



Basic Microbiology

Basics of Infection Prevention
2-Day Mini-Course
2016



Objectives

- Describe role of the laboratory in infection prevention; emphasis on microbiology
- Describe factors that can adversely affect reliable lab results
- Interpret gram stains
- Discuss common HAI pathogens for HAI
- Understand laboratory testing methods to for confirming 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/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

Rule #1: No lab test is 100% accurate 100% of the time

Many factors can affect accuracy of laboratory tests

1. Pre-analysis:

- How was specimen collected, handled, transported, preserved prior to arrival in the lab?

2. Analysis:

- Were correct agar plates used? Was the specimen incubated at correct temp? Lab protocols followed? Skill of the micro tech? Accuracy of biochemicals and instrument system?

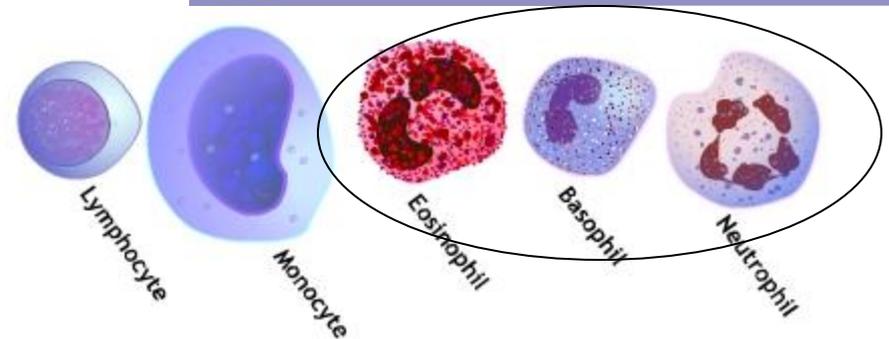
3. Post Analysis:

- Accurate result transcription in computer systems? Did results get communicated to the doctor accurately?

Rule #2: Just because an organism is found does not mean it is causing disease.

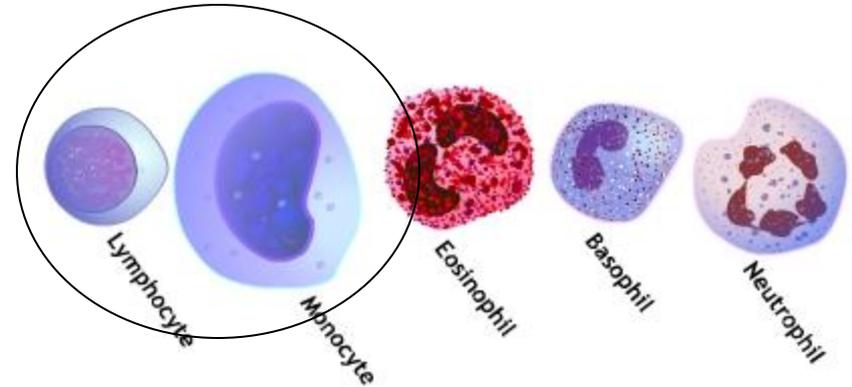
- For normally sterile body sites, growth may indeed be an infection
 - Interpret all cultures in the context of what pathogens would typically/normally grow in that body site
- For some tests such as polymerase chain reaction (PCR), because an organism is present does not mean it is viable (transmissible)
- Pseudo-outbreaks due to lab contamination of samples can occur

White Blood Cell (WBC) Terminology



- PMNs (polymorphonuclear leukocytes) made in bone marrow; provide general response to threat
 - Neutrophils (~50-60% wbc) are 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



