

TO Public Health January 17, 2007

An Introduction to Clinical Microbiology

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Microbiologist & Infectious Disease Consultant

Objectives

1. To provide an introduction to a typical microbiology laboratory
2. To address specific microbiology laboratory test issues as they apply to public health

Who we are

- Shared microbiology service between TML (UHN & MDS) and MSH
- Serve nine Ontario hospitals (~5000 beds) and five non-hospital clients
- Approximately 35 000 specimens processed per month

Who we are

- Site:
 - 14th Floor Mount Sinai Hospital
- Website:
 - www.microbiology.mtsinai.on.ca

What we do

- Clinical Service
 - Routine Diagnostics
 - Infection Control
 - Reference Testing
- Research
- Education

What we do

- Clinical Service
 - Routine Diagnostics
 - Infection Control
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- Education

Clinical Service

A. Routine Diagnostics

- Bacteriology
- Mycology
- Virology
- Serology
- Parasitology
- Mycobacteriology

Terminology

- Bacteriology
 - Prokaryotic, single cell organisms
 - Divided into aerobic and anaerobic
 - Divided into gram-positive and gram-negative
- Mycology
 - Eukaryotic, multi-cellular organisms
 - Divided into yeast and filamentous fungi

Terminology

- Virology
 - Acellular infectious particles consisting of core of RNA or DNA surrounded by a protein coat unable to replicate without a host cell
- Serology
 - Detection of antibodies against infectious agents

Terminology

- Parasitology
 - Eukaryotic organisms
 - Divided into protozoa (e.g. *Plasmodium* spp., *Giardia lamblia*) and nematodes (i.e. worms)
- Mycobacteriology
 - Prokaryotic, single cell organisms
 - Acid-fast bacteria

Process

- Specimen collection
- Specimen receipt
- Specimen processing
- Testing
- Interpretation
- Reporting

Specimen Collection → Receipt


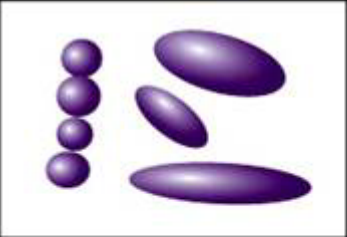
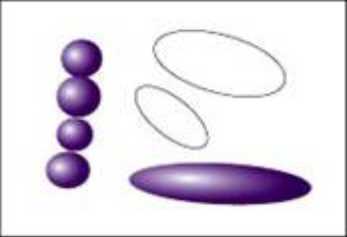
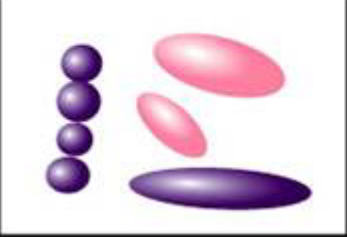
- Transport media
 - Stool cultures (Cary-Blair)
 - *Viral/Mycoplasma/Chlamydia* (transport media)
- Transport temperature
 - Sterile Site Specimens (room temp/incubate)
 - Nonsterile Site Specimens (room temp/4°C)
 - Virology/Serology/NAAT (4°C)

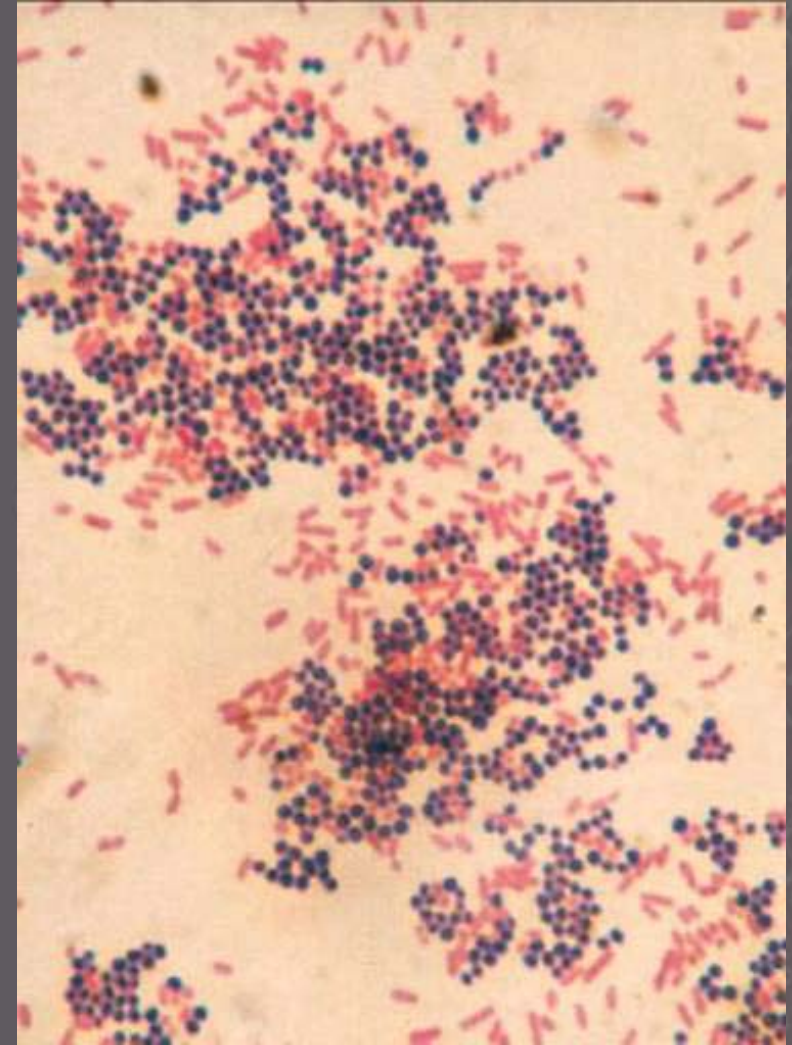
Tests Overview

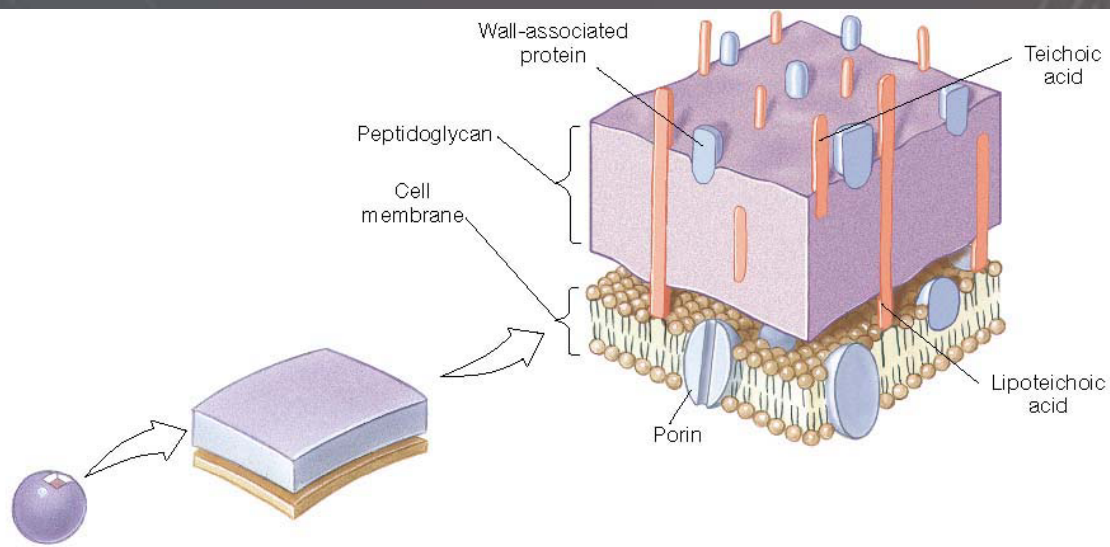
- Direct detection
 - Stained smears, EM, LA, DFA, EIA, NAAT
- Culture
 - Media, Cell lines
- Serology
 - EIA, IFA, Immunoblots
- Susceptibility Testing

Direct Detection

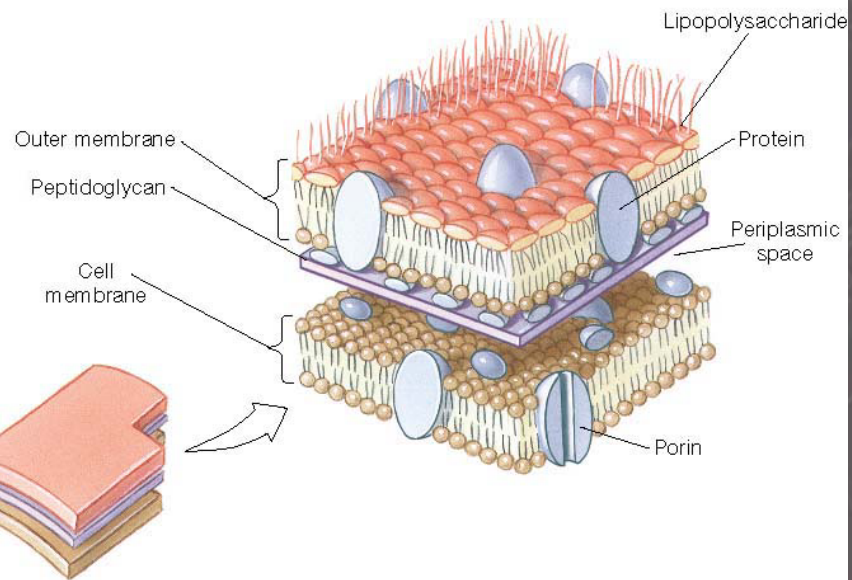
The Gram Stain

Steps in Staining	State of Bacteria
	Step 1: Crystal violet (primary stain) Cells stain purple.
	Step 2: Iodine (mordant) Cells remain purple.
	Step 3: Alcohol (decolorizer) Gram-positive cells remain purple; Gram-negative cells become colorless.
	Step 4: Safranin (counterstain) Gram-positive cells remain purple; Gram-negative cells appear red.



