofluorescent microscopy. "True" yeasts do not form hyphae or mycelia. Direct Microscopic Examination In contrast to gram-positive bacteria, in which microscopic morphology may help provide a presumptive identification, the microscopic characteristics of enterics are indistinguishable from other gram-negative bacilli. fowleri is the causative agent of primary amebic meningoencephalitis (PAM), a rapidly fatal condition involving the CNS. Trichophyton mentagrophytes 3. The symptoms typically persist for at least 2 or more weeks. True germ tubes lack constriction at their bases, where they attach to the mother cell. Because of its small size, it may resemble fecal debris. The capsid and genome associate to form the nucleocapsid. Available at: http:// www.phil.cdc.gov/phil. Life Cycle. Transmission of the organism to humans occurs when the insect vector defecates in the area surrounding its bite site. It is also used as a counterstain in acid-fast staining procedures. Bacteria are prokaryotic, lacking these structures. This alteration can be the cause of a severe necrotizing enterocolitis (C. Other fungal agents are capable of causing systemic disease and include Aspergillus, Fusarium, and Bipolaris spp. Based on carbohydrate utilization, what is the difference between nonfermentative and fermentative organisms? shigelloides in their databases and appear to be able to identify it with a fairly high degree of accuracy. • What is their incubation period? The most transient of carrier states is the inoculation of a person's hands or fingers with an organism (e.g., with Staphylococcus aureus that has colonized the person's anterior nares) that is carried only until the hands are washed. Coeyne T, et al: Advenella incenata gen. Once ingested, the bacteria colonize the small intestine, in which they multiply and produce choleragen. Define the terms bacteriophage, lytic phage, lysogeny, and temperate phage. brasiliensis has a rather narrow range of temperature tolerance, as evidenced by its predilection for growth in cooler areas of the body (nasal and oropharyngeal), dissemination to other organs, particularly the adrenals, occurs with diminished host defenses. Schriefer ME, Petersen JM: Yersinia. Adults live in the biliary passages and gallbladder and rarely cause overt symptoms because infections are light. Available at: mmwr/preview/mmwrhtml/mm5312a4.htm. difficile has largely been replaced by assays designed to detect the toxins in feces produced by the organism. Susceptibility testing of most NTM is not performed routinely. Protothecae spp. ictaluri causes enteric septicemia in fish. pneumophila. This is the typical reaction by Trichophyton mentagrophytes, whereas Trichophyton rubrum causes only surface erosion of hair shaft. Echinococcosis. Echinococcosis (hydatid cyst disease) is an infection by Echinococcus granulosus that normally involves the dog or other member of the family Canidae as the definitive host. Attempts to culture C. When host conditions are altered, however, this isolate is capable of causing disease in almost any site. Urease Test. Another test used to help differentiate T. The larvae reach the liver by migrating through the intestinal wall and peritoneal cavity. The purplish hue produced by some species on this medium may resemble that of a lactose-fermenting bacterium (Figure 21-9). (Nomarski optics, ×450.) FIGURE 27-35 Rhizopus sp. Bengis RG, et al: The role of wildlife in emerging and re-emerging zoonoses, Rev Sci Tech 23:497, 2004. Microscopic evaluation reveals short conidiophores bearing conidia in chains that lengthen in an acropetal fashion (Figure 27-46). The long-term, low-level blood loss will cause this type of anemia, characteristic of iron deficiency anemia, in individuals who are malnourished or lack adequate dietary iron. Human Campylobacter spp. Discuss the methods used to process clinical specimens for mycobacterial culture and the reasons why specimens need to be decontaminated and digested before culture. capsulatum, particularly in infections seen in immunodeficient hosts. or microfilariae, but these methods are rarely used in the clinical laboratory. In clusters In chains In pairs In tetrads BACILLI Coccobacilli Bacilli of various sizes Fusiform bacilli Palisading SPIROCHETES FIGURE 1-5 Diagram of the microscopic shapes and arrangements of bacteria. albicans, C. A reactive tuberculin skin test indicates past exposure to M. Lastly, this chapter describes factors that may make the host more susceptible to microbial infections and how microbes are transmitted and acquired. algae, though infrequent isolates and rarely pathogenic, have been recovered from various human specimens, including abscesses and traumatic ulcers, otitis media, ocular infections, osteomyelitis, peritonitis, and septicemia, but they are usually present in mixed culture. (Nomarski optics, ×650.) tahir99 VRG 610 PART II Laboratory Identification of Significant Isolates FIGURE 27-43 Penicillium sp. mimicus − + − − V V. In contrast to the mild symptoms in an immunocompetent host, patients with a drug-induced immunocompromised state (corticosteroids), lymphoma, malignancy, or other condition that causes T-cell depletion can develop severe infections, referred to as disseminated strongyloidiasis or hyperinfection. tularensis is a highly infectious agent with 50 organisms causing an infection through the cutaneous (ulceroglandular form) or inhalational (pneumonia) routes and has been the cause of many laboratory-acquired infections. H1N1 was found to contain RNA from bird, human, and swine strains of virus. Sputum and Other Respiratory Secretions Although a variety of clinical specimens may be submitted to the laboratory to recover MTB and NTM, respiratory secretions such as sputum and bronchial aspirates are the most common. Eggs may be found in the feces if they have been coughed up and subsequently swallowed. In conventional agar cultures, recovery from smear-positive TB specimens may require 16 days, and from smear-negative specimens, 26 days. Yeasts reproduce by budding or fission. This competition lessens the chance that the pathogen will colonize the host. More than 20% of the U.S. population shows serologic evidence of infection, but in parts of Europe it may be as high as 80%. The larva penetrates the human skin through a hair follicle or break in the skin or through unbroken skin. pauculus in an outpatient clinic in Columbus, Ohio, recently. c. Accessed November 12, 2012. xenopi grows best at 42° C. It grows best at 37° C, with slower growth at 40° C. To do a reverse number lookup, choose a site that offers the service, such as WhitePages, navigate to the phone lookup section and enter the number. In general, non–spore-forming, gram-positive bacilli are difficult to identify because the preformed enzyme system databases often do not contain many of the less commonly isolated species. For better visualization, carbolfuchsin is recommended as a counterstain; if safranin is used, counterstaining should be extended to 2 to 3 minutes. Although almost any tissue or body fluid can be submitted for fungal culture, the most common specimens are respiratory secretions, hair, skin, nails, tissue, blood, bone marrow, and CSF. They contain a number of nitrophenyl and naphthylamide compounds, colorless substances that produce yellow or red products, respectively, in the presence of appropriate enzymes. Case in Point A 52-year-old man was taken to the emergency department in a disoriented and unresponsive state, experiencing shortness of breath. Explain the three ways in which genetic material may be transferred from one bacterium to another. Smaller amounts of molecules, such as phosphate for nucleic acids and phospholipids of cell membranes and sulfur for protein synthesis, make up an additional 4% of the weight. Cedecea The genus Cedecea is composed of five species: C. Isolates are motile by means of polar tufts of flagella. If amebic meningoencephalitis caused by Naegleria fowleri is suspected, the CSF can be cultured. The initial contact of the PMN with an invading organism may be random. Learning Assessment Questions 1. Sheldon GF: Boyd's introduction to the study of disease, ed 11, Philadelphia, 1992, Lea & Febiger. The infection rarely spreads beyond the sinuses in immunocompetent hosts but can be found systemically in those suffering from immune suppression. The organism may also be transmitted congenitally because of a transient parasitemia in pregnant women who have a primary infection. tahir99 VRG CHAPTER 25 Mycoplasma and Ureaplasma Donald C. An example of this in the clinical laboratory is C. If the intestinal mucosa is involved, a stool specimen is most appropriate. The World Health Organization (WHO) and the Pan American Health Organization recommend that E. Hydrogen peroxide can be toxic to cells but not to the degree of the superoxide anion or hydroxyl radical. Cross-reactions with nonspecific antibodies can occur, which makes interpretation of results difficult. Case in Point gram-negative rod that produced pink colonies on MacConkey agar and green colonies on thiosulfate citrate bile salt sucrose agar and required 3% to 6% NaCl for growth. DNA probes have been 28-6 Comparisons of Malarial Species Plasmodium Species Red Blood Cell Morphology P. Which of the following test results is most helpful in categorizing an isolate as a member of the tribe Proteeae? Johnston VJ, Mabey DC: Global epidemiology and control of Trichomonas vaginalis, Curr Opin Infect Dis 21:56, 2008. The trypomastigotes circulate in the blood stream and eventually enter a cell, where they transform into amastigotes. aureus (VISA), tetanus Brucellosis, giardiasis, hepatitis B, hepatitis C, Lyme disease, Rocky Mountain spotted fever, trichinosis Chickenpox, influenza Diseases of significant public health concern— reported by the end of the work week Diseases reported only by number of cases— reported by the end of the work week Report of outbreak, unusual incidence, or epidemic— reported by the end of next working day Blastomycosis, histoplasmosis, scabies, staphylococcal skin infections, toxoplasmosis *These are reporting regulations from the Ohio Administrative Code and may vary from state to state. This damage alters intestinal permeability and can result in decreased uptake of fluids, electrolytes, and nutrients. Treatment. Treatment is administered only for E. Agents such as the West Nile virus, the coronavirus associated with SARS, and the avian influenza virus are three examples of virus-caused emerging diseases. tahir99 VRG CHAPTER 7 Microscopic Examination of Materials from Infected Sites Thin fluid can be prepared by drawing up a small quantity of the fluid or a resuspended sediment of the fluid into a pipette and depositing it as a drop of fluid onto a clearly marked area on the slide (Figure 7-4). Bacteria are blue. psittaci are reported annually. davisae is the most commonly isolated species. Tests for antibody, however, are not particularly useful in distinguishing between past and current infection, because antibodies can persist for years after an infection has resolved. More than one organism should be found if possible. Extensive biomedical research has focused on the potential applications of nanotechnology to medicine—nanomedicine. Cases of rhinoscleroma have been reported in Africa and South America. Symptoms generally occur suddenly and include vomiting, diarrhea, fever and, in many cases, abdominal pain and respiratory symptoms. Fitzgerald C, Nachamkin I: Campylobacter and Arcobacter. Multiple genomes have been identified, and many are found in animals. (Courtesy Charles D. fibroblasts, human embryonal rhabdomyosarcoma (RD) cells, and A549 cells. Mitchison's selective 7H11 contains polymyxin B, amphotericin B, carbenicillin, and trimethoprim lactate. TSP liquefies sputum rapidly but requires a long exposure time to decontaminate the specimen. peregrinum, and M. Most strains are nonpigmented, but occasional isolates may produce a reddish-blue or violet pigment. The HPVs cannot be grown in cell cultures; therefore, laboratory diagnosis of HPV infection often involves cytology sections. The minimum level of respiratory protection is a respirator that contains a National Institute for Occupational Safety and Health–certified N series filter with a 95% efficiency rating (N-95). It is effective against all asexual stages of malarial organisms and all gametocytes, except those of P. cepacia and will be susceptible to aminoglycosides, carbapenems, ciprofloxacin, and SXT, but resistant to aztreonam and the cephalosporins. Reactive or weakly reactive sera should undergo titration and be tested with treponemal tests. a. Antisera reacting against cell wall antigens are commercially available for a number of Candida spp. Rabies is sometimes diagnosed by detecting Negri bodies, which are eosinophilic cytoplasmic inclusions in neurons. Figure 27-10 shows the different layers of tissues in which fungal infections can occur. alvei and H. On physical examination, the patient was observed having a paroxysm of cough with an inspiratory whoop. szulgai S Smooth few 22-37 Rough 22-37 Smooth M. Smith, Baltimore, MD; D, courtesy of Clinical Microbiology Audiovisual Study Units, Health and Education Resources, Inc., Bethesda, MD; E, courtesy of Dr. John E. stutzeri Shewanella putrefaciens H2S-Positive Shewanella putrefaciens Courtesy Anne Morrissey (Cleveland, OH), George Manuselis, and Connie Mahon. Lesions may develop at the site of inoculation or may occur as a result of hematogenous dissemination from the lungs. The modified acid-fast stain has been used to detect C. The organism is 5 to 18 µm in diameter, has four anterior flagella, and an undulating membrane that extends half the length of the body. During this stage, patients often cough so hard they vomit, have difficulty breathing, and may be unable to feed. Before puberty and in postmenopausal women, vaginal flora primarily consists of yeasts, gram-negative bacilli, and gram-positive cocci. longbeachae groups 1 and 2, L. Other water-soluble pigments (e.g., pyorubin [red] and pyomelanin [brown or black]), are occasionally produced by strains of P. Dissemination can occasionally occur; in such cases, antimicrobial therapy is required. The main effect of IS elements in bacteria is that when an IS element inserts itself into the middle of a gene, it disrupts and inactivates the gene. The granular form is similar but possesses granules in the central vacuole and cytoplasm. Dry the material on the slide so that it does not wash off during the staining procedure. They are obligate parasites, and antibiotics are ineffective for treatment of viral infections. Balada-Llasat JM, et al: Pseudo-outbreak of Cupriavidus pauculus infection at an outpatient clinic related to rinsing culturette swabs in tap water, J Clin Microbiol 48:2645, 2010. Enveloped viruses may enter the cell by fusion with the cell membrane, and a third method of penetration is endocytosis, whereby the enveloped virus enters the cell in a cytoplasmic vacuole. Brenner DJ, Farmer JJ III: Enterobacteriaceae. The preparation should be thin enough so that newsprint can be read through it and should not overflow beyond the edges of the coverslip. 711 No vaccines are available, and infection control measures similar to those for RSV are used to prevent spread in health care facilities. Yellow fever, caused by the yellow fever virus, is also considered an emerging infection. Examination of Prepared Material A limited number of microbial pathogens from commonly sampled infected sites are regularly encountered by the technologist or microbiologist. coli) is a bacterium, but Entamoeba coli (Ent. The enlarged areas eventually develop a hard, leathery appearance. The parasite remains compact, has golden brown pigment, and has a range of 6 to 12 merozoites in the mature schizont. Serovars A, B, Ba, and C are associated with the severe eye infection trachoma, whereas serovars D through K, Da, Ia, and Ja are associated with inclusion conjunctivitis, a milder eye infection, and urogenital infections. HFMD is generally a disease that occurs in young children. Establishing a case definition, forming and testing a hypothesis, and communicating findings c. In contrast to both Treponema and Borrelia organisms, the spirals FIGURE 23-1 Dark field image of Leptospira interrogans serotype Sejroe Wolffi 3705. Optimal recovery occurs if fresh specimens are inoculated directly into laboratory media. • Anti-EA/D (antibody to early antigen, diffuse): Antibodies to EA/D appear in the acute phase, and their presence indicates current or recent infection. As many as 67% of the pediatric patients infected with E. The distal end of the conidium is broad or spatulate and reminiscent of a beaver's tail. Most positive results are well above the cutoff value of 10% hybridization. Larval characteristics include the presence or absence of a sheath and the location and arrangement of nuclei in the tail of the microfilariae. Wash smears in running tap water. Mahon* CHAPTER OUTLINE Culture Serologic Diagnosis ■ ANTIMICROBIAL SUSCEPTIBILITY ■ INTERPRETATION OF LABORATORY RESULTS ■ GENERAL CHARACTERISTICS ■ CLINICAL INFECTIONS Mycoplasma pneumoniae Mycoplasma hominis and Ureaplasma Species Other Mycoplasma Species ■ LABORATORY DIAGNOSIS Specimen Collection and Transport Direct Examination OBJECTIVES After reading and studying this chapter, you should be able to: 1. Microorganisms that find their niche colonize various anatomic sites and become the predominant organisms. dysenteriae d. Infection of deep tissue by these fungi is rare, but occasionally extensive inflammation and nail bed involvement or disseminated disease may result. Epstein-Barr Virus Epstein-Barr virus, in the genus Lymphocryptovirus, it causes infectious mononucleosis (Figure 29-8). There are up to four nuclei, and the cytoplasm is often pulled away from the cyst wall. Other anaerobic jars or containers are available from other companies. Another form of the disease, chiclero ulcer—often associated with L. coli O157:H7, when present, produces heavy growth. Patients develop mental status changes, ranging from confusion and agitation to depression and drowsiness. infection. have only two stages, the resistant cyst and the motile trophozoite. trachomatis. Chloroquine-resistant P. Expired fresh-frozen plasma from the blood bank is useful in this test and can be stored at 4° C almost indefinitely. Enzyme immunoassays for IgG or IgA antibody to FHA from a single serum specimen or from nasopharyngeal aspirates have been developed but have not gained widespread acceptance. Molecular methods of detection exist for speciating a number of organisms, including malarial parasites. The brittle, infected hair shafts break off at the scalp, leaving tahir99 VRG CHAPTER 27 Medically Significant Fungi the black dot stubs. penneri can also swarm on nonselective media. The laboratory may be asked to review microbiology data over a previous period of time to determine whether unrecognized cases have occurred. morganii nor M. Hairs are placed directly into a sterile Petri dish. Several serologic assays have been described for detecting antibodies in acute and chronic cases. Some cases of ringworm are subclinical, exhibiting only a dry, scaly lesion without inflammation. diminuta are tahir99 VRG CHAPTER 21 Nonfermenting and Miscellaneous Gram-Negative Bacilli A 489 B C FIGURE 21-12 Flagella stains of gram-negative bacilli. asiaticum M. micdadei is weakly acid-fast in tissue and stains best with the modified Kinyoun procedure (see Chapter 7). The spectrum of illness caused by Mycobacterium spp. If a delay in transport is expected, tissue may be transported in pouches containing an oxygen-free atmosphere. V. Salmonella serotypes Typhi and Paratyphi have no known animal reservoirs, tahir99 VRG CHAPTER 19 Enterobacteriaceae and infections seem to occur only in humans. The number of plasmids present in a bacterial cell may range from one (low copy number) to hundreds (high copy number). The organisms are capable of infecting a wide variety of human organs, including eyes, muscles, liver, kidneys, and the CNS. More significance is often given to isolates of nonfermenters when they are isolated from sterile sites or isolated in large quantities from many different samples. Unless the RBCs on the slide are first lysed in distilled water, Wright stain cannot be used for a thick film because the stain contains methanol, which will fix RBCs. A thick film is best for the detection of parasites (high sensitivity) because of the larger volume of blood and the fact that organisms are concentrated in a relatively small area. Once the organism is confirmed as clinically significant and not a contaminant, treatment is usually penicillin plus an aminoglycoside. material. Bacteroides ureolyticus, Bilophila wadsworthia, or a Fusobacterium sp.; Veillonella Vancomycin* From Mangels JI: Anaerobic bacteriology. jejuni from the other Campylobacter spp.? Growth of the organism can be observed through the pouch within 3 days. Bacteria that grow on humans vary in their atmospheric requirements for growth. *Typical strains in serogroups A through E. Primaquine phosphate, which is effective against hypnozoites that persist in the liver, is used to treat individuals infected with P. Samples that can be collected with viral transport media are respiratory, swab, and tissue samples. In Brenner DJ, et al, editors: Bergey's manual of systematic 473 bacteriology, ed 2, vol 2: the proteobacteria part B, New York, 2005, Springer, p. TABLE Continuous cell cultures are capable of infinite passage and are heteroploid, meaning that they have an abnormal and variable number of chromosomes that is not a multiple of the normal haploid number. This is the pathway used by yeasts when they ferment glucose to produce ethanol. Fungal plates and tubes should be opened only in a biological safety cabinet. These antibody molecules circulate in the bloodstream and lymphatics, bathe body tissues, and bind to infectious agents or substances to aid the host in eliminating them from the body. tahir99 VRG 700 TABLE PB PART II Laboratory Identification of Significant Isolates 29-4 Interpretation of Epstein-Barr Virus Serologic Markers Anti–VCA-IgM Anti–VCA-IgG Anti–EA-IgG Anti-EBNA − + ± − − + + + − ± ± − − − + + − + ± −− Interpretation No previous exposure to Epstein-Barr virus Acute infectious mononucleosis Recent infection Past infection Anti–EA-IgG, Antibody to early antigen; anti-EBNA, antibodies to the nuclear antigen; anti–VCA-IgG, IgG antibodies against the viral capsid antigen; anti–VCA-IgM, IgM antibodies against the viral capsid antigen; PB, Paul-Bunnell antibody; −, negative; +, positive; ± positive or negative. The Svedberg unit is named for Theodor Svedberg, Nobel Prize winner and inventor of the ultracentrifuge. The counterstain must not be left in place too long. In the United States, the most common flavivirus infection is St. Louis encephalitis (SLE). Currently, it is not recommended for sensitivity testing of NTM. The infection control program or managers of the specific area of concern must be familiar with regulations and guidelines addressing water cultures. Keen III, PhD, SM(ASCP) Major, Medical Service Corps, United States Army Chief, Microbiology Department of Pathology and Area Laboratory Services William Beaumont Army Medical Center El Paso, Texas Arun Kumar, PhD Assistant Professor of Medical Laboratory Sciences University of Delaware Newark, Delaware Donald C. The typical branching, filamentous, Gram-stained appearance of an Actinomyces sp., depicted in Figure 22-2, is referred to as Actinomyces-like. Karadag N, et al: Spectrum of Brevundimonas vesicularis infections in neonatal period: a case series at a tertiary referral center, Infection 40:509, 2012. Characteristic clinical features include progressive, nonhealing ulcers and the presence of draining sinus tracts. The capsule is usually composed of polysaccharides but can also be made of proteins or a combination of protein and carbohydrate. As previously mentioned, K. Brushings appear to be more commonly diagnostic than washing or biopsy, possibly because of an inhibitory effect on the mycobacteria by lidocaine Specimens for Processing Respiratory Specimens Spontaneously expectorated sputum Normal saline–nebulized, induced sputum Transtracheal aspirate Bronchoalveolar lavage Bronchoalveolar brushing Laryngeal swab Nasopharyngeal swab Body Fluids Pleural fluid Pericardial fluid Joint aspirate Gastric aspirate Peritoneal fluid Cerebrospinal fluid Stool Urine Pus Body Tissues Blood Bone marrow biopsy/aspirate Solid organ Lymph node Bone Skin used in adults during bronchoscopy or of dilution of the specimen with saline. Organisms are placed within this group when no mode of sexual reproduction has been identified. Notices Knowledge and best practice in this field are constantly changing. It inhibits cell proliferation and tumor growth and enhances phagocytosis by macrophages, the activity of natural killer cells, and the generation of cytotoxic T cells. capsulatum antigen can be detected from a variety of clinical specimens, including blood, CSF, and urine. tahir99 VRG CHAPTER 2 Host-Parasite Interaction TABLE 39 2-7 Summary of Defenses of the Human or Animal Host to Infection and Evasion Mechanisms Attributed to Various Microorganisms Host Defense Mechanism of Evasion Example Hydrodynamic flow Attachment Mucus barrier Attachment Penetration Systems of high-affinity uptake Resistance to lysis Absent or low immunogenicity Antigenic heterogeneity Masking of antigens Destruction Penetration Fimbriae, surface proteins, lipoteichoic acid, pseudomembrane of diphtheria Mannose-sensitive fimbriae Deprivation of essential nutrients Lysozyme in secretions Surface immunoglobulins Unbroken surface (epithelial cell surface) Unknown defenses in lymphatics (intercellular space) Serum defenses Recognition by antibody Complement system Localization Fibrin tapping Abscess formation Secondary immune response Phagocytosis Iron metabolism Substitution of peptidoglycan Hyaluronic acid, capsules Fimbriae, capsules, LPS, M protein Capsules, IgA-binding proteins IgA protease Neisseria gonorrhoeae, Shigella spp. pneumoniae and its orientation of attachment. In