Introduction to Microbiology

Last Updated 2015

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

• Describe role of the laboratory in infection prevention with emphasis on microbiology
• Describe factors that can adversely affect reliable laboratory results
• Interpret Gram stains
• Discuss common HAI pathogens
• Understand laboratory testing methods to confirm infections
Microbiology and Infection Prevention

Microbiology has two important functions related to infections:

• **Clinical**: diagnosis and management of infections
• **Epidemiological**: understand infectious microbes in patients and populations, to find sources and routes of transmission necessary for prevention efforts
Clinical Microbiology

Physician’s perspective:

• What’s growing?
• What antibiotic can be used? Determined either by predictive value of the organism type (e.g., gram negative bacillus) or by complete result with sensitivities

IP or Epidemiologist’s perspective:

• Surveillance for determining clusters and outbreaks, and assessing trends
• Need to know organism so can implement proper transmission-based precautions as needed in a timely fashion
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**: Type of agar plates used, skill of the laboratory technician, accuracy of biochemicals and instrument system
  3. **Post Analysis**: Accuracy of result transcription, results communicated to the doctor accurately
Presence of an Organism Does Not mean there is Disease

• For normally sterile body sites, growth may confirm an infection
  – Interpret all cultures in the context of what pathogens would normally grow in that body site
  – Just because an organism is found does not mean it is causing disease

• For some tests, an organism’s presence does not mean it is viable (transmissible)

• Pseudo-outbreaks due to contamination of samples can occur
White Blood Cell (WBC) Terminology

- Polymorphonuclear leukocytes (PMN): made in bone marrow, provide general response to threat
  - Neutrophils (~50-60% WBC) are the first line of response to infection; may also be called ‘segs’
  - Eosinophils (1-7% WBC); allergic reactions and parasites
  - Basophils (<1%); allergic reactions, help mediate strength of immune response
- Left shift: presence of immature neutrophils (called ‘bands’ or ‘stabs’) in blood count; are indicative of acute infection or inflammatory process
Lymphocytes

- Lymphocytes (‘lymphs’) 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
Immunoglobulins

- 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

Acid-fast stain – distinguishes bacteria that retain stain in the presence of an acid decolorizer. Present with Mycobacterium species (tuberculosis, avium and others)
Sputum Gram Stain

Quality of sputum specimen:

• Squamous epithelial cells (SEC)
  • < 10 excellent, no appreciable contamination
  • 10-25 equivocal but acceptable
  • >25 reject due to unacceptable levels of oral contamination
Sputum Gram Stain - 2

• Quality of sputum specimen: WBC
  • < 10 no infection or poor immune response
  • 10-25 equivocal
  • > 25 purulence indicates presence of infection
Lower Respiratory Cultures

- Sputum and bronchial wash is often contaminated with oral flora
- Protected brush specimen is not contaminated with oral flora
  - Semi-quantitative method recommended
- Tracheal aspirates often show colonizers
Common Lower Respiratory Tract Pathogens

- Community-acquired pneumonia (CAP)
  - *S. pneumoniae, H. influenzae, Mycoplasma*
- Hospital-associated
  - Most often ICU or ventilator-associated:
    - *Pseudomonas aeruginosa*
    - *Stenotrophomonas maltophilia*
- Either CAP or hospital-associated pneumonia
  - *Staphylococcus aureus* (MRSA or MSSA) increase mortality; must be recognized quickly
  - *Moraxella catarhallis* (most often CAP)

Note: Yeast is NOT usually an infecting organism for pneumonia or other lower respiratory tract infections unless it constitutes >70% of organisms in a specimen and specimen is not contaminated with oral flora
Cerebrospinal Fluid (CSF) Bacteria

- Source: often upper respiratory flora
- Meningitis due to gram-negative rods or *Staphylococcus* usually associated with predisposing factors such as trauma
- Adult, most common *Strep pneumo* (gram-positive cocci in pairs)
  - Generates increased WBC response
- Meningococcemia: gram stain showing gram-negative diplococci is diagnostic
  - 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 is collected in two bottles
  - Bottles are designed to recover aerobes and anaerobes
  - Growth may occur in one or both bottles
- In adults, low numbers of bacteria in blood ($\leq 30$/mL) can lead to negative-gram stain and false negatives
- Collecting the appropriate volume of blood (40cc blood for 4 bottles) is important

Less blood is needed for children due to the larger number of bacteria per cc of blood; children do not normally have anaerobes
**Blood Culture Contaminants**

Partial list of common contaminants:

- Coagulase-negative staphylococci
- Diphtheroids
- Bacillus
- Propionibacteria
- Viridans strep
- Aerococcus
- Micrococcus

To be interpreted as causing infection by these organisms:
- Two sets of blood cultures are required
- Must have specific signs and symptoms such as fever
- Refer to your NHSN definitions and for a more comprehensive list of contaminants.
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 UTI Pathogens

• Gram-negative organisms:
  – *E. coli*: Causes 80% of all UTI
  – Proteus, Klebsiella, Enterobacter, Pseudomonas, and Gardnerella cause 5-10%

• Gram-positive organisms:
  – Staph, Enterococcus, *Staph saprophyticus*, 10-20%
Common UTI Pathogens - 2