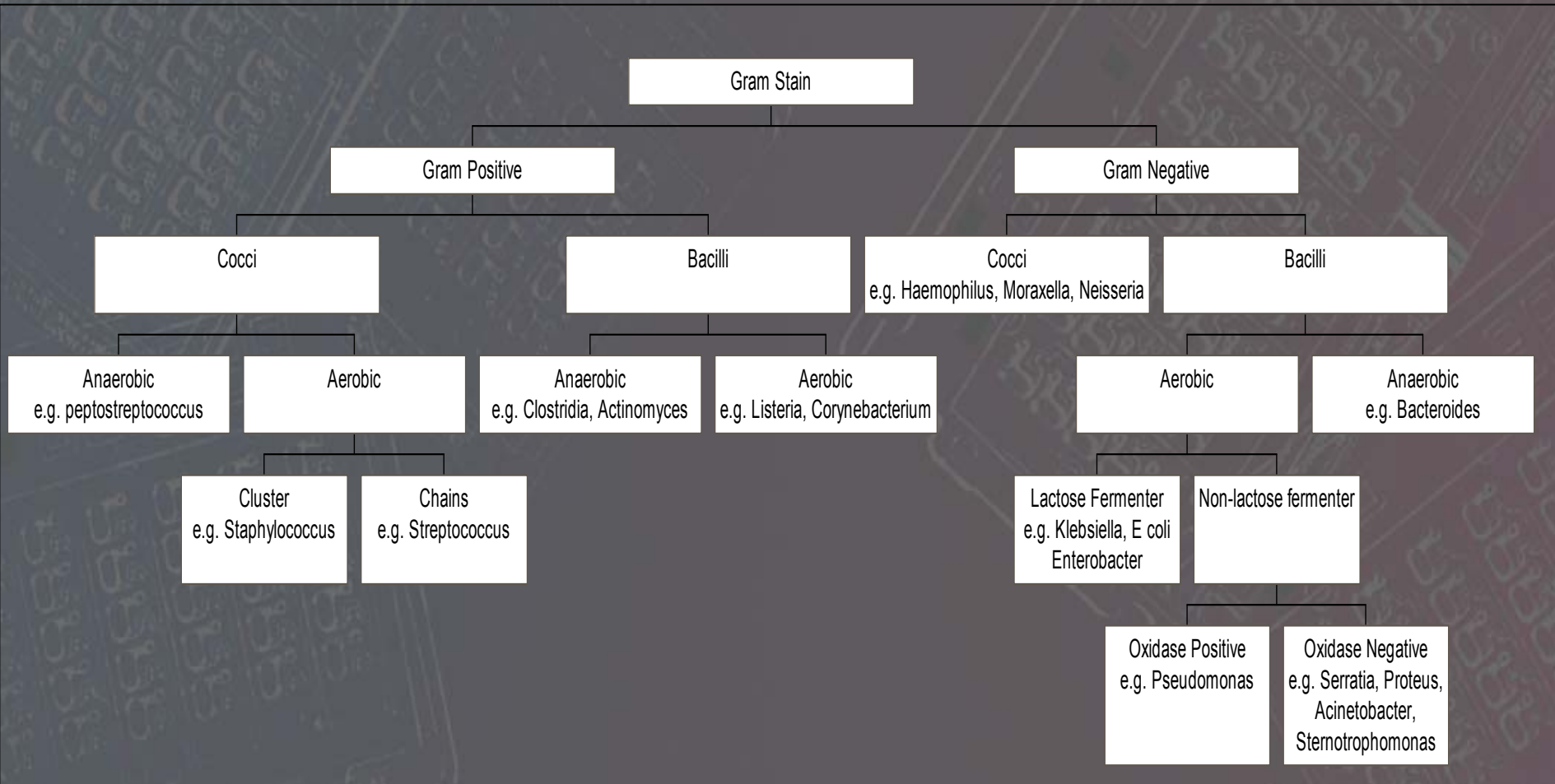


(a) Gram-positive bacteria

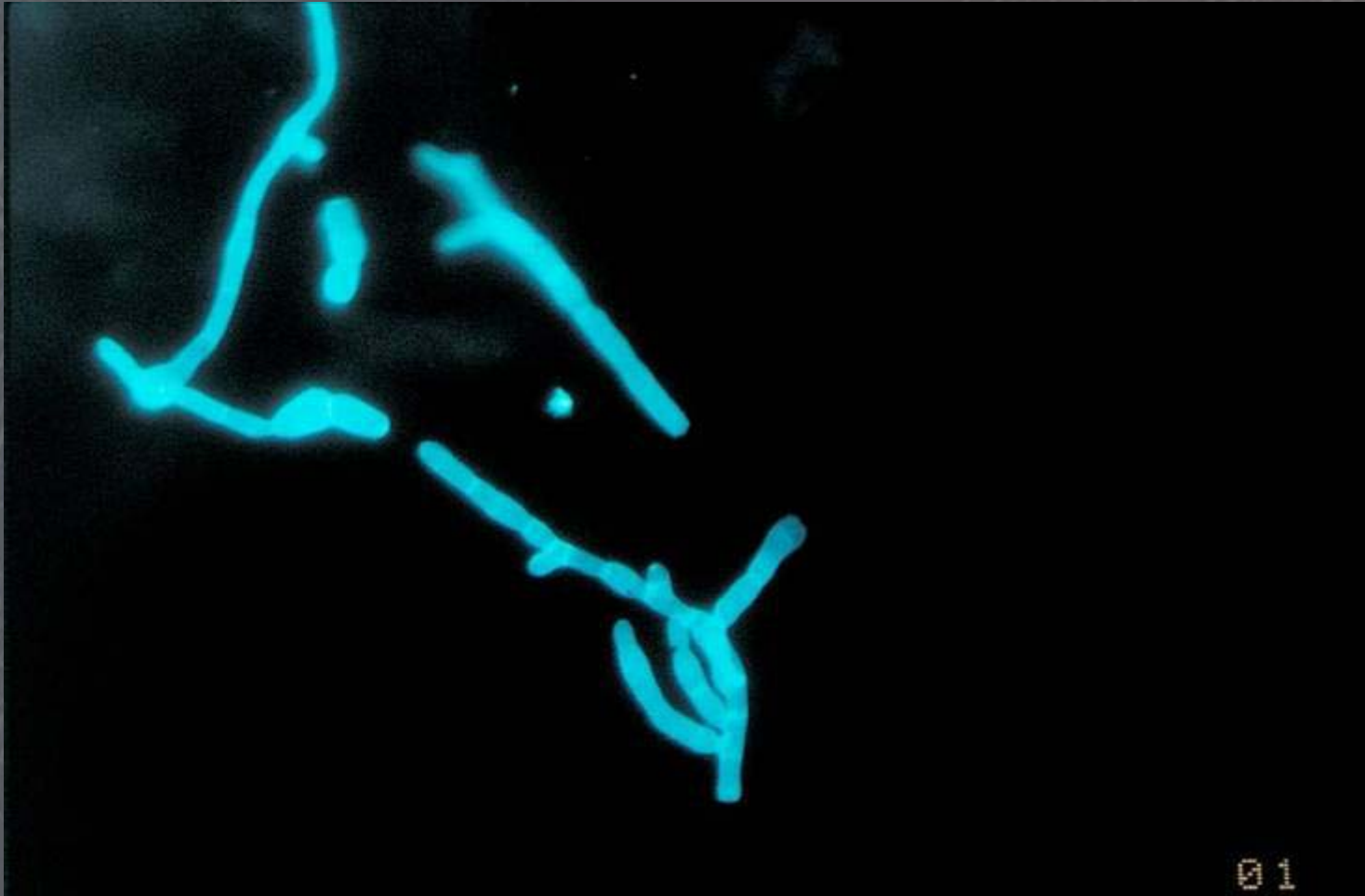


(b) Gram-negative bacteria

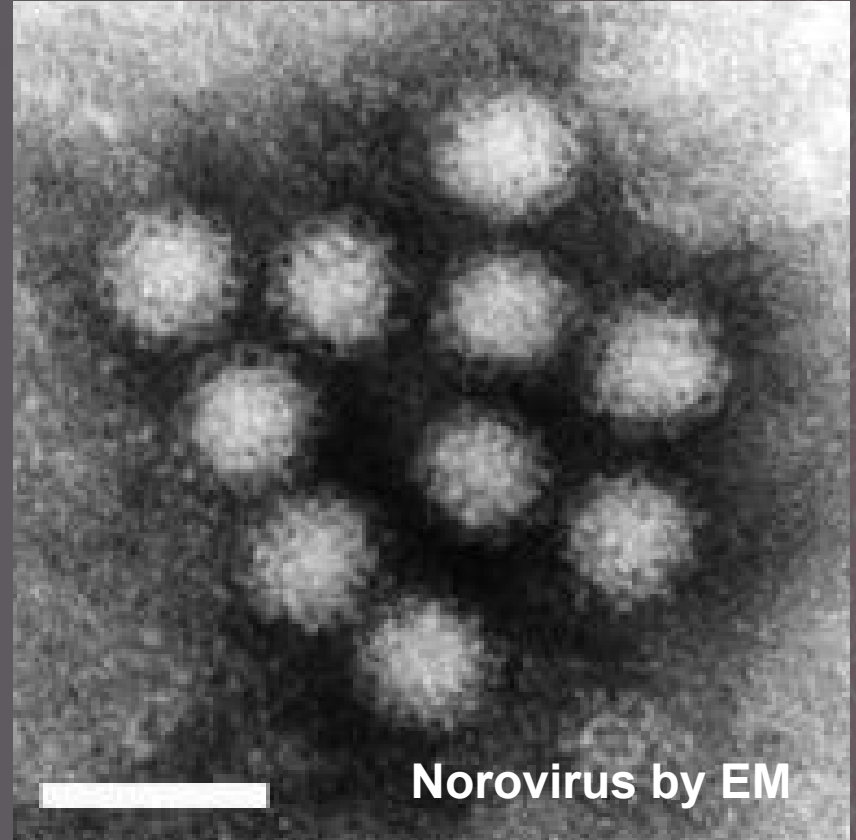
Bacterial Classification



Calcofluor White



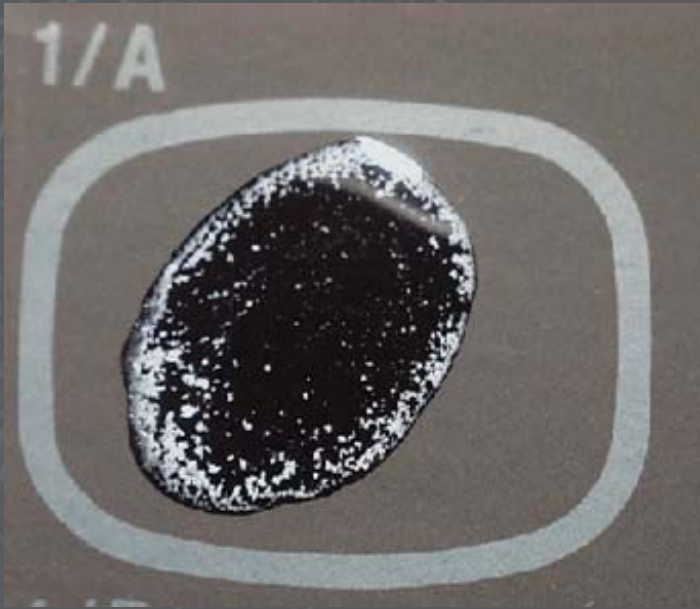
Electron Microscopy



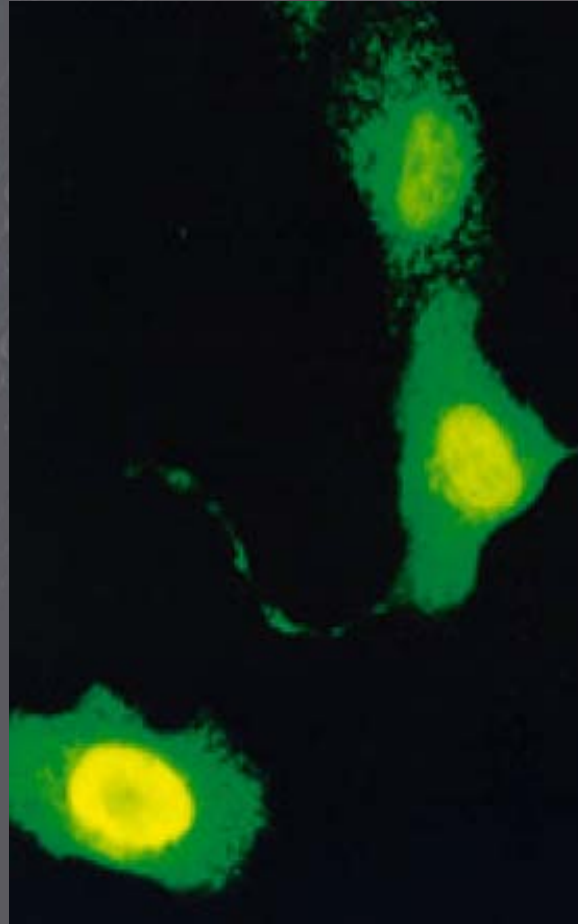
Norovirus by EM

Latex Agglutination

- Cryptococcal Antigen (CRAG)