addition, the nonpathogens Mansonella ozzardi, Mansonella (Dipetalonema) perstans, and Mansonella (Dipetalonema) streptocerca may be seen in clinical specimens. • Sodium hypochlorite is effective at a concentration of 0.1% to 0.5% (e.g., a 1 : 50 to 1 : 10 dilution of most household bleaches). Available at: . The Clinical and Laboratory Standards Institute (CLSI) has interpretive guidelines only for V. Cell viability is maintained by periodically removing cells from the surface, diluting them, and placing them into a new container. baumanii) include UTIs, pneumonia, tracheobronchitis, or both, endocarditis, with up to a 22% mortality, septicemia, meningitis, often as a complication of intrathecal chemotherapy for cancer, and cellulitis, usually as a result of contaminated indwelling catheters, trauma, burns, or introduction of a foreign body. Lesions of chromoblastomycosis frequently appear as verrucous nodules that may become ulcerated and crusted. Methylene blue remains white in the absence of oxygen (reduced) and turns blue in the presence of oxygen; resazurin goes from colorless in the absence of oxygen to pink in the presence of oxygen. However, some aerotolerant anaerobes (e.g., certain Clostridium, Actinomyces, Propionibacterium, and Lactobacillus spp.) can grow on the CO2-incubated chocolate agar, but they usually grow much better on the anaerobically incubated plate. Table 21-1 lists the present taxonomy of many nonfermenters. The incubation period of leptospirosis is usually 10 to 12 days but ranges from 3 to 30 days after inoculation. It is a small intracellular stage found in macrophages or histiocytes around the periphery of the skin lesions (L. Langley JM, et al: Azithromycin is as effective and better tolerated than erythromycin estolate for the treatment of pertussis, Pediatrics 114:e96, 2004. Laboratory Diagnosis. Direct examination of tissue might reveal S. These two pseudomonads are rarely isolated from humans but have been isolated from wounds, abscesses, blood cultures, peritoneal and chronic ambulatory peritoneal dialysis (CAPD) fluids, and other sources. pneumoniae is a frequent cause of lower respiratory tract infections among hospitalized patients and in immunocompromised hosts such as newborns, elderly patients, and seriously ill patients on respirators. The organisms enter the circulation and break out of the capillaries into the lung and then migrate up the bronchial tree, over the epiglottis, and into the digestive tract. psittaci in culture, although diagnostic, is difficult, dangerous, and not routinely used or recommended. These media contain one or more carbohydrates, such as lactose and sucrose, which show the ability of the species to ferment specific carbohydrates. Infants suffering from chlamydial infection can experience conjunctivitis, nasopharyngeal infections, and pneumonia. They have been found in respiratory, urine, and blood cultures. The incidence of syphilis in the United States dropped through the 1990s, and the fewest cases since reporting began in 1941 (31,618) was reached in 2000. It is possible to detect invasiveness using monolayer cell cultures with human epithelial–2 (HEp-2) cells. Specimen processing and plating must be performed in a properly maintained and operating safety cabinet. In addition, C. belli will autofluoresce a bluish color at 365 nm and a bright green at 405 nm. Anaerobic jar systems use an envelope gas generator. Murphy, Centers for Disease Control and Prevention, Atlanta, GA.) and Uganda (2007). An inexperienced observer might misinterpret this as mixed infection rather than as the simple presence of dead pneumococci in a classic infection. This human infection apparently started as a coronavirus that jumped from its normal animal host, possibly a civet cat, into a human. Acid-fast bacteria appear yellow or orange under a fluorescent microscope, making them easier to find. coli may cause several different GI syndromes (Table 19-4). Box 2-3 lists common microorganisms encountered in the nose and nasopharynx. sonnei is unique in its ability to decarboxylate ornithine. In addition, K. In the lytic cycle, replication of the bacterial chromosome is disrupted; phage components are formed and assembled into phage particles. Didier ES, Weiss LM: Microsporidiosis: current status, Curr Opin Infect Dis 19:485, 2006. More serious infections, such as bacteremia and meningitis, usually occur only in severely immunocompromised patients or neonates. When contamination can be controlled with a lower concentration of NaOH, the recovery of mycobacteria is indirectly improved using the milder procedure because fewer mycobacteria are lost in the process. These use an antigen capture immunochromatographic principle to detect soluble proteins from malarial organisms in the blood. Borrelia spp. Probably a Prevotella sp. Most fungi are multicellular, and many can reproduce sexually and asexually. cloacae. The fever does not exhibit the cyclic nature seen in malaria. PAS attaches to polysaccharides in the fungal wall and stains fungi pink. With the GasPak EZ Gas Generating Container System (BD Diagnostic Systems, Sparks, MD), specimen plates along with a sachet are placed into a clear plastic incubation container, sealed, and incubated at the appropriate temperature. An abundance of nonmycobacterial organisms, and possible mycobacteria, make up the microbiota of these specimens. Kaur J, Jain S: Role of antigens and virulence factors of Salmonella enterica serovar Typhi in its pathogenesis, Microbiol Res 167:199, 2012. Other isolates can be identified as "biochemically compatible with Salmonella" and submitted to a reference laboratory for further testing. These tests have relatively high sensitivity when compared with examination of a 645 FIGURE 28-20 Chilomastix mesnili cyst, identified by arrow (trichrome stain, ×1000). Enzyme Immunoassays Problems with developing EIA methods for the detection of antibody to intestinal parasites include difficulty in obtaining parasite antigen appropriate for detection, cross-reactivity of antibodies, and poor sensitivity and specificity of tests. parahaemolyticus is not as well understood as V. Both species have been isolated from urine, wounds, and ear and bacteremic infections. The identification of Legionella from patient specimens is possible for most microbiology laboratories using commercial media and other diagnostic methods. Either of the above d. Matin A, et al: Increasing importance of Balamuthia mandrillaris, Clin Microbiol Rev 21:435, 2008. If the enzyme is present, MUG is cleaved, and a fluorescent product is formed. enterocolitica characterized by tender, red nodules that may be accompanied by itching and burning. bifermentans, C. A 52-year-old man who had recently received a kidney transplant was admitted to the hospital with a low-grade fever, a heart murmur, and neutropenia. Both preparations can be made on the same slide or on separate slides. violaceum bacteremia were young age, presence of localized

serologic evaluation. All species resemble E. trematum. Less Commonly Encountered Nonfermentative, Gram-Negative Bacilli Alcaligenes and Achromobacter The family Alcaligenaceae includes the clinically relevant genera of Alcaligenes, Achromobacter, Bordetella, Advenella, and Kertersia. The region most severely affected by HIV-AIDS is sub-Saharan Africa, which has approximately 22.9 million HIV-infected patients, accounting for 67% of the total worldwide. Aeromonas b. • Identify the cellular components and debris of inflammation and establish the probability of infection. The nature and origin of these cells are described in the following paragraph. Because other toxins present in diarrhea stools can produce similar cytopathic effects, this test must be verified with specific antitoxins to Stx1 and Stx2. neoformans will not. Although the Gram stain performed in the routine microbiology laboratory often gives the first evidence of infections with bacteria and yeast, other direct stains give more specific information about mold infections. They usually cause diarrhea and vomiting in infants, young children, and older patients. The adult filarial worm lives in the lymphatics and lymph nodes, especially those in the lower extremities. Eukaryotes differ from prokaryotes in that they have membraneenclosed nuclei and organelles. Screening tests for blood donors in the United States have been implemented. Gram-positive anaerobes can be further divided between those organisms capable of forming endospores (spore formers) and those unable to form spores (non–spore formers). ? Untreated hyperinfestations are associated with death of the patient. The HACEK group is an important cause of endocarditis, specifically related to poor oral hygiene or dental procedures. fluvialis primarily by production of gas in D-glucose. Describe the pathogenesis and clinical manifestations of Borrelia spp. In severe cases, the patient can develop disseminated intravascular coagulation. The plates can be sealed with tape or parafilm or sealed in semipermeable bags to minimize dehydration and prevent the spread of fungal spores. This is not the same formulation of MacConkey agar typically used for the isolation of enteric bacilli. Growth Curve If bacteria are in a balanced growth state, with enough nutrients and no toxic products present, the increase in bacterial numbers is proportional to the increase in other bacterial properties, such as mass, protein content, and nucleic acid content. This is especially true in cases of pediatric diarrhea or any type of immunocompromised individual. 629 Other Specimens Examined for Intestinal and Urogenital Parasites Cellophane Tape Preparation for Pinworm The life cycle of the pinworm Enterobius vermicularis includes migration of the female from the anus at night to lay eggs in the perianal area. Outside these populations, morbidity and mortality rates remain low, and consideration needs to be given to the possibility of contamination rather than infection when it is isolated in a non-CF or non-CGD individual. They may take place in homes, aboard a plane or ship, in a city or state, or in a foreign country. Extraintestinal Infection Because of the organism’s wide dissemination among animal populations and aquatic environments, it is apparent that occupational exposure can be a source of infections for veterinarians, zookeepers, aquaculturists, fish handlers, and athletes participating in water-related sports. Abe Baalness.) FIGURE 19-14 Salmonella growing on CHROMagar Salmonella differential agar. Subsequent epidemic outbreaks can arise from the respiratory transmission of the organisms. For the remainder of the nonfermenters, for which there remains great variability depending on the system used, decisions need to be made about whether a full identification is needed based on the clinical situation and relevance of the isolate. It is about 58 to 76 µm × 40 to 50 µm and has a small, knoblike protuberance at the end opposite the operculum. floccosum are yellow to yellow-tan, flat with feathered edges, and remain small in diameter. Some organisms produce soluble substances, such as proteases and hyaluronidases that liquefy the hyaluronic acid of the connective tissue matrix, helping to spread bacteria in tissues, promoting the dissemination of infection. Paecilomyces. Paecilomyces lilacinus is extremely refractory to treatment and was recovered in a hospital outbreak, with high associated mortality. Laboratory workers in the microbiology laboratory have contracted typhoid fever while working with the organisms. Automated DNA sequencing, used by some laboratories, is the most accurate method for the identification of mycobacterial isolates. Some tests are relatively nonspecies-specific and detect a protein such as parasite lactate dehydrogenase or aldolase that is common to all four human Plasmodium spp. Although biopsy specimens initially were required to identify life cycle stages, they are not routinely used for laboratory diagnosis. Each B lymphocyte has surface receptors that recognize only one type of antigen. inkin have been implicated in superficial mycoses. capsulatum, Paracoccidioides brasiliensis, Sporothrix schenckii, and Penicillium marneffei. Oocysts are infective when passed and may be ingested in contaminated water or food or passed by person-to-person contact. Sterile forceps should be used to pull affected hair. Once inside the body, it does not enter circulation but wanders through the subcutaneous tissue, creating long, winding tunnels. putrefaciens, whereas S. Urease activity c. Each proglottid, when mature, produces eggs infective for the intermediate host. endemicum and closely resembles yaws in clinical manifestations. An IgM titer greater than 1 : 32 or an IgG single titer greater than 1 : 512 may suggest C. Morganella The genus Morganella has only one species, M. Many Mycobacterium spp. Additionally, liquefying the mucin enables the mycobacteria to contact and use the nutrients of the medium to which they are subsequently inoculated. When placed onto culture media, the abundant nonmycobacterial or ganisms can quickly overgrow the more slowly growing mycobacteria. It forms brown, restricted colonies that remain sterile. The zinc sulfate method is not satisfactory, however, because all eggs except those of schistosomes are operculated. veronii biovar sobria A. Because of the risk of airborne conidia, these slide preparations must be performed in a biological safety cabinet. Therefore, tahir99 VRG 698 PART II Laboratory Identification of Significant Isolates FIGURE 29-6 Advanced cytopathic effect in an A549 cell line caused by herpes simplex virus infection. These structures form conidia, which contain all the genetic material necessary to create a new fungal colony. hartmanni to demonstrate the irregular peripheral chromatin. Fertilization of the macrogamete results in formation of the oocyst, which contains four sporozoites. Compare the macroscopic and microscopic morphology of the following saprobes: Penicillium spp., Aspergillus fumigatus, Fusarium spp., and Curvularia spp. The laboratory technologist, IPCP, and manager should maintain a close working relationship to ensure compliance with culturing requirements. are primarily recovered as laboratory contaminants. Later, a mycoplasma was isolated from humans and was referred to as a pleuropneumonia-like organism (PPLO) and the Eaton agent, after the researcher who first isolated it from humans. The anaerobe to be tested is grown in a tube of peptone–yeast extract–glucose (PYG) medium. FIGURE 19-5 Mucoid, yellow-pigmented colonies of CronoFIGURE 19-4 Yellow-pigmented Pantoea agglomerans on bacter sakazakii growing on brain–heart infusion agar. For instance, a Tzanck smear can detect Cowdry type A bodies from herpes simplex virus (HSV) and varicellazoster virus (VZV) lesions, and Papanicolaou (Pap) smears can reveal human papillomavirus (HPV)–associated koilocytes, which are squamous cells with an enlarged nucleus surrounded by a nonstaining halo. can inhabit the lungs of many mammals. The parasite protein P. Strongyloides stercoralis is also a soil-transmitted helminth but does not have the broad geographic distribution of the other organisms. The mean time to identification of one isolate is 6 minutes, with a cost estimated at 20% to 30% lower than conventional procedures that use phenotypic methods. cepacia complex, but rates up to 30% in some adult CF patients have been reported. Fluid loss has been reported to range from 3 to 6 L/day to as much as 17 L/day. The tight coils and bent ends are characteristic of this organism (×1000). +, Most strains positive; −, most strains negative; +/− or −/+, predominant reaction first. cloacae Test or Substrate Urease Motility Lysine decarboxylase Arginine dihydrolase Ornithine decarboxylase Gelatin (22° C) Adonitol, gas Inositol Acid Gas D-tartrate, Jordan’s Sodium alginate (utilization) Sign %+ +w or − + − + + (+) − or + 65 95 0 97 96 0 21.7 d − − or + − 13 4.1 30 0 E. EIEC infections seem to occur in adults and children alike. ✓ Case Check 18-2 The Case in Point at the beginning of this section describes a cluster of acute respiratory symptoms that led to hospitalization in some patients. The cells of P. Processing clinical 575 specimens or transferring viable cultures outside a safety cabinet should not be permitted. A portion of the hypha on one side of the septation grows out and connects to the hypha on the other side of the septation, thereby bypassing the septation. Lin WR, et al: Cellulitis and bacteremia caused by Bergeyella zoohelcum, J Formos Med Assoc 106:573, 2007. The standard therapy for treating H. Serum antibodies can be detected by indirect fluorescent antibody methods using ameba-coated slides. The vegetative bacteria then multiply and produce toxins. In addition, enteroviruses are a major cause of aseptic meningitis and can also be isolated from urine specimens. On the fifth day of the cruise, several members of the party visited the ship’s doctor because of a worsening cough. Judicial Commission of the International Committee on Systematics of Prokaryotes: Opinion: the type species of the genus Salmonella lignieres 1900 is Salmonella enterica (ex Kauffmann and Edwards 1952) Le Minor and Popoff 1987, with the type strain LT22, and conservation of the epithet enterica in Salmonella enterica over all earlier epithets that may be applied to this species. The SARS-CoV targets the epithelial cells of the GI tract and is transmitted person to person by direct contact, droplet, or airborne routes. Growth Rate. Growth rate and recovery time depend on the species of mycobacteria but are also influenced by the media, incubation temperature, and initial inoculum size. Potassium Nitrate Assimilation. Potassium nitrate (KNO3) assimilation provides valuable information for separating the clinically significant yeasts. ✓ Case Check 19-2 The Case in Point illustrates typical predisposing risk factors for increased colonization of gram-negative bacilli in the respiratory tract of hospitalized patients. The eggs are identical and are reported as “hookworm eggs.” Eggs of S. Acid-Fast Cell Wall. Certain genera, such as Mycobacterium and Nocardia, have a gram-positive cell wall structure but also contain a waxy layer of glycolipids and fatty acids (mycolic acid) bound to the exterior of the cell wall. The oocysts are infective at a low dose (as few as 10 oocysts) and are resistant to common chlorine- and ammonia-based disinfectants. The bodies of multicellular fungi are composed of filaments called hyphae, which interweave to form mats called mycelia. The blood cultures were also negative. This isolate was identified incorrectly as D acidovorans with the Vitek-2 card; however, it was identified with 16S rRNA sequencing. However, at this time, EIAs are not sensitive enough for diagnosing individuals suspected of being infected. Thus, IgG antibodies are said to be bivalent—capable of binding two antigen molecules. grisea, and Exophiala sp. parahaemolyticus can also be serotyped by means of its O and K (capsule) antigens, but this is generally done only during epidemiologic studies conducted by reference laboratories. Stark D, et al: Dientamoeba fragilis as a cause of travelers’ diarrhea: report of seven cases, J Travel Med 14:72, 2007. Lipases hydrolyze triglycerides and diglycerides to fatty acids and glycerol. The organisms might be easily misidentified because of their similarity to shigellae. agglomerans complex, it includes members that are lysine-, ornithine-, and arginine-negative or “triple decarboxylasesnegative.” More than 13 hybridization groups (HGs) have been described in this complex. The organism does not stain with traditional trichrome or iron hematoxylin stains. It would be unacceptable, however, to batch-process improperly submitted specimens (e.g., dry swabs) or clinical specimens apt to contain rapidly growing bacteria. The catarrhal phase of the disease is followed by the paroxysmal phase. metschnikovii Group 2 − + + + + V. There have been more reports of disease attributed to this organism, including endocarditis, especially in a setting of prior intravenous (IV) drug abuse or heart surgery, wound infections, including cellulitis and ecthyma gangrenosum, bacteremia and, rarely; meningitis and UTIs. With rare exceptions, infections have occurred in a nosocomial setting. In addition, freed germinal epithelium may serve as a source of new infection. As with F. freundii endocarditis required aortic valve replacement when antimicrobial therapy failed. Most host cell receptors are glycoproteins, some of which include the immune globulin superfamily molecules (for poliovirus), acetylcholine (for rabies virus), sialic acid (for influenza virus), CD4 (for HIV), and complement receptor C3d (for Epstein-Barr virus [EBV]). However, the presence of ingested erythrocytes in the trophozoite stage will distinguish E. marcescens is the species considered most significant clinically. Prevention includes adequate cooking of water vegetation, fish, and crustaceans. Note intense peripheral staining of the organisms. The ability of organisms to produce exotoxins and extracellular enzymes is another major factor that contributes to the virulence and invasiveness of organisms. During translation, ribosomes containing rRNA sequentially add amino acids to the growing polypeptide chain. Tissue allograft material was found to be the cause of infection in two recipients during repair of their cruciate ligament; the allografts were thought to have become contaminated during processing of the tissue. Microscopically, the mold form produces small (2 to 10 µm in diameter), one-celled conidia, generally indistinguishable from those observed with the mold phase of B. The conventional acid-fast staining methods, Ziehl-Neelsen and Kinyoun stains, use carbolfuchsin as the primary stain, acid-alcohol as a decolorizing agent, and a methylene blue counterstain. ulcerans, and M. This delays the time to detection; it certainly is not the optimal method, but might be necessary in small laboratories lacking resources. are shown in Table 19-5. It commonly appears in childhood and includes symptoms such as a mild febrile illness, rash, and vesicular lesions. The combination of rapid growth, plaque formation, and growth on many different cell types, such as MRC-5, human fibroblasts, Vero, HEp-2, mink lung, and PMK, is presumptive evidence for the identification of HSV. Most eggs have a brown, bile-stained, mammillated outer layer (Figure 28-60). 