Introduction to Microbiology

Infection Preventionist Two-Day Course, 2024

Healthcare-Associated Infections Program
Center for Health Care Quality
California Department of Public Health

Objectives

- Describe role of the microbiology laboratory in infection prevention
- Explain basic laboratory tests for infectious pathogens
- Define common Healthcare Associated Infection (HAI) pathogens

Microbiology and Infection Prevention

Microbiology has two important functions related to the prevention and control of infections:

- **Clinical:** identify pathogens and their susceptibility to antimicrobial treatment
  - Example: Physician reviews a culture, prescribes an antibiotic
- **Epidemiological:** identify pathogens causing disease or outbreak in a population, and looks for potential sources of these pathogens
  - Example: Public health investigates a report of an outbreak of foodborne illnesses

Importance of Micro Lab Results

- Determines need for transmission-based precaution
- Assessment and implementation of Enhanced Barrier Precautions (EBP)
- Reinforces the need for adherence monitoring for:
  - Hand hygiene
  - PPE donning and doffing
  - Environmental cleaning
  - Fluorescent marking

Importance of Micro Lab Results (Continued)

- MDRO cohorting decisions
- Observation of housekeeping practices if transmission has been detected
  - Check if contact time followed for disinfectant
  - Appropriate disinfectant for organism identified is being used
  - Housekeeping staff use standard process to prevent cross-contamination
- Antimicrobial Stewardship

Accuracy of Lab Results

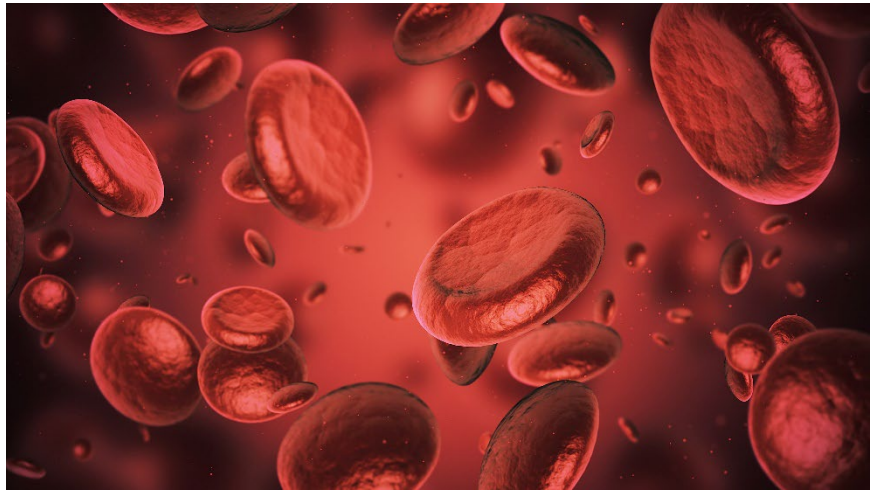
- No lab test is 100% accurate 100% of the time
- Many factors can affect accuracy of laboratory tests
  1. Pre-testing: specimen collection, handling, transportation, and preservation prior to arrival in the lab
  2. During testing: specimen processing, skill of the laboratory technician, accuracy of biochemicals and instrument system
  3. Post-testing: Accuracy of result transcription, results communicated to provider in a timely manner

Interpreting Microbiology and Laboratory Test Results

- Organism presence does not mean it is causing disease
  - Bacterial growth may confirm infection if found in normally sterile sites
- Interpreting cultures
  - Pathogens normally found in that body site without signs and symptoms of an infection may indicate colonization, and may not need treatment
- Contamination of samples at any point can result in inaccurate results and pseudo-outbreaks
- Use blood cell counts to interpret results
  - CBC
  - WBCs
  - Clotting times

Complete Blood Cell Count (CBC) and Clotting Times

- Blood tests used to evaluate overall health
  - Includes detection or absence of infection
- Measuring blood components
  - White blood cells (WBC)
  - Sedimentation rate (sed rate)
  - Clotting times



