Introduction to Microbiology

Last Updated 2017

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

California Department of PublicHealth

Objectives

- Describe role of the laboratory in infection prevention
- Describe factors that can adversely affect reliable laboratory results
- Interpret Gram stains
- Discuss common HAI pathogens
- Discuss laboratory testing methods to confirm infections

Microbiology and Infection Prevention

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

- **Clinical:** identification of pathogens and their susceptibility to treatment
- **Epidemiological:** identification of pathogens causing disease or outbreak in a population, and the potential sources for these pathogens

Assessing Accuracy of Lab Results

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

Presence of an Organism Does Not mean there is Disease

- For sterile body sites, growth may confirm an infection
  - Interpret all cultures in the context of what pathogens are normally found in that body site
  - If an organism is found it does not mean it is causing disease
- Pseudo-outbreaks due to contamination of samples can occur

White Blood Cell (WBC) Types

- **Polymorphonuclear leukocytes** (PMN): provide general response to threat
  - Neutrophils (50-60% of WBC) are the first line of response to infection; may also be called 'segs'
  - Eosinophils (1-7% of WBC); seen with allergic reactions and parasites
  - Basophils (<1% of WBC); seen with allergic reactions, help mediate strength of immune response
- Left shift: presence of immature neutrophils (called 'bands' or 'stabs') indicating acute infection or inflammatory process

White Blood Cell (WBC) Types - 2

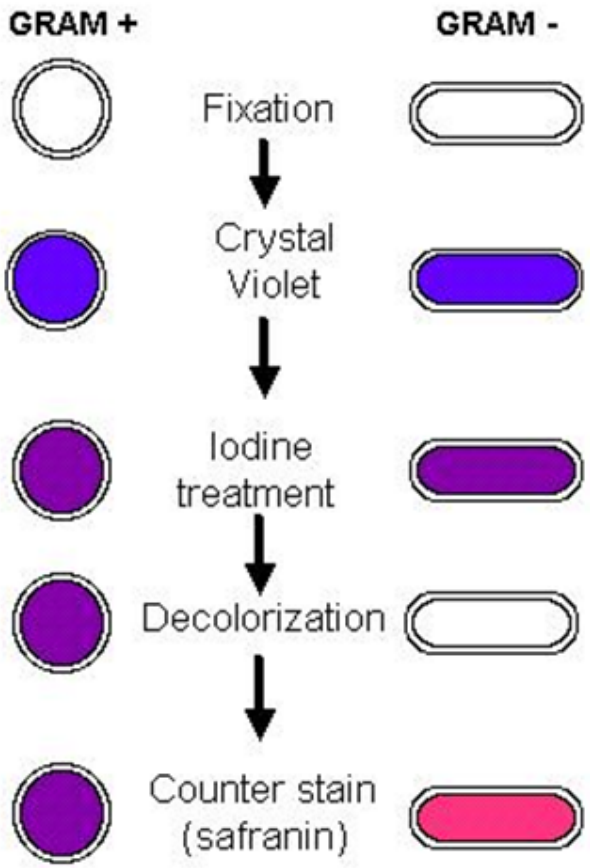
- **Lymphocytes** mature in the lymphatic portion of the immune system
  - Include pathogen-specific immune response (B cells, T cells)
  - Increase may be indicative of viral infection
- **Monocytes** (or macrophages) have phagocytic function and eat cellular debris and foreign pathogens in the immune system

