

Introduction to Microbiology

ACH IP Course, 2022

Infection Prevention Training for ACH
Healthcare-Associated Infections Program
Center for Health Care Quality California
Department of Public Health



Objectives

- Describe basic laboratory tests for identifying infection processes and infectious pathogens
- Discuss common Healthcare Associated Infection (HAI) pathogens

Microbiology Lab and Infection Prevention

Microbiology lab results have two important functions related to the prevention and control of infections:

- **Clinical:** identify pathogens and their susceptibility to treatment
- **Epidemiological:** identify pathogens causing unusual disease occurrences or outbreak in a population and potential sources for these pathogens

Many Microbiology Laboratory Test Methods for Infectious Pathogens and Disease

- **Serology** testing looks for antibodies that demonstrate exposure/infection
- **Cultures** identify causative pathogens
- **Antibiotic susceptibility tests** of bacterial cultures identify the susceptibility or resistance to specific antimicrobial agents
- **Microscopic evaluation** performed for certain fungal, bacterial, and parasitic infections
 - Wet mounts for vaginal organisms, CSF, skin
 - Acid-fast bacillus test for TB
 - Urinalysis

Complete Blood Cell Count (CBC)

- Most commonly performed lab test
- Blood test used to evaluate overall health
- Can detect a wide range of disorders, including infection
- CBC test measures the blood components: red blood cells, white blood cells, and platelets
- White blood cells (**WBC**) are produced to fight infection
 - Higher-than-normal WBC count indicates infection; WBC are multiplying to destroy bacteria or virus pathogen
 - WBC are also called leukocytes

White Blood Cell (WBC) Types

Granulocytes provide general response to threat

- **Neutrophils** (“segs”) – 62% of WBC
 - 1st line of response to infection
 - Left shift, or the presence of immature neutrophils (‘bands’), indicates acute bacterial infection or inflammatory process
- **Eosinophils** - 2-3% of WBC
 - Attack parasites and cancer cells
 - Help with allergic responses
- **Basophils** - Less than 1% of WBC
 - Secrete histamine, a marker of allergic disease
 - Help mediate strength of immune response

White Blood Cell (WBC) Types - 2

Lymphocytes

- **B cells** (B-lymphocytes)
 - Release antibodies to neutralize target pathogen or mark it for attack by T cells
- **T cells** (T-lymphocytes)
 - Can destroy cells taken over by viruses or cancer; can also suppress immune response
- **Natural killer cells:**
 - Quickly respond, to certain foreign bodies

Monocytes (macrophages)

- Longer lifespan; present if chronic infection
- Phagocytic; remove cellular debris and bacteria

Sedimentation Rate (Sed Rate)

- Blood test that checks for inflammation
- May indicate a disease linked to inflammation, such as arthritis or cancer, or an infection, including bones
- Test measures how fast red blood cells (**RBC**) fall to the bottom of a tube.
 - Inflammation creates proteins that make red blood cells fall more quickly
- Full name is erythrocyte sedimentation rate (ESR)
 - Red blood cells are called **erythrocytes**
 - **Sedimentation** refers to the process of falling

Serology

- Serology diagnostic tests identify **immunoglobulins** (also called **antibodies**) in blood serum
- Immunoglobulins (Ig) are proteins that bind to viruses and bacteria; produced by B cells in response to an **antigen**
- Types
 - 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
 - IgE: mediator of allergic or hypersensitivity reactions