555 salivarium orale buccale faucium lipophilum pneumoniae fermentans Large, fried egg, vesiculated peripheral zone Tiny, spherical, fried egg, granular Small, fried egg, spherical *Relative sizes: large, >100 nm; small, 50-100 nm; tiny, 45 +(94) −(99) −(99) − −(99) −(97) −(92) N = 99% −(99) −(93) N −(99) − N = 99% −(99) −(87) + − −(74) +/−(53/47) +(66) +(63) (97) p = 4% −(72) +(75) −(70) +(59) +(60) S = 38% N =58% − N +(99) −(99) −(99) −(99) −(99) −(99) −(99) −(99) +(96) +(98) +(99) −(99) −(99) N = 99% −(99) N (99) Inositol +(97) +(99) −(99) −(99) −(99) Mannitol Catalase Heat Stable (68° C) Arylsulfatase Carbon Sources Iron Uptake + (99) Growth on MacConkey +(96) Niacin −(99) +(99) −(56) −(99) −(99) −(99) −(99) +(52) −(99) −(98) +−Tan(99) +(96) − − − −(95) −(94) +(98) +(99) − −(95) −(51) −(99) +(99) −(92) −(99) Pyrazinamidase† − −(98) +(98) Growth on 5% NaCI −(99) −(99) −(99) − −(52) −(99) −(99) +(90) −(99) +(75) Growth on T2H Tellurite Reduction −(99) Var. fragilis and E. Leptospires General Characteristics Organisms of the genus Leptospira are tightly coiled, thin, flexible spirochetes, 0.1 µm wide and 5 to 15 µm long (Figure 23-1). The zinc sulfate method is the usual flotation procedure. Although transfusion-transmitted babesiosis has been reported, there is no screening test for blood donors. Table 28-8 compares the characteristics of tapeworm eggs. The disease was ultimately termed severe acute respiratory syndrome (SARS), and the causative agent was designated as the SARS-associated coronavirus (SARS-CoV). coli C. Instead, it appears as a clear area (“halo”-like) between or surrounding the stained organism and the stained amorphous background material in a direct smear from a clinical specimen. vivax may be treated with mefloquine or quinine sulfate with doxycycline. These include P. Typhus Group The typhus group of rickettsiae includes the species R. coli are β-hemolytic. However, an early end to the outbreak does not ameliorate the need for communication and a written report. Foulks GN: Acanthamoeba keratitis and contact lens wear: static or increasing problem, Eye Contact Lens 33:412, 2007. Another alternative is to use a disposable collection container that can be fitted under the toilet bowl rim. A MAC agar plate shows no growth. Other agents to which it may demonstrate in vitro susceptibility include ticarcillin-clavulanate, the fluoroquinolone levofloxacin, and tetracyclines, including tigecycline. haemophilum, M. ✓ Case Check 21-2 When a nonfermentative bacillus is isolated from a clinical specimen in the laboratory, it could represent a pathogen, a saprophytic bacterium that has become part of the microbiota of the patient, or contamination of the specimen that occurred during specimen collection, transport, or processing in the laboratory. Explain the advantages and limitations of conventional cell cultures for diagnosing viral infections. freundii on primary selective media can be mistaken for Salmonella when isolated from stool cultures. infections do not respond to most antifungal and antiprotozoan agents. The lesion, known as a chancre, is typically a single erythematous lesion that is nontender but firm, with a clean surface and raised border. When attempting to recover enteric Campylobacter spp., which specimen, media, and incubation conditions should be used? This is achieved by preparing a tease mount or cellophane tape mount. WNV can be present in tissues, blood, serum, and CSF of infected humans or animals. Most outbreaks in endemic areas occur during the rainy season. Colonization of ETEC on the proximal small intestine has been recognized as being mediated by fimbriae that permit ETEC to bind to specific receptors on the intestinal microvilli. Bacteria vary widely in their ability to use various compounds as substrates and in the end products generated. Wuchereria bancrofti. The causative agent of Bancroftian filariasis and elephantiasis, W. The chance for the release of airborne conidia is less likely with tubed media. They are a means of survival, not reproduction. Nearly all former Salmonella spp. The organization and type (either DNA or RNA) of genome of the virus, how the virus replicates, and the virion (a virus outside of a cell) structure of the virus are used to differentiate virus order, family, and tahir99 VRG 6 PART I Introduction to Clinical Microbiology genera. Hemorrhaging in the skin and internal organs may occur as well. Several viral transport systems are commercially available. Lactobacilli produce lactic acid from glycogen, which lowers the vaginal pH and suppresses the overgrowth of organisms such as Mobiluncus, Prevotella, and G. sobria), A. Therefore, an extremely important event in the life of an invading pathogen that wants to survive in the host is avoiding phagocytosis. Individuals who travel to endemic areas are susceptible to traveler’s diarrhea caused by C. Oral Herpes. Oral herpes infections were thought to have been caused by HSV-1, but it is now known that a number of cases are due to HSV-2. capsulatum d. Degand N, et al: Matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of nonfermenting gram-negative bacilli isolated from cystic fibrosis patients, J Clin Microbiol 46:3361, 2008. However, one report of susceptibility testing of more than 50 isolates of Chryseobacterium spp. Over the last several years, a viral antigenemia test has gained wider use by clinical virology laboratories. fowleri as it appears in a CSF specimen stained with iron hematoxylin. Figure 28-39, A, shows an oocyst of C. Entamoeba histolytica, Entamoeba dispar, and Entamoeba moshkovkii. E. Haemophilus influenzae colonizes the upper respiratory tract of healthy individuals without causing disease, but given the opportunity, it may rapidly produce a life-threatening infection. tahir99 VRG 492 PART II Laboratory Identification of Significant Isolates Shewanella FIGURE 21-15 Roseomonas on sheep blood agar (SBA). Presumptive Identification Microscopic Morphology. Campylobacter spp. Cultures and Serology In an outbreak investigation and collection of routine surveillance data, the collection, processing, reporting, and reviewing of pertinent cultures becomes critical. In the Case in Point, the P. Some are able to infect more than one related species. No commercially prepared NAATs are available. Permanently Stained Smears. Permanently stained smear preparations of all stool specimens should be made to detect and identify protozoan trophozoites and cysts. Working with animals or in ratinfested surroundings poses hazards for veterinarians, dairy workers, swine handlers, slaughterhouse workers, miners, sewer workers, and fish and poultry processors. curvus, a strict anaerobic environment does not support the growth of most Campylobacter spp. Eggs serve as the primary diagnostic stage. will fluoresce with a blue-white color when stained and viewed microscopically with ultraviolet light. falciparum are the result of CNS complications. Syncytia are giant multinucleated cells resulting from cell fusion as a consequence of virus infection. A regular microscope slide may be used by marking separate circles with a wax pencil. The microscope’s low-power objective (×2.5 to ×10) should be used first to evaluate the general content of the material on the slide. Caution must be exercised when diagnosis is made by histopathologic means only. All of the following are characteristic of fermentation except: a. and Bordetella spp. Nonfermenting species are differentiated by lack of color change, and colonies retain the original color of the medium. The organism can also be detected in lymphatic fluid and CSF. The wet mount procedure uses a glass slide on which a drop of physiologic saline (0.85%) has been placed at one end and a drop of iodine (Dobell and O’Connor, D’Antoni, or 1 : 5 dilution of Lugol solution) at the other end. Aeromonas c. hormaechei has been isolated from human sources such as blood, wounds, and sputum. The clinical form that mimics acute appendicitis occurs primarily in older children and adults. It is extremely difficult to distinguish C. Spotted Fever Group Rocky Mountain Spotted Fever. The most severe of the rickettsial infections, Rocky Mountain spotted fever (RMSF) is caused by R. In the mild form of the disease, viable organisms remain in the host, quiescent for years, which are the presumed source of reactivation in individuals with abrogated immune systems. Sexual reproduction requires the joining of two compatible nuclei, followed by meiosis (Figure 27-8). Color ranges from pale yellow to orange. They have emerged as a potential cause of enteric disease in humans and have also been isolated from a number of extraintestinal tahir99 VRG CHAPTER 20 Vibrio, Aeromonas, Plesiomonas, and Campylobacter Species infections. If not already harboring Acinetobacter spp., hospitalized patients become easily colonized. Stains can be categorized as simple stains, differential stains, and probe-mediated stains. Sporozoites are produced within the oocyst. Tetanospasmin acts on inhibitory neurons, preventing the release of neurotransmitters. pickettii isolates are slow growers, taking more than 72 hours on primary cultures before colonies are visible. They are in general biochemically inert with regard to carbohydrate oxidation (asaccharolytic). have weak growth at 35° C and no growth at 42° C. This edition of the Textbook of Diagnostic Microbiology, as in the previous editions, incorporates the expertise of contributors along with elements such as full-color photographs and photomicrographs, an engaging and easy-to-follow design, learning assessment questions and answers, opening case scenarios, hands-on procedures, and lists of key terms to strengthen the learning strategy. Common food sources involved in botulism include homecanned vegetables, home-cured meat such as ham, fermented fish, and other preserved foods. Nonfermenters can be more resistant to antimicrobial agents than members of the family Enterobacteriaceae. Unfortunately, molecular methods have not been standardized, and there is a high variability of the samples used for each technique. Furthermore, it is important to remember that swabs with plastic or metal shafts are superior to those with wooden shafts, which are toxic to cells. hominis is also unresolved. The organism may also cause migratory subcutaneous nodules, itching, and pain. The aspirate should be injected into an oxygen-free transport tube or vial, preferably one containing a prereduced, anaerobically sterilized (PRAS) transport medium, such as the one shown in Figure 22-3. Free amastigotes then invade other cells. Occasionally, conidia may be single-celled, but usually they are separated into two to five cells by perpendicular cross walls. Erwinia organisms grow poorly at 37° C and fail to grow on selective media, such as EMB and MAC, and other differential media typically used for the isolation of enterics. 30-45, round Thick, brown, radially striated shell Embryonated, with six-hooked oncosphere when passed Hymenolepis nana 30-47, oval Two membranes—inner has two polar knobs, from which four polar filaments extend into space between inner and outer membranes Embryonated, with six-hooked oncosphere when passed Hymenolepis diminuta 50-75, round to slightly oval Two membranes—inner has very slight polar knobs No polar filaments Embryonated, with six-hooked oncosphere when passed Dipylidium caninum 20-40 (each egg), round; resembles Taenia spp. gondii has two life cycle stages in humans, the tachyzoite and bradyzoite. hoshinae has been isolated from snakes, birds, and water. (From Joklik WK et al: Zinsser microbiology, ed 20, Norwalk, CT, 1992, Appleton & Lange.) tahir99 VRG 16 PART I Introduction to Clinical Microbiology D–Glucose ATP ADP D–Glucose – 6 – PO4 D–Fructose – 6 – PO4 ATP D–Fructose – 1,6 – di PO4 D–Glyceraldehyde–3–PO4 Dihydroxyacetone–PO4 1,3–Diphosphoglycerate ADP 2 ATP 3–Phosphoglycerate 2 NADH 2–Phosphoglycerate Ethanol Lactate Phosphoenolpyruvate ADP Acetaldehyde 2 ATP Pyruvate CO2 FIGURE 1-9 Embden-Meyerhof-Parnas glycolytic pathway. Most microorganisms are susceptible to the acid pH of the stomach and are destroyed, with the exception of spore-forming bacterial species in their spore phase, the cysts of parasites, and Helicobacter pylori. As illustrated in the Case in Point at the beginning of the chapter, the gram-positive control organism, Staphylococcus, stained grampositive, which is an acceptable result. furfur complex causes tinea versicolor, a disease characterized by patchy lesions or scaling of varying pigmentation. bancrofti antigens in blood. A reemergence of measles occurred in 1989 through 1991, attributed to lack of vaccination and immigration. coli) are known to cause intestinal tract infections. Supportive therapy, including iron and protein supplements, may be needed in severe cases, especially if the child shows evidence of anemia or if the infection is in a pregnant woman. Brevundimonas diminuta has been found in blood, CSF, urine, and wounds; it is often considered a contaminant. The results of these microbiologic cultures must be reported to the infection control team and evaluated. Human adenoviruses belong to the family Adenoviridae and the genus Mastadenovirus. are probably the most virulent of all human mycotic agents. holmesii, B. Temperature Studies. Temperature studies offer information for yeast identification. Growth on MAC agar and oxidase production is variable; glucose is oxidized and nitrates are reduced to nitrites, and isolates are ADH positive. Individual eggs are 20 to 40 µm and resemble those of Taenia spp. LPCB is used to fix and stain tease or tape mounts from cultures. Strains harboring these plasmids have been found in healthy volunteers and hospitalized patients. The range in recovery time is wide, from 3 to 60 days. Nutrition, stress, genetic background, and the presence of other diseases are additional influences that have an effect on the outcome of the meeting of human and microbe. Ledeboer NA: Haemophilus. Infection is usually treated with albendazole or mebendazole. Rickettsialpox occurs in Korea and the Ukraine and in the eastern United States, including the cities of New York, Boston, and Philadelphia. Tadesse, DVM, PhD Research Microbiologist Division of Animal and Food Microbiology FDA-CVM Laurel, Maryland Kimberly E. They also seem to be associated with chronic or recurring UTIs. Several strains of DAEC have been associated with pediatric diarrheal disease, particularly in developing nations. may tahir99 VRG 134 TABLE PART I Introduction to Clinical Microbiology 7-4 Gram Stain Morphology and Associated Organisms Morphotype Description Most Common Organisms Bacteria Cocci Gram-positive cocci Gram-positive cocci Pairs Tetrads Groups Chains Clusters, intracellular Encapsulated Gram-positive diplococci (lancet-shaped) Gram-negative diplococci Aerococcus, Enterococcus, Leuconostoc, Pediococcus, Planococcus, Staphylococcus, Stomatococcus, Streptococcus spp. In Brenner DJ, et al, editors: Bergey’s manual of systematic bacteriology: the proteobacteria, vol 2, ed 2, New York, 2005, Springer, p. In a trichrome-stained smear, the cytoplasm of the organism appears clean and free of ingested bacteria and vacuoles. These bacteria have been isolated from the environment and clinical specimens, including blood, CSF, sputum, abscesses, and wounds. In Detrick B, et al, editors: Manual of molecular and clinical laboratory immunology, ed 7, Washington, DC, 2006, American Society for Microbiology, p. The consequences of longer centrifuge time are prolonged exposure to the toxic effects of the digestion-decontamination agents used and the higher temperatures generated by unrefrigerated centrifuges. It can also be separated from the halophilic Vibrio spp. Humans acquire infection by ingesting the infective egg. The microscopic staining characteristics, shapes, and groupings are used in the classification of microorganisms (Figure 1-6). This unique characteristic is a major reason for the applications of nucleic acid amplification by polymerase chain reaction (PCR) assay and identification by hybridization. grow on TCBS, especially Grimontia (formerly Vibrio) hollisae. This organism causes atrophic rhinitis, a tissue-destructive disease restricted to the nose. The virus can also be cultured from urine or respiratory secretions, but because shedding of CMV from these sites is common in normal hosts, isolation from these sources must be interpreted with extreme caution. • Small, translucent or opaque colonies of tiny gram-negative cocci or diplococci can be identified as Veillonella. Coxsackievirus A16 grows in PMK and tahir99 VRG 714 PART II Laboratory Identification of Significant Isolates human diploid fibroblast cells and can be identified by serum neutralization tests. Antibiograms Antibiograms can often be used in the investigation of an outbreak. coli or E. Improperly stored food allows germination of the spores and growth of vegetative bacteria. These organisms will stain as large, boxcar-shaped bacilli. infection may play a role in Guillain-Barré syndrome (GBS), and Helicobacter pylori can cause ulcers and has been linked to gastric carcinoma. Numerous automated systems are available that use liquid media. bongori in Sicily during the period 1984-1997. found in breast-fed infants but have instead a colon flora similar to that seen in older children and adults (see Box 2-5). Buzoni-Gatel D, Werts C: Toxoplasma gondii and subversion of the immune system, Trends Parasitol 22:448, 2006. As the prevalence of Legionella infections declines, the false-positive rate increases, making it more important to confirm results with culture, especially when determining the dynamics of this organism in a given patient population. The laboratory technologist must relearn information once thought to be out of date. pneumoniae (% +) (0.8) (61.2) Sign %+ (% +) + − + − − − d 95.4 0 98 0 0 0 84.4 (0.1) + + + + or (+) 97.2 92.5 95 88.9 (6.3) (0.2) (0.3) (0.9) (1.5) (8.9) Modified from Ewing WH: Edwards and Ewing’s identification of Enterobacteriaceae, ed 4, East Norwalk, CT, 1986, Appleton & Lange. In the interpretation of a smear as positive for AFB, laboratory scientists must realize that organisms other than Mycobacterium might stain at least partially acid-fast (e.g., Nocardia spp., Legionella micdadei, and Rhodococcus spp.). Sporocysts can be seen in wet mounts. Commercial systems usually cannot discriminate the two species and other methods of identification such as MALDI-TOF or DNA sequencing is needed for definitive species differentiation. The rickettsial organism enters the human host following a Scrub typhus is a disease that occurs in India, Pakistan, Burma, eastern Russia, Asia, and Australia. This disease is almost always caused by Phaeoannellomyces werneckii and is characterized by brown or black macular patches, primarily on the palms. furfur the term the spaghetti and meatballs fungus (Figure 27-11). Slides are examined using a ×100 oil immersion objective on a light microscope for 15 minutes, viewing a minimum of 300 fields before a slide is called negative. In the case of a seropositive organ transplant recipient, immunosuppressive treatment to prevent organ rejection may reactivate a latent infection in the transplant recipient. The virus replicates slowly, so it may take up to 3 weeks for the CPE to appear in culture. The mollicutes produce small colonies ranging in size from about 15 µm to over 300 µm in diameter. This is most often a so-called rule-out disease. phenylpyruvica has been reclassified as Psychrobacter phenylpyruvicus. Enteroviruses characteristically produce rather small, round infected cells that spread diffusely on PMK, diploid 29-3 Cell Cultures Commonly Used in the Clinical Virology Laboratory Virus Herpes simplex virus Cytomegalovirus Varicella-zoster virus Enterovirus Adenovirus Respiratory syncytial virus Influenza, parainfluenza PMK HDF HEp2 RK A549 CPE − − − + + ± +++ +++ +++ +++ + ++ ± ± +++ − − ++ +++ +++ − +++ − − − − − − +++ − ± + ++ ++ − Large, rounded cells Large, rounded cells Foci or rounded cells; possible syncytia Refractile, round cells in clusters Large, rounded cells in clusters Syncytia Variable—none to granular appearance Modified from Costello MJ et al: Guidelines for specimen collection, transportation, and test selection, Lab Med 24:19, 1993. Chronic infection caused by these helminths, especially hookworm, can adversely affect physical and mental development in children. Finally, the ability to ferment glucose, with or without the production of gas, distinguishes Aeromonas from oxidasepositive, nonfermenting Pseudomonas isolates. However, if the engulfed organism is not exposed to intracellular killing and digestive processes, it is able to survive and multiply inside the macrophage. A minor outbreak in Indiana, linked to two infected people who visited the Super Bowl Village in February 2012, sparked a debate on vaccination, immigration, and global travel. Hence, most cases of gastroenteritis can be traced to recent consumption of raw, improperly cooked, or recontaminated seafood, particularly oysters. Microscopic characteristics that should be observed are the following: • Septate versus sparsely septate hyphae • Hyaline or phaeoid hyphae • Fruiting structures • The types, size, shape, and arrangement of conidia Tease Mount. For the tease mount, two teasing needles are used to remove a portion of the mycelium from the middle third of the colony. Clinically significant Enterobacter spp. A common stool transport medium, buffered glycerol saline, is toxic to enteric campylobacters and should therefore be avoided. Preformed Enzyme-Based Systems Many of the newer commercial identification systems are based on the presence of preformed (preexisting) bacterial enzymes. They are not, however, classified as L-forms, which are bacteria that have temporarily lost their cell wall as a result of environmental conditions. It also tends to distort protozoan cysts. Gaydos C: Nucleic acid amplification tests for gonorrhea and chlamydia: practice and applications, Infect Dis Clin North Am 19:367, 2005. pertenue. cayetanensis is autofluorescence, exhibiting a bright blue fluorescence (365 nm) or mint green (450 to 490 nm) under ultraviolet light. cepacia selective agar). HSV infections are very common. fermentum, L. Lawler A: Boston University under fire for pathogen mishap, Science 307:501, 2005. +, Positive result; −, negative result; ND, not determined; TSI, Triple sugar iron agar slant; V, variable result; W, weak; −V, variable usually negative result. WNV ELISA assays can cross-react with other flaviviruses and should be confirmed by antibody neutralization. The LCM virus causes an influenza-like illness; about 25% of infected patients develop meningitis. Orthomyxoviridae The influenza viruses are members of the family Orthomyxoviridae. Also, except for the blood flukes, flukes are hermaphroditic, possessing male and female reproductive organs. Available at: . Hydrochloric acid (50 mL HCl in 50 mL of water), sulfanilamide, and N-naphthylenediamine dihydrochloride are then added; if the bacteria reduce the nitrate to nitrite, a red color forms (Figure 26-11). pertussis/B. coli or diarrheogenic E. Brown-Elliott BA, Wallace RJ: Mycobacterium: clinical and laboratory characteristics of rapidly growing mycobacteria. are plant pathogens and are not significant in human infections. Hemolysis is variable on SBA, but most major clinical species, such as A. A delayed positive citrate reaction is a major characteristic of Hafnia. After 24 hours of incubation, the modified Thayer-Martin plate has small opaque colonies that adhere slightly to the medium. Scopulariopsis grows moderately rapidly and forms colonies covered by tan to buff conidia. histolytica infection. sonnei infection is usually a short, self-limiting disease characterized by fever and watery diarrhea. They are classified into one of two groups, the mesophilic group (optimal growth around 37°C) or psychrophilic group (optimal growth around 22°C). Usual Flora of the Genitourinary Tract The kidneys, bladder, and fallopian tubes are normally free of microorganisms, although a few organisms originating from the perineum may be found in the distal centimeter of the urethra, particularly in women. lari. The most severe form of malaria is caused by P. CRAB isolates are usually only susceptible to colistin and tigecycline. The surface of primary or secondary lesions is cleaned with saline and gently abraded with dry, sterile gauze; bleeding should not be induced. perfringens from food or feces during outbreaks. lamblia trophozoite (trichrome stain). osloensis is similar morphologically and biochemically to M. Donors in these cases were asymptomatic and in the latent or chronic state of the disease. Antibodies to the organism show a rapid rise during infection, and tests for antibodies are most commonly used for diagnosis. • Develop epidemiologic data. Christakis GB, et al: Chryseobacterium indologenes non–catheterrelated bacteremia in a patient with a solid tumor, J Clin Microbiol 43:2021, 2005. Metulae attach directly to the vesicle, and attached to each of the metula are phialides (Figure 27-37). It causes an acute illness producing unilateral or bilateral swelling of the parotid glands, although other glands such as the testes, ovaries, and pancreas can be infected. Usually, the lesions appear first on the head and trunk and then spread to the limbs. fallax from Levy-Frebault V, et al: Mycobacterium fallax sp. Indications of the Presence of Anaerobes in Cultures Several clues can alert the clinical microbiologist that anaerobes may be present on the primary plates. Sputum smear demonstrating the presence of acid-fast Mycobacterium species (arrow) stained by the Kinyoun or Ziehl-Neelsen carbolfuchsin method. The IgM molecule has up to 10 antigen-binding sites available. cholerae are susceptible to doxycycline or ciprofloxacin. Historically, pathogenic organisms were identified as Leptospira interrogans and saprophytes were categorized as Leptospira biflexa. mentagrophytes from T. Through August 2013, there were 340 cases in the United States. Numerous gene amplification techniques are available for the amplification and detection of viral genomes, primarily the bloodborne pathogens, such as HIV-1, HBV, hepatitis C virus (HCV), and WNV. Each manufacturer provides a mixture of antimicrobial agents to be added to each culture vial at the time of inoculation. A positive germ tube has no constriction at its base. ✓ Case Check 21-3 Laboratories often implement protocols when a susceptibility test would be performed on a nonfermenter. Urine specimens should be refrigerated during the interval between collection and processing; specimens should be processed promptly. brasiliensis is the causative agent of paracoccidioidomycosis (South American blastomycosis, Brazilian blastomycosis, Lutz-Splendore-Almeida disease, paracoccidioidal granuloma), a chronic, progressive fungal disease endemic to Central and South America. capsulatum in blood or bone marrow (Figure 27-56). Based on nucleic acid studies, they have been reclassified as subspecies of K. Found worldwide, this fungus is frequently recovered from grass, leaves, and decaying vegetation. The egg is embryonated, with a six-hooked oncosphere when passed in the feces (Figure 28-51). parahaemolyticus, V. (Nomarski optics, ×650.) and begin dirty gray, becoming phaeoid with age. parahaemolyticus strains produce a heat-stable hemolysin that is able to lyse human erythrocytes in a special, high-salt mannitol medium (Wagatsuma agar). are oxidase negative, catalase positive, and nonmotile. On certain selective and differential media, such as MAC or EMB agars, E. RBCs in the unfixed thick smear will lyse during the staining procedure. These changes may predispose an individual to infection by the indigenous flora, a type of infection referred to as an opportunistic infection. Since 1988, the polio vaccine has been crucial to the WHO’s effort to eradicate polio wor