White Blood Cell (WBC) Types

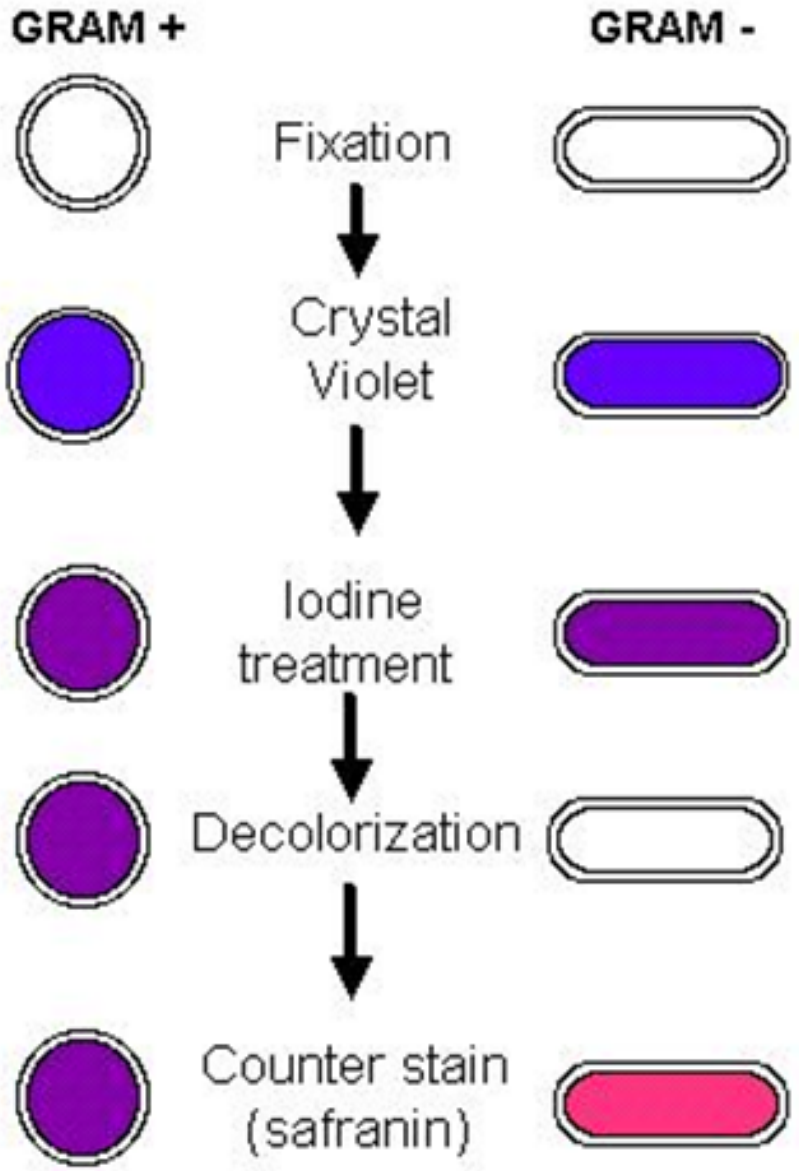
- **Polymorphonuclear leukocytes** (PMNs): General response to threat
  - **Neutrophils** (50-60% of WBC)
    - First line of response to infection
    - Also called 'segs'
    - Left shift = presence of **immature neutrophils**
      - Bands or Stabs
      - Indicates acute infection or inflammatory process
  - Eosinophils (1-7% of WBC)
    - Allergic reactions and parasites
  - Basophils (<1% of WBC)
    - Allergic reactions
    - Help mediate the strength of the immune response
- **Lymphocytes:** play a crucial role in the immune system.