DFA/IFA



Membrane EIAs

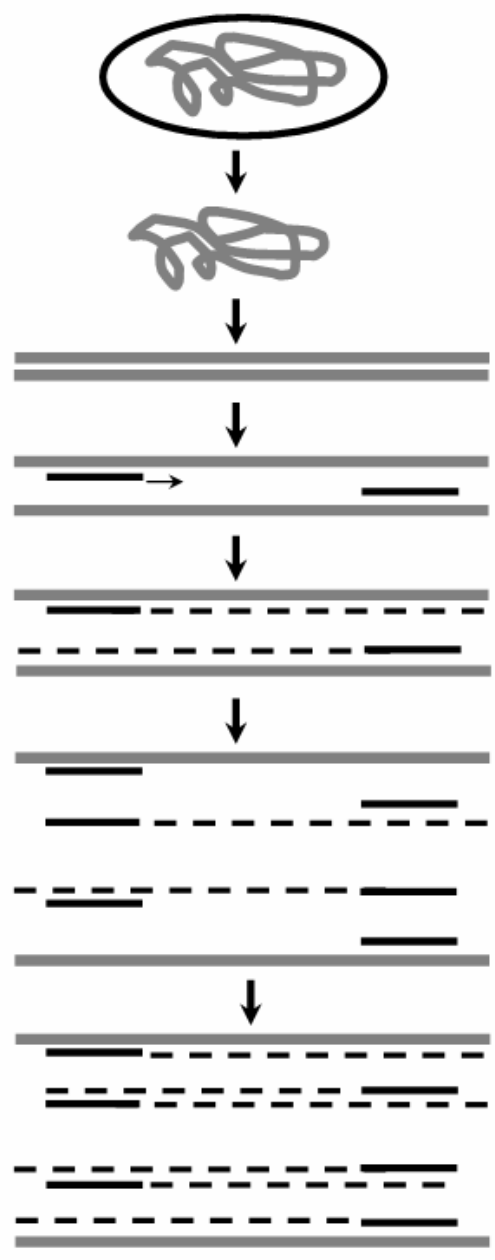


NAAT

- PCR most common
- Real-time instruments



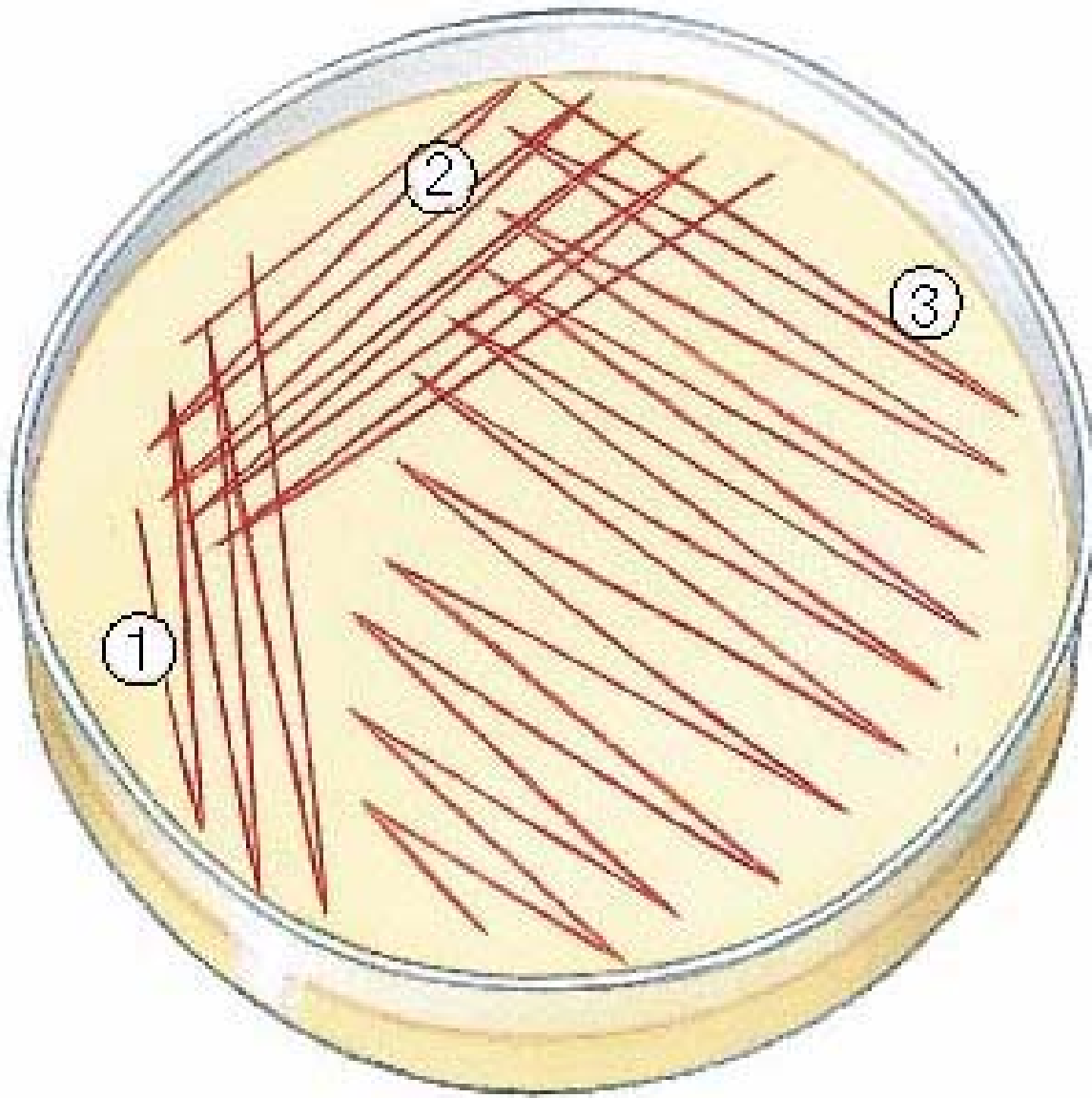
PCR



Culture – Media

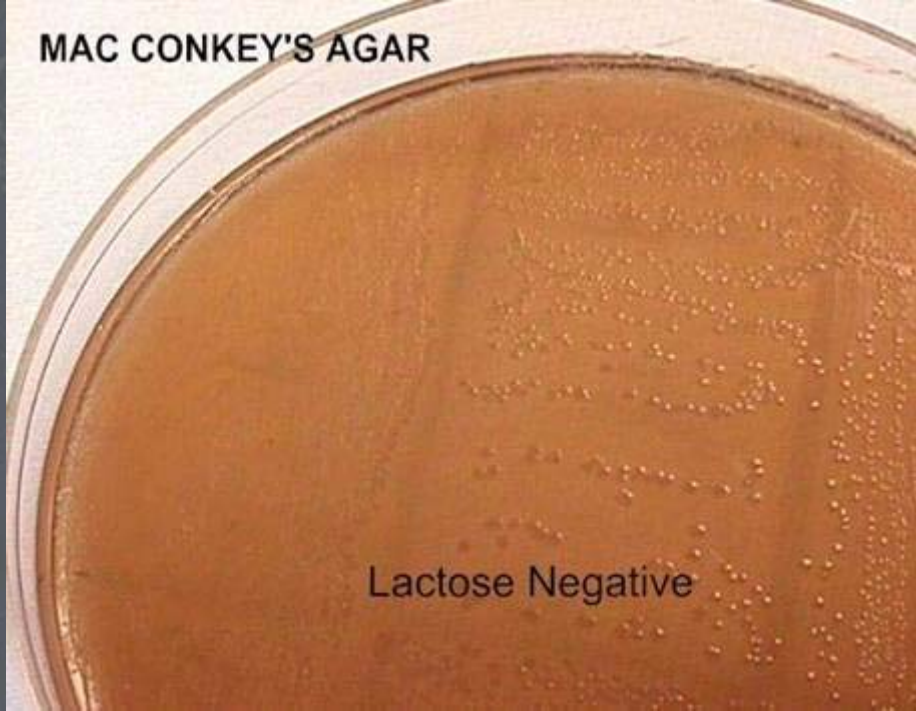
Blood Agar





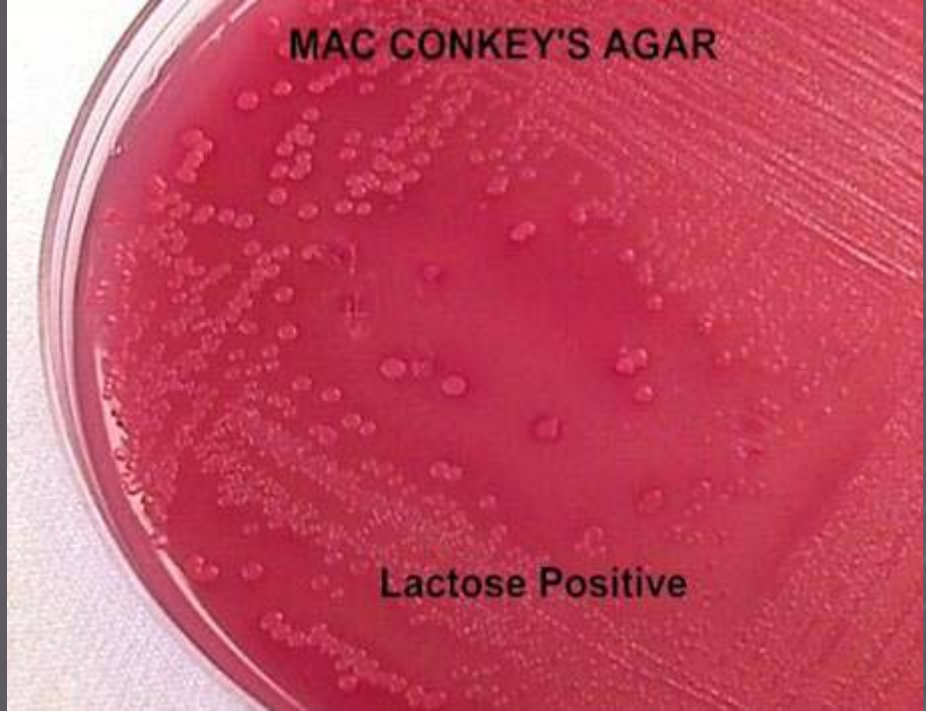


MAC CONKEY'S AGAR



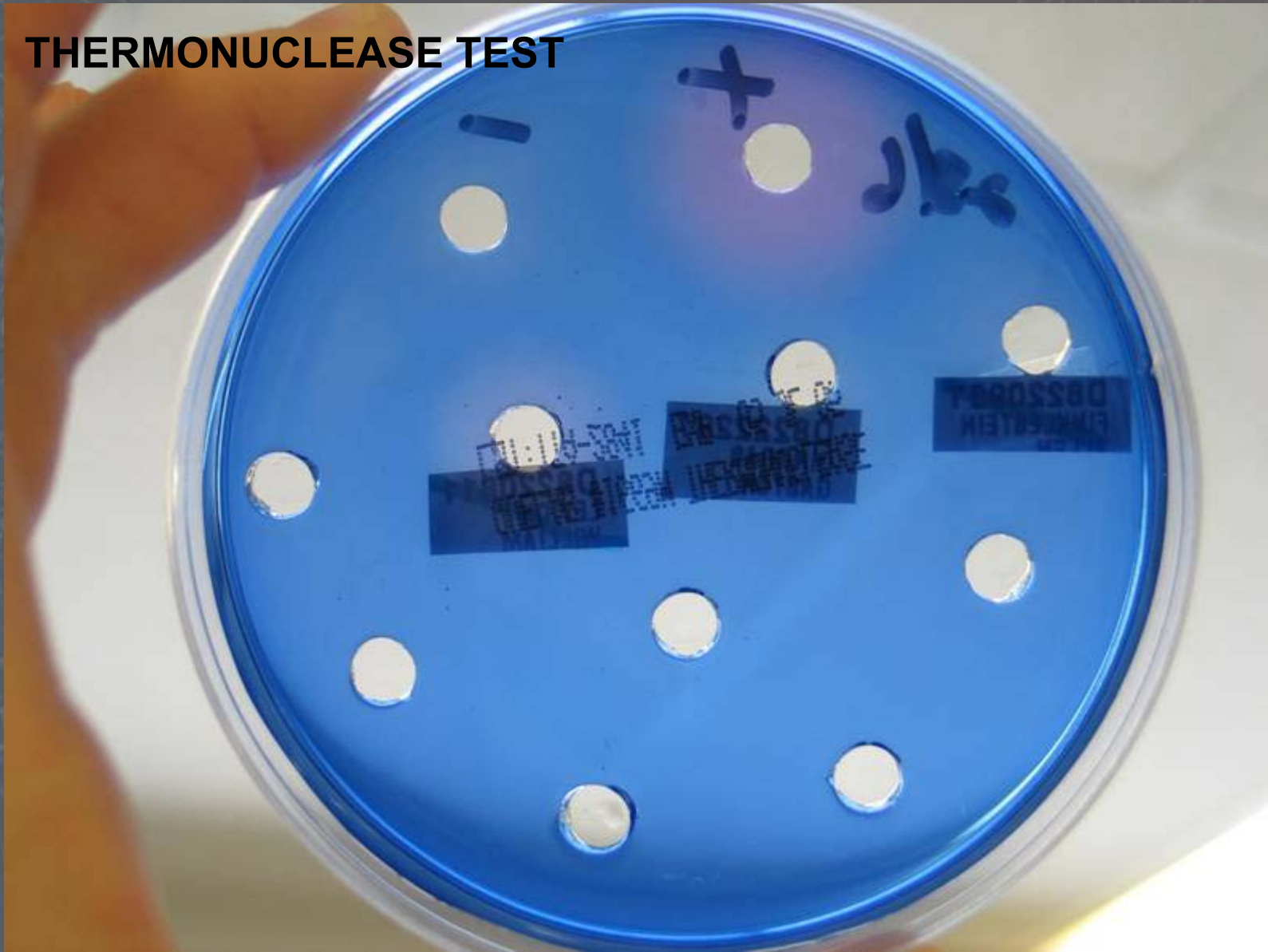
Lactose Negative

MAC CONKEY'S AGAR



Lactose Positive

THERMONUCLEASE TEST



COAGULASE TEST

Rabbit Plasma



Nutrient Broth



Pipette



COAGULASE TEST

Positive

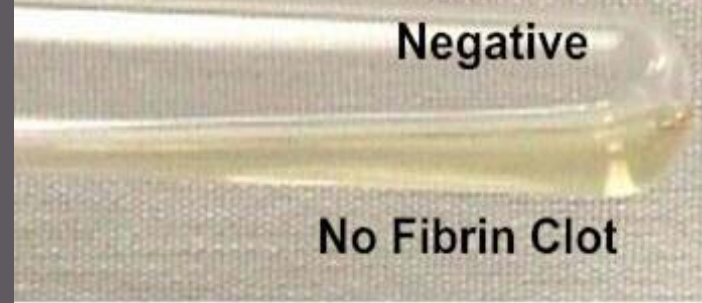
Fibrin Clot

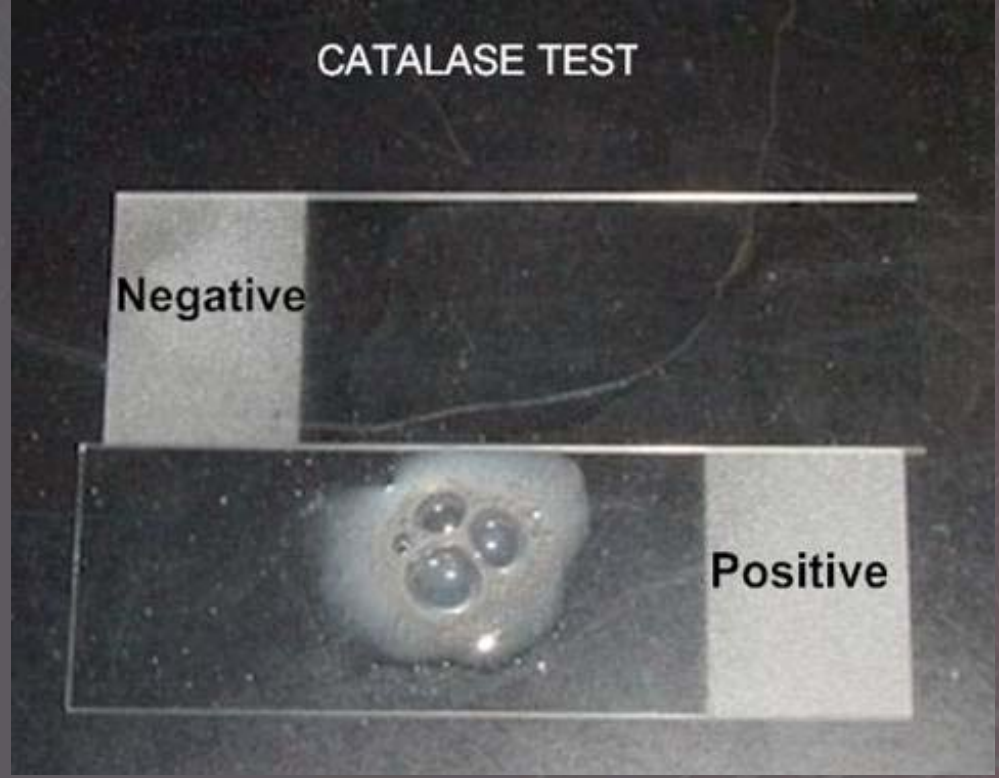
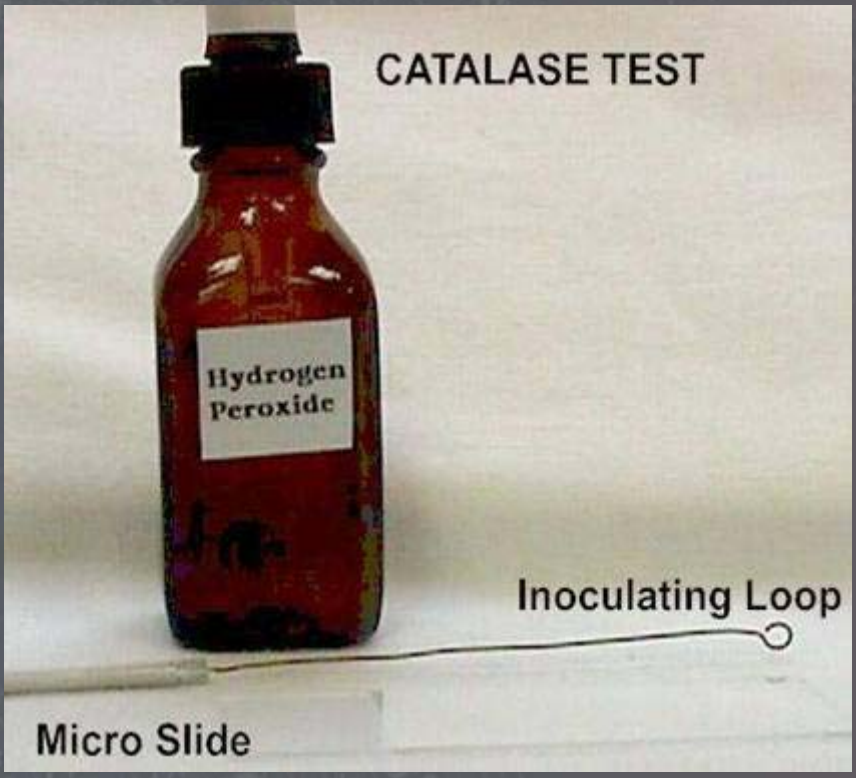