common causes of diarrheal disease related to the consumption of raw shellfish or related to aquatic wound infections with serious sequelae, such as septicemia and death. The microscopist must have experience with the morphologic and staining features of Legionella spp. Mucorales The traditional Zygomycota have undergone taxonomic changes. Although isolates have been found to produce esterases, endotoxin, lipases, and phosphatases, inherent virulence is limited, and most isolates should be considered as colonizers or contaminants initially unless repeatedly isolated from a patient's samples. The incubation period varies from 2 days to 2 weeks. Thiamine Requirement. Some dermatophytes cannot synthesize certain vitamins and therefore do not grow on vitaminfree media. bronchiseptica, B. Congenital infections and infections in immunocompromised patients are often symptomatic and can be serious; however, a congenital infection is unlikely to occur if the mother was seropositive at the time of conception. In a Giemsa-stained preparation, the pear-shaped organism shows the presence of an axostyle extending the length of the organism, a single nucleus near the anterior end, and chromatic granules extending the length of the axostyle. Gram-negative rods (fermenters and nonfermenters) Moraxella spp. Other hybridization tests can detect CMV from blood and HBV from plasma and serum. The donor tahir99 VRG CHAPTER 1 Bacterial Cell Structure, Physiology, Metabolism, and Genetics Bacterial chromosome Plasmid DNA ready to be taken into the cell A Viral DNA being injected into the cell 21 their own cell. Jaundice is usually absent. Tachyzoites are capable of invading any nucleated cell and are the actively motile and reproducing forms. plantarum, and others have also been recovered. Another factor that contributes to the virulence of salmonellae is their ability to traverse intestinal mucosa. Plaque also results in a low oxidation-reduction potential at the tooth surface; this supports the growth of strict anaerobes, particularly in crevices and in the areas between the teeth. The genome can be doublestranded (ds) or single-stranded (ss). Because most (80%) C. Depending on the manufacturer, the EIA detects the outer membrane LPS chlamydial antigen or the MOMP. The microscopic morphology of these organisms varies, ranging from very short rods to long branching filaments. Available at: . Students and other readers are provided with valuable learning tools to help them sort through the vast amount of information—background theoretic concepts, disease mechanisms, identification schemas, diagnostic characteristics, biochemical reactions, and isolation techniques—to produce clinically relevant results. fluvialis V. Examples of nucleic acid–based assays include the hybridization assay, polymerase chain reaction (PCR) assays, traditional and real-time, branched DNA, nucleic acid sequence–based amplification, and a combination of PCR and flow cytometry, such as the Luminex system (Luminex, Austin, TX) for multiplex detection. pacificus. BOX 22-3 Unacceptable Specimens for Anaerobic Bacteriology* • Throat swabs, nasopharyngeal swabs; sputum obtained by nasotracheal or endotracheal suction; bronchial washings or other specimens obtained via a bronchoscope (unless a protected double-lumen catheter is used); expectorated sputum • Gingival swabs or any other intraoral surface swabs • Large bowel contents†; feces†; ileostomy and colostomy effluents; rectal swabs; gastric and small bowel contents • Voided or catheterized urine • Vaginal, cervical, or urethral swabs; female genital tract specimens collected via the vagina‡; swabs of a vaginal discharge • Surface swabs from decubitus ulcers, perirectal abscesses, foot ulcers, exposed wounds, eschars, pilonidal sinuses, and other sinus tracts • Any material adjacent to a mucous membrane that has not been adequately decontaminated Modified from Engelkirk PG et al: Principles and practice of clinical anaerobic bacteriology, Belmont, CA, 1992, Star. Specimens should be collected aseptically. Isolation of Acanthamoeba may be performed in a manner similar to that for N. This procedure of examining each specimen submitted is time-consuming and labor-intensive. Another superficial infection, piedra, is confined to the hair shaft and is characterized by nodules composed of hyphae and a cement-like substance that attaches it to the hair shaft. baumanii, 39% were caused by S. Issues to Consider After reading the patient's case history, consider: ■ Risk factors for acquiring infectious disease for the patient Agents that cause influenza-like illness and methods to identify or rule out those agents ■ Effective prophylaxis, if available, for influenza-like illness ■ Empiric therapy options Key Terms Chancre Endemic relapsing fever Endemic syphilis Epidemic relapsing fever Erythema migrans (EM) Gummas Jarisch-Herxheimer reaction Leptospirosis Lyme borreliosis Pinta tahir99 VRG Endemic Relapsing fever Rapid plasma reagin (RPR) test Spirochetes Syphilis Venereal Disease Research Laboratory (VDRL) test Weil disease Yaws Zoonoses 529 530 PART II Laboratory Identification of Significant Isolates T he order Spirochaetales contains two families: Leptospiraceae and Spirochaetaceae. Bogaerts P, et al: IMI-13–producing Pseudomonas monteilii recovered in a hospital environment, J Antimicrob Chemother 66:2434, 2011. The taxonomy of this group of bacteria continues to change and expand. The life cycle of this tahir99 VRG CHAPTER 28 Diagnostic Parasitology 675 Deposition of eggs in perianal areas Human ingestion of infective eggs Female migration from anus at night Egg maturation within several hours Adults in intestine Reentry into intestine Hatching of larvae Autoinfection Release of larvae in intestine Complete maturation FIGURE 28-56 Life cycle of Enterobius vermicularis. For many years, there were questions about the pathogenicity of D. have been implicated in rhinocerebral zygomycosis in addition to disseminated disease. Symbiosis as a biological relationship between two or more organisms where both (host and organism) benefit from one another may be described as mutualism. They differ from the other members of the rickettsiae in that they multiply in the phagosomes of host leukocytes and not in the cytoplasm of endothelial cells. Good growth on a BBE plate or growth in 20% bile indicates bile tolerance. The stain is a chemofluorescent agent, such as calcofluor white M2R (American Cyanamid, Princeton, NJ) or Fungi-Fluor (Polysciences, Washington, PA). tahir99 VRG Sexual and asexual All Present in some Present in some; contain hydrolytic enzymes Present in all; lipid synthesis, transport Present in most Present in all Present in algae and plants Present in all 80 S in size, consisting of 60 S and 40 S subunits In the inner membrane of mitochondria and chloroplasts Present Also contains glycolipids and glycoproteins Cellulose, phenolic polymers, lignin (plants), chitin (fungi), other glycans (algae) Present; some animal cells Present; see description of flagella Complex cilia or flagella; composed of MTs and polymers of tubulin with dynein connecting MTs; movement by coordinated sliding microtubules Absent CHAPTER 1 Bacterial Cell Structure, Physiology, Metabolism, and Genetics 5 Division septum Outer membrane Peptidoglycan Mesosome (Capsule) layer (Pili) (Capsule) Cytoplasmic membrane Inclusion body Inclusion body Peptidoglycan layer Cytoplasmic membrane Ribosome Ribosome (Flagellum) Surface proteins Chromosome A GRAM-POSITIVE Porin proteins Periplasmic space (Flagellum) GRAM-NEGATIVE Centrosome Ribosomes Centrioles Smooth endoplasmic reticulum Mitochondria Smooth endoplasmic reticulum Cilia Mitochondrion Lysosome Rough endoplasmic reticulum Free ribosomes Peroxisome B Golgi apparatus Vesicle Nuclear Nucleus Nucleolus envelope FIGURE 1-1 Comparison of prokaryotic and eukaryotic cell organization and structures. After the insect bites, there is 649 an incubation period that ranges from 2 to 4 weeks. In 2011, 501 the Centers for Disease Control and Prevention (CDC) reported 24 cases of foodborne botulism, 97 cases of infant botulism, and 32 cases of wound or other botulism infections. • Identify specific infectious agents using direct visual detection of characteristic shape, size, and Gram stain reaction. The bacteria methylate their own DNA at these same sequences so that the restriction enzymes do not cut the DNA in ■ There are many organisms that inhabit our environment. Most diseases are caused by a handful of species making identification, at least to the genus level, fairly easy. El Tor has been the predominant biogroup in the last two pandemics. Sethi S, et al: Probably congenital babesiosis in infant in New Jersey, USA, Emerg Infect Dis 15:788, 2009. In 2012, there were reports of another possible SARS outbreak in China in Baoding City in the province of Hebei. RMSF is a zoonosis, and humans typically acquire the infection by tick bites. Commercial systems using a plastic pouch containing culture medium are available for detection of the organisms. Gastroenteritis. One of the most common forms of "food poisoning," GI infection caused by salmonellae results from the ingestion of the organisms through contaminated food. (Giemsa stain.) B, B. (Courtesy Dr. G. are morphologically similar and produce similar clinical disorders, the infective dose of EIEC necessary to produce disease is much higher (106) than that of shigellae (about 100 bacterial cells). This species grows very slowly but is initially white, and becomes yellow, olivaceous, or brown, with a characteristic diffusible brown pigment with age. aeruginosa infection are otitis externa, in particular, in swimmers or divers, a necrotizing skin rash, referred to as Jacuzzi or hot tub syndrome that develops in users of these recreational facilities, and infections of the nail beds in people with artificial nails, keratitis, and endophthalmitis. capsulatum (teleomorph Ajellomyces capsulatus). Colony Morphology On BCYE agar, colonies appear as grayish white or blue-green, convex, and glistening, measuring approximately 2 to 4 mm in diameter. tahir99 VRG 536 PART II Laboratory Identification of Significant Isolates Pinta Pinta, caused by T. Mahon*, Steven Mahlen A. Incubate in moist chamber (without CO2) at 35° C for 7 days. Many members of this family possess antigens that can be used in the identification of different serologic groups. These microorganisms are also associated with BOX 18-1 Grouping of Clinically Significant Legionella Species Based on Colony Autofluorescence* Yellow-Green I n 1976, during an American Legion convention in Philadelphia, 221 persons became ill with pneumonia, and 34 of them died of a mysterious disease. The causative agent of Hansen disease: a. Accessed June 30, 2013. Alternatively, CSF can be assayed by PCR for HSV. Anaerobes, on the other hand, are particularly susceptible to these toxic derivatives of oxygen because they lack the protective enzymes superoxide dismutase and/or catalase, or the enzymes are present in low concentrations. ozzardi microfilariae have tails with nuclei that do not extend to the tips, whereas the nuclei in the tail of an M. (From Kumar V, Abbas AK, Fausto M: Robbins and Cotran pathologic basis of disease, ed 7, Philadelphia, 2005, Saunders.) Ability to Survive Intracellularly and Proliferate Some pathogens are able to survive within the phagocytic cell after they have been engulfed. infection is acquired early in life; serologic studies have shown that most humans have antibodies or antigens by 2 to 4 years of age. These antibody levels rise after tissue invasion but are not protective. trachomatis; and (2) Chlamydophila to include C. Cutaneous lesions should be sampled below the edges of the ulcer; surface samples do not yield infected cells. The central body form is the most commonly identified. Because the hydroxyl radical is a product of the further reduction of the superoxide anion, elimination of the superoxide anion by superoxide dismutase will inhibit the formation of the hydroxyl radical. Cerebrospinal Fluid Viable organisms in suspected cases of amebic meningitis or sleeping sickness can occasionally be seen in a cerebrospinal fluid (CSF) specimen. Dip slide in solution I five times for 1 second each time. tahir99 VRG CHAPTER 29 Clinical Virology 703 Monkeypox Virus Monkeypox was first described in primates in 1958, with the first case of human monkeypox in 1970. Centers for Disease Control and Prevention: Investigation update: human Salmonella Typhimurium infections associated with exposure to clinical and teaching microbiology laboratories. Kennedy PGE: The continuing problem of human African trypanosomiasis (sleeping sickness), Ann Neurol 64:116, 2008. Pull to spread. The host-microbe relationship, depending on the circumstance, may be one of symbiosis, commensalism, or parasitism. Move slides to slide rack on acid alcohol collection container. No vaccine or antiviral agent was available to fight the pandemic, which tahir99 VRG CHAPTER 29 Clinical Virology 707 sera sampled from 1986 to 1999. Complications of EBV infections are splenic hemorrhage and rupture, hepatitis, thrombocytopenia purpura with hemolytic anemia, Reye syndrome, encephalitis, and other neurologic syndromes. Gomes JP, et al: Polymorphisms in the nine polymorphic membrane proteins of Chlamydia trachomatis across all serovars: evidence for serovar Da recombination and correlation with tissue tropism, J Bacteriol 188:275, 2006. Each sporocyst contains four elongated sporozoites. Flood smears with potassium permanganate counterstain for 4 minutes. The laboratory should be cautious in processing such a specimen. Bunyaviridae The family Bunyaviridae includes the genera Orthobunyavirus, Phlebovirus, and Nairovirus, which are classified as arboviruses. Yersinia infections include GI disease, mediastinal lymphadenitis, fulminant septicemia, and pneumonia. Such an investigation may involve collecting environmental samples (e.g., searching for sources of airborne fungi). The generation time of a bacterium in culture can be 20 minutes for a Stationary log10 Number of bacteria 14 9 7 Death phase Exponential 5 3 Lag 1 4 8 12 16 20 FIGURE 1-7 Typical growth curve of a bacterial culture. Both coagulase-negative staphylococci and A. Table 1-1 compares prokaryotic and eukaryotic cell organization; Figure 1-1 shows both types of cells. Centers for Disease Control and Prevention: Brucellosis. One patient on hemodialysis suffered from C. Pseudomonas aeruginosa, hepatitis C, lice, Giardia spp. This isolate grows slowly, produces olive brown to black colonies, and may produce a reddish brown pigment. Primary Plating Media for Anaerobic Cultures. Table 22-8 lists the primary media recommended for the recovery of anaerobes. Biochemically, K. Differential features of Klebsiella spp. aerogenes, and K. The reservoirs are primarily humans and flying squirrels located in the eastern United States. Microscopically simple, wide-based, single-celled conidia are produced on nonspecialized cells (Figure 27-39). Biotype 1 grows in the beer wort of breweries and has not been isolated clinically. flexneri, they do not produce gas from glucose. The size range is 130 to 150 µm × 60 to 90 µm. These fungi are called dimorphic. A positive test for antibody would help confirm a diagnosis. Carriers are often the source of infection. Eikenella corrodens Fusobacterium nucleatum Candida albicans Usual Flora of the Respiratory Tract The respiratory tract, commonly divided into upper and lower tracts, is responsible for the exchange of oxygen and carbon dioxide as well as for the delivery of air from the outside of the body to the pulmonary tissues responsible for that exchange. Nonmotile Blue-green Acinetobacter spp. A kit can detect and distinguish between influenza A and B viruses, detect both influenza A and B but not distinguish between them, or detect only influenza A viruses. perfringens type C food poisoning (enteritis necroticans) is associated with strains that produce β-toxin and, less commonly, α-toxin. These granules are storage deposits and may consist of polysaccharides such as glycogen, lipids such as poly-β-hydroxybutyrate, or polyphosphates. Describe the characteristics of viruses and how they differ from bacteria. An online phone book, like the Telkom phone book, provides a quick way to look up numbers of people and businesses you want to call or locate. A total of 33,097 cases were reported in the United States in 2011. These small, ssRNA, round viruses of 27 to 30 nm in diameter were, until recently, called Norwalk-like viruses, caliciviruses, and small round structured viruses. morganii P. pneumoniae are shown in Table 19-6. Infection of the cells in the respiratory tract leads to cell death and an inflammatory reaction in the upper and lower respiratory tracts. Available at: . The infection is accompanied by sudden high temperature, rigors, severe headache, muscle pains, and weakness. It may be necessary to collect fluid containing fibrinogen (e.g., pleural, pericardial, peritoneal) into a container with an anticoagulant. The entire smear should be scanned at ×100 for detection of large organisms such as microfilariae; then at least 100 oil immersion fields (×1000) must be examined for the presence of organisms such as Trypanosoma or for intracellular organisms such as Plasmodium or Babesia. tahir99 VRG 624 PART II Laboratory Identification of Significant Isolates BIBLIOGRAPHY Ahmed AOA, de Hoog GS: Fungi causing eumycotic mycetoma. Once inside the RBC, the organism feeds on hemoglobin and initiates the erythrocytic phase. Gram-Positive, Spore-Forming Anaerobic Bacilli All spore-forming anaerobic bacilli are classified in the genus Clostridium and are collectively termed clostridia. rubidaea, S. Explain the clinical significance of Bordetella spp. coli or 24 hours for a slowgrowing bacterium such as Mycobacterium tuberculosis. They are 70S in size and dissociate into two subunits, 50S and 30S in size (see Table 1-1). Growth is moderate to slow, and colonies are velvety to woolly and gray-brown to olivaceous black. In Brenner DJ, et al, editors: Bergey's manual of systematic bacteriology, ed 2, vol 2: the proteobacteria part C, New York, 2005, Springer, p. Also, so that the holding jar can maintain as low an oxygen concentration as possible, care should be taken to minimize convection currents whenever freshly inoculated plates are added to the jar. However, these hosts, called carriers, are capable of transmitting the infection. jejuni Campylobacter jejuni subsp. As a result of this immune response, the tachyzoites convert to bradyzoites, and eventually the cystlike structure contains hundreds to thousands of the slowly growing and reproducing bradyzoites. Once the oxygen removal system is activated, the specimen is transported to the laboratory in the bag, thereby minimizing exposure to oxygen. schubertii. The drugs are not effective against bradyzoites in cysts located in muscle and brain tissue. Cellular fatty acids are coded for on bacterial chromosomes, as opposed to plasmids, and are not affected by simple mutations or plasmid loss. For example, certain coxsackie A viruses require suckling mice for isolation of the virus. Routine fungal media can be used, but selective chromogenic agars such as CHROMagar Candida (CHROMagar, Paris, France) and Candida ID (bioMérieux, Durham, NC) provide a rapid preliminary identification. Was it submitted in an appropriate transport container or medium? NLF species such as Salmonella enterica, which produces lysine decarboxylase, produce red colonies with black centers on XLD medium (Figure 19-13). Although no link could be found between the New York case and the other cases, all isolates were identical by hexon and fiber gene sequencing. The Case in Point describes two teammates who were on doxycyline for malaria prophylaxis, which is also effective against many bacterial agents, including Leptospira. They have few growth requirements and thus are capable of growing on most laboratory media, including MAC agar. Serologic typing with pooled antisera may be performed to identify EPEC serotypes, but this is generally used for epidemiologic studies rather than for diagnostic purposes. Pigmented species of Porphyromonas and Prevotella produce substances that fluoresce under long-wave UV light prior to becoming darkly pigmented. would you expect to be most likely isolated from a blood culture? Terminology referring to the various life cycle forms, however, is reflected in the fact that it was first considered a protozoan. Many of the organisms listed in Table 2-2 are common isolates, which is not surprising because they have developed a means to interfere with phagocytosis, increasing their pathogenicity. aureus both produce exfoliatin, which causes rash and massive skin peeling or exfoliation. Discuss key biochemical reactions that will help differentiate among the different genera and identify various species. APPLICATION Calcofluor white may be used as a specific stain for rapid screening of clinical specimens for fungal elements. Frequently, the physician must decide between treating the fungal infection and risk damaging the patient's kidneys. In the United States, the condition is often seen in Hispanic immigrants from endemic Eggs in human feces Hymenolepis nana or Hymenolepis diminuta Insect ingestion of infective egg Hymenolepis nana autoinfection Scolex release Migration back to intestine Human ingestion of egg Hexacanth embryo release in intestine Penetration into tissue to form cysticercoid Embryo release into intestinal tissue Migration to bowel Human ingestion of insect Cysticercoid formation Freeing of scolex in intestine and attachment to wall FIGURE 28-52 Life cycle of Hymenolepis spp. The plate should be incubated at 30° C, the optimal temperature for recovery of this organism and for M. Bacteremia. Many of the clostridia have been recovered from blood cultures, but C. This fungus is found in the soil and decaying vegetation. The young trophozoite is characterized by its ameboid appearance; by maturity, it usually fills the RBC, and golden brown malarial pigment is present. In some cases, this connective tissue manifestation occurs without the prodrome or rash stage. Most infections occur in immunocompromised and elderly patients. However, lactosefermenting species such as V. stercoralis, known as the threadworm, inhabits the small intestine but is also capable of existing as a free-living worm. Detection times are 581 shorter than with conventional agar but significantly longer than with the BACTEC system. It appears that the leech Hirudo medicinalis has a symbiotic relationship with this aeromonad species within its gut, wherein the organisms aid in the enzymatic digestion of the blood ingested by the leech. malariae Normal size May have dark hue Ziemann's dots (rare) P. morganii is a documented cause of UTI. Describe each of the following as they relate to the culture and isolation of anaerobes: • Atmospheric requirements • Isolation media • Identification systems 7. Patients with V. The standard procedure, (see Appendix D), requires the use of serum or plasma, such as fetal bovine serum. Cangelosi JJ, et al: Transmission of Babesia microti by blood transfusion in Texas, Vox Sang 95:331, 2008. by a positive-oxidase, the characteristic Gram-stained microscopic morphology, and the characteristic motility. Members of the family Flavobacteriaceae are ubiquitous in soil and water and are not considered part of the normal human microbiota. Certain fungi can assume both morphologies (yeast and hyphae/mycelial forms), growing as yeast at incubator or human temperature and as the filamentous form at room 1-1 Comparison of Prokaryotic and Eukaryotic Cell Organization Characteristic Prokaryote Eukaryote Typical size 0.4-2 µm in diameter 10-100 µm in diameter Nucleus 0.5-5 µm in length No nuclear membrane; nucleoid region of the cytosol >10 µm in length Classic membrane-bound nucleus Location Chromosomal DNA In the nucleoid, at the mesosome Circular; complexed with RNA Genome: extrachromosomal circular DNA Plasmids, small circular molecule of DNA containing accessory information; most commonly found in gram-negative bacteria; each carries genes for its own replication; can confer resistance to antibiotics Asexual (binary fission) Absent Absent in all Absent in all Absent in all Absent in all Absent in all Present in all In the nucleus Linear; complexed with basic histones and other proteins In mitochondria and chloroplasts Genome Reproduction Membrane-bound organelles Golgi bodies Lysosomes Endoplasmic reticulum Mitochondria Nucleus Chloroplasts for photosynthesis Ribosomes: site of protein synthesis (nonmembranous) Size Electron transport for energy Sterols in cytoplasmic membrane Plasma membrane Cell wall, if present Glycocalyx 70S in size, consisting of 50S and 30S subunits In the cell membrane if present; no mitochondria present Absent except in Mycoplasma spp. coli O157:H7 may be performed using MAC agar containing sorbitol (SMAC) instead of lactose. These organisms produce watery diarrhea, vomiting, dehydration, and occasionally abdominal pain, mostly in children. werneckii. Most Erwinia spp. Some are able to detect more than one parasite in the specimen by detecting antigens of the organism in the stool using organism-specific antibodies immobilized on a membrane. This organism is nonmotile and grows best at 22° C to 25° C. Laboratory Diagnosis Specimens collected for the detection of C. meningitidis) produce an immunoglobulin A (IgA) protease that degrades the IgA found at mucosal surfaces. Members of the newly named genus, Wautersiella, have been isolated from blood and surgical wounds. They may expect these to be delineated by office practice, physician, site, or patient type. Others begin recovering after about 10 days of illness. Trichophyton tonsurans and Trichophyton violaceum are the most common fungi implicated in this form of dermatophytosis. The hookworm rhabditiform larva is 250 to 300 µm long and has a small, inconspicuous, genital primordium (Figure 28-63, A) and a long buccal capsule (see Figure 28-63, B). One clostridial infection of endogenous origin is antibiotic-associated pseudomembranous colitis caused by C. Penicillin c. Unclassified organisms usually are referred to by the encompassing term microsporidia. Add Fungi-Fluor solution B if quenching of nonspecific staining is desired. fragilis with other organisms. A cervical culture for possible gonococcal infection is sent to the microbiology laboratory. parahaemolyticus and, to a lesser degree, V. Laboratory Safety Considerations The serious nature of TB disease and the usual airborne route of infection require that special safety precautions be used by anyone handling mycobacterial specimens. Plesiomonas colonies are white to pink on this medium, and most coliform colonies are green or pink. are fastidious and require supplementation with cysteine, cystine, or thiosulfate for growth on successive passage. For example, HSV grows rapidly on many different cell lines and frequently produces CPE within 24 hours. Examine the stained smear using the low-power objective, and select an area to examine more closely using a 40 to 60 oil objective. pylori can be recovered from gastric biopsy materials. pallidum Virulence Factors Treponema pallidum subsp. Their halflife in serum is about 3 to 4 weeks. With respect to any drug or pharmaceutical products identified, readers are advised to check the most current information provided (i) on procedures featured or (ii) by the manufacturer of each product to be administered, to verify the recommended dose or formula, the method and duration of administration, and contraindications. The cabinet should be checked daily to see that none of the airflow inlets or outlets is blocked by supplies, incinerators, or waste disposal containers. Mycoses Mycoses (singular, mycosis) are diseases caused by fungi. Many species of the viridans group can be isolated, including S. influenzae should be treated with which of the following? One system uses the reactions of three common tests: (1) growth on MAC agar; (2) oxidase reaction; and (3) glucose O-F test. Sporangiospores are formed in sporangia on erect sporangiophores (Figure 27-34). ornitholytica, K. Urine can also be collected, but yield is much higher after the first week of illness, and shedding can occur intermittently for weeks. capsulatum (Gomori methylene stain, ×1200.) in the Ohio, Missouri, and Mississippi river deltas. Seroconversion occurs in 90% of children between the ages of 6 months and 2 years. stutzeri, which can also appear as wrinkled colonies, does not use lactose, in contrast to B. Cysts often have a punched-out ping-pong ball appearance. If culture is attempted, blood should be collected as early in the disease as possible. Ecofix (Meridian Diagnostics, Cincinnati, OH), Parasafe (Scientific Device Laboratory, Des Plaines, IL), and Prot-fix (Alpha-Tec, Vancouver, WA) are single-vial fixatives for wet mounts and permanent stained slides that do not use formaldehyde or mercury compounds. Second, these organisms have different life cycle stages for diagnosis. Once the material is flattened and sufficiently thinned, pull the glass slides smoothly away from each other to produce two smears. by Chlamydia trachomatis serovars L1, L2a, L2b, or L3—that is, lymphogranuloma venereum. Figure 28-69 depicts a cross section of tissue containing these organisms. cytoplasm and a diffuse chromatin mass that stains pale pinkpurple. Certain precautions should be followed in the preparation, storage, and incubation of Middlebrook media. This teleomorph does not occur in the routine laboratory because the species is heterothallic, requiring two mating strains to produce the sexual form. It should be fixed in methanol for 1 minute and airdried before staining with Giemsa stain. Acridine Orange Acridine orange is a fluorochrome dye that stains both grampositive and gram-negative bacteria, living or dead. They occur in three basic shapes (Figure 1-5): • Cocci (spherical) • Bacilli (rod-shaped) • Spirochetes (spiral) Individual bacteria may form characteristic groupings. buski live in the duodenum, where they cause mechanical and toxic damage. novyi type A, and C. Tissue parasites such as Trichinella spiralis, Leishmania spp., and Toxoplasma gondii can be identified by examination of tissue biopsy. Gastrointestinal Pathogens E. Miscellaneous Tests for the Identification of Yeasts Germ Tube Production. The germ tube test is probably the most important and easiest test to perform for the identification of yeasts. rubrum, which may be morphologically similar to T. 539. An exception to this pattern occurs with Rickettsia prowazekii. HEp2 (derived from a human laryngeal epithelial carcinoma), A549 (derived from a human lung carcinoma), and Vero (derived from monkey kidney) are examples of continuous cell lines used in diagnostic virology. In this methodology, enzyme-digested chromosomal fragments of bacteria are separated electrophoretically. Organisms that can establish infection with a relatively low infective dose are considered more virulent than organisms that require high numbers for infection. They are free-living, or survive in association with animal and human hosts as normal biota or pathogens. Eggs of these species are indistinguishable and must be reported as "Taenia sp. In addition, these tests provide limited information in patients from endemic areas. maltophilia, followed by Achromobacter spp. hydrophila A. The tick-borne borreliae are transmitted by a large variety of soft ticks of the genus Ornithodoros. fragilis group (Figure 22-19). violaceum is a fermentative, gram-negative bacillus that may be oxidase positive, so it may be mistaken initially as a nonfermenter in the laboratory. Table 27-7 shows important differentiating characteristics among Candida spp. Human infections most often occur after the ingestion of contaminated food, often pork, and vacuum-packed deli meat, beef, lamb, chicken, and possibly chocolate milk and water. The two simultaneous outbreaks of Ebola fever were caused by two different species, EBO-Z and EBO-S; EBO-Z is the more virulent. A tache noire, similar to that of boutonneuse fever, forms at the site of inoculation. They are unsheathed and have nuclei that extend to the end of the so-called shepherd's crook tail. The most common vehicle of transmission is the ingestion of contaminated water or food, particularly uncooked or undercooked seafood such as oysters, clams, or shrimp. albicans and therefore can be easily differentiated. With few exceptions, paired sera (acute and convalescent) demonstrating seroconversion or a fourfold rise in titer are required to establish a diagnosis of recent infection. Any rinsing between the crystal violet and the iodine steps must be very brief. He was still recovering from multiple abrasions from the jungle trekking and mountain biking. diminuta, B. are environmental microorganisms infrequently isolated from clinical specimens. Most vibrios are also susceptible to gentamicin, tetracyclines, chloramphenicol (except P. Peritoneal (ascitic fluid) smears are also rarely positive for AFB. tuberculosis—absolute concentration, resistance ratio, and agar and broth-based proportional methods. No systemic signs or symptoms are evident in the primary stages of the disease. (A, ×1000; B, original magnification, ×1000.) FIGURE 28-11 Iodamoeba bütschlii trophozoite, identified by arrow (trichrome stain, ×1000). demonstrated reduced activity to vancomycin compared with what had been previously reported. What organisms should be considered as possible causes of neonatal conjunctivitis? The adult tapeworm is 20 to 60 cm long. If viral antigens are present in the sample, the labeled antibody will bind and fluorescence will be seen tahir99 VRG CHAPTER 29 Clinical Virology microscopically (see Chapter 10 for a more detailed description). Curvularia. Curvularia spp. nonliquefaciens have been reported. The practice of Standard Precautions further extends infection control to the microbiology laboratory. The clinical course tends to be more severe if the patient has undergone splenectomy, is immunosuppressed, or is older. p. The mumps virus can be isolated from infected saliva and swabs rubbed over the Stensen's duct from 9 days before onset of symptoms until 8 days after parotitis appears. The bacterial cells are removed by centrifugation and then saponified to release the fatty acids from the bacterial lipids. Most studies have shown increased sensitivity when multiple specimens are examined. The average size of the cyst is 10 to 20 µm (Figure 28-4). vaginalis, L. When fungi are atypical or are a species not routinely recovered, a slide culture should be prepared. The word sterile is a term that is relevant to the method used. Subcutaneous Mycoses Subcutaneous mycoses involve the deeper skin layers, including muscle, connective tissue, and bone. gladioli oxidizes glucose, is mannitol positive and decarboxylase negative, grows on MAC agar, and is 100% resistant to polymyxin B. Pulmonary toxoplasmosis may be present in conjunction with the CNS infections or can be the presenting condition; however, the organism is capable of disseminating to any organ of the body. The best specimens for culture are aspirates from vesicles, open lesions, or host cells collected from infected sites. A pyrimidine consists of a single ring of six atoms of carbon and nitrogen. EBV infects circulating B lymphocytes and stimulates them to produce multiple heterophile antibodies, including antibodies to sheep and horse RBCs. The Paul-Bunnell heterophile antibody test is an excellent rapid screening test for these antibodies, although some false-positive reactions occur. These characteristics do not allow for definitive identification but are presumptive and help in the selection of other, more definitive tests. The cell wall is treated to allow penetration of the dye either by heat (Ziehl-Neelsen method) or by a detergent (Kinyoun method). Stevens DL, et al. shimoidei 35-45 2 Weeks S = 99% N = 85% −(99) +(51) S = 12% +(76) +(99) S P = 3% N = 80% S = 20% − − S N Pigment Photo, Scoto, Nonphoto Smooth Rough Trans. from clinical specimens. The organism is saccharolytic, oxidizing glucose, mannose, fructose, and other sugars. that cause enteric illness are isolated from stool samples and rectal swabs, the less preferred specimen. A typical microscopic picture of T. Molecular methods are also used in bone marrow transplant patients to detect infection. coli O157:H7 does not ferment sorbitol in 48 hours, a characteristic that differentiates it from most other E. mirabilis — — Providencia — — — — K/K Salmonella* Edwardsiella Salmonella P. Right, Lactose-positive appearance of S. tahir99 VRG 620 PART II Laboratory Identification of Significant Isolates Yeast Germ tube + – + Growth at 42°C – Candida albicans Candida Cornmeal dubliensis Blastoconidia only Cryptococcus sp. List the three most common causes of atypical pneumonia. marinum. Late manifestations, or late persistent infections (stage 3), focus on the cardiac, musculoskeletal, and neurologic systems. Only a few microorganisms are capable of penetrating normal, healthy skin; these include Leptospira spp., Francisella tularensis, Treponema spp., and some fungi. These organisms may appear as coccobacilli in smears prepared from older cultures. Most cases are identified in the upper Midwest and Northeast United States. Simmon KE, et al: Genotypic diversity of anaerobic isolates from bloodstream infections, J Clin Microbiol 46:1596, 2008. parahaemo lyticus Group 6 − + + + − − + V. boydii and P. Eukaryotic ribosomes, where protein synthesis occurs, are 80S in size and dissociate into two subunits: 60S and 40S. include the following: • They are nonmotile. They grow on MAC agar and are variable in the tahir99 VRG 484 PART II Laboratory Identification of Significant Isolates reduction of nitrates to nitrites or nitrogen gas. belli, and C. stercoralis can persist in the host for decades after initial infection and may progress to hyperinfection if the host becomes immunocompromised. enterocolitica infections. In addition, respiratory protection must be used when performing procedures outside the biological safety cabinet when aerosolization could result. The Flukes Flukes (trematodes) are members of the phylum Platyhelminthes, or flatworms. Name the clinical specimens from which the mycoplasma species are most likely to be isolated. Pathologic mechanisms associated with G. Subsequently, there have been isolated outbreaks associated with imported vegetables such as mesclun lettuce, basil, and snow peas. Sena AC, et al: Trichomonas vaginalis infection in male sexual partners: implications for diagnosis, treatment, and prevention, Clin Infect Dis 44:13, 2007. (Courtesy S.W. Joseph.) If a selective medium is warranted because of the clinical history (exposure to seafood or seawater) or for geographic reasons (coastal area resident or recent foreign travel), thiosulfate citrate bile salt sucrose (TCBS) agar is recommended. mirabilis, can produce "swarming" colonies on nonselective media, such as SBA (Figure 19-7). The nebulous syndrome commonly referred to as traveler's diarrhea (similar to enterotoxigenic E. Chitin is a distinct component of fungal cell walls. FIGURE 23-2 Appearance of Borrelia recurrentis (arrows) in blood (Giemsa stain, ×850). lacunata is a common conjunctival isolate. If the S. ovale is primarily confined to western parts of Africa; P. School-age children and young adults are especially susceptible to infection. As our knowledge of the nucleotide sequences grows, this also becomes a valuable tool for the taxonomic placement of viruses. Ehrlichiosis cases have increased from about 200 in the year 2000 to 961 in 2008, although cases may be underreported. Classification by Phenotypic and Genotypic Characteristics The traditional method of placing an organism into a particular genus and species is based on the similarity of all members in numerous phenotypic characteristics. tahir99 VRG 424 PART II Laboratory Identification of Significant Isolates A Escherichia coli (lactose-fermenting) Escherichia coli Escherichia coli (non–lactose-fermenting) B C FIGURE 19-1 A, Typical dry, lactose-positive Escherichia coli growing on MacConkey (MAC) agar. Clinically, it is believed that pneumonia in infants younger than 6 months is associated with C. tahir99 VRG 428 PART II Laboratory Identification of Significant Isolates Infection may also result if contamination of the amniotic fluid occurs. C, Polar, multitrichous (e.g., Comamonas spp.) (×1000). These cells have hairlike extensions (cilia) that sweep particles and organisms upward toward the oropharynx. Most aeromonad wound isolates are A. The viruses can also cause epidemic keratoconjunctivitis, acute hemorrhagic cystitis, and pharyngoconjunctival fever. The Gram stain is regularly used to characterize bacteria growing on culture media. Aeromonadaceae. Fermentation is indicated by a color change on the medium, which results from a decrease in pH detected by a pH indicator incorporated into the medium. This is a typical smear presentation for early pneumococcal pneumonia. EAEC causes diarrhea by adhering to the surface of the intestinal mucosa. Benzalkonium Chloride. Another digestant-decontamination procedure uses benzalkonium chloride (Zephiran) combined with trisodium phosphate (Z-TSP). List the major considerations in the collection and handling of specimens for the identification of intestinal and blood and tissue parasites. The result of sexual reproduction is the production of an oocyst, which is passed in cat feces. Most members of the rickettsial group are arthropodborne, obligately intracellular pathogens that can grow only in the cytoplasm of host cells. pseudomallei has been placed on the list of organisms considered potential agents of bioterrorism (see Chapter 30). Cultures of serovar Paratyphi and some rare serotypes may fail to produce H2S. (Courtesy Anaerobe Systems, Morgan Hill, CA.) TABLE 22-12 Interpretation of Special Potency Antimicrobial Disk Results Kanamycin† Colistin‡ Interpretation S V R S R R R R R R R R R R S V S S Probably a pink-staining, gram-positive bacillus such as Clostridium ramosum or C. 1027. furfur is a common endogenous skin colonizer. After 18 hours of incubation, a MacConkey (MAC) agar plate showed *My comments are my own and do not represent the view of Health Resources and Services Administration of the Department of Health and Human Services. Differentiate the functions and purposes of a disinfectant and an antiseptic. violaceum infections were reported in a clinical review article. or anaerobic grampositive cocci as a presumptive identification rather than identifying these organisms with the newer nomenclature. Using calcium alginate, Dacron flexible wire swabs or flocked swabs, collect two pernasal specimens from the posterior pharynx. Other Vibrio spp. Paired sera taken as little as 4 to 5 days apart can demonstrate a diagnostic or fourfold rise in titer. They are neither prokaryotic nor eukaryotic. aeruginosa, but does not produce pyoverdin and is acetamidenegative. Lecithinase, Lipase, and Proteolytic Reactions. An EYA plate can be used to determine the activities of lecithinase, lipase, and proteolytic enzymes. parapertussis. pestis Y. Table 28-5 shows the characteristics of the trophozoite and cyst stages of the intestinal and genitourinary flagellates. Paromomycin and azithromycin, which can suppress the infection, have been used, with m

rule out the involvement of anaerobes. Adults in intestine circulation to develop as a cysticercus in any tissue or organ. Nails become thick, discolored, and flaky. Tick vectors include Ixodes scapularus and I. The primary method of diagnosis is finding the egg in a stool specimen. Inglis TJ, et al: Use of matrix-assisted laser desorption/ionization time-of flight mass spectrometry analysis for rapid confirmation of B. pneumophila (Genetic Systems, Seattle, WA) and L. Colony Morphology. Colonies of mycobacteria are generally distinguished as having a smooth and soft or rough and friable appearance. To prevent a potentially infectious aerosol, an alcohol-soaked gauze pad can be placed over the needle while air is expelled. The larva can live up to 7 years and elicits a host tissue reaction, resulting in production of a fibrous capsule. Wound botulism is the result of contamination of wounds with spores of C. Biochemical reactions are interpreted by measuring the pH with a probe or by color changes based on a bromthymol blue indicator. Antibiotic therapy selects for bacterial strains containing plasmids encoding antibiotic resistance genes; this is one reason antibiotics should not be overprescribed. Cysts of E. fragilis has a worldwide distribution, with prevalence varying from less than 1% to greater than 40%. The spot indole test also can be performed directly on a pure culture plate. The combined affects of rapid demographic, environmental, societal, technologic, and climatic changes, as well as changes in the way we live our lives, have affected the occurrence of infectious disease. difficile Certain Clostridium C. beigelii is no longer a valid species but rather a complex of at least 19 distinct species. This leads to an accumulation of cyclic adenosine monophosphate (cAMP) along the cell membrane, which stimulates hypersecretion of electrolytes (Na+, K+, HCO3−) and water out of the cell and into the lumen of the intestine, as shown in Figure 20-3. The usual flora of the oropharynx is listed in Box 2-4. Pseudomonas mendocina Pseudomonas mendocina can be found in soil and water but is rarely isolated from human specimens; when it is, it is often considered a contaminant. Cell Culture. Until the development of PCR assays, chlamydial cell culture was considered the gold standard for detecting C. test positive. can often be recovered from urine and genital cultures, in which their role in the infectious process is doubtful. Obligate aerobes must have oxygen to grow; incubation in air or an aerobic incubator with 10% carbon dioxide present satisfies their oxygen requirement. Advantages of nucleic acid–based detection assays include a much faster turnaround time (TAT), better sensitivity than cell culture and DFA, assays that can be quantitative, detection of viruses nonculturable by cell culture (e.g., norovirus, hepatitis viruses), ability to detect multiple viruses simultaneously (multiplex), and potentially characterize the virus genetically (genotype). Antibodies bind to antigens via their Fab or antigen-binding portion. Different HPV types exhibit different tissue tropism based on the type of epithelial cells that the viruses preferentially infect, cutaneous or mucosal. are relatively inactive. The trypomastigote is 15 to 20 µm long with a single large nucleus and a posterior kinetoplast to which is attached the flagellum of the undulating membrane (Figure 28-25). • Translucent colonies with a black fish eye center observed on BBE agar (usually at 48 to 72 hours) can be used to presumptively identify Bilophila wadsworthia (Figure 22-20). stutzeri, identification of the organism and in vitro susceptibility testing can be unreliable. Stool Examination of stool specimens for the presence of AFB can be useful in identifying patients, such as those with AIDS, who may be at risk for developing disseminated mycobacterial disease resulting from MAC. equinum, which does not penetrate hair. They are distinguished from living cells by the following characteristics: • Viruses consist of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), but not both. The virus was ultimately characterized as a new hantavirus and was named Sin Nombre (“no name”) virus (SNV). Microscopic examination from culture reveals thin, delicate hyphae bearing conidia developing in a rosette pattern at the ends of delicate conidiophores. Most M. Most patients experiencing acute disease recover after about 4 days. FIGURE 27-15 Microsporum gypseum showing fusiform, moderately thick-walled macroconidia containing several cells. C. Rigid control measures have reduced that number to fewer than 100 cases annually. They are also free of bias that might be associated with reading and interpreting the tuberculin skin test. Reproduction Fungi can reproduce asexually (imperfect) or sexually (perfect). sporogenes. Detection methods include electron microscopy, ELISA (EUROIMMUN, Lübeck, Germany), and RT-PCR. Because of the adherent nature of colonies of P. spp. Septicemia c. A different organism may require a low infective dose but produces a relatively mild disease. Taxonomy Originally, viruses were classified by the diseases they caused and their host range. Subsequent manipulation of cultures may be performed with bare hands, if preferred. Clinical manifestations develop as early as 2 hours or as late as 3 to 8 days following the ingestion of food containing the botulinum toxin. The tests come in a variety of formats, including EIA, strip immunoblot, and even simple membranebased, point-of-care assays. Mycoplasma spp. Serology. It generally is considered sufficient for most clinical laboratories below the level of reference laboratory simply to screen their presumptive V. A fifth species, Plasmodium knowlesi, which causes malaria in the macaque monkey, has also been implicated in cases of zoonotic human malaria in Malaysia and the Philippines. They can infect the proximal kidney tubules and can cause acute glomerulonephritis, particularly in patients with urinary tract defects or catheterization. cayetanensis. tahir99 VRG 504 TABLE PART II Laboratory Identification of Significant Isolates 22-6 Acceptable Specimens for Anaerobic Bacteriology Anatomic Source Central nervous system Dental, ear-nose-throat specimens Localized abscesses Decubitus ulcers Sinus tracts or draining wounds Deep tissue or bone Pulmonary Intraabdominal Urinary tract Female genital tract Other Specimens and Recommended Methods of Collection Cerebrospinal fluid, aspirated abscess material, tissue from biopsy or autopsy Aspirated abscess material, biopsied tissue Needle and syringe aspiration of closed abscesses Aspirated pus Aspirated material Specimens obtained during surgery from depths of wound or underlying bone lesion Aspirate obtained by direct lung puncture; pleural fluid obtained by thoracentesis; open lung biopsy; sulfur granules from draining fistula Aspirate from abscess, ascites fluid, peritoneal fluid, tissue Suprapubic bladder aspiration Aspirate from loculated abscess; culdocentesis specimen Blood, bone marrow, synovial fluid, biopsied tissue from any normally sterile site Modified from Engelkirk PG et al: Principles and practice of clinical anaerobic bacteriology, Belmont, CA, 1992, Star. Phase 1 flagellar antigens occur only in a few serotypes and determine the immunologic identity of the particular serotype. When the female insect takes a blood meal, she ingests the amastigote stage. Reporting to Committees and Programs Depending on the setting served by the microbiology laboratory, there may be expectations of reports of infectious diseases to TABLE Class A1 A2 A3 B C 3-7 Examples of Reportable Diseases* Description Examples Diseases of major public health concern—reported immediately on recognition of a case, suspected case, or positive laboratory results Diseases of public health concern needing timely response—reported by the end of the next business day after recognition of a case, suspected case, or positive laboratory results Anthrax, botulism (foodborne), diphtheria, plague, rabies, smallpox, cholera, meningococcal disease, measles, tularemia, yellow fever Encephalitis (viral), foodborne disease outbreaks, hepatitis A, Legionnaires’ disease, pertussis, syphilis, tuberculosis, typhoid fever, vancomycin-resistant S. Both organisms attach to the intestinal mucosa of the small intestine. Citrobacter Earlier classifications of the family Enterobacteriaceae included the genus Citrobacter within the tribe Salmonelleae, which formerly consisted of the genera Salmonella, Citrobacter, and Arizona. Figure 22-11 depicts the appearance of colonies as seen through a dissection microscope. Bifidobacterium spp., brevibacteria Bordetella, Haemophilus spp. A case of communityacquired lobar pneumonia and pulmonary abscess was described in a 65-year-old patient; the lung biopsy and thoracentesis fluid grew R. Anaerobic chambers are available as sealed glove boxes and gloveless chambers. The most common mechanism for evading phagocytosis that is used by many different microorganisms is that of having a polysaccharide capsule on the surface. True pathogens are organisms recognized to cause disease in healthy immunocompetent individuals. Noninvasive diagnostic procedure; EIA antigen detection is 80% accurate, PCR, 98% Tissue culture isolation and direct immunofluorescence are relatively new and need further evaluation. The latex agglutination test is recommended for routine use in most clinical microbiology laboratories. Leo M, et al: Evaluation of Entamoeba histolytica antigen and antibody point-of-care tests for rapid diagnosis of amebiasis, J Clin Microbiol 44:4569, 2006. The rash appears on all parts of the body, but lesions are present in greater concentration on the head and limbs (centrifugal distribution), including the palms and soles. This will lead to faster treatment and better patient outcomes. Because obligate anaerobes can be overgrown by more rapidly growing facultative organisms present in the specimen and killed by their toxic, metabolic byproducts, THIO serves only as a backup source of culture material (e.g., in case there is no growth on plated media because of a jar failure or presence of antimicrobial agents in the specimen); chopped meat carbohydrate broth can be used in place of THIO; broth cultures should never be relied on exclusively for isolating anaerobes from clinical material Supports growth of almost all obligate and facultative anaerobes, best for gram-negative bacteria Supports growth of Bacteroides and Prevotella spp.; yeasts and kanamycin-resistant, facultative, gram-negative bacilli will also grow Supports growth of almost all obligate anaerobes (gram-positive and gram-negative) and gram-positive, facultative anaerobes Supports growth of almost all obligate anaerobes (gram-positive and gram-negative) and gram-positive, facultative anaerobes Supports growth of almost all types of bacteria; in THIO, obligate aerobes and microaerophiles grow