Laboratory Testing Methods for Infectious Pathogens and Disease

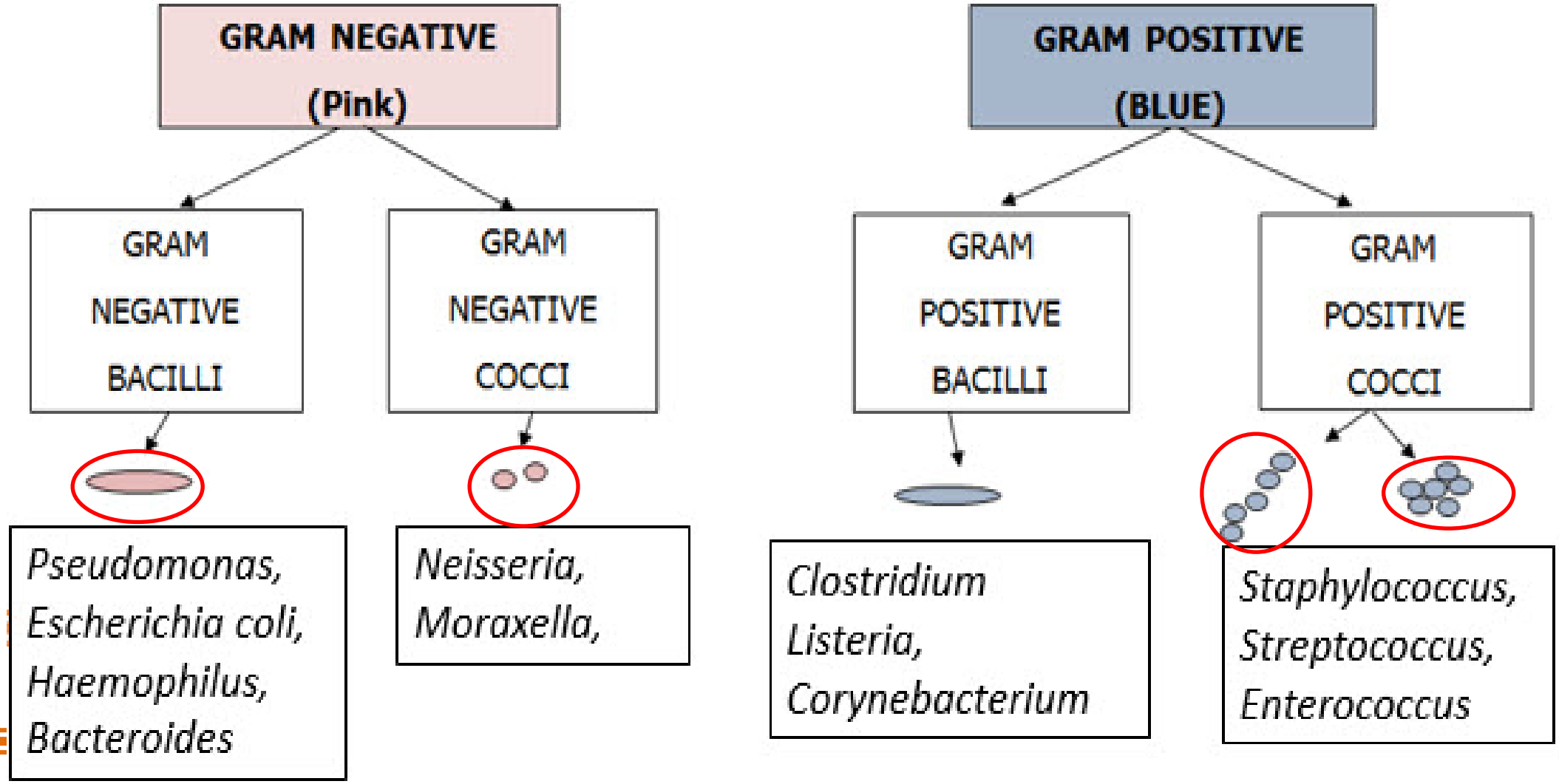
- Serology testing looks for antibodies that demonstrate exposure/infection
- Cultures identify causative pathogens and categorize them
- Antibiotic susceptibility tests of bacterial cultures identify the susceptibility or resistance to specific antimicrobial agents

Gram Stain

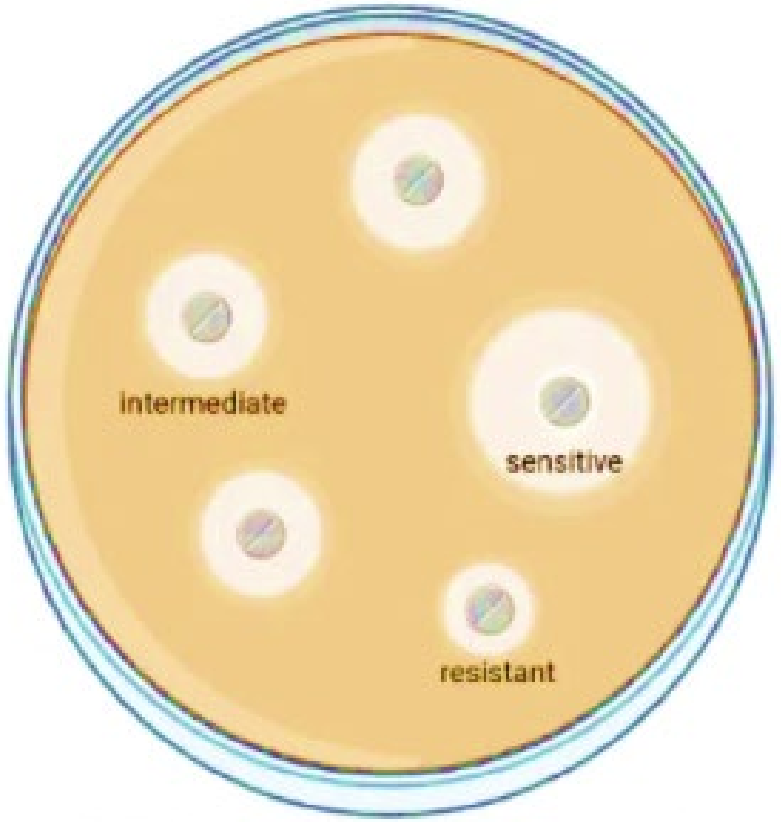
- Microbiology lab method of classifying bacteria into 2 large groups: positive (+) and negative (-)
- Differentiates bacteria by the chemical and physical properties of their cell walls
  - Lipid, non-lipid, thick or thin walled
- Guides initial antibiotic therapy