Assessing Accuracy of Microbiology Lab Results

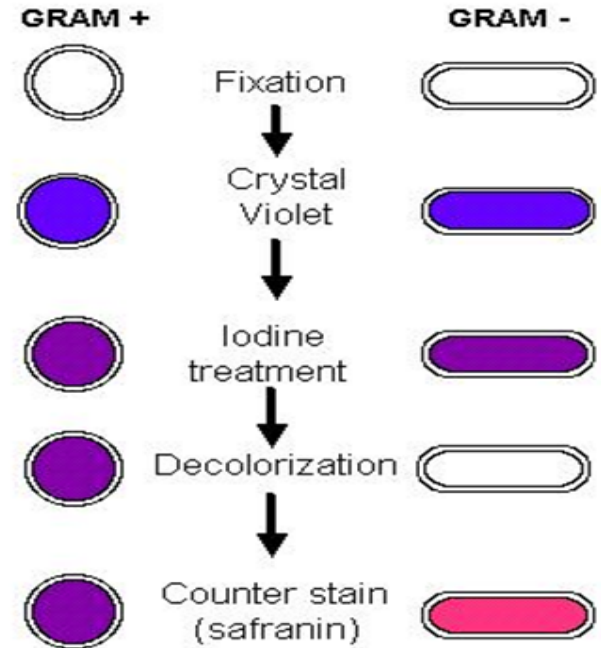
- No lab test is 100% accurate 100% of the time
- Many factors can affect accuracy of microbiology laboratory tests
 1. Pre-testing: specimen collection, handling, transportation, and preservation prior to arrival in the lab
 2. During testing: specimen processing, skill of the laboratory technician,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

Interpreting Microbiology Test Results

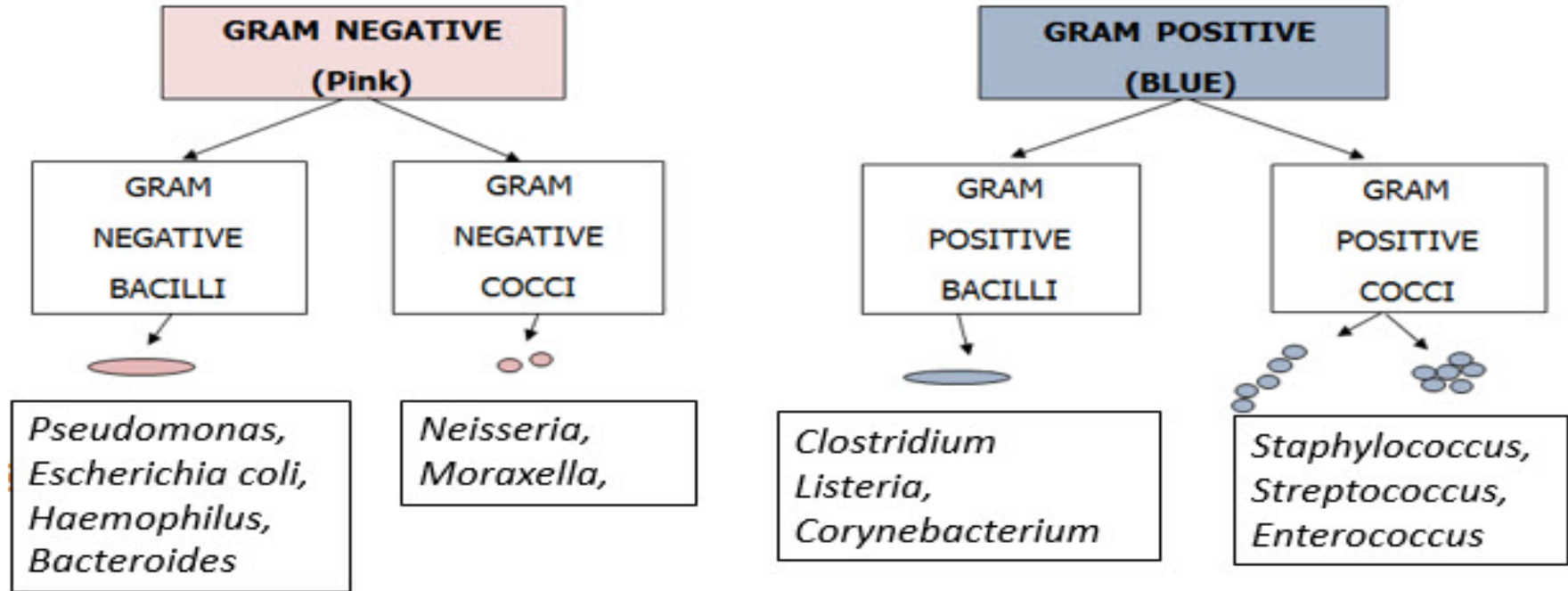
- Presence of an organism does not mean it is causing disease
 - For sterile body sites, bacterial growth may confirm an infection
- Interpret all cultures in the context of what pathogens are normally found in that body site
- Contamination of samples can result in inaccurate results and pseudo-outbreaks
- To interpret microbiology test results, use in conjunction with blood cell counts