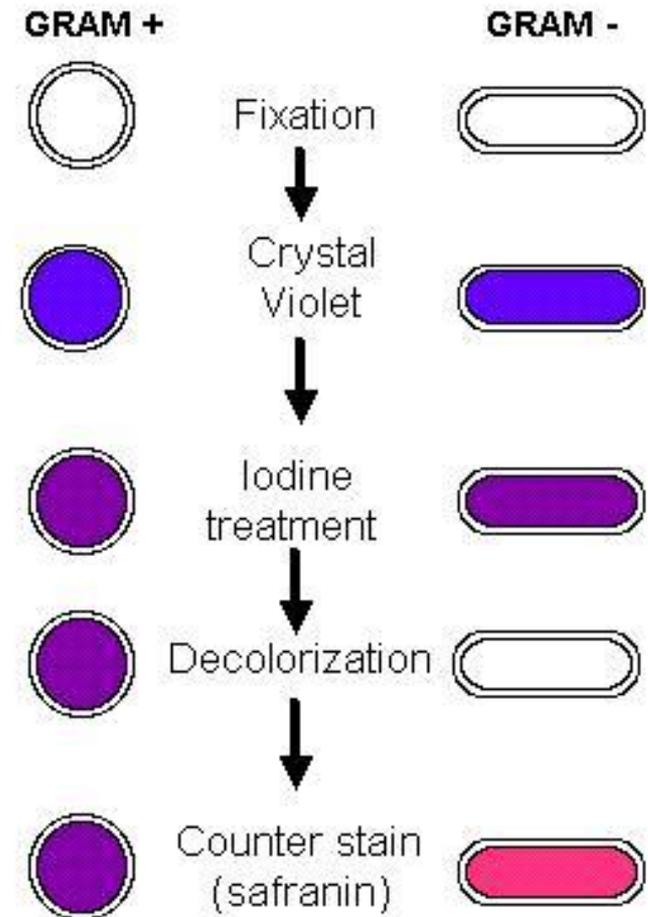
- Lymphocytes (lymphs) mature in the lymphatic portion of the immune system
 - Include pathogen-specific immune response (B cells, T cells)
 - Increase may be indicative of viral infection
- Monocytes (or macrophages) phagocyte function (or eat) cellular debris and foreign pathogens from the immune system

Immunoglobulins are Specific Lymphocytes

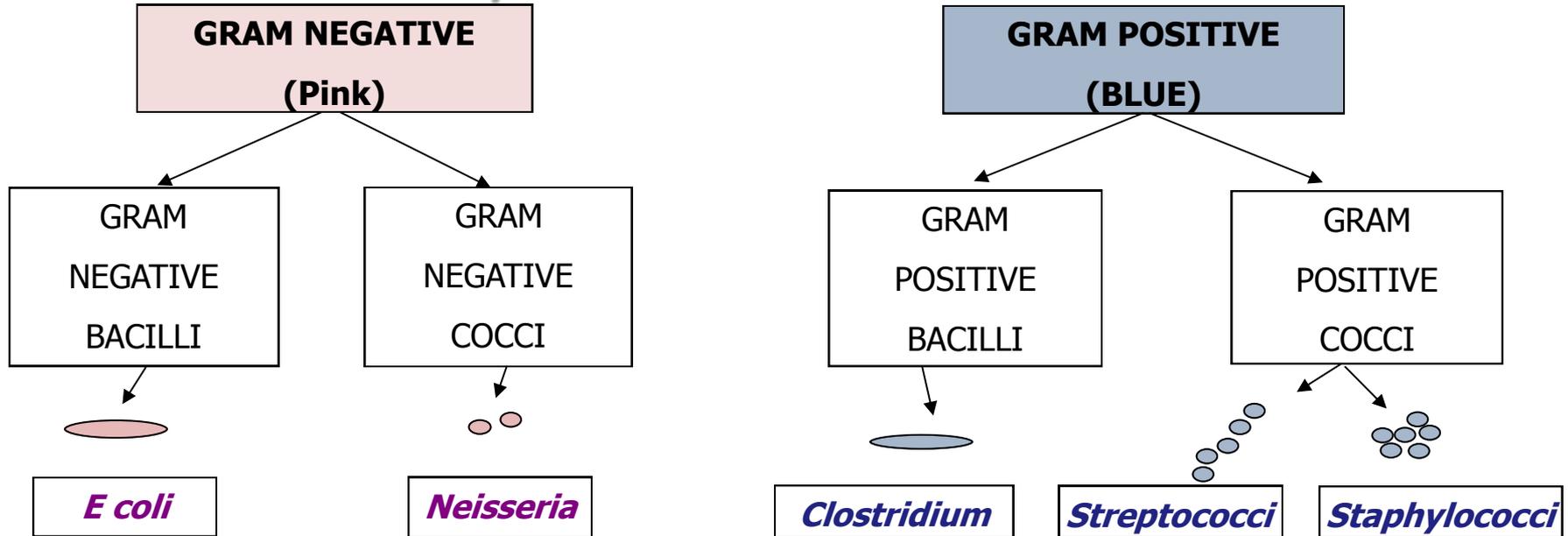
- Immunoglobulins (antibodies) are proteins that bind to viruses and bacteria
 - IgM – produced immediately after exposure
 - IgG – most abundant, is long term response to disease
 - IgA – secretory, present in mucosal linings
 - IgE – plays a role in hypersensitivity reactions

Gram Staining

- 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
 - results should get to physician ASAP



Bacterial Groups



Gram stain identifies four basic groups of bacteria:

1. Gram positive cocci (*Staphylococcus*, *Streptococcus*, *Enterococcus*)
2. Gram negative cocci (*Neisseria*, *Moraxella*)
3. Gram positive bacilli (*Clostridium*, *Listeria*, *Corynebacterium*)
4. Gram negative bacilli (*Pseudomonas*, *Escherichia coli*, *Haemophilus*, *Bacteroides*)

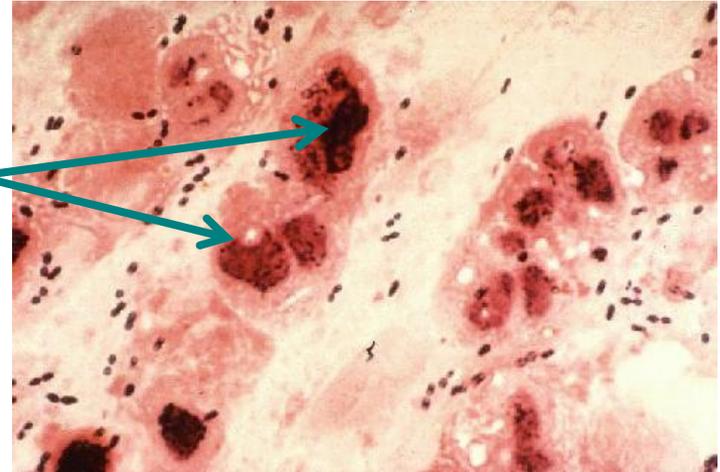
Acid-fast stain

Distinguishes bacteria that retain the stain even in the presence of an acid decolorizer. Used to show the presence of 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
- WBC
 - <10 no infection (or poor immune response)
 - 10-25 equivocal
 - >25 purulence indicates presence of infection



Lower Respiratory Cultures

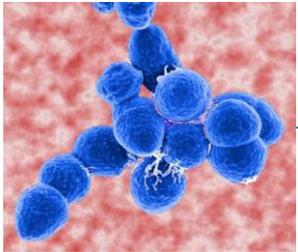
- Sputum and bronchial wash: often contaminated with oral flora
- Protected brush specimen: not contaminated with oral flora
 - semi-quantitative method recommended
 - put brush into 1.0mL TSI* broth; vortex; inoculate agar with urine loop
 - reported as number of CFU/ml**
- Tracheal aspirates: often show colonizers



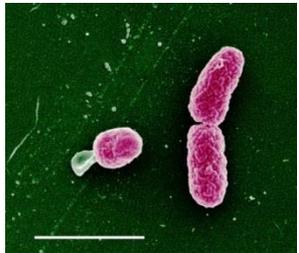
*TSI (triple sugar iron) helps distinguish between certain enteric pathogens

**CFU/ml = colony forming units per milliliter

Common Lower Respiratory Tract Pathogens



- Community-acquired pneumonia (CAP)
 - *S. pneumoniae*
 - *H. influenzae*
 - *Mycoplasma*
- Hospital-acquired, most often ICU or ventilator-associated
 - *Pseudomonas aeruginosa*
 - *Stenotrophomonas maltophilia*

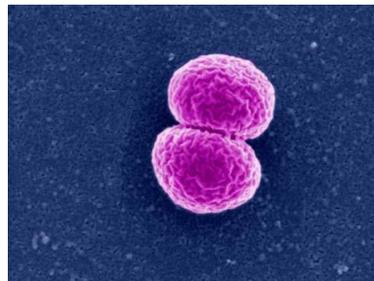


- Either CAP or hospital-acquired pneumonia
 - ***Staphylococcus aureus*** (MRSA or MSSA)
 - ↑ mortality; must be recognized quickly
 - *Moraxella catarrhalis* (most often CAP)