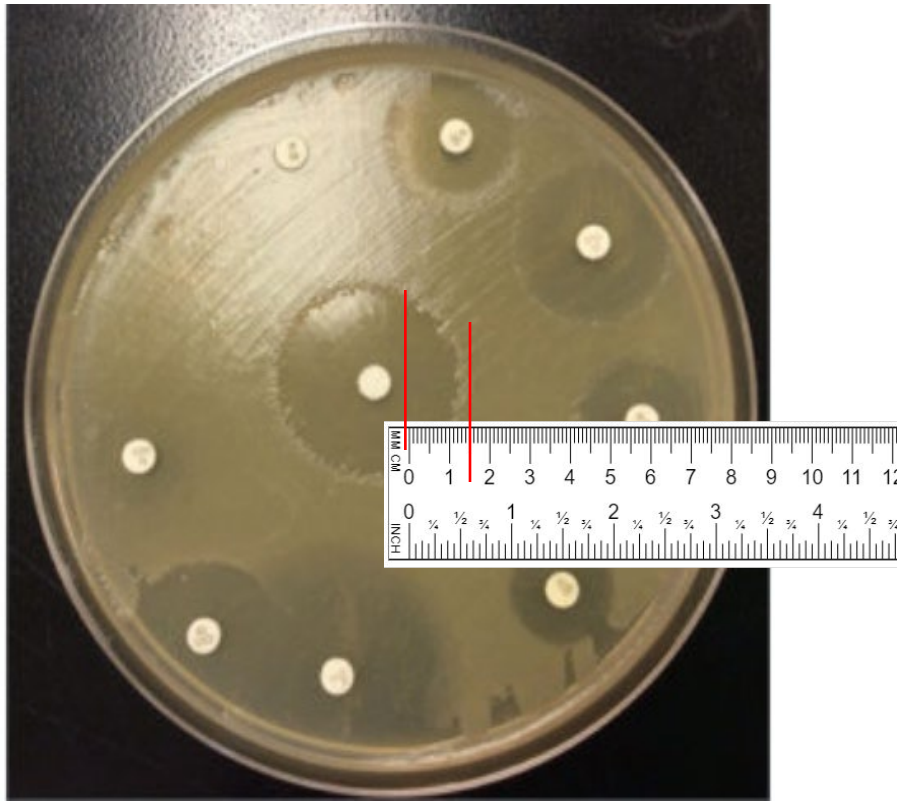
Gram Stain Identifies Four Basic Bacteria Groups



What Does Susceptible and Resistant Mean?



Antimicrobial susceptibility testing



Kirby-Bauer Disc Diffusion

MIC = minimum inhibitory concentration: Smallest amount of antibiotic on those white discs pictured above, that kills the bacteria on the culture plate

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Antibiotic Resistance (AR)

- AR is when some or all of a species or subspecies of bacteria survive exposure to an antibiotic
- Multi-drug resistance organisms (MDRO) are organisms resistant to multiple antibiotic classifications
- An antibiogram shows the number of types of bacteria susceptible or resistant to specific antibiotics in a facility or region
  - % of Staphylococcus aureus sensitive or resistant to penicillin, for example, in a facility or community
  - Used for clinical decision-making and antibiotic selection if a resident is positive for staph aureus and has symptoms
- Antibiograms reduce the risk of creating antibiotic resistance

Antimicrobial Resistance Types & Mechanisms

Types

- Natural or Intrinsic
- Acquired or Transferred

Mechanisms

- Limiting uptake of the drug
- Altering binding site for antibiotic
- Producing enzymes, e.g., Lactamases, Carbapenemases

Antibiotic Stewardship and Microbiology

- Antibiotics are essential, but
  - Leads to *C. difficile* infection (CDI) or resistant organism formation if used inappropriately
- Microbiology culture results impact treatment
  - Antibiotics given for a gram-negative organism if organism causing symptoms is gram-positive
    - Will not affect the infection
    - Sets up the resident for antibiotic resistance risk