B-Lymphocytes

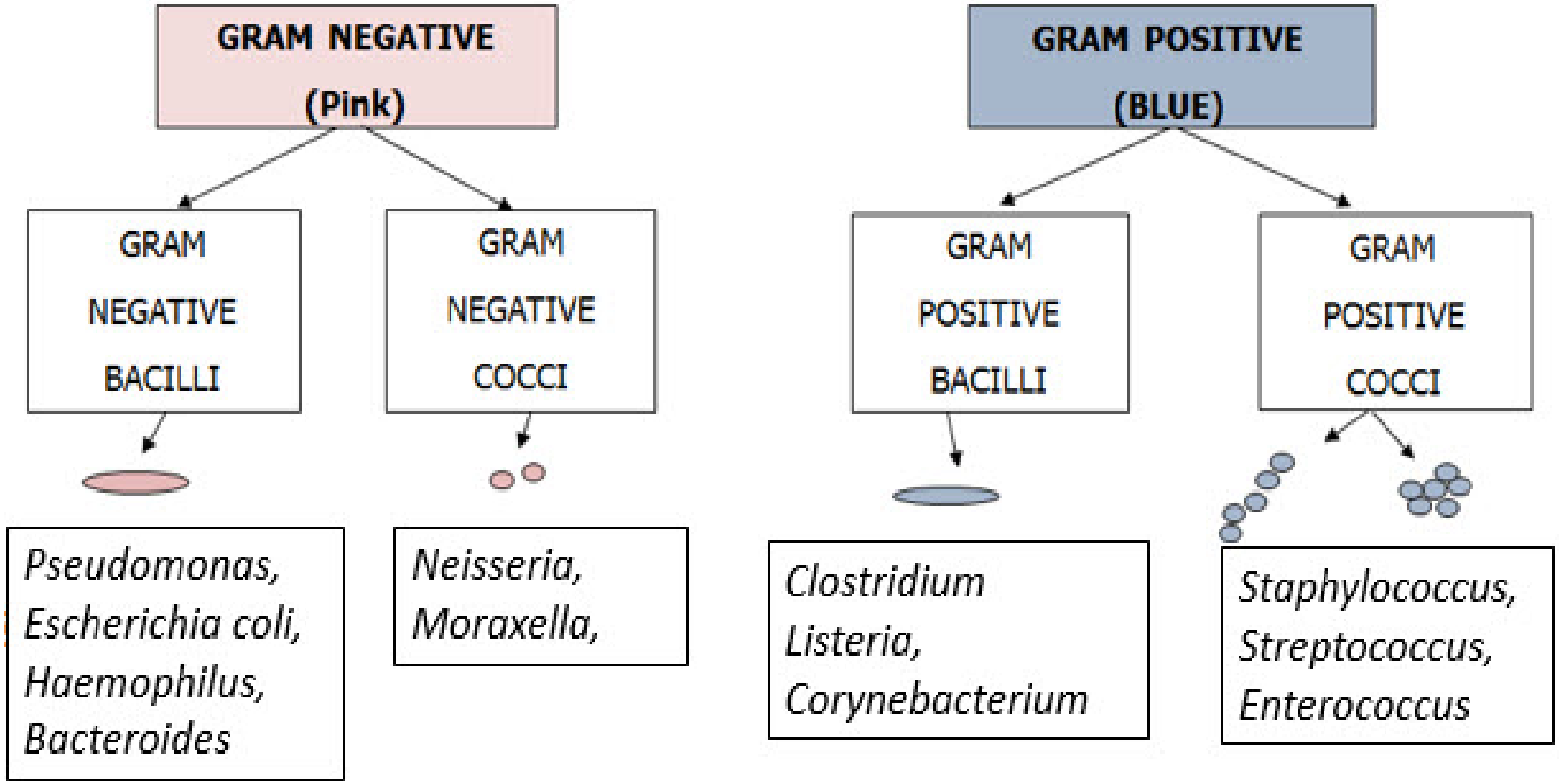
- Immunoglobulins (antibodies) are proteins that bind to viruses and bacteria:
  - IgM: produced immediately after exposure
  - IgG: most abundant, is long term response to disease
  - IgA: secretory, present in mucosal linings
  - IgE: plays a role in hypersensitivity reactions

Gram Stain

- 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



Gram Stain Identifies Four Basic Groups of Bacteria



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, no appreciable contamination
- 10-25 equivocal but acceptable
- >25 reject due to unacceptable levels of oral contamination

Quality of Sputum Specimen

Number of WBC:

- < 10 no infection or poor immune response
- 10-25 equivocal
- > 25 purulence indicates presence of infection

Lower Respiratory Cultures

- Sputum and bronchial wash are often contaminated with oral flora
- Protected brush specimen is not contaminated with oral flora
- Tracheal aspirates often identify the colonizers for ventilator-associated pneumonia

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 catarhallis* (most often CAP)

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

Meningitis

Onset of Symptoms

↓

Patient presents for medical evaluation

Lumbar Puncture (LP)

↙ ↘

Bacterial

CSF cloudy
elevated protein
decreased glucose
WBC; positive neutrophils
organisms on gram stain

Viral (aseptic)

CSF clear
normal or elevated protein
normal glucose
no organisms on gram stain

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 numbers of bacteria in blood (≤30/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

Common Pathogens of Deep and Organ Space SSI

- **Anaerobic –** does not require O_2 for growth
  - *B. fragilis*
  - Clostridium
  - Peptostreptococcus
  - Propionibacterium (septic arthritis, endocarditis, suture sites for craniotomy)
- **Aerobic**
  - Staphylococcus
  - Streptococcus
  - Gram-negative rods (GNR)