near the top, obligate anaerobes at the bottom, and facultative anaerobes throughout the broth Engelkirk PG, et al: Principles and Practice of Clinical Anaerobic Anaerobic Bacteriology, Belmont, CA 1992, Star Publishing Company, Inc. Conidiophores occur singularly or can be in clusters (Figure 27-44). Six to 12 room air changes per hour effectively remove 99% or more of airborne particles within 30 to 45 minutes. Repeated or heavy infections cause inflammation because of mechanical irritation, fever, diarrhea, pain, fibrotic changes, or obstruction of the bile duct. In this case, the arthropod vector, the body louse, can die of the rickettsial infection, and humans act as a natural reservoir. Eastern Maine Medical Center Bangor, Maine Lester Pretlow, PhD, C(ASCP), NRCC(CC) Associate Professor and Chair Department of Medical Laboratory, Imaging, and Radiologic Sciences Georgia Regents University Augusta, Georgia Connie R. Prevotella, Veillonella spp. cholerae non-O1, all of which share a common flagellar (H) antigen and somatic (O) antigen. On Bordet-Gengou agar, colonies of B. The Anoxomat (Advanced Instruments, Norwood, MA) is a gas evacuation system that creates an anaerobic environment within 3 minutes (Figure 22-9). Nucleic acid hybridization tests can detect viruses from various clinical specimens. This usually is accomplished by meticulous preparation of the venipuncture site with a bactericidal agent, such as tincture of iodine, iodophor or, more recently, chlorhexidine gluconate in TABLE 505 combination with 70% isopropyl alcohol. Standard therapy for all leishmanial infections is pentavalent antimony compounds. Antigenic variation in the spirochetes that cause relapsing fever makes the serodiagnosis of their diseases difficult and impractical. The actual incidence may greatly exceed these numbers. enterocolitica, have caused intestinal disease has not been found. Clinically, they have been isolated from the eye of a newborn, who had acquired the infection nosocomially via a water source. Is the necrotic tissue or exudate black? It places bacteria into one of two main groups: gram-positive (blue to purple) or gram-negative (pink) (see Figure 1-6, A and B). Some data suggest human to human transmission and the possibility of human reservoirs. The operculated, unembryonated egg, passed in human feces, must reach water to mature. The epimastigote and promastigote stages are seen in the insect vectors. Infections Caused by Legionella Clinical manifestations of Legionella infections include febrile disease with pneumonia (legionnaires’ disease), febrile disease without pulmonary involvement (Pontiac fever), and asymptomatic infection. • When do the maintenance personnel need to worry about fungi? Anaerobic Bags. An alternative to an anaerobic chamber or jars is the use of anaerobic bags or pouches (Figure 22-10). but occurs within the cytoplasm of epithelial cells of the small intestine. Flood the slide with crystal violet, and allow it to stand for 30 seconds. The genus Advenella contains one species, A. Wang CM, et al: Clinical characteristics of infections caused by Roseomonas species and antimicrobial susceptibilities of the isolates, Diagn Microbiol Infect Dis 72:199, 2012. Miscellaneous Species The remaining Bordetella spp. Adenoviruses also produce cell rounding (Figure 29-4) on a number of cell types, including diploid fibroblasts, HEp-2, A549, and PMK, but this is usually larger than that caused by enteroviruses. DNA is then transferred from one cell to the other. • Ground-glass or breadcrumb-like colonies of long, slender, gram-negative rods with pointed ends are usually Fusobacterium nucleatum (Figure 22-21). This material is expectorated or swallowed. The genitourinary tract is cleansed by voiding urine. Serratia marcescens 5. Western blot confirmation of IgM antibody presence includes reactivity for two of the three following bands—24, 39, and 41 kDa. Confirmation of IgG antibody presence is acceptable when five of the scored bands are present—18, 21, 28, 30, 39, 41, 45, 58, 66, and 93 kDa. If serology is negative 533 and symptoms are consistent with Lyme disease, a convalescent serum should be obtained and tested. They were obligately intracellular, arthropod-borne, gram-negative coccobacilli. pneumoniae causes approximately 20% of reported pneumonias in the general population and up to 50% in confined populations, such as those in military settings. Humans become infected when they inhale the aerosolized virus or come into contact with fomites. Because S. The conidiogenous cell disintegrates or breaks to release the conidia. Shigellae may also be transmitted by flies, fingers, and food or water contaminated by infected persons. aeruginosa was isolated from four blood cultures and grew relatively quickly, indicating that this could be the pathogen responsible for the patient’s symptoms. Mucor. As with other Mucorales, Mucor spp. A reassortment occurs when the genome of different influenza viruses becomes mixed into a single virion, resulting in a new strain of influenza virus. Most cases of Lassa fever are community-acquired, primarily by contact with excretions from the multimammate rat Mastomys natalensis, which sheds the virus throughout its life once infected. necrophorum in penetrating the tissue to reach the jugular veins. The secondary stage can last for several weeks and can relapse. Care should be taken to ensure that inoculated plates do not remain in the holding jar at room temperature for extended periods (not longer than 1 hour). In utero infection can cause hydrops fetalis, resulting from anemia. These organisms are rapidly growing Zygomycetes that form a cottony colony that is initially white but becomes gray with age. Although not considered a common cause of diarrheal disease, this organism nevertheless has been found in patients with abdominal pain and diarrhea who have no other intestinal pathogens present. This fungus is a known insect pathogen and is found worldwide on vegetation and in the soil. wisconsensis. Although it is not conclusively proven, fresh groundwater is the likely source of many infections. Typhoid fever develops approximately 9 to 14 days after ingestion of the organisms. Discuss the unique growth cycle of Chlamydia, describing elementary and reticulate bodies. damsela), monobactams, carbapenems, and fluoroquinolones. A common one is the nematode parasite Strongyloides stercoralis, which can be seen in the sputum and bronchoalveolar fluid of patients with a hyperinfestation syndrome. In endemic areas, individuals can acquire low-level immunity that increases resistance to infection and/or decreases severity of symptoms. Overall, however, most provided a satisfactory substitute for the standard PVA containing mercuric chloride. Saprobic fungi are most problematic in the immunocompromised host. The Gram stain reaction and morphologic appearance of the organism aid in the presumptive identification of isolates, as shown in an algorithm in Figure 22-12. bovis M. (See also the section on ability to resist phagocytosis in Chapter 2B, Pathogenesis of Infection.) Cell Appendages. The flagellum is the organ of locomotion. More than 2000 descriptions of viruses can be found in the Universal Virus Database of the International Committee on Taxonomy of Viruses (VirusClassification.asp). An interesting association between A. smegmatis group *Some M. mosselii, P. Salpingitis can lead to scarring and dysfunction of the oviductal transport system, resulting in infertility or ectopic pregnancy. Acyclovir was administered after culture results were received. As new techniques become available or old techniques are replaced, the technologist needs to relay that information to the IPCP. Colonies of Shewanella spp. Presumptive Identification of Gram-Negative Anaerobes Using Gram stain results, growth characteristics on primary plating media (e.g., nonselective anaerobic blood, BBE, KVLB), and a few rapid tests, many gram-negative anaerobes can be presumptively identified, often within 24 hours of inoculation (Table 22-14). have been linked with amebic keratitis in soft contact lens wearers and with cutaneous infections in patients with acquired immunodeficiency syndrome (AIDS). A CF test had been the traditional assay most often used for C. Phase 2 flagellar antigens occur among several strains. Steroids are given only in rare cases. metschnikovii, and some V. Babesia spp. To appreciate and understand the concepts involved in the pathogenesis of infectious diseases, knowledge and understanding of the host-pathogen relationship is important. nov., Cronobacter turicensis sp. Failure to isolate mycobacteria from patients with signs and symptoms of classic mycobacterial disease may indicate that the decontamination is too harsh. b. These mosquitoes infest more than 100 countries and bring the risk of DF to 2.5 billion people. Retrospective serologic testing of sera from patients with acute respiratory disease have shown that many people previously thought to have C. It is distributed worldwide but especially in warm, arid areas such as Mexico and also in moist, humid regions such as Brazil, Uruguay, and South Africa. diminuta is acquired by ingesting fleas that contain the infective cysticercoid. ovale, and P. neoformans, targeting primarily compromised patients. Compare and contrast the different levels of mycobacterial laboratory testing, and cite the reasons why smaller volume laboratories should consider not performing full identification and susceptibility testing on mycobacterial isolates. Doubleimmunodiffusion is probably the most commonly used method. FIGURE 27-8 Sexual reproduction occurs by the fusion of compatible nuclei and subsequent production of a zygospore. The WHO estimates that more than 30 million cases occur annually, with approximately 500,000 deaths in Africa alone. In most laboratories, a combination of several methods is used to optimize detection and reduce cost. tuberculosis complex, the MAC, M. A Gram stain of the wound specimen revealed numerous large, rectangular-shaped, gram-positive bacilli, with no spores and very few leukocytes. The microfilariae of W. Serum immunoglobulin G (IgG) and other antibodies effective against microorganisms would be suppressed, which would make the individual more susceptible to pathogenic species. coli; although EIEC do not decarboxylate lysine, more than 80% of E. Wiliams BAP: Unique physiology of host-parasite interactions in microsporidia infections, Cell Microbiol 11:1551, 2009. The microbiology laboratory may be involved in providing data for these reports. Other disks, as described later, may be used for identification purposes. colonize the ducts of hair follicles because these bacteria are able to break down the skin lipids to fatty acids. These amino acids are brought to the ribosome by transfer RNA (tRNA) molecules that “translate” the codons. General Characteristics Case in Point A group of two dozen retirees from the tobacco industry arranged to go on a 2-week cruise of the Caribbean islands. Because they lack enzymes, ribosomes, and other metabolites, they “take over” host cell function to reproduce. The term cellular fatty acid refers to fatty acids and related compounds (e.g., aldehydes, hydrocarbons, dimethylacetals) present within organisms as cellular components. testosteroni bacteremia, accompanied by endocarditis and signs of peripheral embolism. Describe two other bacterial cell wall types, and give microbial examples of each. Plasmids are located in the cytoplasm of the cell and are self-replicating and passed to daughter cells, similar to chromosomal DNA. catarrhalis is discussed in Chapter 18 because of its phenotypic similarity to the Neisseria. Table 20-6 summarizes the clinical significance of Campylobacter, Arcobacter, and Helicobacter organisms. With the acid-fast stain, the organism stains as a bright red sphere, which distinguishes it from yeasts, which stain green. When such an error occurs, the results may not be reported until the discrepancy is resolved and the procedure is repeated with acceptable quality control results. Other groups at risk include closed-in populations such as prisoners, college students, and m c mv FIGURE 25-3 Electron micrograph of Mycoplasma pneumoniae attaching by specific attachment features to ciliated trachea (×100,000). A coinfection with HIV can result in false-negative serologic test results. tuberculosis • Level 3. The key terms include abbreviations used in the text; this places abbreviations where students can easily find them. Conidiophores bear dark, multicelled conidia on sympodial conidiophores (Figure 27-52). At least 18 humans acquired the disease via contact with birds that year, and six deaths were reported. Epidemiology P. Issues to Consider After reading the patient’s case history, consider: ■ Factors that predisposed this patient to his current condition ■ Role of the host innate and acquired immunity in protecting the host from infection ■ Significance of microbial virulence factors in promoting infection Key Terms Adaptive immunity Adhesin Anamnestic immune response Antibody Bactericidal effect Bacteriocin Chemotactic agent Chemotaxis Complement Diapedesis Dissemination Endotoxin Exotoxin Fimbriae Glycolysis Humoral immune response Iatrogenic infection Immune response Immune system Immunoglobulin A (IgA) Immunoglobulin E (IgE) Immunoglobulin G (IgG) Immunoglobulin M (IgM) Immunoglobulins Innate immunity Interferon Lactoferrin Leukocidin Lymphocyte Lymphokine Lysozyme Opsonin Opsonization Panton-Valentine Pathogenicity Phagocyte 5. An example of a commonly used primary cell culture is primary monkey kidney (PMK) cells. At maturity the spherules (30 to 60 µm) produce endospores by a process known as progressive cleavage; rupture of the spherule wall releases the endospores into the bloodstream and surrounding tissues. Evaluate the vaccine program for influenza. Higher antibody titers can be seen with heat-treated organisms; however, less cross-reactivity occurs with formalin preparations. Compare and contrast each group of organisms with respect to macroscopic and microscopic morphology, biochemical reactions, media, epidemiology, clinical infection, specimen collection, and specimen transport. Care must be taken when using this process because much variability exists among manufacturers and even in different lots of the stain prepared by the same manufacturer. Although this species is the most common mold causing disease in the immunocompromised patient, there are dozens of other fungi, such as Fusarium in the Case in Point, that give the same appearance. Acute Q fever generally has an abrupt onset of an undifferentiated febrile disease consisting of high fever that can be accompanied by headaches, myalgia, arthralgia, cough and, rarely, a rash. In culture at 22° C, the organism can produce a variety of colony morphologies. hartmanni is 4 to 12 µm; trophozoites with an average size larger than 12 µm are identified as E. In some diseases caused by exotoxins (e.g., botulism, staphylococcal food poisoning), adherence is not important. It is found in two forms. cryocrescens, and K. This is sometimes used in conjunction with rapid antigen kits. Most epidemics occur in developing countries, in which it is endemic; in particu- lar, cholera is prevalent in the Bengal region of India and Bangladesh. dermatitidis is named Ajellomyces dermatitidis. Examine plates at 2 days; thereafter use stereomicroscope for typical colonies. Anaerobic cultures are routinely held 5 to 7 days to allow growth of particularly slow-growing anaerobes and at least 10 days whenever Actinomyces is suspected. In the United States, it is primarily seen in the areas around the Great Lakes. Apparently, only a very narrow range of conditions supports its growth. Erwinia and Pectobacterium Both Erwinia and Pectobacterium spp. The diagnosis of tapeworm infection is usually made by finding the eggs in feces, although proglottids can be used if passed intact. The white blood cell count was 10.2 × 103/µL (reference range, 4.8 to 10.8 × 103/µL), and the differential showed 14% eosinophils (reference range, 0% to 4%). parahemolyticus, and V. tuberculosis has the longest replication time, at 20 to 22 hours. One or two inoculated plates are placed into a bag, an oxygen removal system is activated, and the bag is sealed and incubated. Today, the most common cause of bacterial gastroenteritis worldwide is Campylobacter jejuni. Mobile Genetic Elements Certain pieces of DNA are mobile and may jump from one place in the chromosome to another place. If yes, describe the typical patient symptoms and possible complications. A positive KNO3 assimilation result turns the medium blue, and a negative result turns the medium yellow. All except C. jejuni and other enteric campylobacters. The organisms appear as small, delicate, pleomorphic ring-form trophozoites, about 1 to 2 µm in length, with a prominent chromatin dot and faintly staining cytoplasm. Deckers N, Dorny P: Immunodiagnosis of Taenia solium taeniosis/ cysticercosis, Trends Parasitol 26:137, 2010. Although most treated patients completely recover, long-term disability and even death can occur. Determine whether the implemented activities have an impact on the outbreak. The sediment is inoculated onto culture media. furfur can be identified by visualizing skin scrapings from characteristic lesions in a potassium hydroxide (KOH) preparation or by observing yellow fluorescence with a Wood’s lamp on examination of the infected body site. dumoffii Data from Edelstein PH: Legionella. The MOMP is a transmembrane protein that contains both species-specific and subspecies-specific epitopes that can be defined by monoclonal antibodies. antigen extract is used for suspected allergic bronchopulmonary aspergillosis, atopic dermatitis, and allergic asthma. avium, M. Add 1 to 2 drops of working calcofluor white solution or solution A (Fungi-Fluor) to fixed smear or imprint for 1 to 2 minutes. Ten species of Vibrio have been implicated in human infection and most are agents of diarrheal disease. This was problematic, because most adult patients have antibodies to HSV-1. grow readily on most media routinely used in the clinical laboratory. For example, human immunodeficiency virus (HIV) continues to devastate entire continents, effectively reducing large portions of generations. Both have lower sensitivities in the primary stage, but approach 100% in the secondary stage of syphilis. Infection is often asymptomatic, but the very young, older adults, and immunosuppressed patients are at greatest risk of symptomatic infection. An SPS-sensitive, gram-positive anaerobic coccus can be presumptively identified as Peptostreptococcus anaerobius, whereas an SPS-resistant, spot indole–positive, gram-positive anaerobic coccus can be tahir99 VRG 514 PART II Laboratory Identification of Significant Isolates Gram reaction Positive Negative Cocci Bacilli Clostridium-like SPS disk Branching or diphtheroid Cocci Bacilli Nitrate disk V + Nitrate disk + No disks Nitrate disk K + CL + Nitrate disk + Bile disk FIGURE 22-14 Disks to add to the pure culture-subculture plate. The broth method gives results more quickly than the agar method and can be determined by several of the continuous monitoring systems. Embryonated eggs are passed in human feces and ingested by the intermediate host. The Salmonella strains associated with gastroenteritis are usually strains found in animals; most such strains in the United States are members of S. In 2011, there were 190 cases from broiled chicken livers, 136 from ground turkey, 106 from imported papaya, and 43 from pine nuts. tahir99 VRG CHAPTER 22 Anaerobes of Clinical Importance Robert C. pecorum have been associated with human disease. valuable with isolates from India and Bangladesh, in which resistance to O/129 is common in V. The PMN also has receptors on the cell membrane for some complement components that stimulate cell motion, the metabolic burst, and secretion of the lysosome contents into a phagosome. They are not essential for bacterial growth, so they can be gained or lost. However, the absence of leukocytes does not rule out the possibility that anaerobes are contributing to the process because some anaerobes, such as Clostridium perfringens, produce enzymatic virulence factors that destroy neutrophils, macrophages, and other cells. Detection is accomplished by addition of a reagent, which is cleaved by the enzyme produced in virus infected cells and results in the formation of a blue color, which is easily seen by light microscopy. Opsonization can be accomplished by three different types of responses: (1) IgG1 or IgG3 binds to the organism; (2) the antibody response is insufficient for opsonization, but complement is fixed on the surface of the organism; or (3) the alternative complement pathway is activated by the endotoxin or polysaccharides of the organism. The shigellae are nonmotile; therefore they lack H antigens. One or both ends of the organism have hooks rather than tapering off. micdadei is suspected. Antimicrobial Susceptibility Although most cases of plesiomonad gastroenteritis are selflimiting, antimicrobial therapy is indicated for patients with severe or chronic disease. Laboratory personnel must use appropriate safety equipment and following established procedures. (Courtesy Jean Barnishan.) sheep blood agar (SBA). Therefore administration of additional antimicrobials such as azithromycin and ciprofloxacin may be necessary. These larvae enter the insect proboscis and are introduced into human circulation when the insect feeds. ozaenae is highly associated with the presence of plasmid-mediated ESBLs, contributing to the large numbers of antimicrobial-resistant hospitalacquired infections seen today. NAATs, such as the PCR assay, have also been successful in diagnosing infections; whole blood and buffy coats are often successful in detecting the organism. Genera in this group include Trichophyton, Microsporum, and Epidermophyton. West African sleeping sickness, caused by T. Conidia are somewhat barrelshaped, formed singly on simple short conidiophores (Figure 27-51). The ameboid form, which may be seen in diarrheic stools, is 3 to 8 µm and often lacks a vacuole but possesses several pseudopods. Blastomyces dermatitidis Clinical Manifestations. Blastomycosis is most prevalent in middle-aged men, as are other systemic mycoses, presumably because of men’s greater occupational and recreational exposure to the soil. Healthy individuals have CD4+ counts gp120 gp41 p24 p18 Lipid envelope RNA Reverse transcriptase FIGURE 29-16 Human immunodeficiency virus (HIV). It is imperative that the laboratory technologist knows which infectious diseases are reportable, to what agency they are to be reported, and in what time frame they are to be reported. cepacia complex is difficult and when a commercial system is used, confirmation should follow with supplemental biochemical, MALDI-TOF, or molecular methods. The stock solution is stable for 1 year at room temperature. Semiselective BCYE agar contains polymyxin B, anisomysin, and either vancomycin or cefamandole. The oocyst wall does not stain but often shows a faint outline because of precipitated stain, and the sporoblasts or sporocysts stain dark red. Gray patch ringworm is a common childhood disease that is easily spread among children. endemic relapsing fever infection? The contents of the spore (sporoplasm containing a nucleus, ribosomes, Golgi apparatus) are then transferred into the cell. The Salmonella spp. perstans microfilaria extend to the tip. Pigment production is typically a characteristic in those strains of environmental origin. dysenteriae type 1 remains the most virulent species, with significant morbidity and high mortality. The interferons are a group of cellular proteins induced in eukaryotic cells in response to virus infection or other inducers. denitrificans c. The general flow of information in a bacterial cell is from DNA (which contains the genetic information) to messenger RNA (mRNA) (which acts as a blueprint for protein construction) to the actual protein itself. Metagonimus yokogawai and Heterophyes heterophyes are two small flukes found in the Far East and Middle East. Cell Culture. The term cell culture is technically used to indicate culture of cells in vitro; the cells are not organized into a tissue. Name the characteristics of infectious agents that enable them to cause disease in the host. Two major types of serologic tests exist, nontreponemal tests and treponemal tests. rettgeri M. typhi, and R. can cause dysentery, species vary in epidemiology, mortality rate, and severity of disease. parahaemolyticus c. A complete blood count revealed a marked increase in neutrophils and a total white blood cell count of 33,000/mL. Some species of bacteria produce metabolic by-products that result in a microenvironment hostile to potential pathogens. Molecular techniques were subsequently developed to detect many new hantaviruses in the Americas (Table 29-6), sometimes called the New World hantaviruses. falciparum have become resistant to the drug. Immunoglobulin A (IgA) antibodies represent 15% to 20% of the total serum immunoglobulin pool. The kidneys gradually regain function as the patient recovers. In this edition, considerable changes have been made to show the vital nature of the field of diagnostic microbiology. Organisms are uniquely adapted to adhere to and replicate on ciliated respiratory epithelial cells. Antimicrobial Susceptibility Although a few strains of erythromycin-resistant B. Human milk has a high lactose concentration and maintains a pH of 5.0 to 5.5, an environment supportive of Bifidobacterium spp. The cellular elements are deposited within a discrete area for easy viewing (Figure 7-5). Box 2-1 lists the microorganisms most commonly found on the skin. Activation can cause lesions to reappear. floccosum produces only one size of conidia, these conidia are described as macroconidia because of their size. Both a and b BIBLIOGRAPHY The mycobacteria are important causes of human diseases, such as tuberculosis and Hansen disease. do not readily convert to the spherule stage at 37° C in the laboratory, they produce a variety of mold morphologies at 22° C. Benzalkonium