MDRO Acronyms on a Microbiology Lab Report

- CR = carbapenem resistant
- CRE = carbapenem resistant Enterobacteriaceae (Enterobacterales)
- CRO = carbapenem resistant organism
- CPO = carbapenemase producing organism
- KPC, IMP, VIM, OXA, NDM = genetic mechanism of carbapenem resistance

Classification of Common Organisms

- **Anaerobes**
  - Bacteria that die in the presence of oxygen
  - Usually not found in the respiratory tract
- **Aerobes**
  - Oxygen loving
  - Will be found in respiratory tract but not in urine
- **Non-lactose fermenters**: Bacteria that on special medium (McConkey) will not turn color
  - Differentiate gram-negative bacteria from each other
    - Example: Pseudomonas and Salmonella are non-lactose fermenters
    - E. Coli and Serratia are lactose fermenters

Blood Cultures

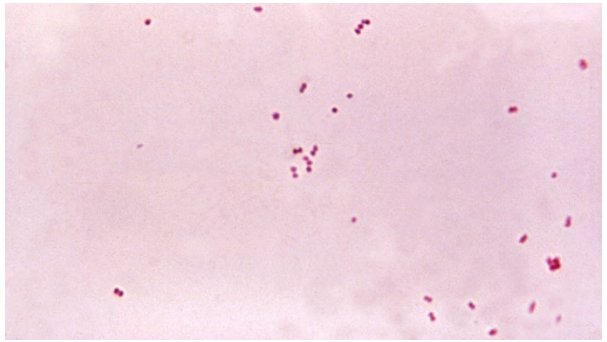
- A single blood culture specimen is collected in two bottles
  - Bottles are designed to recover either aerobes or anaerobes
  - Growth may occur in one or both bottles
- In adults, low volume of blood in the bottle (≤8 mL) can mean low numbers of bacteria in the sample
  - Leads to negative results on gram staining and false negative culture
  - Collecting the appropriate volume of blood (8-10 mL in each bottle, or 20 mL of blood divided into two bottles) is important
- Poor specimen collection technique can introduce contaminants
  - These are often common skin (commensal) flora

Urinalysis (UA)

- Odor or cloudiness may or may not mean a UTI is present
- Blood may or may not be present in a culture from resident with catheter-associated or symptomatic UTI
- Increase in WBCs with symptoms, with or without leukocyte esterase or nitrite, may indicate the urine should be cultured in microbiology lab
  - Positive leukocyte esterase or nitrite found on UA can be helpful in determining presence of WBCs
  - Increased WBC in urine with negative urine bacterial cultures may indicate infection with a sexually transmitted disease (STD) Chlamydia or Gonorrhea

Common Urinary Tract Infection (UTI) Pathogens

- Organisms may come from the resident's perineal area (GI, GU) or poor hand hygiene
- Gram-negative organisms: *Eschericia coli:* Causes 80% of all UTIs
  - *Proteus, Klebsiella, Enterobacter, Pseudomonas, and Gardnerella* are others
- Gram-positive organisms: *Staphylococcus, Enterococcus*
- Yeasts: *Candida glabrata, Candida albicans*



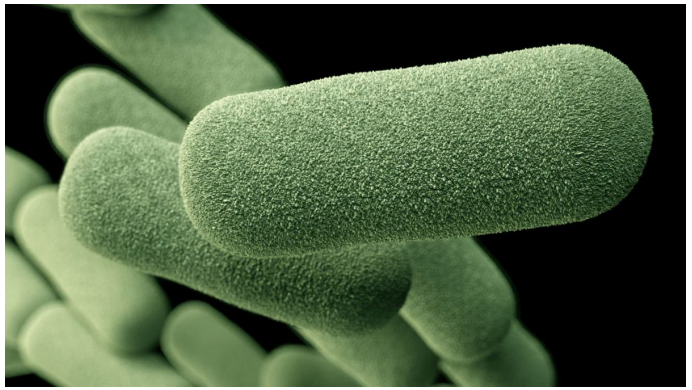
Gram stain of gram-negative bacteria
https://www.cdc.gov/gram-negative-bacteria/media/images/gram-negative-bacteria.jpg

Testing for Lower Respiratory Bacterial Pathogens

- Sputum and bronchial washes are often contaminated with oral flora
- Tracheal aspirates and protected brush specimens are not contaminated with oral flora
- Bronchoscopy suctioned and BAL are the most accurate specimen collection for lower respiratory testing

Common Lower Respiratory Tract Pathogens

- Community-acquired pneumonia (CAP)
  - *Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasmas*
- Healthcare-associated pneumonia, most often ventilator-associated
  - *Pseudomonas aeruginosa*
  - *Stenotrophomonas maltophilia*
- CAP or healthcare-associated pneumonia caused by
  - *Staphylococcus aureus* (MRSA or MSSA)
  - *Moraxella catarhallis* (most often CAP)