COAGULASE TEST

Negative

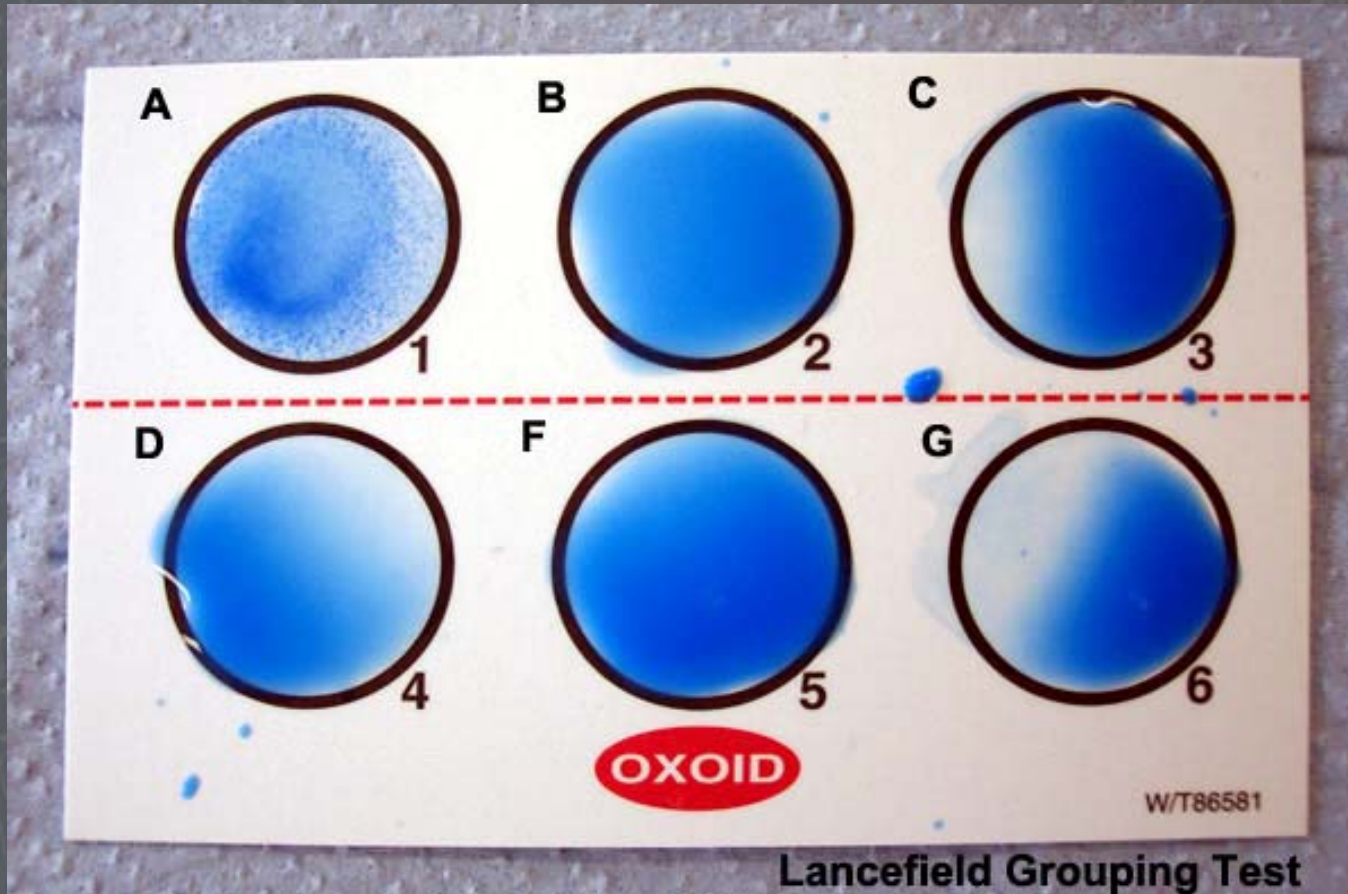
No Fibrin Clot



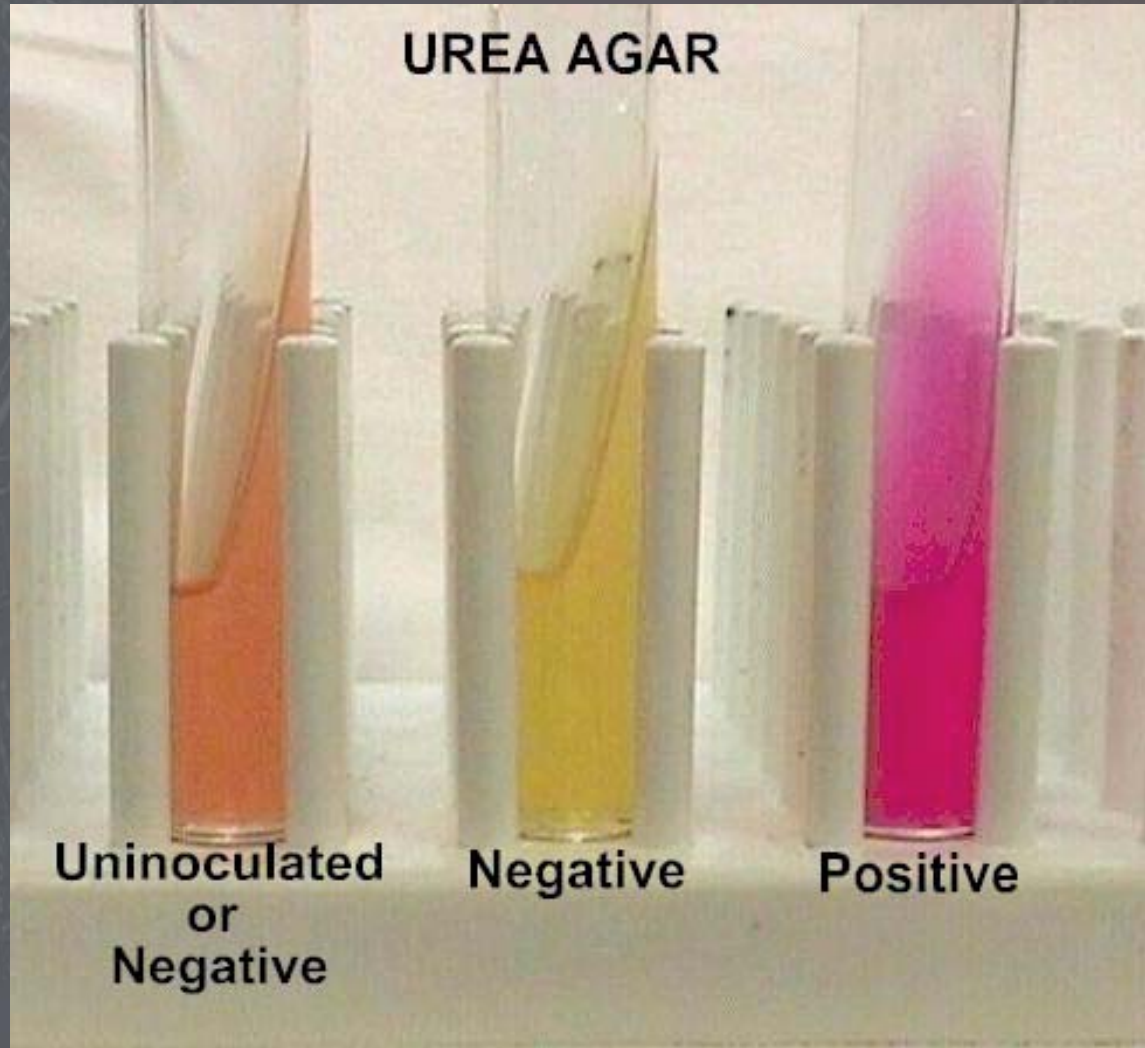






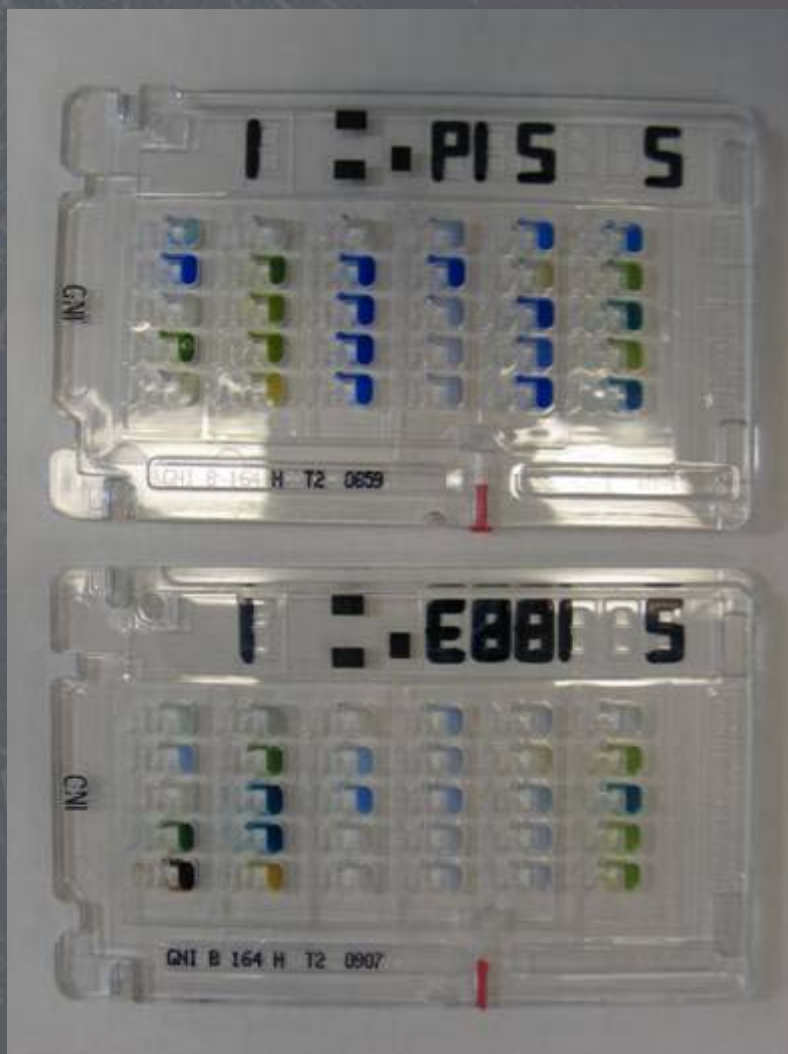






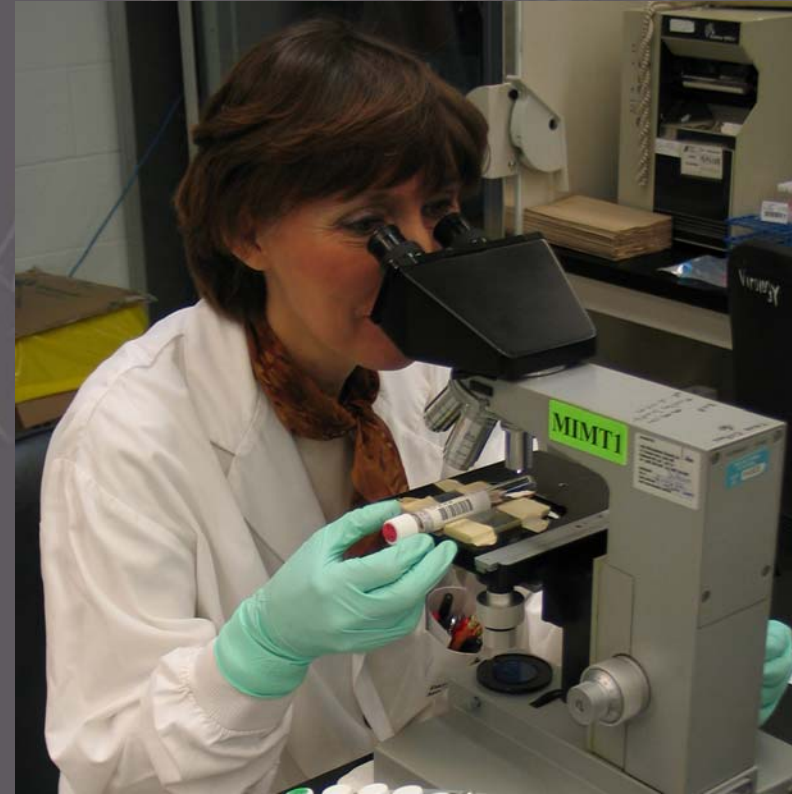
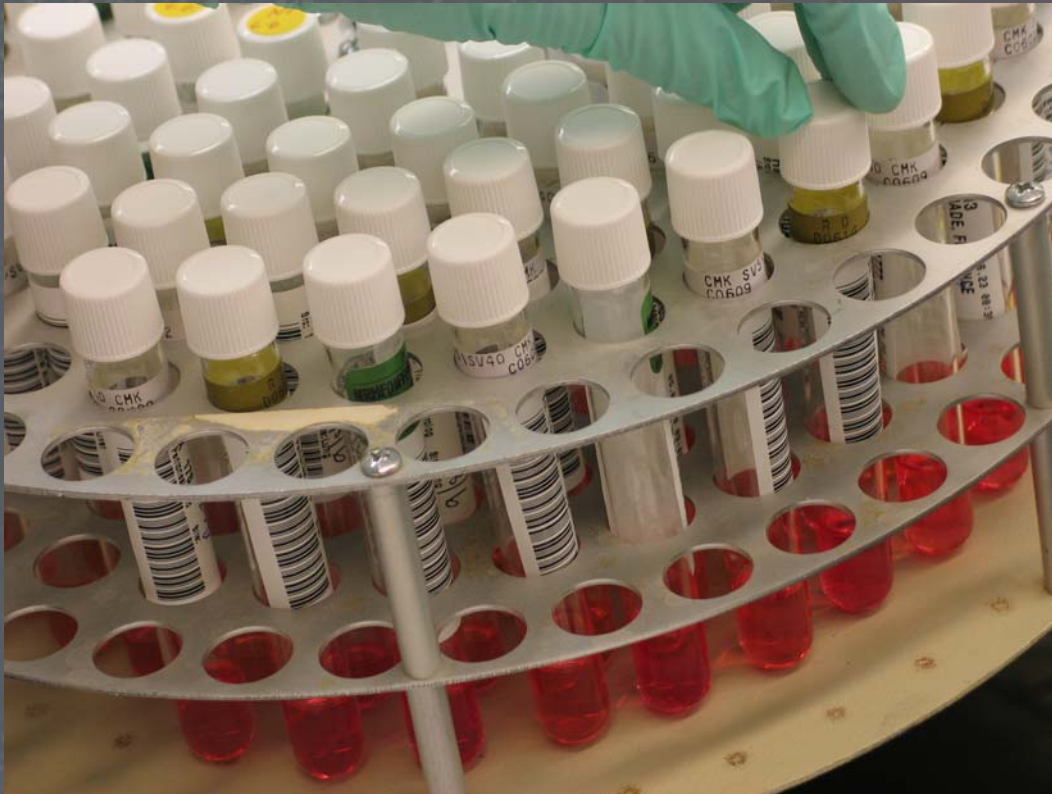


Automated Identification

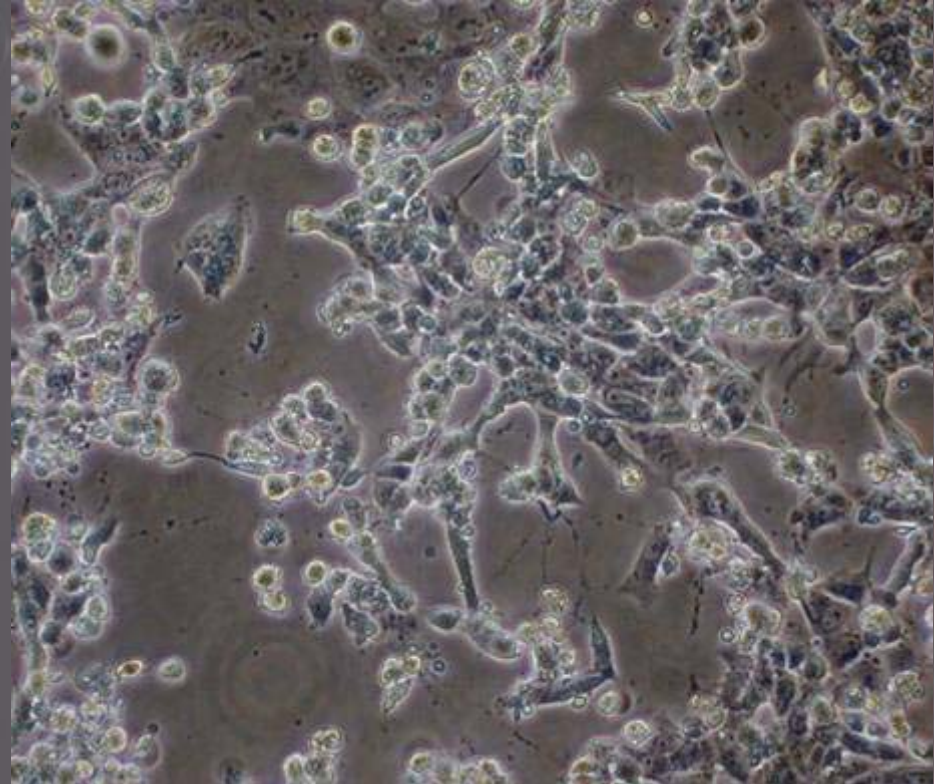
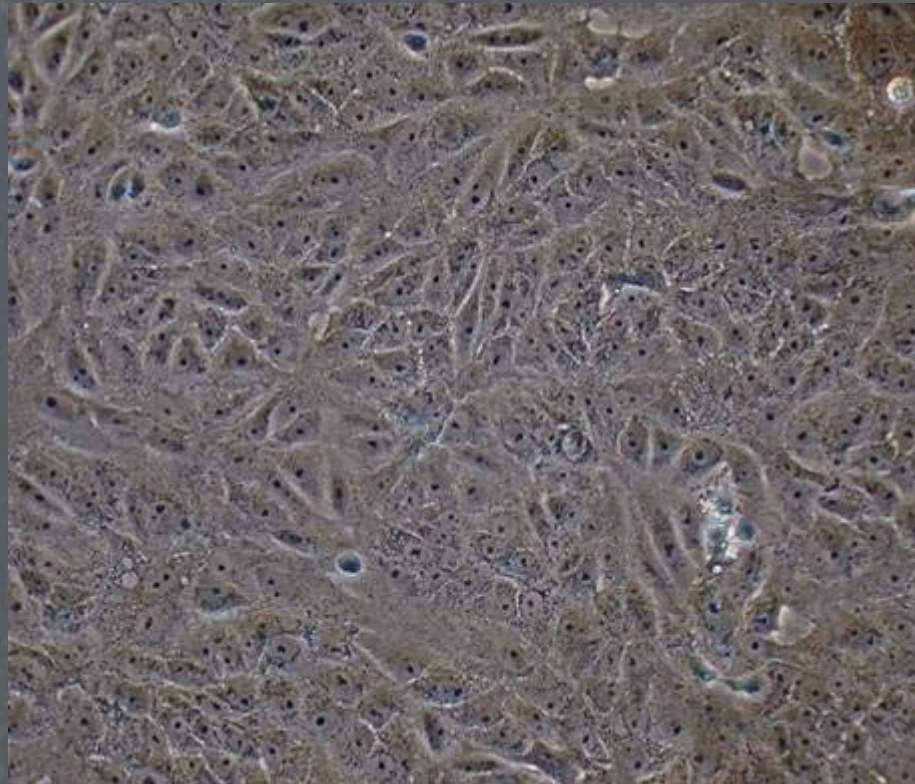


Culture – Cell Lines

Tube Culture



Vero Cells – SARS-CoV



Shell Vial



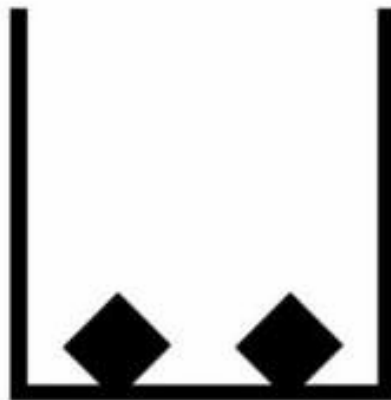
Serology

Serologic Tests

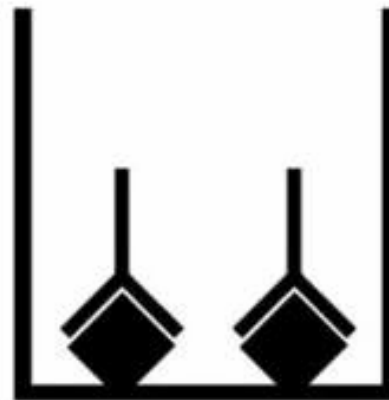
- Enzyme Immunoassay (EIA)
- Immunofluorescent Assays (IFA)
- Complement Fixation (CF)
- Hemagglutination Inhibition Assays (HAI)
- Western Blot
- Neutralization Tests

EIA

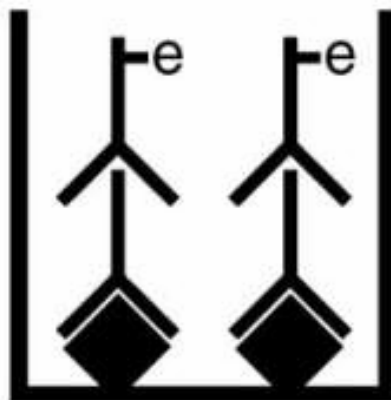
Step 1
Specific antigen is attached to a solid-phase surface



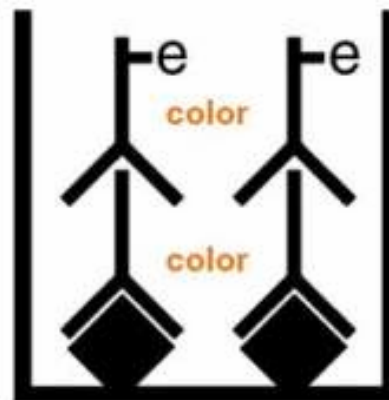
Step 2
Test specimen is added, which may or may not contain the antibody



Step 3
An enzyme-labeled antibody specific to the test antibody is added (conjugate)



Step 4
Chromogenic substrate is added, which in the presence of the enzyme, changes color.



IFA

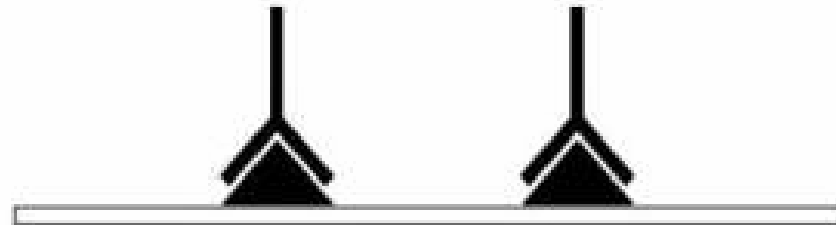
Step 1

Microbial antigen is dried on a glass slide and treated with a chemical fixative



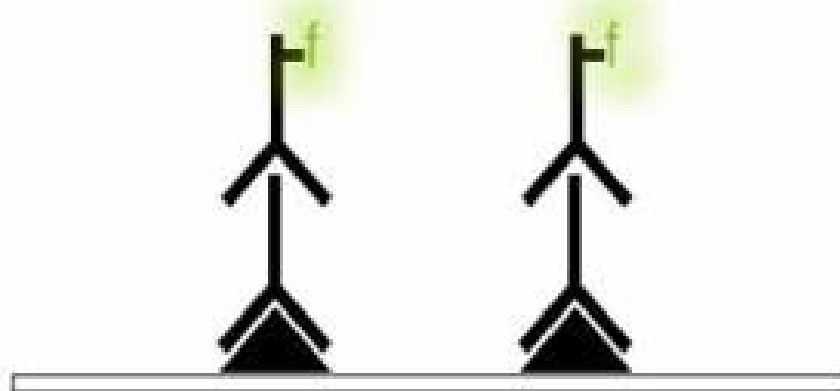
Step 2

Dilutions of patient serum are incubated with the antigen on the slide, and then rinsed