chloride shortens the exposure time and effectively destroys many contaminants, with little bactericidal effect on the tubercle bacilli. Describe the different heat methods and their respective applications. faecalis, Int J Syst Evol Microbiol 62:671, 2012. Table 22-11 lists the fluorescence characteristics of commonly isolated anaerobic bacteria. Some organisms are gram-variable or do not stain at all. (The term fermentation is often used loosely in the diagnostic microbiology laboratory to indicate any type of utilization— fermentative or oxidative—of a carbohydrate—sugar—with the resulting production of an acid pH.) Respiration (not an act of breathing) is an efficient energygenerating process in which molecular oxygen is the final electron acceptor. When the meront ruptures, merozoites are released and penetrate other cells to continue asexual reproduction or to transform into a gamete: a microgamete or macrogamete. Colonies are moderately rapid to rapidly growing ×450). are potentially serious and difficult to treat; care should be taken when encountering fungi that are mauve. pneumoniae as a recent causative agent, warranting further evaluation. These specimens are examined immediately for motile trophozoites, and a portion of the sample is placed in PVA fixative so that permanently stained smears can be prepared for examination. Nowicki MJ, et al: Prevalence of antibodies to Trypanosoma cruzi among solid organ donors in Southern California: a population at risk, Transplantation 81:477, 2006. The sediment is used for wet mounts and permanent stained slides. Centers for Disease Control and Prevention: Update: cutaneous leishmaniasis in U.S. military personnel—Southwest/Central Asia, 20022004, MMWR 53:264, 2004. Escherichia vulneris Escherichia hermannii FIGURE 19-2 Comparison of the colony morphology of Escherichia vulneris and a yellow-pigmented Escherichia hermannii on MacConkey (MAC) agar. pneumoniae in respiratory secretions; the antibody reacts with the MOMP (Figure 24-8). Most everyone is familiar with James Watson and Francis Crick, who described the three-dimensional structure of the DNA molecule in the 1950s. Cysticercosis. Cysticercosis results when a human ingests the infective eggs of T. In normal patients, this decrease results in a short-lived anemia. The vesicular lesions on the foot (ground itch) represent sites at which filariform larvae have penetrated the skin and entered circulation. Reducing agents such as thioglycollate, cysteine, and dithiothreitol often are added to microbiologic media to obtain a low redox potential. pdf. Some additional tests necessary to identify eight clinical Vibrio spp. Infections of the glabrous skin range from mild, with only minimal scaling and erythema, to severely inflamed lesions. Horneman AJ, Ali A: Aeromonas. prowazekii (epidemic louse-borne typhus and Brill-Zinsser disease). However, some strains fluoresce colors other than brick red (e.g., brilliant red, yellow, orange, pink-orange). Pulsed-Field Gel Electrophoresis Pulsed-field gel electrophoresis (PGFE) is a strain typing technique that can be an important adjunct to epidemiologic investigations. Fusarium is a rapidly growing hyaline fungus that can develop various colors with age, ranging from rose to mauve to purple to yellow. putida cannot reduce nitrate to nitrogen gas, but they can produce acid from xylose, characteristics that separate them from the other fluorescent pseudomonads. These pathways are designed to generate pyruvic acid, a key three-carbon intermediate. The capsule offers the organism protection against phagocytosis and antimicrobial absorption, contributing to its virulence. Centers for Disease Control and Prevention: Yersinia enterocolitica gastroenteritis among infants exposed to chitterlings—Chicago, Illinois, 2002, MMWR 52:956, 2003. veronii complex, which includes A. The major reason for this development is the growing number of persons with defects in their immune systems. Lice are infected with R. The atmospheric oxygen in the container is quickly absorbed, with the simultaneous generation of carbon dioxide. (Courtesy American Society for Clinical Laboratory Science, Education and Research Fund, Inc, 1982.) appear as colorless colonies only. bovis may be positive, although this occurs rarely. The Vi antigen often blocks the O antigen during serologic typing but may be removed by heating. Evolve Student Resources are provided free with each book purchase only. A tan or slightly pink coloration is observed in some enteric campylobacter colonies. Another is the A. *DAEC causes both UTIs and gastrointestinal infections. Reasons for this low number of cultures include the following: (1) the diarrhea lasts 24 to 48 hours and people may not seek medical help; (2) people not from the immediate area may not know of the potential outbreak and the need to provide culture material; (3) people do not want to be bothered with the expense and time spent to collect cultures; and (4) the specimens may be collected too late in the infection or not transported properly, so no organisms are recovered. However, mortality rates for untreated pneumonic and typhoidal forms before the introduction of antimicrobial agents were 30% to 60%. The PMN is an end-stage cell and has a circulating half-life of 2 to 7 hours. However, the response to phagocytosed antigens is primarily by T lymphocytes that produce chemicals that enhance the activities of the phagocytic cells. Citrobacter freundii Edwardsiella tarda K/A H2S– Proteus vulgaris Citrobacter freundii Salmonella Arizonae Providencia rettgeri Morganella morganii Providencia stuartii Citrobacter spp. Bacteria that grow optimally at moderate temperatures are called mesophiles (optimal growth at 20° to 40° C). Eggs of Fasciola hepatica and Clonorchis sinensis, as well as oocysts of Cryptosporidium and Cystoisospora belli, can also be recovered. marneffei resemble those of H. Figure 28-14 shows a trophozoite of N. Variola Virus Variola virus causes smallpox, a disease that was common throughout early history (Figure 29-11). Clostridium difficile B. Fluorescent antibody stains that detect viral antigen, like those used directly on clinical specimens, can also be used to screen cell cultures before a final negative result is reported. The pathogenesis of V. Vibrio cholerae Epidemiology Vibrio cholerae O1 is the causative agent of cholera, also known as Asiatic cholera or epidemic cholera. hollisae Group 4 − + + + − + V P. Growth on MAC agar is sparse to none. Dumler JS, et al: Reorganization of the genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of Ehrlichia with Anaplasma, Cowdria with Ehrlichia and Ehrlichia with Neorickettsia, description of six new species combinations and designation of Ehrlichia canis and “HGE agent” as subjective synonyms of Ehrlichia phagocytophila, Int J Syst Bacteriol 51:2145, 2001. pneumoniae Test or Substrate Urease Indole Methyl red Voges-Proskauer Citrate (Simmons) Gelatin (22° C) Lysine decarboxylase Malonate Mucate Sodium alginate (utilization) Gas from glucose Lactose Dulcitol Organic acid media Citrate D-Tartrate K. Many children become infected with EBV and show few signs of infection. ramosum are shown in Figure 22-15. Michael Janda.) tahir99 VRG 458 TABLE PART II Laboratory Identification of Significant Isolates 20-2 Key Features for the Identification of Vibrio,

eggs. Table 19-7 shows the differentiating characteristics of medically important Proteus, Providencia, and Morganella. A facility’s selection of a level of service depends on the volume of specimens submitted, patient populations served, ability to perform the requested tests according to comfort, biological safety level, and training in performance of each requested test, and the time, effort, and funds allocated for the service. The fluid should not be spread over a larger area of the slide unless it is turbid. Initial isolation of pathogens and saprobes Recovery of pathogenic fungi Dermatophytes not usually recovered Initial isolation of pathogens except dermatophytes Primary recovery of dermatophytes At 37° C SDA BHI with blood The yeast form of dimorphic fungi and other organisms grow Dermatophytes grow poorly. To prepare a trichrome-stained smear on a fresh specimen, applicator sticks are used to smear a thin film of stool across a 1- by 3-inch slide. For each of the viral agents presented in this chapter, discuss how the virus is transmitted or acquired, infection produced by the virus, and most effective method of laboratory diagnosis. The organism is grown in pure culture in PYG broth or another standardized medium. Until breakpoints are established, the CLSI has provided Epidemiological Cutoff Values (ECVs). In severe cases, bronchitis and asthma may result. There is far less vomiting and fever than with rotavirus infections. The choice of system is influenced by a number of factors, including tahir99 VRG 508 PART II Laboratory Identification of Significant Isolates “Wound” aspirate containing the following: Escherichia coli Bacteroides fragilis Pigmented species of Prevotella Staphylococcus aureus Peptostreptococcus anaerobius Growth on plates incubated in ambiant air or CO2 incubator Growth on plates incubated anaerobically E. gondii in several ways, such as ingestion or inhalation of the oocyst from soil or water, ingestion of undercooked meat containing the cystlike structure containing bradyzoites, and congenital transmission. from nasopharyngeal swabs. In addition, the low pH resulting from long-chain fatty acids secreted by sebaceous glands ensures that relatively few organisms can survive and prosper in the acid environment of the skin. The trophozoite ranges in size from 5 to 12 µm, with the average being less than 10 µm (Figure 28-9). Recently, it has become evident that microbial biofilms are involved in the pathogenesis of several human diseases. What once was a large diverse group of gram-negative, weak to nonfermentative bacilli belonging to a single genus has been separated into several genera. Amorphous debris usually is the remains of tissue mixed with the breakdown products or fluids of acute inflammation and always should be searched for organisms. lamblia. Humans ingest the infective cysts, which excyst in the intestinal tract, and the emerged trophozoites multiply by binary fission. Examples include halophiles (salt-loving cells) in Utah’s Great Salt Lake, thermophiles (heat-loving cells) in hot springs and deep ocean vents, and the anaerobic methanogens that give off swamp gas and inhabit the intestinal tracts of animals. Enterobacter sp. A new vaccine, whose development was directed by the military which had experienced outbreaks among their personnel, became available October 2011. Uropathogenic DAEC strains are closely associated with cystitis in children and acute pyelonephritis in pregnant women. Humans acquire the infection by ingesting a copepod containing the procercoid larva, by ingesting reptiles, amphibians, or other animals containing the plerocercoid larva, or through invasion by the plerocercoid larva when the raw tissue from the second intermediate host is used as a poultice. Consequently, only the outermost portions of the urethra have a microbial population. Lake Victoria marburgvirus hemorrhagic fever begins with a febrile prodrome. Strains lacking the phage do not produce the toxin and do not cause disease. There are many types of stains, each with specific applications. ✓ Case Check 1-1 The differential ability of the Gram stain makes it useful in classifying a bacterial organism as gram-positive or gram-negative. In addition, patients only need to be seen once by a health care provider and do not need to return, as is necessary for reading a tuberculin skin test. 433 Primary Intestinal Pathogens of the Family Enterobacteriaceae Salmonella and Shigella organisms produce GI illnesses in humans; neither is considered normal biota of the human intestinal tract. gonorrhoeae, Shigella spp. Move slides to rack on acid collection container. Other pathogens (influenza virus, Borrelia spp.) circumvent host antibodies by shifting key cell surface antigens. 22-10 Interpretation of Aerotolerance Test Results Aerobe Capnophilic Aerobe Facultative Anaerobe Obligate Anaerobe + − + − + + + − − −* + + Blood agar plate incubated aerobically in a non-CO2 incubator Chocolate agar plate incubated aerobically in a CO2 incubator Blood agar plate incubated anaerobically *Haemophilus influenzae will grow on an anaerobically incubated Brucella blood agar plate but can be differentiated from an anaerobe by its growth on the chocolate agar plate incubated in the CO2 incubator. These are sometimes referred to as jumping genes. *Salmonella serovars Typhi and Paratyphi and some rare serovars fail to use citrate in Simmons medium. Patients have watery diarrhea, moderate cramps or vomiting, and little if any fever. Trauma at these sites allows anaerobes of the endogenous biota (or in some cases, soil anaerobes) to gain access to deeper tissues. For this reason, a suitable holding system should ideally be used in conjunction with anaerobic jars. The rhabditiform larvae are then ingested by the copepod. have been placed as serotypes below the level of S. It is unknown if there is a genetic predisposition or if the type of work in which the individuals engage increases exposure risk. Correlations between clinical outcome and in vitro test results have not been shown. Symptoms of tertiary syphilis include the development of granulomatous lesions (gummas) in skin, bones, and liver (benign tertiary syphilis), degenerative changes in the central nervous system (neurosyphilis), and syphilitic cardiovascular lesions, particularly aortitis, aneurysms, and aortic valve insufficiency. Bacteroides spp. Estimates indicate that 25% of the world’s population is infected FIGURE 28-57 Enterobius vermicularis egg (unstained, ×400). Cysts can persist 2 to 4 weeks in a moist environment but may be killed by drying, temperatures over 55° C, superchlorination, or addition of iodine to drinking water. Gametocytes are rounded and fill the cell. Case in Point was obtained. Clinically Significant Species A number of borreliae, including Borrelia recurrentis and Borrelia duttonii, cause relapsing fever. Infection may be manifested by headaches, symptoms resembling those seen with meningitis or a brain tumor, convulsions, or a variety of motor and sensory problems. baumannii is saccharolytic, and A. Figure 2-4 illustrates the components involved in acute and chronic inflammatory responses. Abe Baalness.) P. The incubation period of leptospirosis is usually 10 to 12 days but ranges from 3 to 30 days. Other sites of infection have been noted in cancer patients to include cutaneous, subcutaneous, and systemic disease. typhi is the oriental rat flea Xenopsylla cheopis, and the rat (Rattus exulans) is the primary reservoir. The purpose of these mechanisms is to prevent infectious agents and other particles from reaching the bronchioles and lungs. However, specimens that may contain commensal bacteria should be decontaminated and then concentrated. Lecithinase cleaves lecithin found in egg yolk, releasing insoluble fat (diglyceride) that produces an opaque zone around the colony. (these infections are often polymicrobial) Peptostreptococci, Bacteroides spp., Clostridium spp.; Prevotella bivia, P. Explain how the following factors determine the composition of the microbial flora at various body sites: • Amounts and types of nutrients available in the environment • pH • Oxidation-reduction potential • Resistance to antibacterial substances 3. pertenue, the causative agent of yaws; T. The best specimens for viral culture are aspirated secretions and nasopharyngeal washes. Pontiac Fever The nonpneumonic form of legionellosis, Pontiac fever, usually has a short incubation period of about 2 days. Most infections are mild and require no specific treatment. By definition, there are no degrees of sterilization—it is an all-or-nothing process. Slides of material from difficult sample sites, scant samples, or patients with critical illnesses should not be discarded until the culture evaluation is complete. The organism was first linked to human disease in the 1990s, when it was found in human stool specimens. Describe the current recommendations for the identification of Mycobacterium tuberculosis in the clinical laboratory. They also reach the intestinal tract via the biliary tract. Stool specimens should be collected as early as possible in the course of the illness and preferably before the administration of antimicrobial agents. Most are designed to identify anaerobes that are most frequently encountered in clinical specimens. Assist in an outbreak investigation c. Neisseria gonorrhoeae is a distant third, with 321,849 confirmed cases in 2011 and an estimated 600,000 new cases annually. • Finally, the Gram stain can serve as a valuable quality control tool. H2S, Hydrogen sulfide; KCN, Potassium cyanide; ONPG, o-nitrophenyl-β-D-galactopyranoside; TSI, triple sugar iron. Transmission of mycoplasmas and ureaplasmas in humans can occur via direct sexual contact, from mother to child during delivery or in utero, and by respiratory secretions or fomites in cases of M. typhi (endemic typhus, also referred to as murine typhus) and R. Hookworms Hookworm is one of the most common human parasites. Because many of the anaerobic organisms associated with BV are members of the endogenous microbiota of the female GU tract, culture of vaginal secretions is not performed as part of the diagnosis. Influenza B virus infections, which also can occur seasonally, are usually less common than influenza A virus infections, although epidemics of influenza B virus can occur every few years. Therefore, serologic studies are usually retrospective. tahir99 VRG CHAPTER 27 Medically Significant Fungi 607 This agent, along with other fungi in the order Mucorales, is usually found in diabetic patients suffering from ketoacidosis. pneumoniae, a distinctive feature, accounts for the moist, mucoid colonies observed in this species. parapertussis using agglutinating or fluorescein-labeled antisera. They can progress to gummas of the skin, bones, and nasopharynx. tahir99 VRG CHAPTER 19 Enterobacteriaceae 443 FIGURE 19-12 Clear, green colonies of Shigella growing on Hektoen enteric (HE) agar. The oval cyst is 6 to 15 µm (average, 9 to 10 µm) and contains a single large karyosome and a large, well-defined glycogen vacuole. Gram Stain The Gram stain is the most commonly used stain in the clinical microbiology laboratory. or Panstrongylus sp.). Smears from Thick Liquids or Semisolids Swabs also can be used as the tool for preparation of smears from thick liquid or semisolid specimens such as feces (Figure 7-2). Clostridium spp. Compare the genomes and mode of transmission of the human hepatitis viruses. Salmonella Based on the common O antigens, Salmonella may be placed into major groups designated by capital letters. In culture, HSV replicates rapidly, and the CPE can be seen within 24 hours (Figure 29-6; also see Figure 29-2). Foods are often heated, which B FIGURE 22-1 Classification of some clinically encountered clostridia by endospore location. On SBA or chocolate (CHOC) agar, vibrios produce medium to large colonies that appear smooth, opaque, and iridescent with a greenish hue. Some laboratories prefer not to use polyvalent reagents for direct specimen examination because of these cross-reactions. As with other members of the Apicomplexa, molecular methods are often used for identification during outbreaks. Figure 28-34, B, shows lung tissue containing free tachyzoites and a number of B FIGURE 28-34 A, Toxoplasma gondii tachyzoites, identified by arrow. The next 2 years saw only a slight decrease from the peak number of cases at 44,830 in 2009 and 46,042 in 2011. Brito, MD, FACP Assistant Professor of Medicine Vice Chair for Urban Global Health, Department of Medicine Director, Infectious Diseases Fellowship Training Program Chief of Infectious Diseases Fellowship, Jesse Brown VA Medical Center Division of Infectious Diseases University of Illinois at Chicago Chicago, Illinois Nina M. fetus. Aeromonad sepsis appears to be the most invasive type of Aeromonas infection and similarly has a strong association with the species A. The causative agent of plague is: a. braziliensis complex is found primarily in Mexico and Central and South America. Tissue Infections with Cestodes Cysticercosis, sparganosis, and hydatid cyst disease are the major diseases caused by the tissue stage of tapeworms. Common risk factors for development of these infections are listed in Box 21-1. The availability of commercially prepared monoclonal antibodies has made the identification of HHV-8 infected cells in various types of lesions by immunohistochemistry more common. Grinding of tissue has been recommended, but this process might destroy fragile fungal elements, particularly if a zygomycete is present. This frequently occurs when the organ recipient is seronegative for T. Further immunologic and DNA probe studies supplied addi tional evidence, such as differences in epitopes of the galactosespecific binding lectin, differences in surface antigens, and differences in gene expression. Most of the cases were in Indiana and Ohio and linked to farm activities, livestock shows, or county fairs. The patient had a diagnosis of acquired immunodeficiency syndrome (AIDS). are pleomorphic, weakly staining, gram-negative bacilli that are approximately 1 to 2 µm × 0.5 µm in size. The potential for the virus to adapt to the human host, through genetic reassortment or adaptive mutation, remains an important concern for future influenza seasons. Samples that should be collected without viral transport media include blood, bone marrow, CSF, amniotic fluid, urine, pericardial fluid, and pleural fluid. Pyrazinamidase data (Wayne method) from both Wayne and Hawkins. neoformans. Although much debate exists regarding antifungal susceptibility testing, it is gaining popularity with more laboratories. Common morphotype descriptions and the most prevalent associated species are listed in Table 7-4. Although still considered rare in the United States, the number of reported cases of babesiosis recently has increased, and the disease may threaten the U.S. blood supply. Options for electronic data management are also available. Laboratories wishing to use MIF for C. Without activation by microorganisms and the supporting action of antigenpresenting cells and cytokines, cell-mediated immunity would not develop normally. Wright-Giemsa (modified) is a rapid stain for smears and imprints to stain fully background materials and cells and a wide variety of microorganisms. difficile–associated disease (CDAD). shigelloides can be presumptively differentiated from similar genera with several key tests (see Table 20-2). Other laboratories limit their virology services to viral serology—determining the patient’s immune response to viruses—rather than detecting the viruses directly. Free verotoxins present in stool specimens have been detected in samples that yielded negative culture results. Note that the dark hyphae seen in the tissue section in Figure 27-4, B, is dark-colored because of stains that enhance visualization of fungal elements in tissue and not because of melanin. The viruses cannot be grown in culture, so diagnosis relies on electron microscopy, immune electron microscopy, and RT-PCR. winghamensis. In addition, many serologic tests used by reference laboratories such as the Centers for Disease Control and Prevention (CDC) are not commercially available. Note the coccoid nature of the cells as well as the pleomorphic forms (×1000). Toxoplasma gondii Toxoplasma gondii is an obligate intracellular parasite found in mammals worldwide. B. Examples of phenotypic characteristics include macroscopic (colony morphology on media) and microscopic (size, shape, arrangement into groups or chains of organisms) morphology, staining characteristics (gram-positive or gram-negative), nutritional requirements, physiologic and biochemical characteristics, and susceptibility or resistance to antibiotics or chemicals. Once the organism dies and releases larval antigens, there is an intense host inflammatory reaction that leads to tissue damage in the area. Reiter syndrome (urethritis, conjunctivitis, polyarthritis, and mucocutaneous lesions) in adults is believed to be caused by C. This information may be helpful in determining whether a GI disease is toxin-mediated or an invasive process. Some investigators have identified genetic influence, poor nourishment, and excessive sweating as other factors that contribute to the overgrowth of the organism on the skin. Once in the vasculature, they shed their forked tails and are referred to as schistosomula. Studies have shown a concurrence between the ELISAs and MIF test results. Table 29-2 lists recommended specimens to be collected for viral diagnosis according to the body site affected. shigelloides infection in patients with human immunodeficiency virus (HIV) infections are increasing, as are associations with inflammatory bowel disease. These structures subsequently develop into sporoblasts that differentiate into sporonts. tahir99 VRG 576 PART II Laboratory Identification of Significant Isolates BOX 26-2 Acceptable Specimen Collection Mycobacteria may be recovered from a variety of clinical specimens, including respiratory specimens, urine, feces, blood, CSF, tissue biopsies, and aspirations of any tissue or organ. This method is reported to be better than other alkali decontamination procedures when P. It is believed that antibodies produced during a Campylobacter infection bind to gangliosides found on peripheral nerves. Today, the most common diagnostic approach for the identification of coronaviruses is amplification and detection of virus-specific RNA. Centrifugation-Enhanced Shell Vial Culture. The shell vial culture technique can more rapidly identify viruses than the traditional cell culture method. DFA should not be used as a replacement for culture. The treponemal tests detect antibodies specific for treponemal antigens. The eye is continually exposed to microorganisms, which means this organ has some highly developed antimicrobial mechanisms. Antigenic Structure Little is known about the antigenic structure of Vibrio except for V. Epidemiology Treponema pallidum subsp. Sorbitol-negative and MUG-negative colonies are subsequently subcultured for serotyping using E. Purulence Criteria Less than 25 PMN leukocytes (WBCs) per LPF and none or few (i.e., 1000 bp for DNA or 1000 bases for RNA) are typically separated best in lowpercentage agarose gels, such as 0.8% to 1%. Infections caused by Yersinia spp. Role of the Microbial Flora in the Pathogenesis of Infectious Disease Some organisms that make up the usual microbial flora are actually parasites that live off the host’s nutrients, but in most cases they provide some benefit to the host, creating a symbiotic relationship with the host, as mentioned earlier. Samples must be transported quickly to the laboratory. A summary of the principles of the various biochemical identification methods for gram-negative bacteria is described in Chapter 9. Opportunistic saprobes 8. Measles Virus The measles virus is an enveloped virus classified in the genus Morbillivirus. SLE is transmitted to humans by the bird-biting Culex mosquitoes. For example, antibodies are produced by B lymphocytes in response to bloodborne organisms and aid in their elimination. Many laboratories establish an algorithm that takes into account the types of parasitology examinations routinely performed (e.g., concentration and permanent stained smear), patient population, and specific criteria (e.g., travel, symptoms, immune status, inpatient or outpatient classification) to determine if a single specimen or multiple stool specimens should be examined. Those making these requests may be in home health care, extended care, or communal living settings. They include lactoferrin, which chelates iron and prevents bacterial growth; lysozyme; and several basic proteins. Following the secondary stage, patients enter latent syphilis, when clinical manifestations are absent. The clinician and laboratory scientists should collaborate to make sure that the appropriate specimen is properly collected, handled, and examined. It kills these cells by contact-dependent lysis, and the subsequent release of lysozymes, superoxides, and collagenases from the neutrophil granules produces additional damage to the intestinal mucosa. tahir99 VRG CHAPTER 2 Host-Parasite Interaction require microscopic breaks in the skin surface. Use of Proper Disinfectant Covering the work surface with a towel or absorbent pad soaked in a disinfectant reduces the accidental creation of infectious aerosols. tahir99 VRG CHAPTER 19 Enterobacteriaceae Issues to Consider After reading the patient’s case history, consider: ■ The significance of this patient’s health status and medical history ■ The colony morphology feature that provides clues about the identity of the organism ■ The biochemical tests that are the most specific for identification of this organism Key Terms Buboes Diffusely adherent Escherichia coli (DAEC) Enterics Enteroaggregative Escherichia coli (EAEC) Enterohemorrhagic Escherichia coli (EHEC) Enteroinvasive Escherichia coli (EIEC) Enteropathogenic Escherichia coli (EPEC) Enterotoxigenic Escherichia coli (ETEC) H antigen K antigen O antigen Shiga toxin (Stx) Traveler’s diarrhea Verotoxin Vi antigen T he family Enterobacteriaceae includes many genera and species. The dilute iodine solution can be used to wash away the crystal violet, and no water rinse is employed. Because the skin snip is painful and poses a risk of infection, researchers are trying to develop immunochromatographic tests for parasite-specific antigens in body fluids such as urine and tears. 