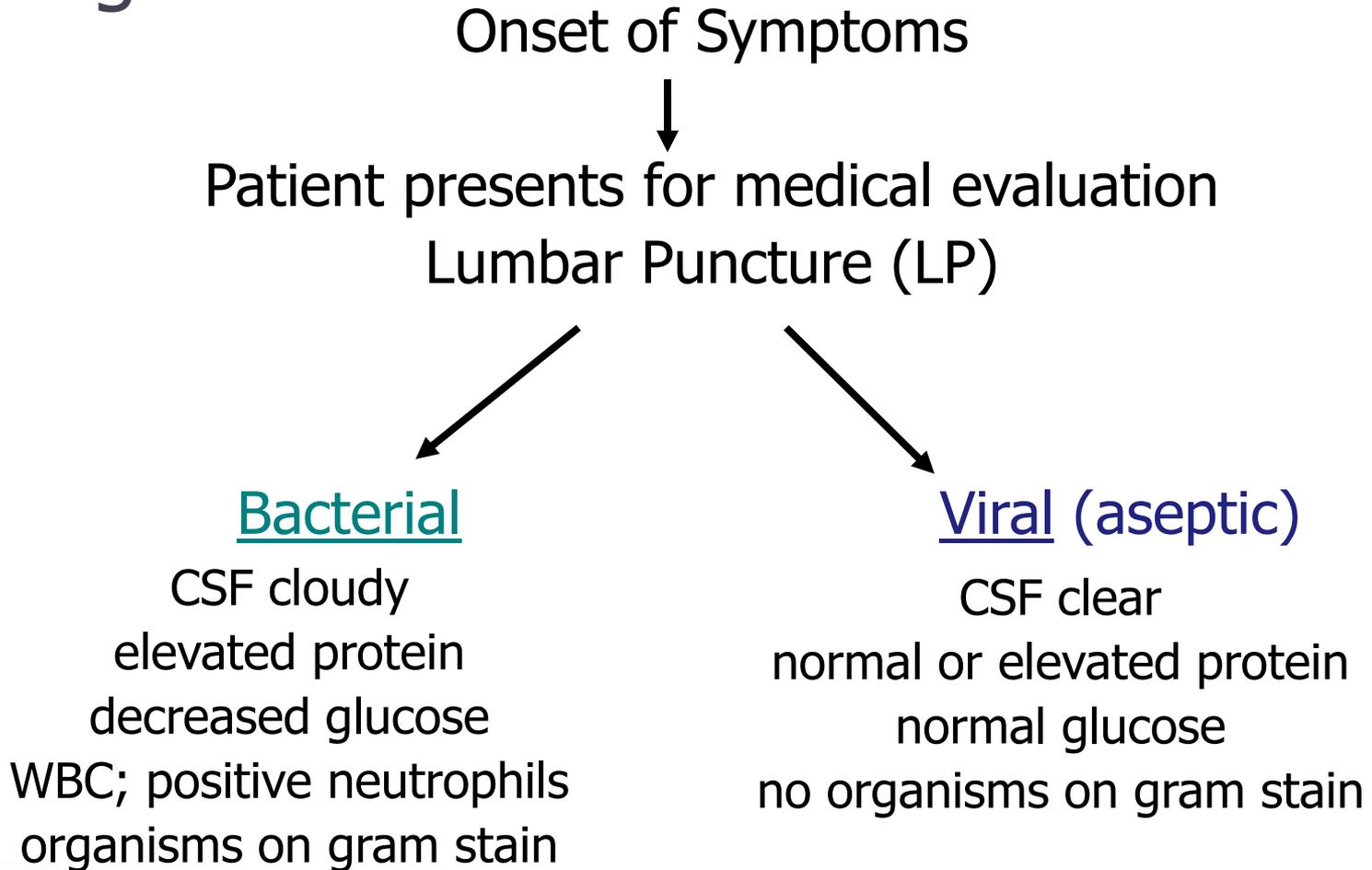
Note: Yeast is NOT usually an infecting organism for pneumonia or other lower respiratory tract infections unless it constitutes >70% of organisms in a specimen and specimen is not contaminated with oral flora

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



Blood Cultures



- A single blood culture consists of two bottles
 - Bottles designed to recover aerobes and anaerobes
 - Irrelevant which bottle has growth or if both or only one bottle has growth
- Adults: low numbers of bacteria in blood ($\leq 30/mL$)
 - Can lead to negative gram stain and false negative
 - Volume is important; usual 4 bottles/40cc blood
 - Less blood needed for children due to larger number of bacteria per cc of blood/don't normally have anaerobes



Blood Culture Contaminants

Partial list of common contaminants

- Coag neg staphylococci
- Diphtheroids
- Bacillus
- Proprionibacteria
- Viridans strep
- Aerococcus
- Micrococcus

For these bacteria to be interpreted as causing infection, two sets of blood cultures are required PLUS 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 O₂ for growth)
 - *B. fragilis*
 - Clostridium
 - *Peptostreptococcus*
 - *Propionibacterium* (septic arthritis, endocarditis, suture sites for craniotomy)
- Aerobic examples
 - Staphylococcus
 - Streptococcus
 - Gram negative rods (GNR)

Common UTI Pathogens

- Gram negatives
 - *E. coli*: Causes 80% of all UTI
 - Proteus, Klebsiella, Enterobacter, Pseudomonas, Gardnerella cause 5-10%
- Gram positives
 - Staph, Enterococcus, *Staph saprophyticus*, 10-20%
- Positive leukocyte esterase and/or nitrite found on a UA can be helpful in determining infection status.
- Increased WBC in urine w/ negative cultures may indicate infection w/ chlamydia or gonorrhea.