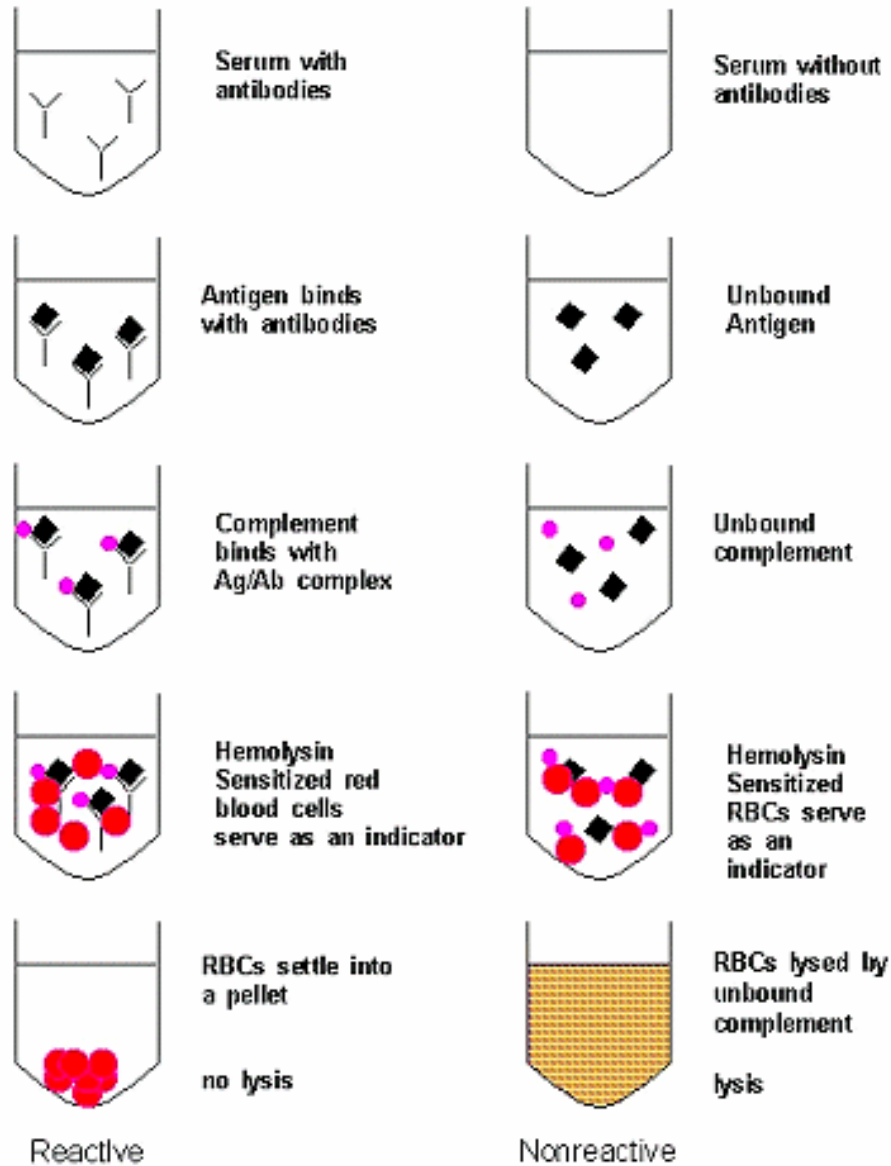
Step 3

A fluorescein-labeled antibody (conjugate) is added

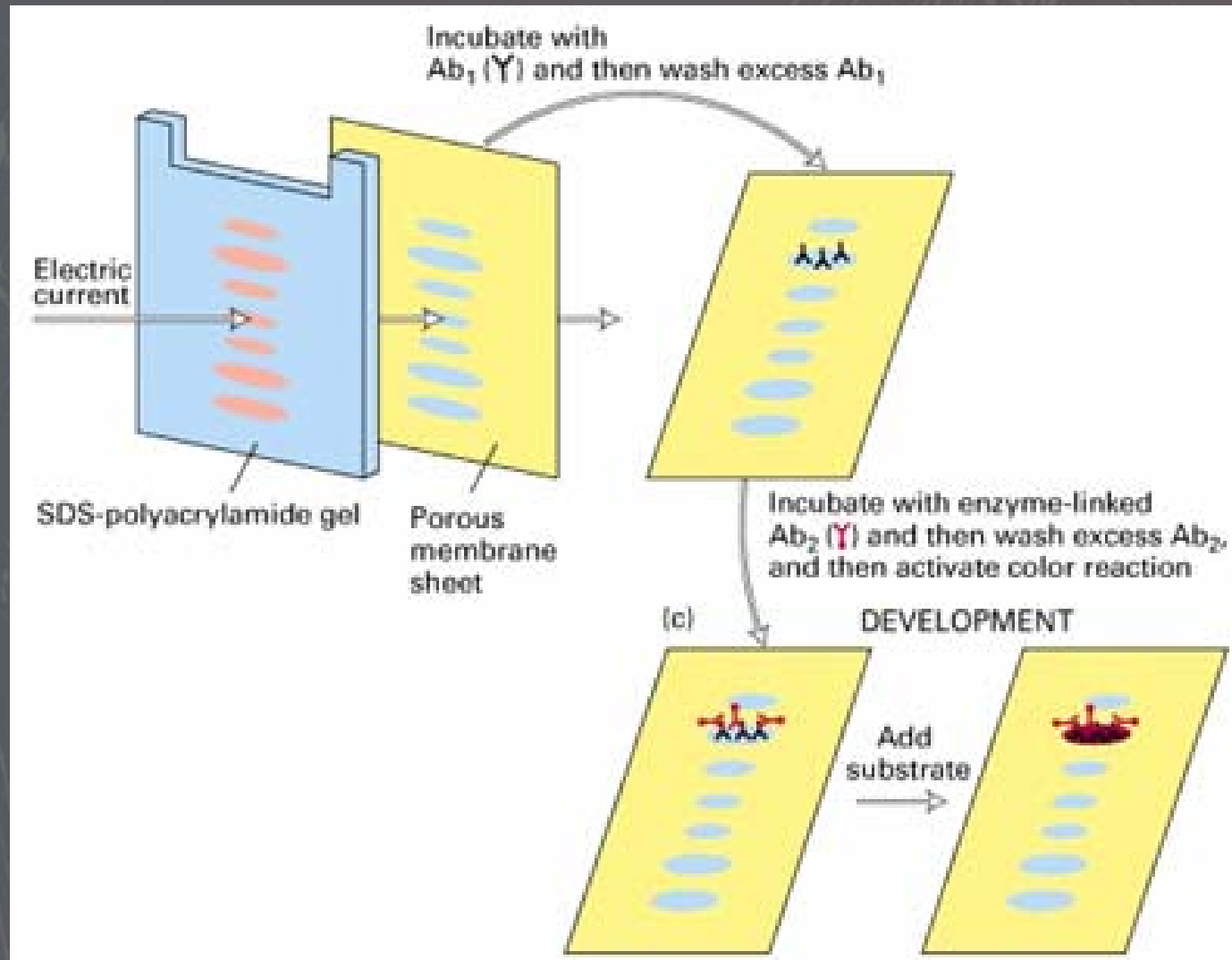


CF

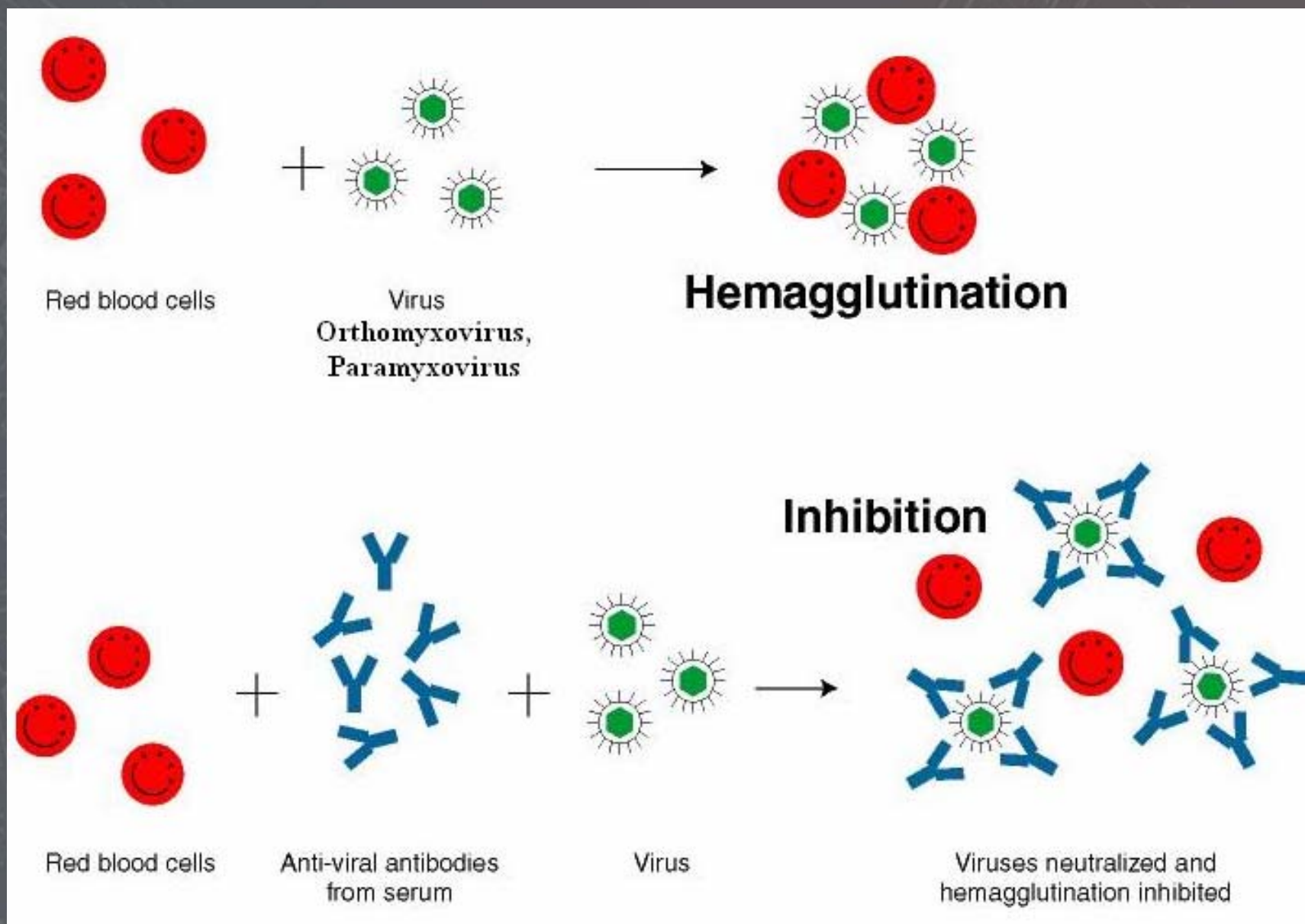
Complement Fixation Test



W. Blot



HAI



Neutralization Tests

- Neutralization of a virus is defined as the loss of infectivity through reaction of the virus with specific antibody
- Virus and serum are mixed under appropriate condition and then inoculated into cell culture, eggs or animals

Titres

- Dilute specimen to determine how concentrated antibody titre is
- Expressed as 1:8, 1:16, 1:32, 1:64 etc.
- Positive
 - +IgM test
 - >set cutoff (specific to each agent)
 - ≥ 4 fold rise between acute and convalescent specimens

Susceptibility Testing

Definitions

- MIC (Minimum Inhibitory Concentration)
- MBC (Minimum Bactericidal Concentration)
- Tolerance
 - $MBC/MIC \geq 32$
 - Clinical relevance not established
 - Mostly related to beta-lactam drugs

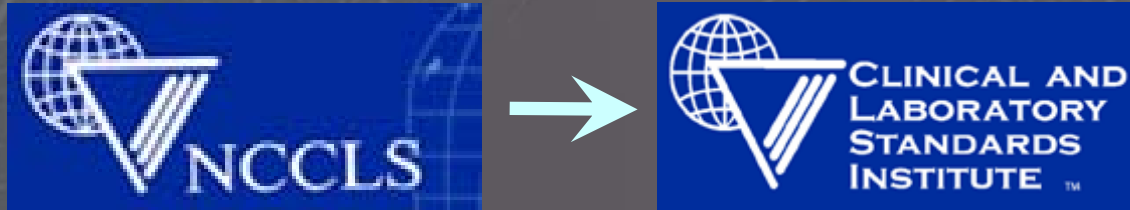
Definitions

Combination Testing

- MCBT (multiple combination bactericidal testing)
- Synergy Testing (synergy, indifference, antagonism)
 - Checkerboard Titration
 - Time Kill Curves

MIC

- Interpretive Standards
 - NCCLS (changed to CLSI in Jan 2005)



- Susceptible (S), Intermediate (I), Resistant (R)
 - MIC breakpoints based on studies assessing:
 - PK/PD based on systemic antibiotic delivery
 - Clinical efficacy studies
 - » Clinical resistance vs. biologic resistance

M100-S15

Vol. 25 No. 1

Replaces M100-S14

Vol. 24 No. 1

January 2005

Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement

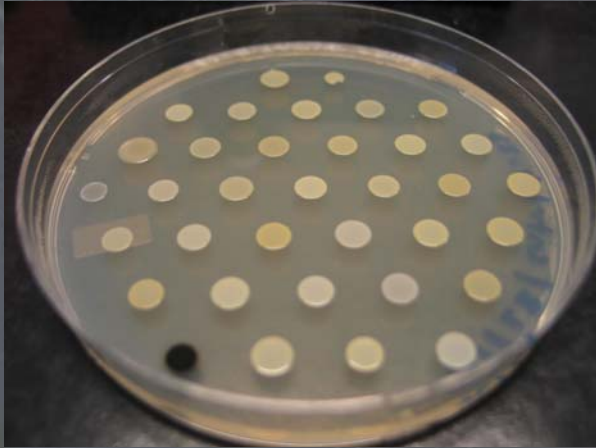
Susceptibility Testing

- Bacterial
 - Agar dilution, broth macrodilution, broth microdilution
 - Automated broth microdilution
 - Disk diffusion
 - E test
 - Screening Plates
 - Molecular (latex agglutination, NAAT)
- Fungal
 - Macrodilution, microdilution
- Mycobacteriology
 - Macrodilution

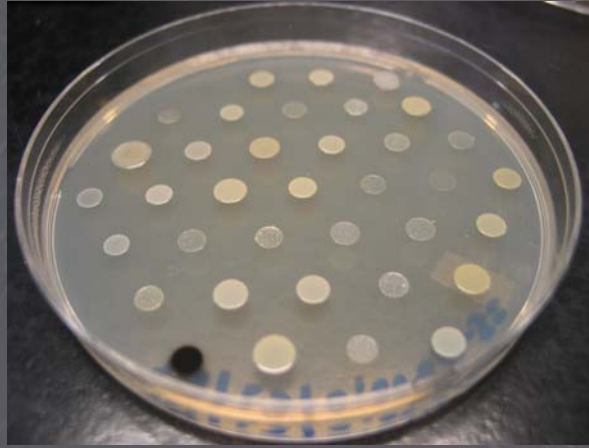
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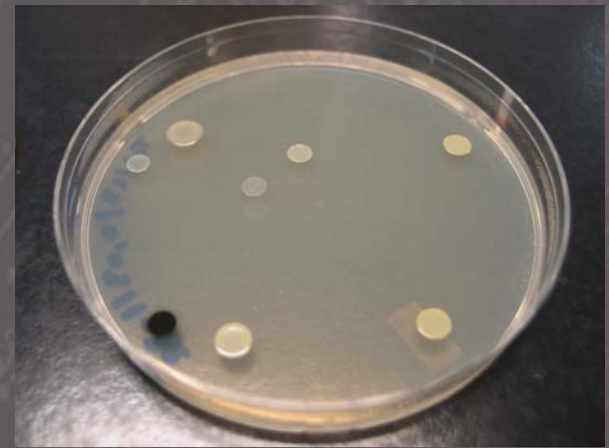
Agar Dilution