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
- Helpful in guiding initial empiric therapy



Morphology of Common Bacteria



Blood Culture



- Detects and identifies type of bacteria in the blood
- 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 numbers of bacteria in blood ($\leq 30/\text{mL}$) can lead to negative-gram staining and false negatives
- Collecting the appropriate volume of blood (40cc blood for 4 bottles) is important
- Poor specimen collection technique can introduce contaminants to the specimen which are often common skin commensal flora

Specimens for Identifying Lower Respiratory Bacterial Pathogens

- Detect pathogens causing pneumonia or lower respiratory infection
- Sputum and bronchial wash are often contaminated with oral flora
- Tracheal aspirates and protected brush specimens are not contaminated with oral flora

Common Lower Respiratory Tract Pathogens

- Community-acquired pneumonia (CAP)
 - *S. pneumoniae*, *H. influenzae*, *Mycoplasma*
- Hospital-associated pneumonia; most often ICU or ventilator-associated
 - *Pseudomonas aeruginosa*
 - *Stenotrophomonas maltophilia*
- CAP or hospital-associated pneumonia
 - *Staphylococcus aureus* (MRSA or MSSA)
 - *Moraxella catarrhalis* (most often CAP)

Interpreting Results from Sputum Specimens

- Microbiology lab results are affected by **quality of sputum** specimen
 - **Squamous epithelial cells (SEC)** shed from the lining of the mouth and pharynx; presence indicates saliva and oral flora
 - <10 - excellent specimen, no appreciable contamination
 - 10-25 - equivocal but acceptable
 - >25 - reject due to unacceptable levels of oral contamination
 - Assess number of **WBC**
 - < 10 - no infection or poor immune response
 - 10-25 - equivocal
 - >25 - purulence indicates presence of infection