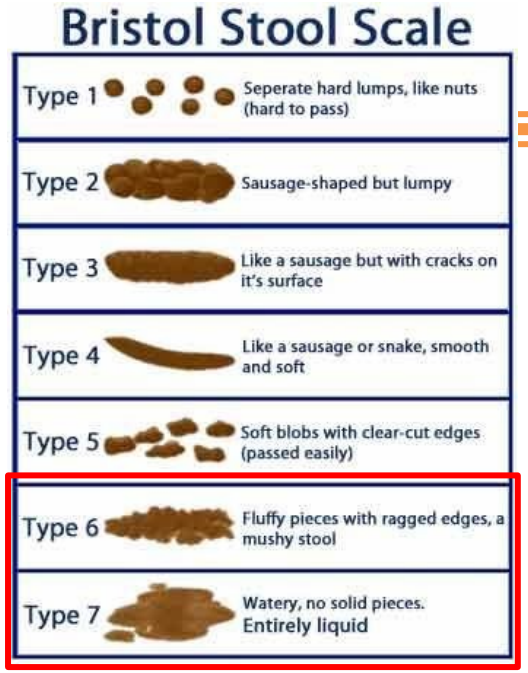
H. influenzae, a gram-negative bacterium
https://www.cdc.gov/hi-disease/media/images/Hinfluenzae.png

Common Bowel Flora

- A normal mix of bacterial flora maintains our gut health
- There are more than 400 species in the bowel
  - 95-99% are Enterobacter, Enterococcus, Proteus, Morganella, Peptostreptococcus, Bacteroides, Clostridium species
- Presence of altered conditions (colitis, diarrhea), yeast, *C. difficile,* pseudomonas species, VRE, and others can pathogenically change bowel flora
- Presence of bowel organisms found in non-GI sites may need to be investigated for possible contaminated specimen if resident is asymptomatic
  - Should not be routinely found in blood or urine, for example

Testing Stool

- C. difficile testing: Bristol Stool Scale Type 6 or 7
  - Toxin test: Toxins A/B, results same day
    - C. diff toxin degrades after one hour at room temperature
  - Molecular tests: PCR, results 24-48 hours
  - Antigen tests: Rapid, results < 1 hour, used with toxin testing
  - Stool cultures: Slow growth, 48-96 hours for results
- Norovirus: RT-PCR from whole stool sample
- Both requires a fresh stool sample
  - Avoid collecting from stool collection device
  - Must be taken to the lab within an hour



Clinical Testing and Diagnosis for CDI
(https://www.cdc.gov/c-diff/hcp/diagnosis-testing/index.html)

Viral Testing

- Viruses are tested differently than bacteria
- Viral testing types
  - PCR
  - Direct fluorescence (DFA) or immunofluorescence (IFA)
  - Serological (EIA)
  - Immunoassays (rapid tests)
    - RSV
    - Influenza
- Antibody testing (IgM, IgG)

Serology – Antibody Testing

- Diagnostic test that identifies immunoglobulins (antibodies) in blood serum
  - Immunoglobulins (Ig) are proteins that bind to viruses and bacteria

Types of antibodies

  - IgM: produced immediately after exposure (acute phase of disease)
  - IgG: most abundant; long term response to disease (chronic disease)
  - IgA: secretory, present in mucosal linings (respiratory, GI, GU tracts)
  - IgE: plays a role in hypersensitivity reactions

Laboratory Tests for Respiratory Viruses

- Direct fluorescent antibody (DFA) tests identify respiratory viruses
  - RSV
  - Influenza or parainfluenza
  - COVID 19
- Detected from nasal wash samples of patient/residents with suspected infection
  - Collected by a swab used for nasal sampling
    - Cotton or calcium alginate swab and/or wood handle does not collect viruses, resulting in false negative results

Hepatitis A Virus Test Results

- Hepatitis A Virus (HAV)
  - Hepatitis A Total
    - Result indicates current or past HAV infection
    - Cannot determine if acute or not on total result
  - Hepatitis A, IgM positive
    - Acute HAV infection
    - Patient/resident in infectious stage of disease
  - Hepatitis A, IgG positive
    - Recovering from infection
    - No longer infectious