Penicillin
1 mg/L

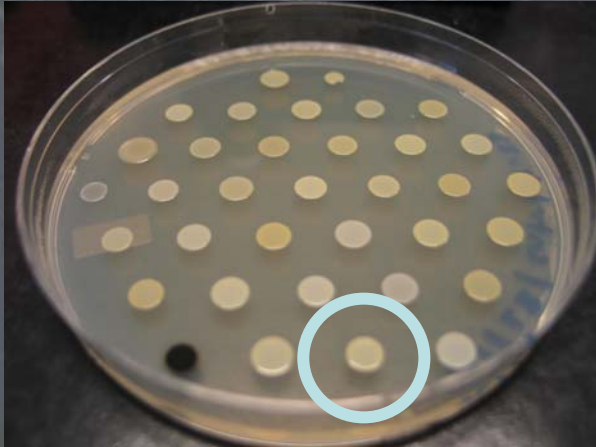


Penicillin
2 mg/L

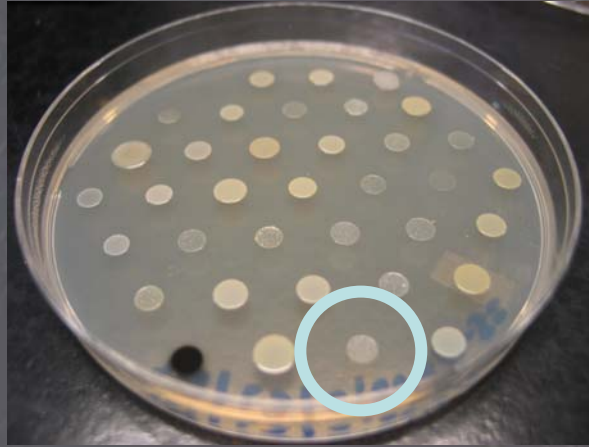


Penicillin
4 mg/L

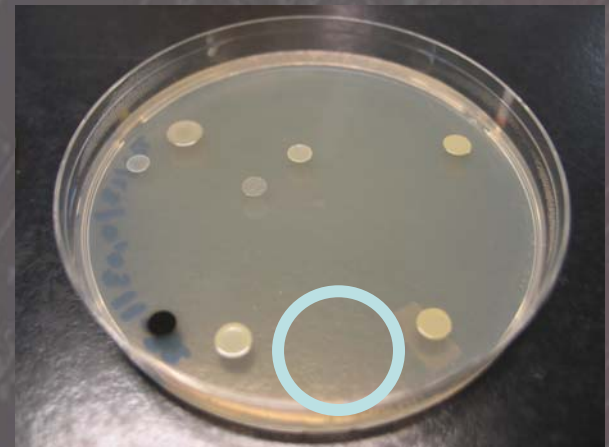
Agar Dilution



Penicillin
1 mg/L



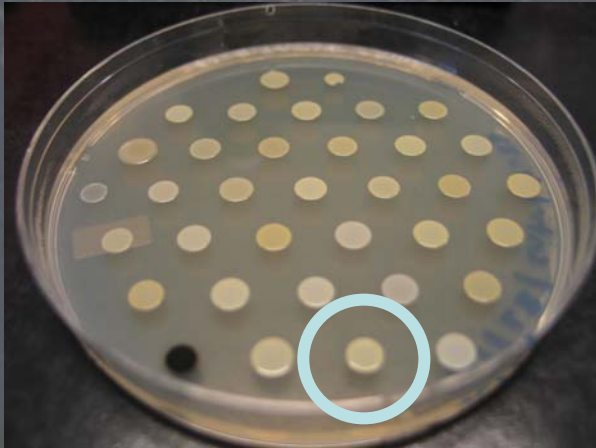
Penicillin
2 mg/L



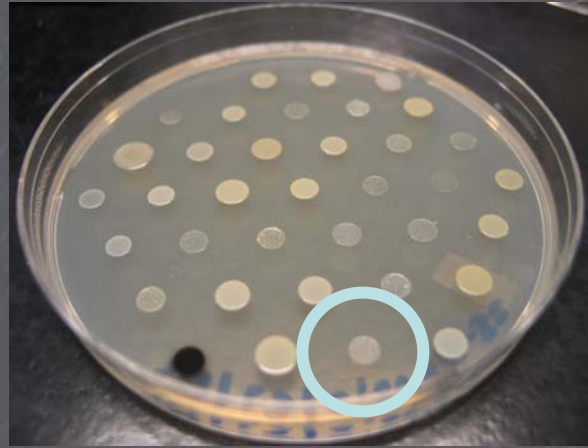
Penicillin
4 mg/L

Agar Dilution

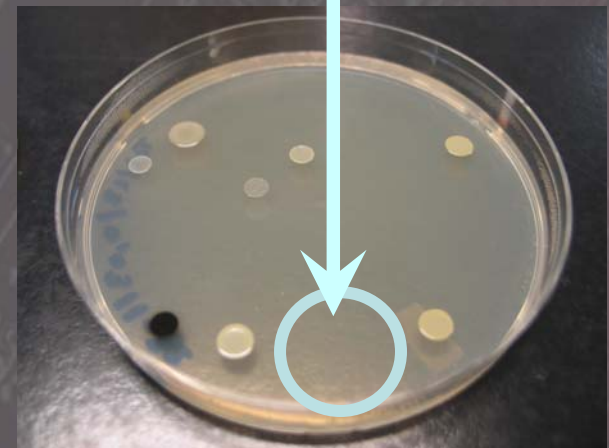
Pen MIC
= 4 mg/L



Penicillin
1 mg/L



Penicillin
2 mg/L



Penicillin
4 mg/L

Broth Macrodilution Testing



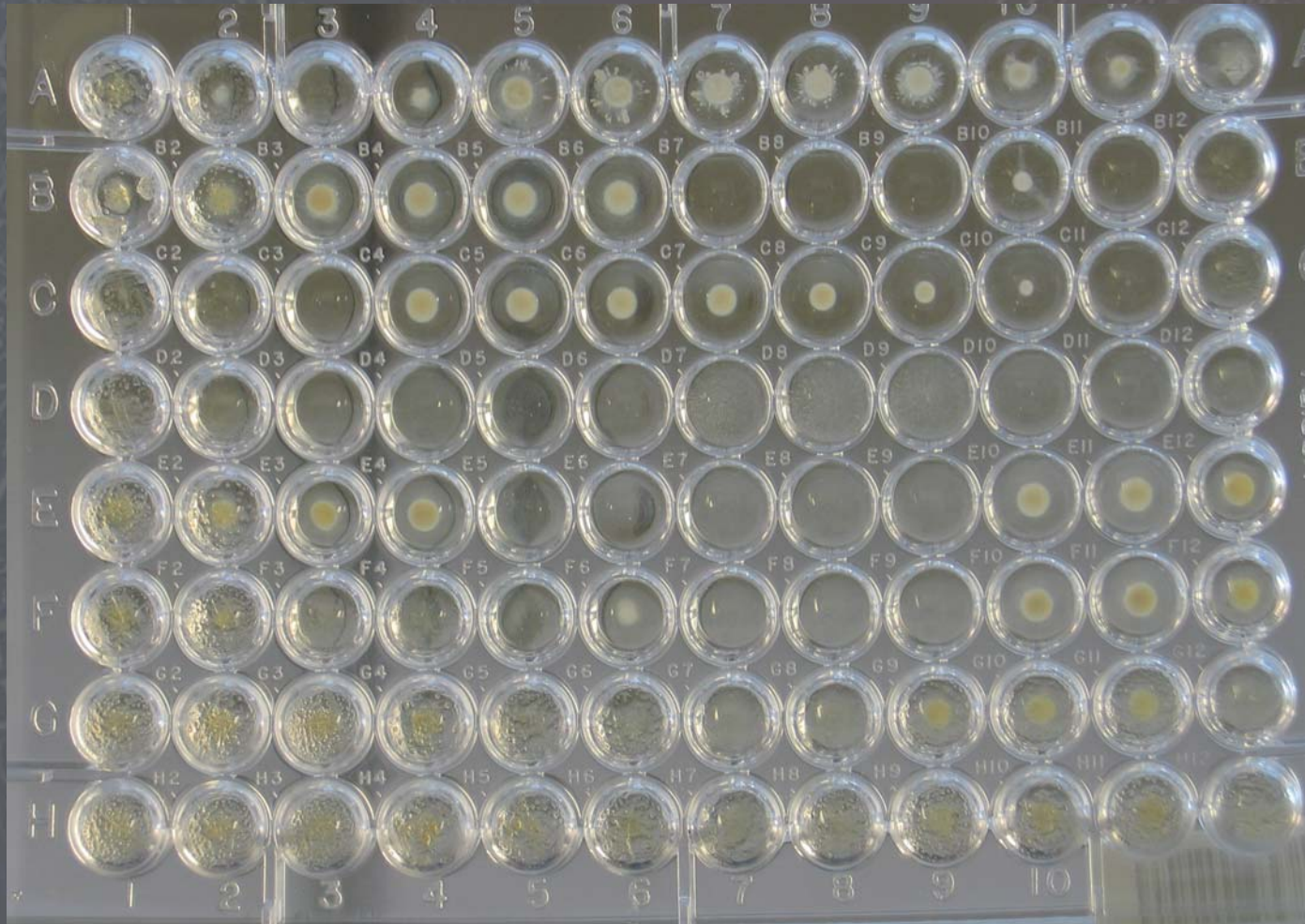
1 2 4 8 16 32 64 128 256 512
Penicillin (mg/L)

Broth Macrodilution Testing



1 2 4 8 16 32 64 128 256 512
Penicillin (mg/L)

Broth Microdilution Testing



Broth Microdilution Testing

ISOLATE #: _____

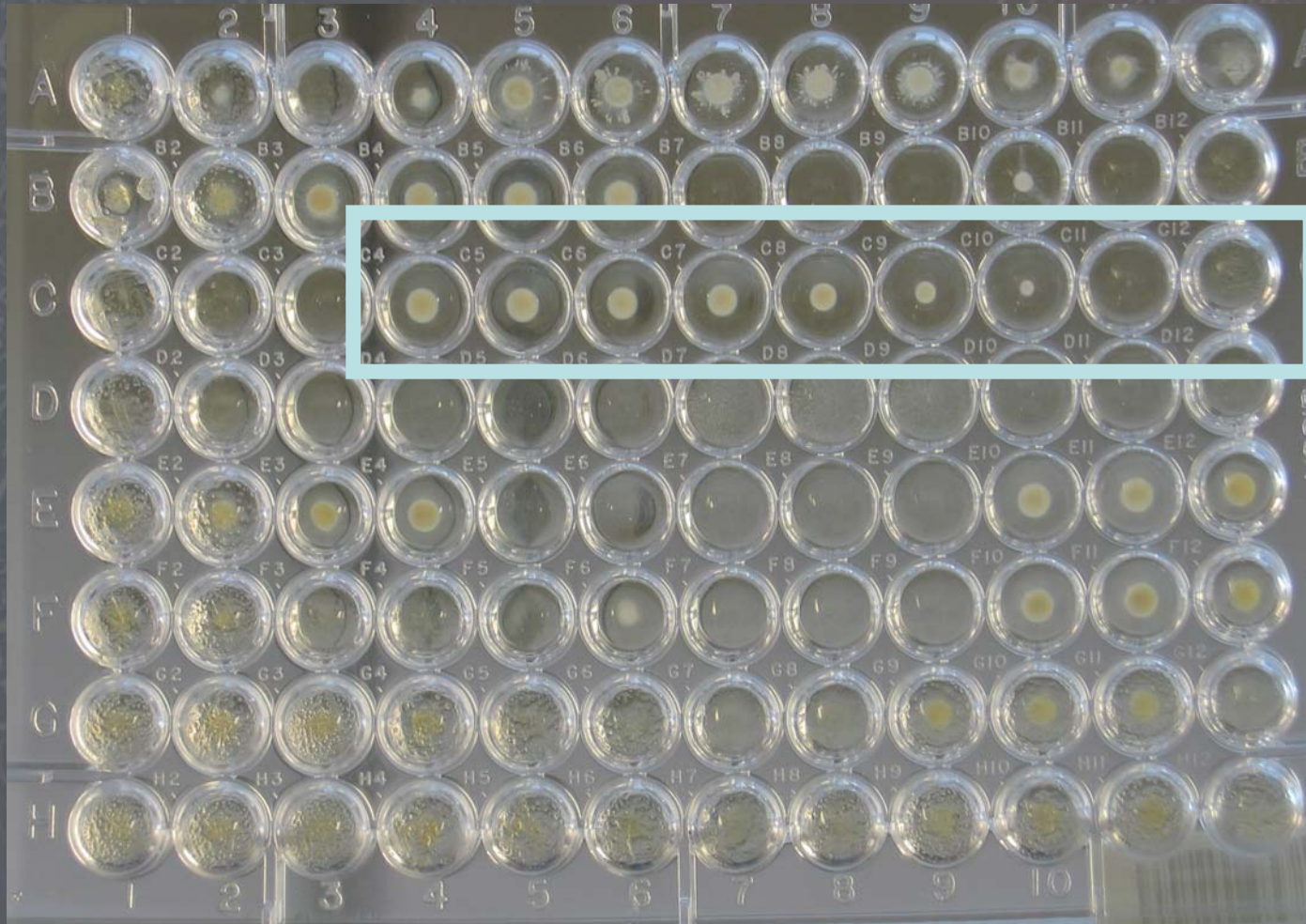
	1	2	3	4	5	6	7	8	9	10	11	12
A	MHB CONT.	Vanco 2	Vanco 4	Vanco 8	Oxacillin 0.25	Oxacillin 0.5	Oxacillin 1	Oxacillin 2	Oxacillin 4	Oxacillin 8	Oxacillin 16	Oxacillin 32
B	MHB 2%NaCl	Ery 0.5	Ery 1	Ery 2	Ery 4	Ery 8	Clinda 2	Clinda 4	Clind/ery 4/0.25	Tetra 4	Tetra 8	Tetra 16
C	Doxy 4	Doxy 8	Doxy 16	Mup 1	Mup 2	Mup 4	Mup 8	Mup 16	Mup 32	Mup 64	Mup 128	Mup 256
D	Mino 4	Mino 8	Mino 16	Rif 1	Rif 2	Rif 4	TMP/SMX 2	TMP/SMX 4	TMP/SMX 8	Genta 4	Genta 8	Genta 16
E	Cipro 1	Cipro 2	Cipro 4	Cipro 8	Fucidic 0.5	Fucidic 1	Fucidic 2	Fucidic 4	Fucidic 8	Gemi 0.25	Gemi 0.5	Gemi 1
F	Linezo 1	Linezo 2	Linezo 4	Linezo 8	Linezo 16	Synercid 1	Synercid 2	Synercid 4	Synercid 8	Gemi 8	Gemi 4	Gemi 2
G	Dapto 0.03	Dapto 0.06	Dapto 0.12	Dapto 0.25	Dapto 0.5	Dapto 1	Dapto 2	Dapto 4	BMS 756 0.25	BMS 756 0.5	BMS 756 1	Genta 500
H	Cefoxitin 0.5	Cefoxitin 1	Cefoxitin 2	Cefoxitin 4	Cefoxitin 8	Cefoxitin 16	Cefoxitin 32	Cefoxitin 64	BMS 756 8	BMS 756 4	BMS 756 2	NG CONT.

Broth Microdilution Testing

ISOLATE #: _____

	1	2	3	4	5	6	7	8	9	10	11	12
A	MHB CONT.	Vanco 2	Vanco 4	Vanco 8	Oxacillin 0.25	Oxacillin 0.5	Oxacillin 1	Oxacillin 2	Oxacillin 4	Oxacillin 8	Oxacillin 16	Oxacillin 32
B	MHB 2%NaCl	Ery 0.5	Ery 1	Ery 2	Ery 4	Ery 8	Clinda 2	Clinda 4	Clind/ery 1/0.25	Tetra 4	Tetra 8	Tetra 16
C	Doxy 4	Doxy 8	Doxy 16	Mup 1	Mup 2	Mup 4	Mup 8	Mup 16	Mup 32	Mup 64	Mup 128	Mup 256
D	Mino 4	Mino 8	Mino 16	Rif 1	Rif 2	Rif 4	TMP/SMX 2	TMP/SMX 4	TMP/SMX 8	Genta 4	Genta 8	Genta 16
E	Cipro 1	Cipro 2	Cipro 4	Cipro 8	Fucidic 0.5	Fucidic 1	Fucidic 2	Fucidic 4	Fucidic 8	Gemi 0.25	Gemi 0.5	Gemi 1
F	Linezo 1	Linezo 2	Linezo 4	Linezo 8	Linezo 16	Synercid 1	Synercid 2	Synercid 4	Synercid 8	Gemi 8	Gemi 4	Gemi 2
G	Dapto 0.03	Dapto 0.06	Dapto 0.12	Dapto 0.25	Dapto 0.5	Dapto 1	Dapto 2	Dapto 4	BMS 756 0.25	BMS 756 0.5	BMS 756 1	Genta 500
H	Cefoxitin 0.5	Cefoxitin 1	Cefoxitin 2	Cefoxitin 4	Cefoxitin 8	Cefoxitin 16	Cefoxitin 32	Cefoxitin 64	BMS 756 8	BMS 756 4	BMS 756 2	NG CONT.

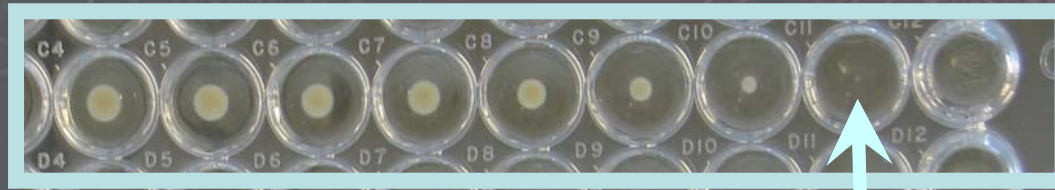
Broth Microdilution Testing



Broth Microdilution Testing

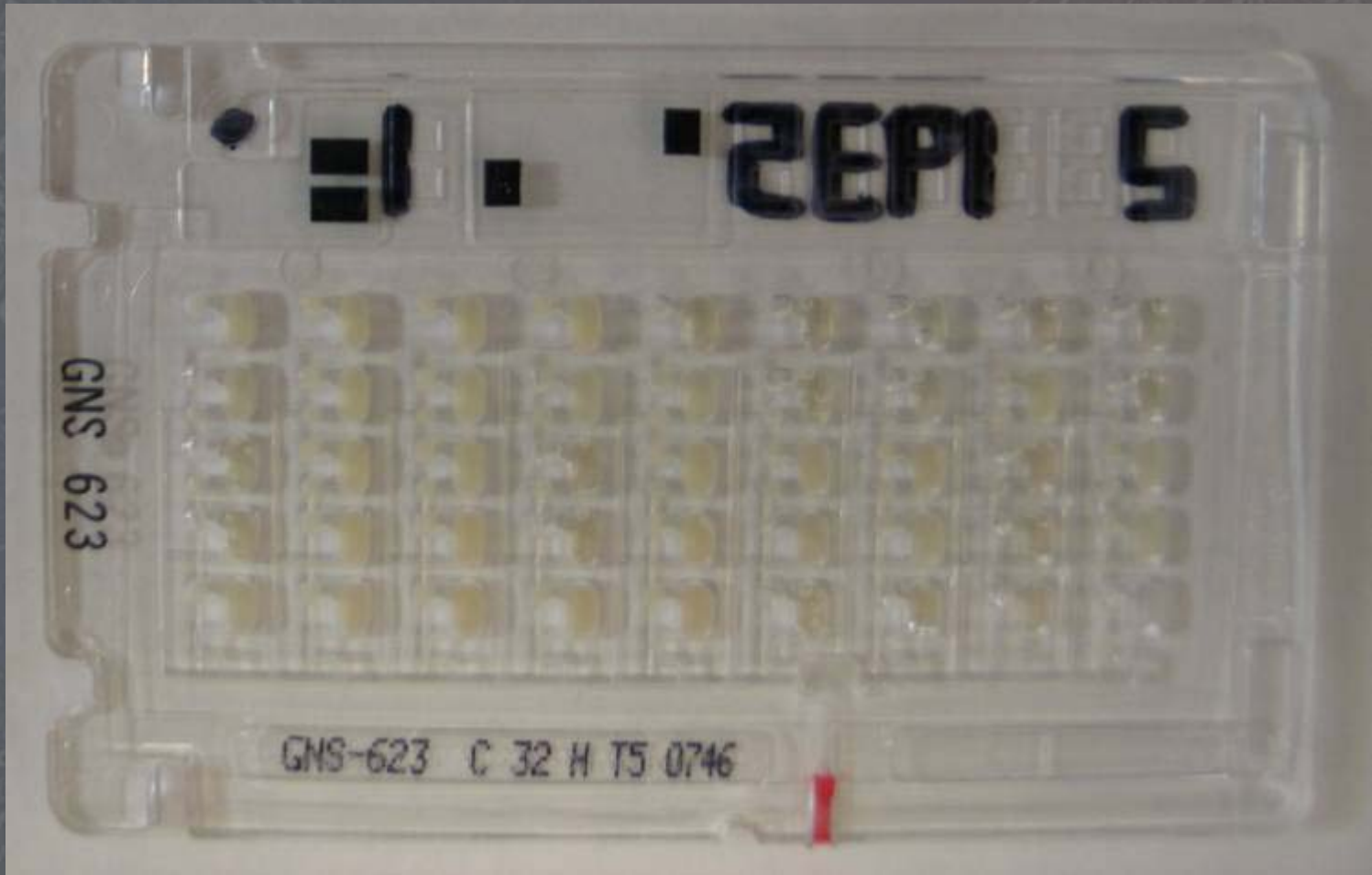
Mupirocin (mg/L)