• Presence of yeast are not part of the NHSN definition for a urinary tract infection

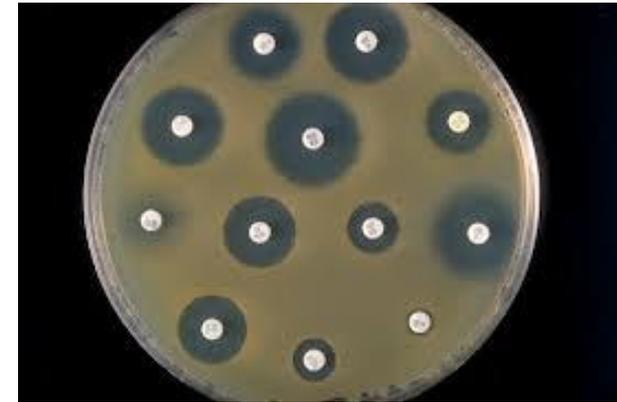
Common Bowel Flora

- Normal mix of bacterial flora keeps numbers of yeast, *C. difficile*, and other potential pathogens in the gut in check
- With altered flora, yeast, *C. difficile*, pseudomonas species, VRE, and others can proliferate

Of note: Stool samples contain digestive enzymes; enzymes continue to work after collection, necessitating addition of a preservative and/or prompt processing of specimens

Antibiotic Resistance

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



Kirby-Bauer Disk
Diffusion Susceptibility
Plate

Table 2.

Antibiotic	Susceptible		Intermediate		Resistant	
	n	%	n	%	n	%
Amoxicillin-Clavulanate	116	100.0	0	0.0	0	0.0
Penicillin	93	80.2	23	19.8	0	0.0
Ceftriaxone	116	100.0	0	0.0	0	0.0
Clarithromycin	104	89.7	2	1.7	10	8.6
Cefuroxime	115	99.1	1	0.9	0	0.0

Extended Spectrum Beta-lactamase (ESBL)-producing Gram-negative Bacteria

- Cephalosporins: class of antibiotics developed to combat emergence of β -Lactamase producing GNR
- Resistance to cephalosporins began in \sim 1990s
- ESBLs now resistant to 3rd generation Cephalosporins (eg: cefotaxime, ceftazidime, ceftriaxone) and monobactams (e.g.: aztreonam)
- ESBL remain susceptible to cephamycins (cefoxitin, cefotetan, cefmetazole) and carbenapenems (meropenem, imipenem)

ESBL (continued)

- Carbapenems are the last β -Lactam antibiotic class for treatment of ESBL infections
 - e.g. imipenem, meropenem, doripenem, ertapenem
- New Delhi metallo-beta-lactamase 1 (ndm-1) CRE detected in 2008; susceptible only to polymyxins and tigecycline
- Carbapenemase-resistant Enterobacteriaceae (CRE) beginning to emerge, leaving few treatment options
 - Seen in 47 states by Feb 2014

See 2013 CDC guidance for management of CRE
infected patients at

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 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

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 RIBA (**recombinant immunoblot assay**)
 - 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 of Interest to IP

- Acid Fast Bacillus (AFB) test of sputum for diagnosis of TB
 - First morning specimen or bronch lavage are best
 - Rarely negative smear, positive culture (must follow up exposures)
- Direct fluorescent antibody (DFA) tests for identification of respiratory viruses such as legionella
- Rapid diagnostic testing: provides quick diagnosis
 - HIV: detects antibodies, has high sensitivity/specificity but because of false positives, confirmatory testing should be done
 - Influenza: very fast antigen detection; false positives 51-82% of time, so should not be used alone
 - Strep: antigen detection w/ 95% sensitivity; will also detect carriers

Nucleic Acid Amplification Tests (NAAT)

Molecular technique that detects viruses or bacterium

- Polymerase chain reaction (PCR) assays amplify gene segments specific to organism of interest; available for a number of bacterial and viral pathogens
 - Uses alternating step and temperature cycle process to detect molecules
 - Highly sensitive; may not indicate viability of organism
 - Expensive but getting cheaper, more rapid
- 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
- 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 of Interest to IP - continued

- Serology testing to look for antibodies (see Slide 9) 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 (see Kirby Bauer, Slide 22)
- 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 (antibiogram)
- Interpretation of microbiological results
- Education of health care staff

Questions?

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

Thank you