Hepatitis B Viral Testing Terminology

Test / Term	Definition
antigen	Foreign microbe causing an immune response
antibody	Immune (proteins) response to an antigen
IgM	**I**mmune **g**lobulin **M**, 1st antibody to appear after exposure to an antigen
HB	**h**epatitis **B** virus
HBsAG	**s**urface **a**ntigen test; detects a current infection
anti-HBc	**c**ore **anti**body test; detects if ever been infected
anti-HBs	**s**urface **anti**body test; past infection or vaccination (immune)
IgM anti-HBc	**anti**body response due to initial exposure to **HB c**ore antigen
HbeAG	**HB e anti**gen; acute HB infection marker indicates highly infectious

Hepatitis B Viral Testing Results

#	Interpretation Person is:	HB(s) AG	anti-HBc	anti-HBs	IgM anti-HBc	HB(e) AG
1	Susceptible to HBV infection	neg	neg	neg		
2	Immune due to prior HBV infection	neg	pos	pos		
3	Immune due to hepatitis B vaccination	neg	neg	pos		
4	Acutely infected with HBV	pos	pos	neg	pos	
5	Chronically infected with HBV	pos	pos	neg	neg	
6	***Highly Infectious***					***pos***

CDC Interpretation of Hepatitis B Serologic Test Results (PDF)
(cdc.gov/hepatitis/HBV/PDFs/SerologicChartv8.pdf)

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Hepatitis C Viral Testing

Hepatitis C Virus (HCV)

- Hepatitis C antibody (Anti-HCV)
  - Exposure to hepatitis C
  - Active, chronic, or resolved
- Hepatitis C Qualitative (RNA PCR)
  - Identifies genetic material of the virus, detectable earlier than antibody tests
  - Used to screen after exposure
  - Confirmatory test of antibodies to the virus

When the Lab Calls…

- Does the organism sound odd?
  - Question if certain organisms found in sterile sites and patient/resident is not symptomatic
    - Culture from blood results with a resistant organism
    - Organism not usually found in that site
- Cross-contamination is possible during collection and in the lab
- Administration of antibiotics for colonization or cross-contamination increases risk of developing resistance
- Missed opportunities: unrecognized *Candida auris* as a pathogen
  - Some labs may report *Candida auris* as "yeast"

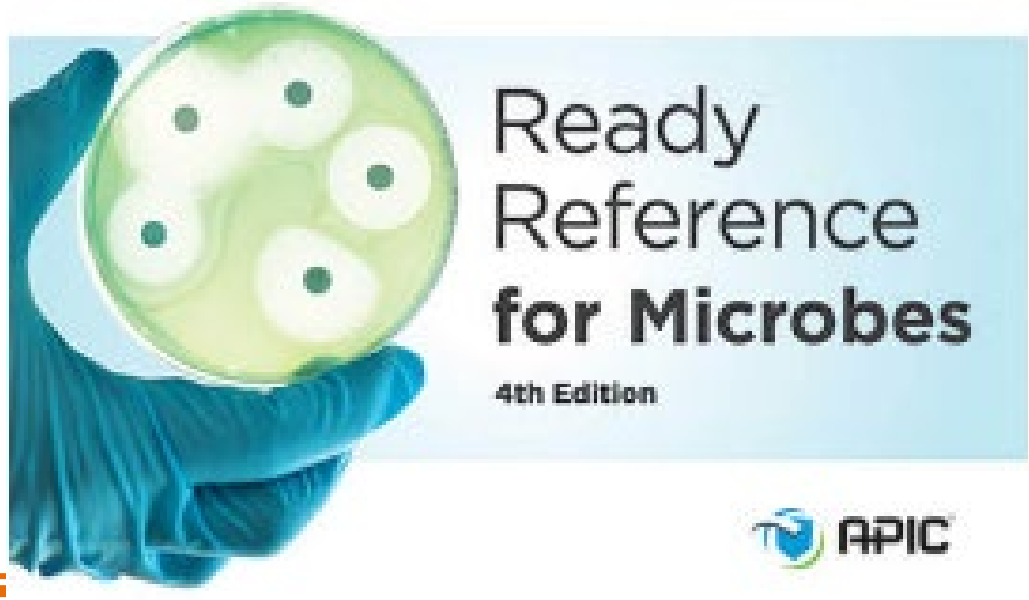
The Role Of The Laboratory In Outbreak Investigation

- How do we identify the relatedness of bacteria in an outbreak

Additional Resource

Brooks, K. *Ready Reference for Microbes*, 4rd Ed., 2018

Available on the apic.org website:
secure.apic.org/web/ItemDetail?iProductCode=SLS6005&Category=BOOKS