1 2 4 8 16 32 64 128 256

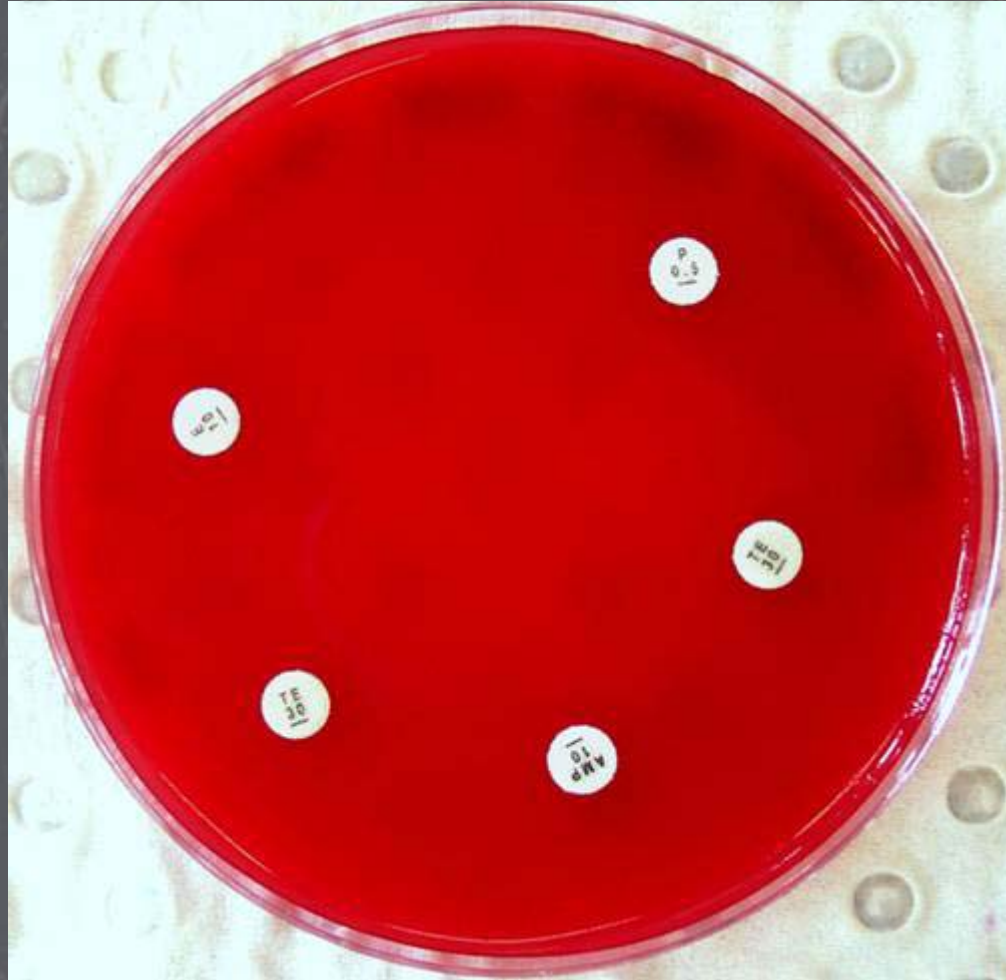


Mupirocin MIC = 128 mg/L

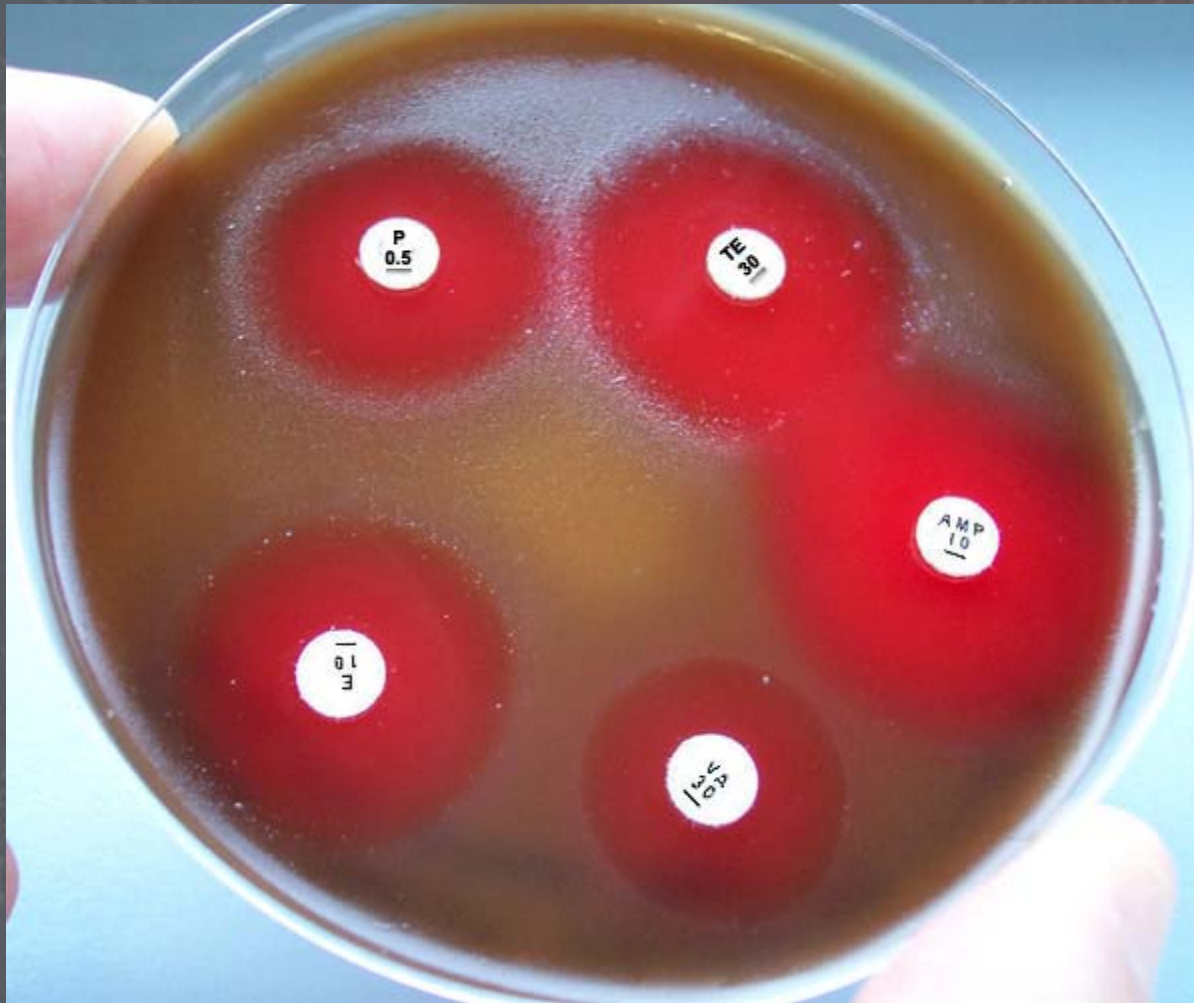
Automated Broth Microdilution



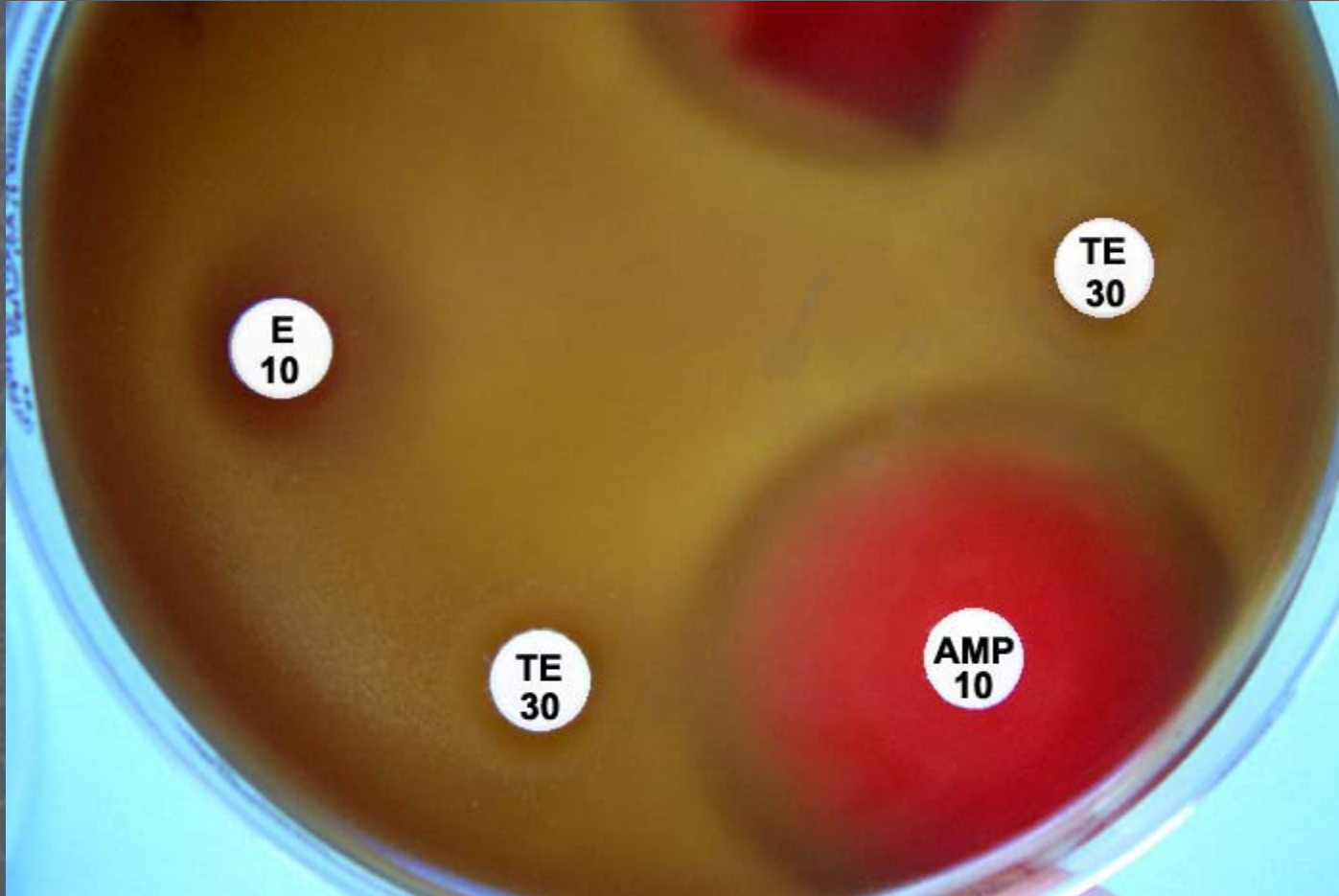
Disk Diffusion Testing



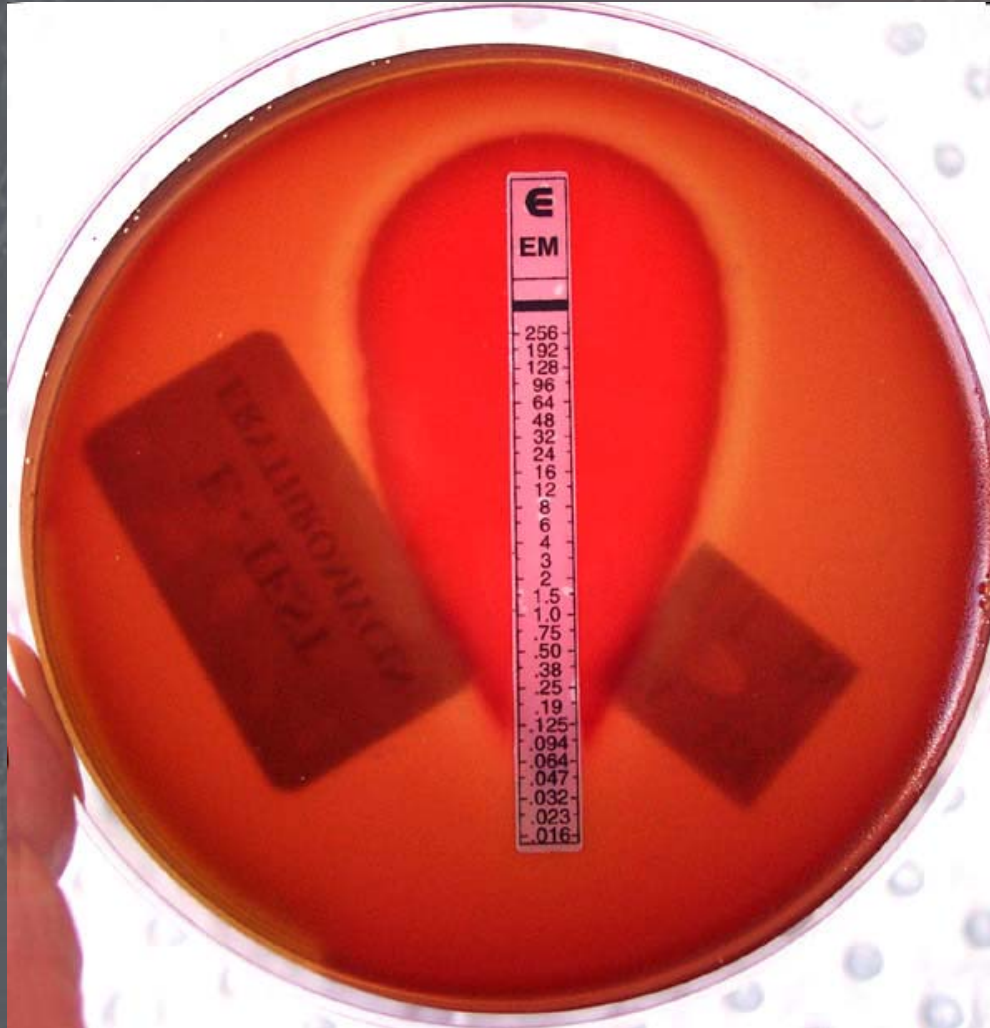
Disk Diffusion Testing



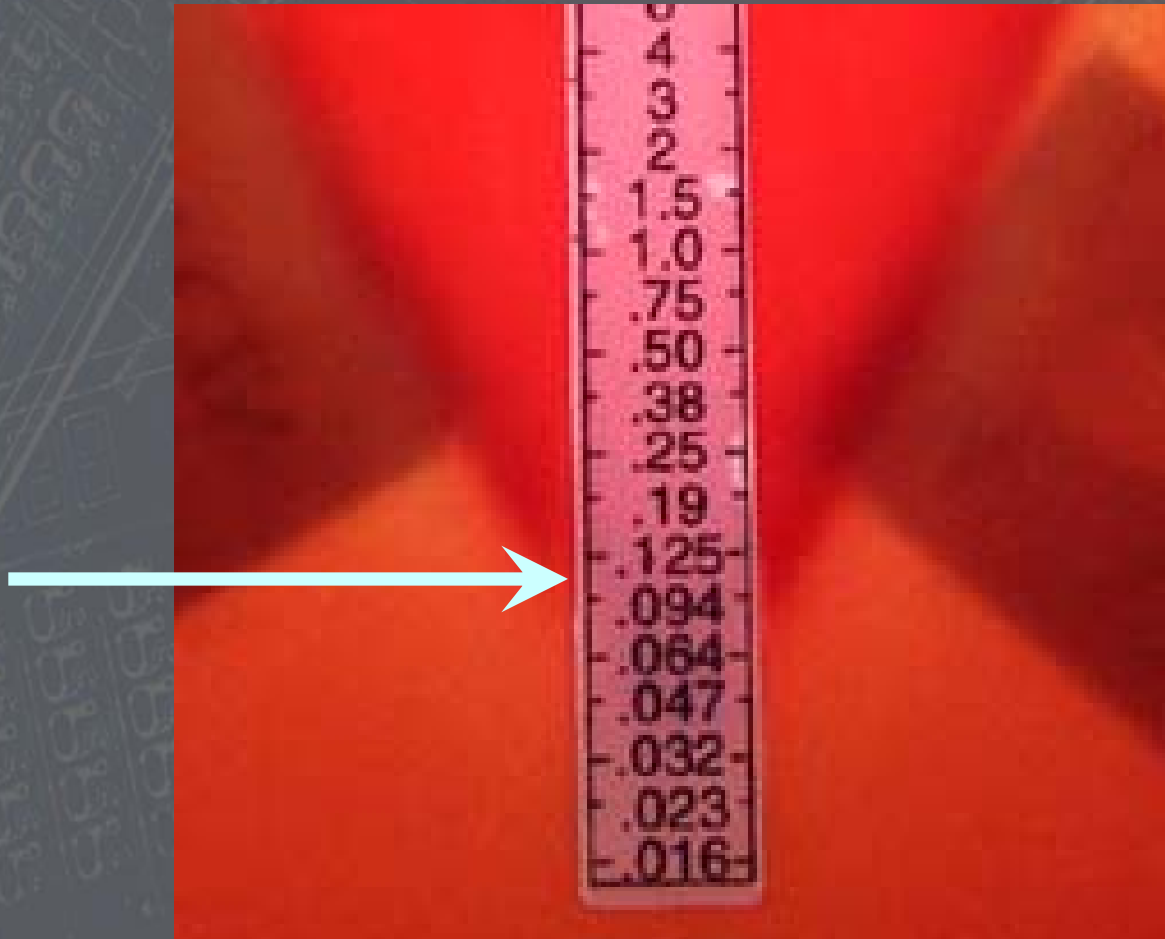
Disk Diffusion Testing



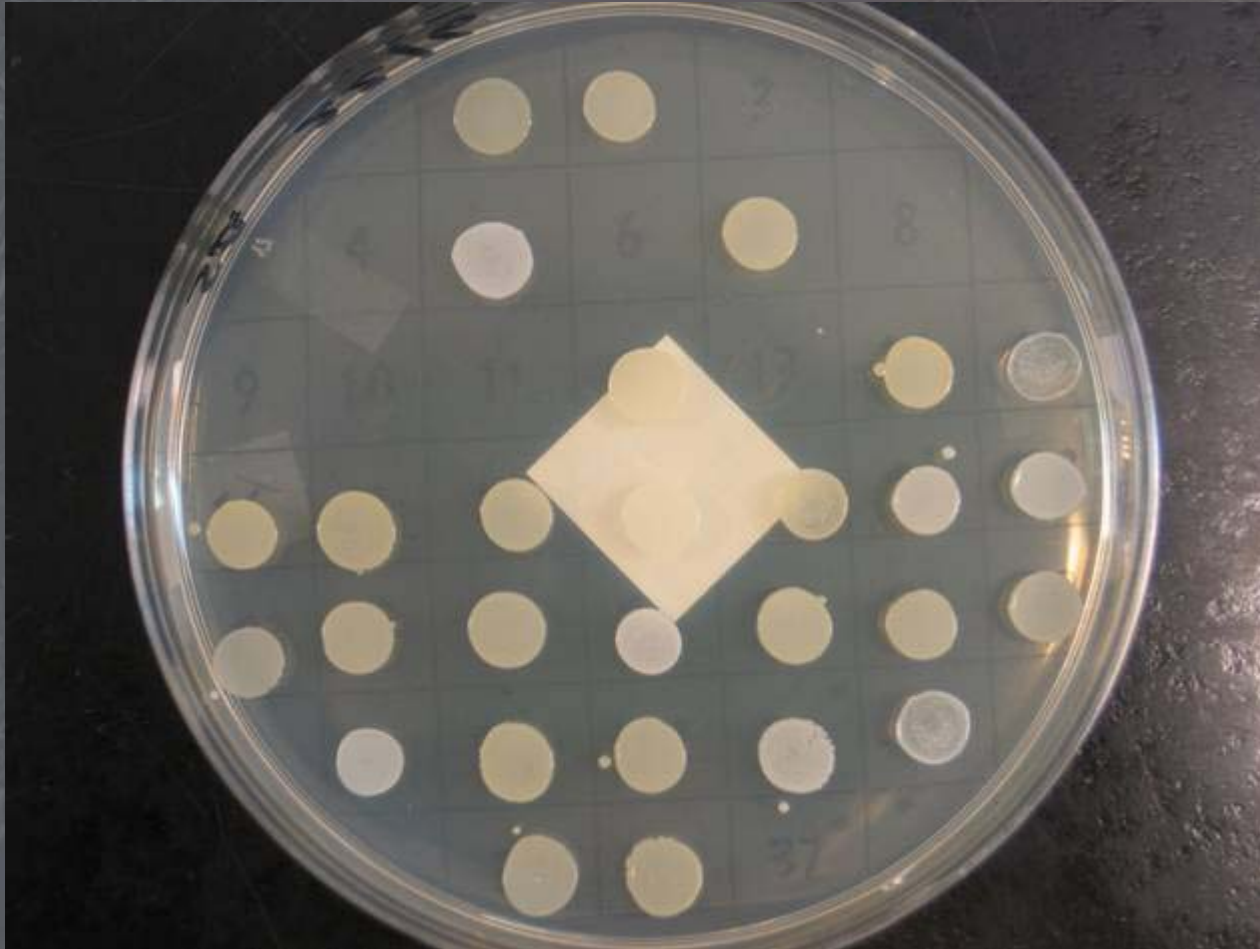
E test



E test



Screening Plates (DIRECT FROM ISOLATE)



Screening Plates (DIRECT FROM SPECIMEN)



Latex Agglutination



NAAT



Limitations of Susceptibility Tests

- Interpretative guidelines
- Cost (NAAT)
- New resistance determinants
 - MRSA
 - VRE
 - ESBL
 - VRSA, VISA
- Turn-around-times

Turn-around-times (TATs)

TATs

- Direct detection
 - STAT or within 24 hours
- Culture
 - Varies
- Serology
 - Usually within 24 hours (excluding weekends)
- Susceptibility Testing
 - Varies (typically requires positive culture)

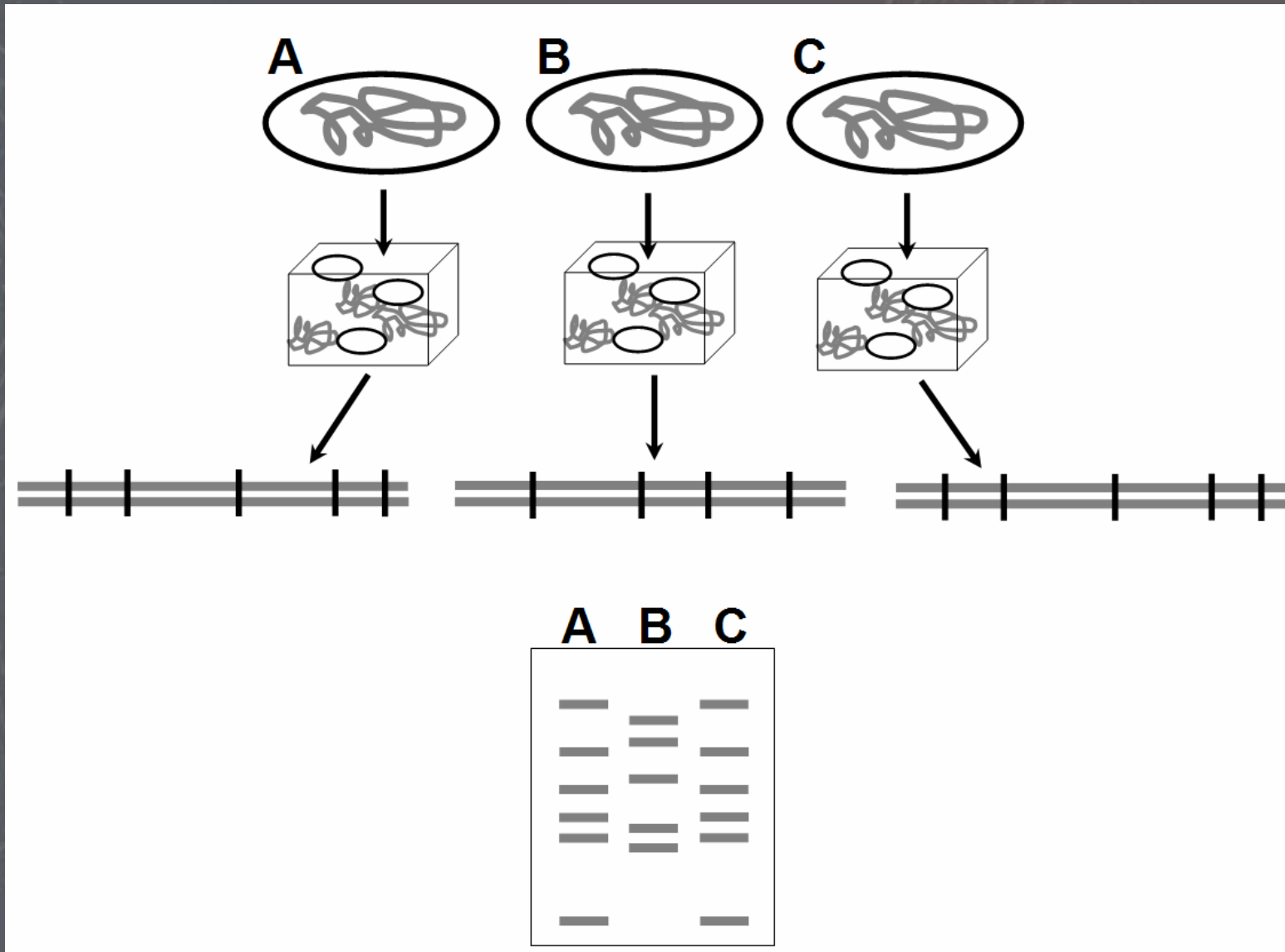
Culture TATs

- Bacteriology
 - Routine: 24-48 hours
 - BC: 5 days (21 days if endocarditis)
- Mycology
 - 2-6 weeks
- Virology
 - 1-2 weeks
- Mycobacteriology
 - 6 weeks

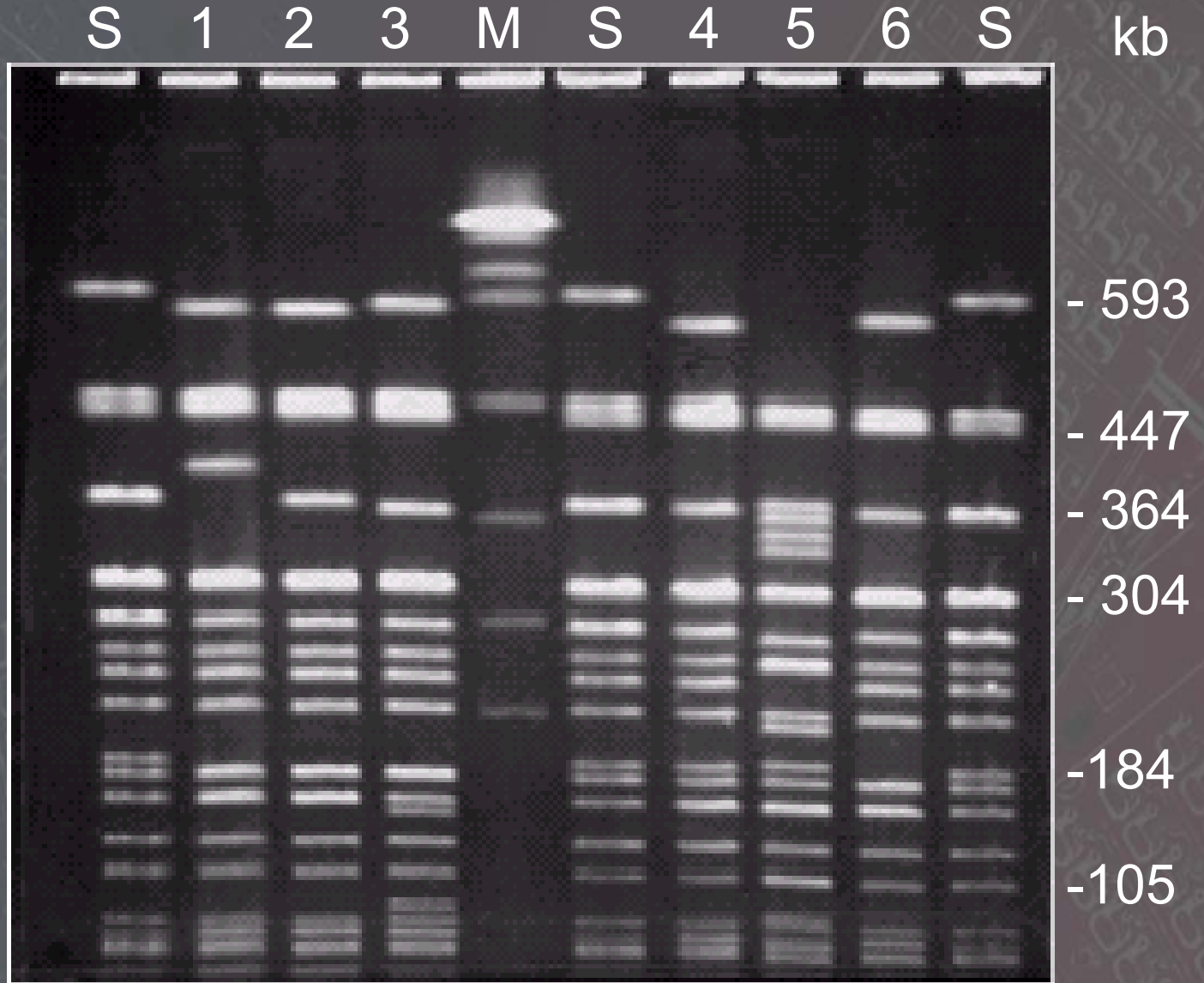
B. Infection Control

- Epidemiology of Infectious Disease
 - Reportable diseases
 - Tracking rates of select pathogens
 - e.g. *C. difficile*, AROs
- Epidemiology of Antimicrobial Resistance
 - Annual antibiogram
 - Antibiotic Subcommittee
 - Formulary, guidelines
- Outbreak investigation
 - Epidemiology typing, treatment options

PFGE



eg.



C. Reference Work

- Susceptibility testing
- Identifying resistance determinants
- Epidemiologic typing (esp. AROs)
- NAAT

Research

Research

- Collaborative studies
- Surveillance studies
- Mechanisms of resistance
- Animal model
 - PK/PD
 - MPC

Education

Education

- Undergraduate lectures
- Postgraduate lectures
- Plate rounds
 - ID team with pharmacists
- Internships
 - Students
 - Pharmaceutical industry representatives

Resources

Specimen Collection Guide : Testing Guideline 2006

The Testing Guideline provides an overview of the laboratory testing available through the Ontario Public Health Laboratories (OPHL).

The guideline is listed in alphabetical order by disease, syndrome and/or causal agent.

Information includes :