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:
  - Staph, Enterococcus, *Staphylococcus saprophyticus*

Urinalysis

- Positive leukocyte esterase or nitrite found on a UA can be helpful in determining presence of WBC
- Increased WBC in urine with negative cultures may indicate infection with chlamydia or gonorrhea

Common Bowel Flora

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

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

Resistance: Extended Spectrum Beta-Lactamase (ESBL) Producing Gram-Negative Rods (GNR)

- Each new generation of Cephalosporins have greater activity on GNR through new forms of beta-lactam
  - Resistance develops to new beta-lactams by new forms of beta-lactamases
- GNR are now resistant to 3rd generation Cephalosporins (e.g., cefotaxime, ceftazidime, ceftriaxone) and Monobactams (e.g., aztreonam) by ESBLs
- ESBL producing GNR remain susceptible to cephamycins (e.g., cefoxitin, cefotetan, cefmetazole) and carbapenems (e.g., meropenem, imipenem)

Resistance: Carbapenem Resistant Enterobacteriaceae (CRE)

- Carbapenems are becoming the last β-Lactam antibiotic class for treatment of ESBL infections
- New Delhi metallo-beta-lactamase 1 (NDM-1) carbapenemase-resistant Enterobacteriaceae (CRE) was detected in 2008; susceptible only to polymyxins and tigecycline.
- Few treatment options are available

CDC guidance for management of CRE infected patients, 2015
https://www.cdc.gov/hai/organisms/cre

Hepatitis A Virus Testing

- Hepatitis A Virus (HAV)
  - Hepatitis A Total: current or past HAV
  - Hepatitis A, IgM: acute HAV infection

Hepatitis B Virus Testing

Interpretation of the Hepatitis B Panel		
Tests	**Results**	**Interpretation**
HBsAg	negative	Susceptible
anti-HBc	negative	
anti-HBs	negative	
HBsAg	negative	Immune due to natural infection
anti-HBc	positive	
anti-HBs	positive	
HBsAg	negative	Immune due to hepatitis B vaccination**
anti-HBc	negative	
anti-HBs	positive	
HBsAg	positive	Acutely infected
anti-HBc	positive	
IgM anti-HBc	positive	
anti-HBs	negative	
HBsAg	positive	Chronically infected
anti-HBc	positive	
IgM anti-HBc	negative	
anti-HBs	negative	
HbeAG	positive	Highly infectious

Ag = antigen c = core
Ab = antibody s = surface

CDC Interpretation of Hepatitis B Serologic Test Results
https://www.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

Laboratory Tests – AFB and DFA

- Acid Fast Bacillus (AFB)
  - Distinguishes bacteria that retain stain in the presence of an acid decolorizer.
    - Present with Mycobacterium species (tuberculosis, avium and others)
  - Very few structures are acid-fast; which makes acid-fastness particularly useful in diagnosis
- Direct fluorescent antibody (DFA) tests for identification of respiratory viruses

Laboratory Tests – Rapid Diagnostic Testing

- Human immunodeficiency virus (HIV): detects antibodies, has high sensitivity and specificity, use confirmatory testing to verify false positives
- Influenza: fast antigen detection; 44-60% false positives, use confirmatory testing
- Group A Streptococci: antigen detection with 95% specificity; will also detect carriers

Laboratory Tests - Rapid

- Loop-mediated isothermal amplification (LAMP) uses a constant temperature and fewer primers
  - Newer, faster, expensive, less versatile, best for use with a single target
- Polymerase chain reaction (PCR) assays
  - Makes thousands of copies of a DNA segment specific to the 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

Laboratory Tests

- Serology testing to look for antibodies that demonstrate exposure/infection
  - Indicates patient immunity
  - Testing can also look for antigens
- Antibiotic susceptibility testing performed on bacterial cultures to test the susceptibility or resistance to specific antimicrobial agents
- Viral load testing for HIV, HCV
- Microscopic evaluation for fungal infections such as wet mounts for vaginal organisms, CSF, skin
- Antigen tests for Cryptococcal meningitis

Role of Microbiology in HAI Prevention

Microbiology can support the Infection Prevention Program with:

- Managing outbreaks
- Performing additional tests for epidemiologic analyses
- Infection surveillance
- Alerts to unusual pathogens or changes in antibiotic susceptibility in the population
- Development of a local Antibiogram
- Assistance with interpretation of microbiological results

Additional Resources

- Brooks, K. *Ready Reference for Microbes*, 3rd Ed., 2012

Questions?

For more information,
please contact any
HAI Liaison IP Team member

Or email
HAIProgram@cdph.ca.gov