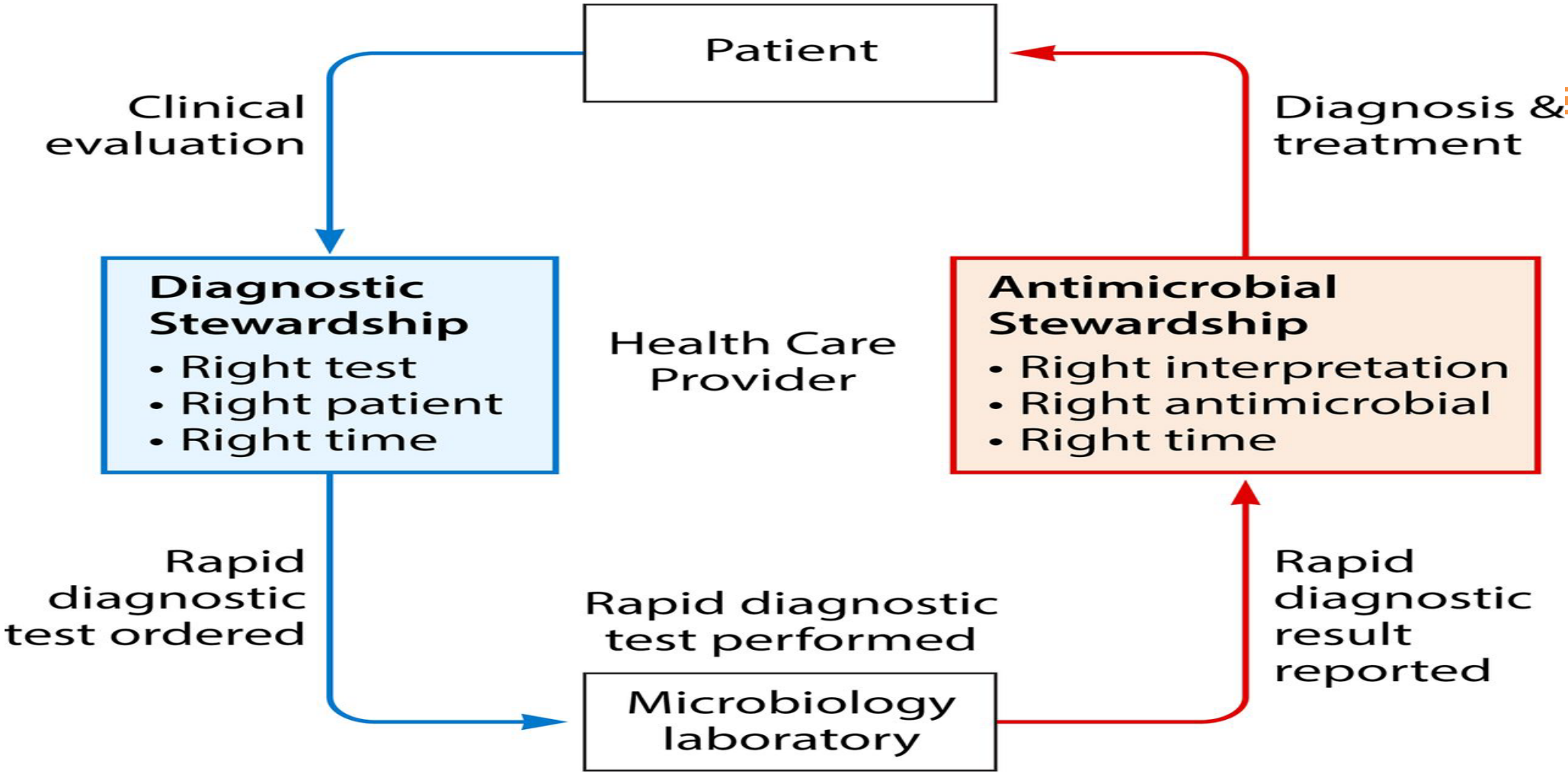


Summary

- Microbiology laboratory is important for HAI Prevention to
  - Provide results to help manage outbreaks
  - Indicate the need to perform additional screening and confirmatory tests for epidemiologic investigations
  - Target infection surveillance
  - Alert that unusual pathogens or changes in antibiotic susceptibility in the population may be occurring
  - Local antibiogram development and antimicrobial stewardship
  - Give assistance with interpretation of test results

Questions?

For more information,
please contact
HAIProgram@cdph.ca.gov


Patient
Clinical evaluation
Diagnosis & treatment
Diagnostic Stewardship
• Right test
• Right patient
• Right time
Health Care Provider
Antimicrobial Stewardship
• Right interpretation
• Right antimicrobial
• Right time
Rapid diagnostic test ordered
Rapid diagnostic test performed
Rapid diagnostic result reported
Microbiology laboratory