- Laboratory tests available
- Laboratory test code
- Appropriate specimens
- Collection kit numbers
- Section / location where test is performed
- Turn-around-times for negative and for positive or confirmatory results
- Additional information as required

Please note that the turn-around-times are based on Monday to Friday business working days.

For further assistance, please use the OPHL HELPLINE at **1-800-640-7221** and your call will be appropriately directed.

Document download

This Publication requires knowledgeable interpretation and is intended primarily for professional health care practitioners, health care organizations and public health units.

[Specimen Collection Guide - August 2006
Testing Guidelines](#)

74 pages | 306 Kb | PDF format

<http://www.health.gov.on.ca/english/providers/pub/abs/specimen.html>

Specimen Collection Guide - Testing Guidelines

*Public Health Laboratories
Ministry of Health and Long-Term Care*

August, 2006



<http://www.health.gov.on.ca/english/providers/pub/labs/specimen.html>

Disease / Syndrome / Causal Agent / Test	Test Code	Specimens	Collection Kit	Test Available	Section	TAT Negative Results Reported	TAT Positive or Confirmatory Results Reported	Notes *
Ascariasis <i>Ascaris lumbricoides</i>	P04	Faeces in SAF preservative	Para	Microscopy	Parasitology	3 days	Within 3 days	
	P02	Adult worm (passed in vomit or faeces)	Sterile container with normal saline	Microscopy	Parasitology	2 days	Within 2 days	
	S06	Blood, clotted or serum	BL-S	EIA	Immunodiagnosics	7 days	Within 7 days	
Aspergillosis (Invasive)	S05	Blood, clotted or serum	BL-S	Immuno-diffusion	Immunodiagnosics	7 days	Within 7 days	
Astrovirus Infections Astrovirus	V06	Faeces	Sterile container	Electron microscopy	Virus Detection	3 days	Within 3 days	If transportation is delayed, refrigerate at 4°C.
Avian Flu	V23	Nasopharyngeal aspirate/swab	Virus-Resp	Virus isolation	Virus Detection	Preliminary 7 days	Within 10 days	Contact the local Health Unit and Head, Virus Detection at 416-235-5730 prior to submitting specimen. Submit travel history and clinical information.
				Direct antigen Detection (Flu A, B)		1 day	1 day	
				rRT-PCR	Molecular Diagnostics	1 day	Preliminary 1 day, confirmation sent to NML, Winnipeg, MB.	

OPHL

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