437 Humans are the only known reservoir of Shigella spp. (see Figure 1-6, H). Evaluate the diagnostic tests used to identify Treponema pallidum in the clinical laboratory. More serious complications, usually from infections with A. Enteroviruses can cause respiratory infections and may be recovered from the stool after the respiratory shedding has ceased. botulinum as an agent of bioterrorism. Therapy for tetanus requires the injection of antitoxin, muscle relaxants, and intensive therapy. multivorum are very similar biochemically, but S. stain faintly or not at all, giving a beaded appearance because of irregular uptake of the stain caused by the increased lipid content of the cell wall. These disks—kanamycin (1000 µg), vancomycin (5 µg) and colistin (10 µg)—are used for identification purposes and are not meant to predict treatment options for the physician (Figure 22-14). However, certain members of the usual flora are opportunists; they cause disease when their habitat is damaged, disturbed, or changed or when the host’s immune system is weakened or compromised. Most of these positive reactions occur within 24 hours. It is estimated that 20 to 40 million individuals are infected in the United States alone. Some yeasts are butter-like, and others range in texture from velvety to wrinkled. The organisms may completely erode the intestinal mucosa and enter the circulation. influenzae. Role of the Usual Microbial Flora CHAPTER OUTLINE ■ ORIGIN OF MICROBIAL FLORA Characteristics of Indigenous Microbial Flora Factors That Determine the Composition of the Usual Microbial Flora ■ COMPOSITION OF MICROBIAL FLORA AT DIFFERENT BODY SITES Usual Flora of the Skin Usual Flora of the Mouth Usual Flora of the Respiratory Tract Usual Flora of the Gastrointestinal Tract Usual Flora of the Genitourinary Tract ■ ROLE OF THE MICROBIAL FLORA IN THE PATHOGENESIS OF INFECTIOUS DISEASE ■ ROLE OF THE MICROBIAL FLORA IN THE HOST DEFENSE AGAINST INFECTIOUS DISEASE OBJECTIVES After reading and studying this chapter, you should be able to: 1. cannot be differentiated on a blood smear; therefore, the organism is reported as Trypanosoma sp. Identify the preferred stain for demonstration of the mycoplasmas. First, C. Each step must be carried out with precision. The rapidly growing species generally form colonies in 2 to 3 days, whereas most pathogenic mycobacteria require 2 to 6 weeks of incubation. (non-ureolytica) Pseudomonas aeruginosa Violet Chromobacterium violaceum (fermenter) Lavender to lavender-green (blood agar) Oxidase-Negative Stenotrophomonas maltophilia Acinetobacter spp. cholerae O1 and V. Horneman AJ, Morris JG: Plesiomonas shigelloides infections, 2012. cholerae Group 1 20-3 Key Differential Tests for the Six Groups of 12 Vibrio Species That Occur in Clinical Specimens* Growth in nutrient broth with: No NaCl added‡ 1% NaCl added Oxidase production Nitrate reduced to nitrite Myo-Inositol fermentation Arginine dihydrolase Lysine decarboxylase Ornithine decarboxylase TABLE 462 PART II Laboratory Identification of Significant Isolates tahir99 VRG TABLE CHAPTER 20 Vibrio, Aeromonas, Plesiomonas, and Campylobacter Species 463 20-4 Key Differential Biochemicals to Separate Vibrio Species in Groups 1, 5, and 6* Group 1 V. Kemple SK, et al: Fatal Naegleria fowleri infection acquired in Minnesota: possible expanded range of a deadly thermophilic organism, Clin Infect Dis 54:805, 2012. These cells are widely distributed in the body and play a central role in specific immunity and nonspecific phagocytosis (Table 2-6). DNA Detection. Several in-house DNA detection systems have been described. Serum IgA occurs primarily as two subunits (each similar to an IgG molecule) linked together by a J chain. Diarrheal outbreaks caused by EPEC have occurred in hospital nurseries and daycare centers, but cases in adults are rarely seen. Lesions become especially evident in warm months, when sun exposure is more likely. The Case Check highlights a particular point in the text that intends to help the learner connect the dots between the content under discussion, as illustrated by the case study. Endemic syphilis is transmitted by direct contact or sharing contaminated eating utensils. In cases of Pseudomonaslike infections of the eye, aminoglycosides are often the first line of therapy; therefore, it is important to obtain the correct identification as quickly as possible and/or its antimicrobial susceptibility for appropriate treatment. boydii and S. The same occurs in the eukaryotic cell, where the two subunits 60S and 40S add up to 80S. Stenotrophomonas maltophilia Stenotrophomonas maltophilia is the third most common nonfermentative, gram-negative bacillus isolated in the clinical laboratory. It is found in the Middle East and the arid, hot areas of the world. pylori infections should be administered for 7 to 14 days to eradicate the infection. Most species are generally susceptible to the vibriostatic compound O/129 (2,4-diamino-6,7diisopropylpteridine), exhibiting a zone of inhibition to a 150-µg Vibriostat disk (Oxoid, Cambridge, England) on Mueller-Hinton or trypticase soy agar (Figure 20-2). Shigella is also seen in people living in crowded and inadequate housing and in people who participate in anal-oral sexual activity. Variability in inhibition exists, depending on the concentration of the inhibitory agent and the temperature of incubation. He had a history of homosexual activities and for several years had received steroid therapy. APPLICATION The direct smear examination is a valuable diagnostic procedure for the detection of mycobacteria in clinical specimens. Temperature. The optimal temperature and range at which a mycobacterial species can grow may be extremely narrow, especially at the time of initial incubation. These viruses have a characteristic brick shape and contain a dsDNA genome. Large granules, grains, or fungal forms such as spherules or fungal mats can best be recognized at low power. The body site of the sample and the classification of the smear together determine the extent of culture evaluation. Infection of the cell occurs only if attachment is the initial event. Move slides to original staining rack. The other Bordetella spp. veronii biovar sobria, and A. Serology Clinical, radiologic, and microbiologic tests are currently used for the diagnosis of M. A concomitant striking lymph node involvement is also evident. The specimen should not be placed in a 37° C incubator, which increases the rate of disintegration of any organisms present and enhances overgrowth by bacteria. The association of K1 antigen was established when E. Compare the morphology and clinical infections of Naegleria fowleri, Acanthamoeba spp., and Balamuthia mandrillaris. The host secretes proinflammatory cytokines, leading to an acute inflammatory response and migration of neutrophils and macrophages into the tissue. • Inoculate a pure subculture plate and add appropriate identification disks. A rounded, refractile body called a nucleolus is also located within the nucleus. Weight loss, increased white blood cell counts, or elevated liver enzyme levels may be present. Dissemination to muscles may result in weakness and pain. Then the preparation is washed for about 30 seconds to remove the primary stain. holmesii have been infrequently associated with pertussis syndrome and other respiratory tract infections. Initially, a painful, blister-like, inflammatory papule appears on the leg in the area of the gravid female. ✓ Case Check 20-3 Although Aeromonas and Plesiomonas are similar to Vibrio spp., the key biochemical results used in the Case in Point were able to rule out Aeromonas and Plesiomonas as the infecting organism and aided in definitively identifying Vibrio as the pathogen causing the disease. Although diseases caused by the organisms are discussed, the emphasis is on the characteristics and methods used to recover and identify each group of organisms. There have been some reports of oseltamivir-resistant influenza A (H1N1) but it is still recommended as a primary treatment. Antibiotics may reduce a particular population of bacteria, allowing the proliferation of other organisms, such as Clostridium difficile or Candida albicans. The latent period develops as the trypomastigotes disappear from the circulation and invade the cells of the cardiac or GI system. Adenoviruses are naked icosahedral viruses with dsDNA (Figure 29-5). The trypomastigote form first circulates in the blood and lymphatic system, ultimately invading the CNS. tahir99 VRG 410 PART II Laboratory Identification of Significant Isolates nosocomial infections. In wet mounts, the oocyst appears nonrefractile, spherical, and unsporulated, with multiple internal globules or granules. Kaposi sarcoma lesions were noted, especially on the lower extremities along with thrush and herpes lesions in the A 36-year-old man was admitted to the hospital after presenting at the emergency department with a self-reported, 7-month history of numbness and weakness in his right leg. veronii biovar sobria from septicemia and meningitis, A. What is the best nonrespiratory specimen for rapid detection of Legionella? However, growth on the anaerobic subculture plate will exhibit much heavier growth than on the aerotolerance test plate. The intestinal mucous layer, host proteases and lipases, peptides such as defensin and lactoferrin, the presence of secretory IgA, and influx of mast cells that produce interleukins (ILs) such as IL-6 help prevent colonization by the organism. If at least two of the first three sputum direct smears are positive, then three specimens are often sufficient to confirm a diagnosis. influenzae may be present in large numbers, hidden within the mucus, in acute exacerbations of chronic bronchitis. Table 29-5 lists some HPV types and their clinical significance. Eggs are laid on the perianal area when the female migrates out the anus at night. pertussis are less fastidious and grow on MAC agar or media containing blood. oryzihabitans. (Giemsa stain, ×1000.) asynchronous life cycle, with rupture of the RBCs taking place at irregular intervals ranging from 36 to 48 hours. are fastidious gram-negative coccobacilli causing zoonoses and are considered potential bioterrorism agents. The number of bacteria in the intestine increases as the distance from the stomach increases. The tests currently available to determine the invasive property of EIEC are not performed in most clinical microbiology laboratories. 18. Because cell wall–damaged bacteria, antibiotic-treated bacteria, or dead bacteria may appear 135 falsely gram-negative, their shapes and sizes are critical “cocharacteristics.” The classic misleading smear example is the observation of gram-negative, lancet-shaped diplococci mixed with the predominant gram-positive forms. Children share this burden; in 2011, approximately 3.4 million children younger than 15 years had HIV-AIDS and about 330,000 newly infected children were reported. The eukaryotic cell is usually larger and contains membrane-encased organelles (“little organs”) or compartments that serve specific functions; the prokaryotic cell is noncompartmentalized. Direct transmission of EIEC from person to person via the fecal-oral route has been reported. Besides respiratory tract specimens, what clinical specimen is useful for the sensitive detection of Legionella antigen? have the ability to grow well at temperatures as high as 45° C. In Versalovic J, et al, editors: Manual of clinical microbiology, ed 10, Washington, DC, 2011, ASM Press. Rapid Tests Fluorescence. Many strains of pigmented Porphyromonas and Prevotella fluoresce brick red under long-wave (366-nm) UV light (Figure 22-13, A). falciparum cause more than 95% of infections. However, the anaerobes most frequently isolated from infectious processes in humans are those of endogenous origin. *A low-risk population is defined as one with a 10% incidence, such as those in sexually transmitted disease clinics, university or college student health centers, and emergency department patients. dematiaceous • Septate vs. All lesions change at the same time—thus, the term synchronous. R, Resistant; S, sensitive; V, variable. The most common nonfermenters isolated in clinical microbiology laboratories are P. lacunata, M. However, disseminated Pneumocystis spp. marinum, and M. saginata, and eggs of the pork tapeworm, T. An interesting aspect is introduced. Other pathogenic bacteria, such as Haemophilus influenzae and the anaerobes, are fastidious, requiring additional metabolites such as vitamins, purines, pyrimidines, and hemoglobin supplied in the growth medium. RotaTeq is a series of three oral vaccines beginning at 6 to 12 weeks of age. Rapid culture assays can be performed using IF staining of infected monolayers grown in shell vials or flat-bottomed wells of microtiter plates. Trichoderma spp. areas. It has also been shown that some individuals with Aspergillus spp. and Elizabethkingia spp. pylori infection resulting in chronic gastritis is an important risk factor for gastric carcinoma resulting in H. Viruses Single or multinuclear cells with intranuclear inclusions Enlarged cells with intranuclear inclusions or cytoplasmic inclusions Cells with dark, “smudged” nuclei Herpesvirus, measles virus Cytomegalovirus Adenovirus tahir99 VRG CHAPTER 7 Microscopic Examination of Materials from Infected Sites TABLE 7-5 Descriptions of Background Material Cells and Structures Amorphous debris (light, moderate, or heavy) Black particulate debris Charcot-Leyden crystals Epithelials with contaminating bacteria Curschmann’s spirals present (sputum) Epithelial cells (light, moderate, or heavy) Intracellular organisms Local material (light, moderate, or heavy) Mononuclear cells present Mucus (light, moderate, or heavy) Purulence (none, light, moderate, or heavy) Red blood cells Associations Necrosis, heavy protein fluid Smoke inhalation, crack cocaine Eosinophils Passage of specimen through contaminated area during collection Bronchospasm, obstruction, asthma Epithelial surface involved or adjacent to collection site Inferred association in infection Reflection of collection site Chronic inflammation Irritation of glandular surface Acute inflammation, exudation Trauma, hemorrhage elicit few polymorphonuclear neutrophils (PMNs). Sporogony is the formation of spores containing sporozoites that results from the division of a zygote. At least two slides should be prepared; slides are dried, heat-fixed, and stained on the same day of collection or stored at −70° C and heat-fixed immediately before staining. The ameboflagellate N. LPS contains three regions: an antigenic O–specific polysaccharide, a core polysaccharide, and an inner lipid A (also called endotoxin). When the carbon dioxide content of an aerobic incubator is increased to 10%, the oxygen content of the incubator is decreased to approximately 18%. External proficiency testing is available from the College of American Pathologists to assist with the laboratory’s overall quality assurance program. Bradyzoites are the slowly growing and reproducing forms found in a cystlike structure during the dormant phase of the infection. Like B. fragilis group, Porphyromonas, Prevotella, and Fusobacterium. pestis Y. The O antisera used for serogrouping are polyvalent, containing several serotypes within each group (with the exception of group D, which contains only a single serotype). Additionally, secretory antibody and phagocytic cells lining the mucosa defend the gastrointestinal tract against infection. The toxin is absorbed through the small intestine and enters the systemic circulation to reach the nervous system. Humans are the main reservoir for T. Hantaviruses endemic to Europe and Asia are called Old World hantaviruses. The College of American Pathologists requires that laboratories performing anaerobic cultures verify daily that anaerobiasis is achieved. The central portion of young colonies has a “ground-glass” appearance, light gray and granular, whereas the periphery of the colony has pink or light blue or bottle green bands with a furrowed appearance. fowleri causes an acute condition called primary amebic meningoencephalitis, which is rapidly fatal. Many anaerobic bacteria can be identified in the laboratory using presumptive identification criteria, thereby providing physicians with timely information concerning the presence of anaerobes in clinical specimens. Intestinal infections are most often caused by Enterocytozoon bieneusi or Encephalitozoon intestinalis. nonliquefaciens is the second most commonly isolated member of the genus. Treatment includes trimethoprim-sulfamethoxazole or pentamidine isethionate. duboisii is endemic in Central Africa. After release by the adult female, the eggs secrete enzymes and begin to penetrate vessel walls and tissue. alginolyticus 3. The morphology of D. This phenomenon has been seen primarily in kidney transplant patients and is thought to have spread from athlete’s foot or onychomycosis. Culture Members of the family Enterobacteriaceae are facultative anaerobes, and most clinically significant species grow at an optimal temperature of 35° C to 37° C. The citrobacters are considered inhabitants of the GI tract and are associated with hospital-acquired infections, most frequently UTIs. The three species most often isolated are C. One of the mycobacteria pathogenic for humans, M. Characteristic symptoms appear 2 to 5 days after infection. Also, the secretions of the mucous membranes and the ciliated epithelial cells of the respiratory tract promote trapping and removal of microorganisms. As previously mentioned, CIN agar can serve the dual purpose of screening for both Y. Currently, the virus cannot be recovered in cell culture. The amebic form colonizes the nasal cavity, invades the nasal mucosa, attaches to olfactory nerves, penetrates the cribriform plate, moves along the olfactory nerve to the olfactory bulb, and moves into the arachnoid space. Each year, antigenic drifts occur, caused by RNA replication errors of the virus. The analysis of ribosomal RNA (rRNA) has proved particularly useful for this purpose. gormanii, L. Sporothrix schenckii 2. A pleomorphic gram-positive bacillus that is spot indole– and catalase-positive can be presumptively identified as Propionibacterium acnes. In an outbreak investigation, surface culturing would be needed; however, such cultures should not be routinely tahir99 VRG 56 PART I Introduction to Clinical Microbiology obtained. Other virulence factors have been proposed, but their roles in disease are unclear. Strains of C. veronii biovar veronii and surgical wound infections involving the use of leeches for medicinal therapy following plastic surgery to relieve venous congestion has been noted. Salmonella gastroenteritis occurs when a sufficient number of organisms contaminate food that is maintained under inadequate refrigeration, allowing growth and multiplication of the organisms. and Cronobacter dublinensis subsp. cholerae serogroups O75 and O141 harbor the cholera toxin gene and have been associated with sporadic, cholera-like diarrhea and bloodstream infections in the United States. Colonies turn whitish gray as they age. Virulence and Antigenic Factors The virulence of the Enterobacteriaceae genera is affected by many factors, such as the ability to adhere, colonize, produce toxins, and invade tissue. This has not appeared to be the case in adults. This situation is uncommon in the United States and other developed countries, where water is purified and treated, and handling of waste is standardized. Serologic Tests. Serology is the primary method used for the laboratory diagnosis of syphilis. ✓ Case Check 24-1 In the Case in Point, the neonate presented with conjunctivitis and symptoms of pneumonia. odorifera biogroup 1 is isolated predominantly from the respiratory tract and is positive for sucrose, raffinose, and ornithine. IgA constitutes the predominant immunoglobulin class in certain body secretions, such as saliva, tears, and intestinal secretions. Compared with culture, the sensitivity of DFA testing for direct specimen examination is approximately 25% to 80%, so a negative result does not rule out Legionella infection. Rapidly growing organisms such as the Mucorales usually appear within 1 to 3 days, whereas intermediate growers may take 5 to 9 days and slow growers up to 2 weeks. are commonly isolated from nail specimens and have been implicated in pulmonary disease in immunocompromised patients. In this test, the patient is given 13C- or 14 C-labeled urea orally. Microbial pathogens of potential bioterrorism activity include: a. Tatumella Tatumella ptyseos is the only species of the genus Tatumella. An outbreak investigation b. • They all ferment glucose. Stool specimens for parasites should be collected before a barium enema, certain procedures using dyes, or the start of antimicrobial therapy. aeruginosa in frequency of isolation of all nonfermenters in the clinical microbiology laboratory. tahir99 VRG 495 496 PART II Laboratory Identification of Significant Isolates Case in Point A 45-year-old male farmer was admitted to the hospital for complications resulting from a tractor accident that caused traumatic injury to his left leg. avium-intracellulare isolates are scotochromogens. Within the species S. They can be found in soil, in freshwater and saltwater sediments, and as components of the endogenous microbiota of humans and other animals. granulosus containing the hexacanth embryo. mimicus P damsela V. These organisms are referred to as aerotolerant, or moderate, anaerobes. The papule ulcerates and when the person’s body comes in contact with water, the female worm exposes her uterus through the ulceration and releases larvae into the water. Aerotolerance Testing. The aerotolerance test determines whether a microorganism isolated under anaerobic conditions is a strict or facultative anaerobe. • They reduce nitrate to nitrite except for Photorhabdus and Xenorhabdus. Primary disease usually resolves without therapy and confers a strong, specific immunity to reinfection, which is detected by the coccidioidin skin test. Adult hookworms are rarely seen in stool specimens; the egg and rhabditiform larva are the usual diagnostic stages. bronchiseptica. In the case of trauma, either accidental or surgical, enough of the usual microbial flora found in the traumatized area may reach other areas in the body where these organisms are not part of the usual microbial flora. The life cycle of malaria is complex, with asexual reproduction taking place in human RBCs and sexual reproduction

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