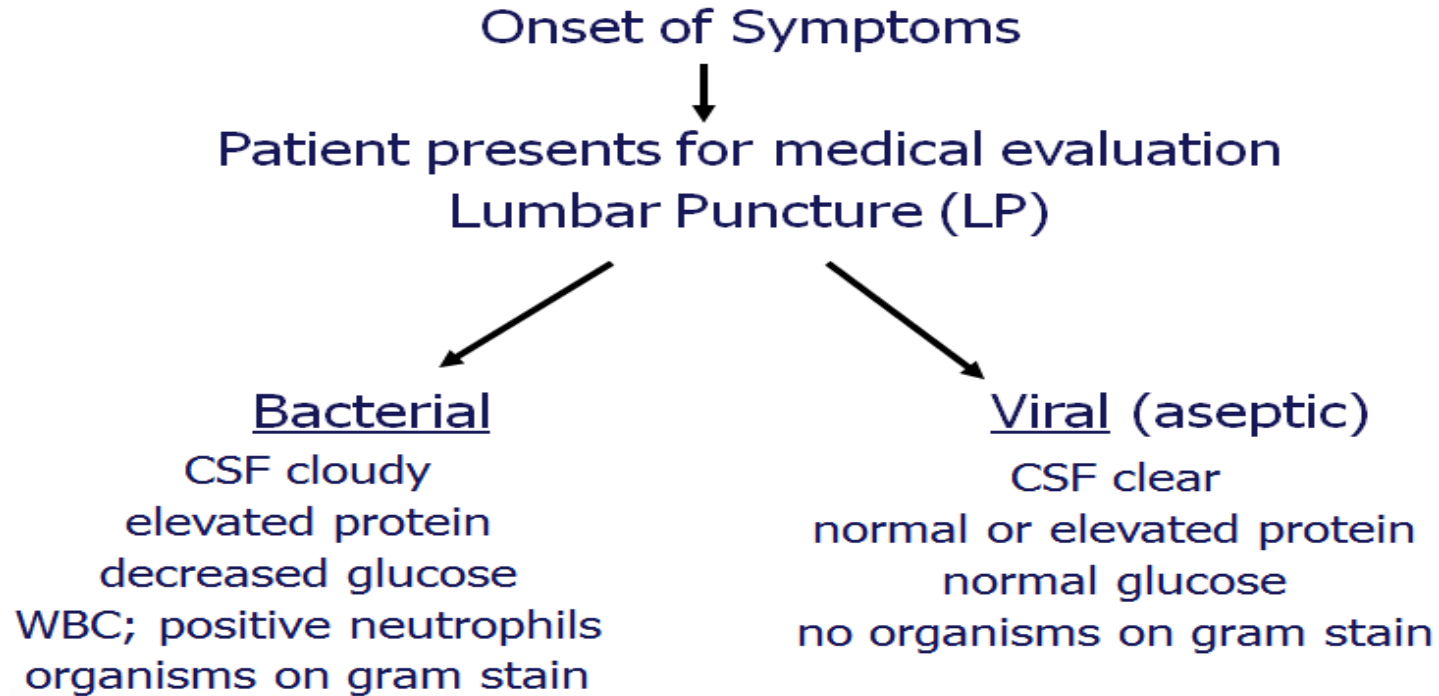
Laboratory Test for Respiratory Viruses

- Direct fluorescent antibody (DFA) tests identify respiratory viruses
- Detected from nasal wash samples of patients with suspected infection

AFB Stain for Tuberculosis

- Acid fast Bacillus (**AFB**) tests are ordered for people with symptoms of active TB
 - Test sputum for the presence of Bacillus, a type of rod-shaped bacteria, that retains a stain in the presence of an acid decolorizer
- AFB are present with **Mycobacterium** species
 - M. tuberculosis
 - M. avium
 - Other Mycobacteria species
- Very few structures are acid-fast; which makes acid-fastness particularly useful in diagnosis and management of MTB

Tests for Meningitis



Cerebrospinal Fluid (CSF) Pathogens

- Meningitis often from viruses or upper respiratory flora
- Meningitis due to gram-negative rods or staphylococcus usually associated with predisposing factors such as trauma
- Most common meningitis in an adult, *Streptococcus pneumoniae* (gram-positive cocci in pairs)
 - Generates increased WBC response
- Meningococcus (gram-negative cocci in pairs) is diagnostic of Neisseria
 - A single case is a true infection emergency

Tests for Urinary Tract Infection

Urinalysis (UA)

- Test for the evaluation of potential urinary tract infection (UTI)
- Positive leukocyte esterase or nitrite found on a UA can be helpful in determining presence of WBC

Urine culture

- Identifies bacterial pathogens in a urine specimen

Common Urinary Tract Infection (UTI) Pathogens

- Gram-negative organisms
 - *E. coli*: Causes 80% of all UTI
 - Proteus, Klebsiella, Enterobacter, Pseudomonas, and Gardnerella
- Gram-positive organisms
 - Staphylococcus and Enterococcus

Common Bowel Flora

- A normal mix of bacterial flora maintain gut health
- Enterobacter, Enterococcus, Proteus, Morganella, Peptostreptococcus, Bacteroides, Clostridium and Bifidobacterium species constitute 95-99% of the more than 400 species in the bowel
- With altered conditions, yeast, *C. difficile*, pseudomonas species, VRE, and others can pathogenically dominate the flora

Common Pathogens of Deep Incisional and Organ/Space SSI

- **Anaerobic** – do not require oxygen to grow
 - *B. fragilis*
 - Clostridium
 - Peptostreptococcus
 - Propionibacterium (septic arthritis, endocarditis, suture sites for craniotomy)
- **Aerobic**
 - Staphylococcus
 - Streptococcus
 - Gram-negative rods (GNR)

Antibiotic Resistance (AR)

- AR emerges when some or all of a species or subspecies of bacteria survive exposure to an antibiotic
 - Can be intrinsic or transferred
 - Multi-drug resistance organisms (MDRO) are resistant to multiple antibiotic agents
- An antibiogram shows the proportion of bacteria resistant to specific antibiotics in a hospital or region
 - Used for clinical decision-making