- Positive leukocyte esterase or nitrite found on a UA can be helpful in determining infection status
- Increased WBC in urine with negative cultures may indicate infection with chlamydia or gonorrhea
- Presence of yeast is not part of the NHSN definition for a UTI
Common Bowel Flora

- A normal mix of bacterial flora (e.g., yeast, C. difficile) maintain gut health
- With altered flora, yeast, C. difficile, pseudomonas species, VRE, and others can proliferate

Note: Stool samples contain digestive enzymes which continue to work after collection, necessitating addition of a preservative or prompt processing of specimens
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
Extended Spectrum Beta-Lactamase (ESBL)-Producing Gram-Negative Bacteria (GNR)

- Cephalosporins are a class of antibiotics developed to combat emergence of β-Lactamase producing GNR
- Resistance to cephalosporins began in ~1990s
- ESBL now resistant to 3rd generation Cephalosporins (e.g., cefotaxime, ceftazidime, ceftriaxone) and monobactams (e.g., aztreonam)
- ESBL remain susceptible to cephamycins (e.g., cefoxitin, cefotetan, cefmetazole) and carbenapenems (e.g., meropenem, imipenem)
Extended Spectrum Beta-Lactamase (ESBL)-Producing Gram-Negative Bacteria (GNR) - 2

- Carbapenems are 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
- CRE are beginning to emerge, leaving few treatment options

See [2015 CDC guidance for management of CRE infected patients](https://www.cdc.gov/hai/organisms/cre/)
Hepatitis A Viral Markers

- Hepatitis A Virus (HAV)
  - HAV, total: current or past HAV
  - HAV, IgM: definitive diagnosis of active HAV infection

- All hepatitis (acute and chronic) are reportable communicable diseases via local public health
  - Acute hepatitis A requires immediate notification
# Interpretation of a Hepatitis B Panel

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>negative</td>
<td>Susceptible</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>negative</td>
<td>Immune due to natural infection</td>
</tr>
<tr>
<td>anti-HBs</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>negative</td>
<td>Immune due to hepatitis B vaccination**</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>positive</td>
<td>Acutely infected</td>
</tr>
<tr>
<td>HBsAg</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>anti-HBc</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>IgM anti-HBc</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>positive</td>
<td>Chronically infected</td>
</tr>
<tr>
<td>anti-HBc</td>
<td>positive</td>
<td></td>
</tr>
<tr>
<td>IgM anti-HBc</td>
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<td></td>
</tr>
<tr>
<td>anti-HBs</td>
<td>negative</td>
<td></td>
</tr>
<tr>
<td>HbeAG</td>
<td>positive</td>
<td>Highly infectious</td>
</tr>
</tbody>
</table>

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

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**Susceptible**

**Immune due to natural infection**

**Immune due to hepatitis B vaccination**

**Acutely infected**

**Chronically infected**

**Highly infectious**
Hepatitis C Viral Markers

Hepatitis C Virus (HCV)

- Anti-HCV
  - Presence of antibodies to the virus, indicating exposure to HCV
  - Active vs. chronic vs. resolved

- HCV recombinant immunoblot assay (RIBA)
  - Confirmatory test of antibodies to the virus
  - Demonstrates if HCV was true positive (present or past is unanswered)

- All Hepatitis (acute and chronic) are reportable communicable diseases via local public health
Laboratory Tests

• Acid Fast Bacillus (AFB) test of sputum for diagnosis of TB
  – First morning specimen or bronch lavage are best
  – Rarely there may be a negative smear followed by positive culture (must follow up exposures)

• Direct fluorescent antibody (DFA) tests for identification of respiratory viruses such as legionella
Laboratory Tests - 2

• Rapid diagnostic testing: provides quick diagnosis
  – HIV: detects antibodies, has high sensitivity and specificity, use confirmatory testing to verify false positives
  – Influenza: fast antigen detection; 51-82% false positives, use confirmatory testing
  – Strep: antigen detection with 95% sensitivity; will also detect carriers
Laboratory Tests - 3

Nucleic Acid Amplification Tests (NAAT)

– Molecular technique that detects viruses or bacterium

• Polymerase chain reaction (PCR) assays
  – Amplifies gene segments specific to organism of interest
  – Available for a number of bacterial and viral pathogen
  – Uses alternating step and temperature cycle process to detect molecules
Laboratory Tests - 4

- Polymerase chain reaction (PCR) assays (continued)
  - Highly sensitive; may not indicate viability of organism
  - Expensive but getting cheaper
  - Ligase chain reaction (LCR) uses DNA polymerase (enzymes that build DNA and an enzyme that helps repair DNA. Because two targets are used, the test has greater specificity
Laboratory Tests - 5

• Loop-mediated isothermal amplification (LAMP) can be performed using a constant temperature and fewer primers
  – Newer, faster, expensive, less versatile, best for use with a single target
Laboratory Tests - 6

- 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 support is critical to:

- Outbreak management
- Performing additional tests for epidemiologic analyses
- Infection surveillance
- Knowledge of new microbes or unusual resistance
- Design of antibiotic formulary (e.g., antibiogram)
- Interpretation of microbiological results
- Education of health care staff
Questions?

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

Thank you.