Hepatitis A Virus

- Hepatitis viruses infect the liver, causing inflammation and damage
- Hepatitis A virus causes a short-term infection transmitted via the oral fecal route
 - Ingestion of contaminated food and water
 - By direct contact, such as from contaminated food handler hands
- **Hepatitis A serological test results**
 - Positive **Total Antibody** test = past or current infection
 - Positive **IgM** = current or acute HAV infection

Hepatitis B Virus

- Hepatitis B is a vaccine-preventable liver infection caused by the hepatitis B virus (HBV)
 - Transmitted by blood, semen, or other body fluids
 - Through sexual contact, sharing needles, syringes, or other drug-injection equipment, or from mother to baby at birth.
- Not all people newly infected with HBV have symptoms
- Hepatitis B virus can cause a short-term or a long-term illness
 - Chronic infection can lead to serious, even life-threatening health issues like cirrhosis or liver cancer
- Best way to prevent hepatitis B is to get vaccinated

Hepatitis B Virus Test Terminology

Test / Term	Definition
antigen	Foreign microbe causing an immune response
antibody	Immune (proteins) response to an antigen
IgM	Immune g lobulin M , 1st antibody to appear after exposure to an antigen
HB	hepatitis B virus
HBsAG	surface antigen test; detects a current infection
anti-HBc	core antibody test; detects if ever been infected
anti-HBs	surface antibody test; past infection or vaccination (immune)
IgM anti-HBc	antibody response due to initial exposure to HB core antigen
HbeAG	HB e antigen ; acute HB infection marker indicates highly infectious

[CDC Interpretation of Hepatitis B Serologic Test Results](#) (PDF)

(www.cdc.gov/hepatitis/HBV/PDFs/SerologicChartv8.pdf)

Hepatitis B Virus Test Results

#	Interpretation	HBsAG	anti-HBc	anti-HBs	IgM anti-HBc	HbeA G
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	<i>Highly Infectious</i>					<i>pos</i>

[CDC Interpretation of Hepatitis B Serologic Test Results \(PDF\)](http://www.cdc.gov/hepatitis/HBV/PDFs/SerologicChartv8.pdf)
 (www.cdc.gov/hepatitis/HBV/PDFs/SerologicChartv8.pdf)

Hepatitis C Virus

- Hepatitis C virus (HCV) is spread through blood contact
- **Hepatitis C serological test results**
 - Positive **antibody (Anti-HCV)** = active, chronic, or resolved infection
- **Hepatitis C qualitative test results (RNA PCR)**
 - Identifies genetic material of the virus, detectable earlier than antibody tests
 - Used to screen after exposure
 - Confirm with antibody test

Rapid Diagnostic Laboratory Tests

- Rapid human immunodeficiency virus (HIV) test detects antibodies with high sensitivity and specificity
 - Use confirmatory testing to verify false positives
- Fast antigen detection for influenza but 44-60% false positives
 - Use confirmatory testing to verify
- Rapid Group A Streptococci antigen detection with 95% specificity
 - Will also detect carriers

Rapid Laboratory Tests - 2

- Polymerase chain reaction (PCR) assays
 - Makes thousands of copies of a DNA segment specific to an organism so it can be detected by identifying tests
 - Available for a number of bacterial and viral pathogens
 - Highly sensitive; may not indicate viability of organism
 - Expensive, but getting less so

Summary

Microbiology laboratory is important for HAI prevention by providing much-needed information for

- **Infection surveillance**
- **Detecting and controlling outbreaks**
 - Alerts to unusual pathogens or changes in antibiotic susceptibility in the population
 - Screening and confirmatory tests for epidemiologic investigations
- **Tracking antimicrobial resistance**
 - Local antibiogram development
- **Expert resource**
 - Assists with interpretation of test results

Additional Resource

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

Questions?

For more information,
please contact

HAIProgram@cdph.ca.gov

Include “ACH IP Basics Course” in
the subject line

Post Test

Now that you have completed this
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