

Welcome to *California*



Basic Microbiology



Basics of Infection Prevention
2-Day Mini-Course
2013

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 laboratory 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? Incubated at correct temp? 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?



Robert Berg, 2009, Micro for ICPs.
www.apicsierra.org/page6.html



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

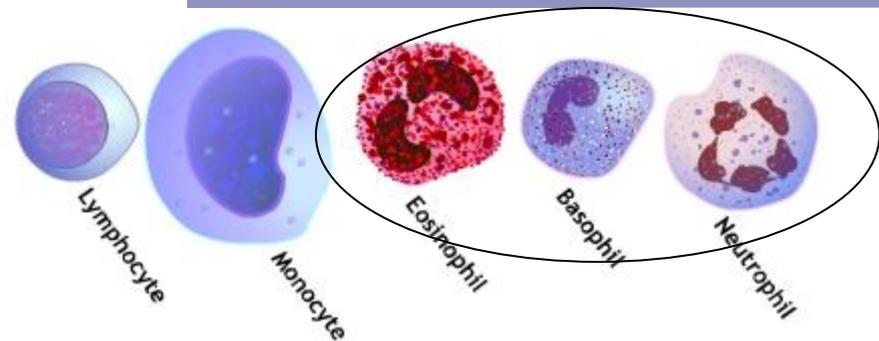
- For normally sterile body sites, growth may indeed be an infection
 - Interpret all cultures in the context of what pathogens would typically/normally grow in that body site
- For some tests such as PCR, because an organism is present does not mean it is viable (transmissible)
- Pseudo-outbreaks due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



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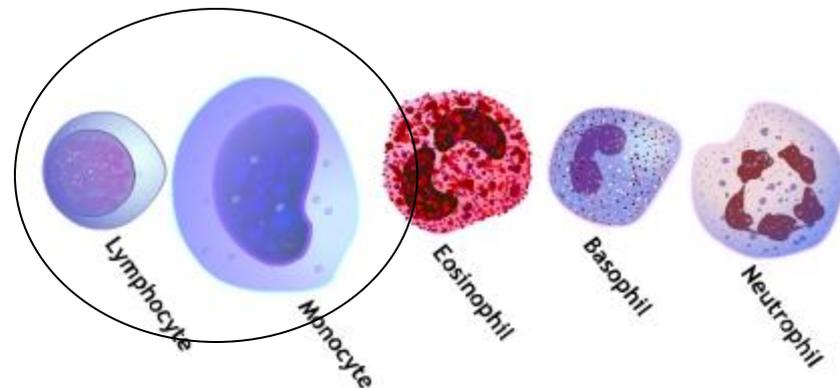


WBC Terminology



- 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

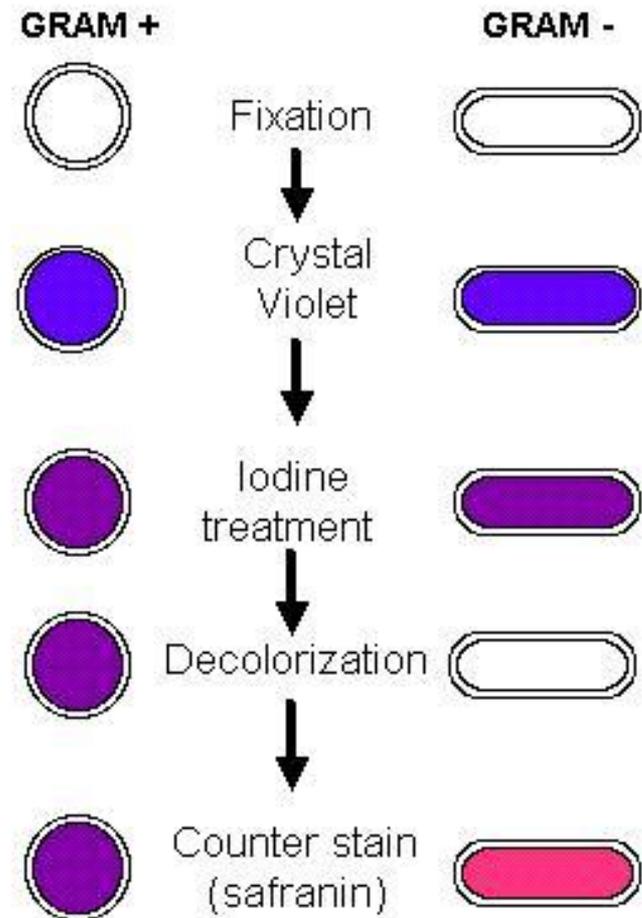
Lymphocytes



- 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) phagocytosis function (or eat) cellular debris from the immune system

Gram Stains

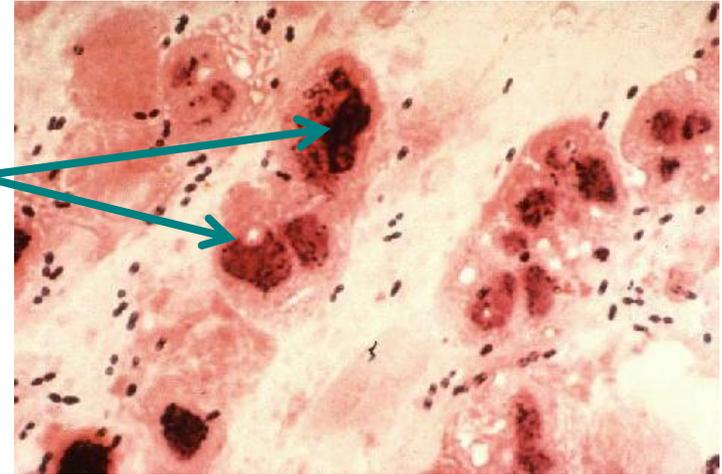
- Helpful in guiding initial empiric therapy
- Helpful in evaluating quality of culture result
- Does not improve patient outcome if the results don't get to the physician ASAP



Sputum Gram Stain

Will see

- 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
- Bacteria



Lower Respiratory Cultures

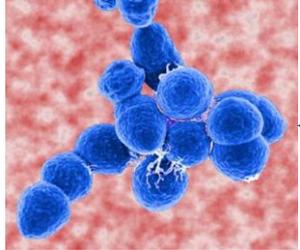
- Sputum and bronchial wash: often contaminated with oral flora
- Protected brush specimen: not contaminated with oral flora
 - semi-quantitative method recommended
 - put brush into 1.0mL TSI broth; vortex; inoculate agar with urine loop
 - reported as number of CFU/ml*
- Tracheal aspirates: often show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



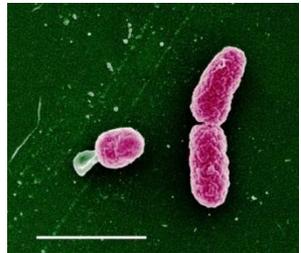
*CFU/ml = colony forming units per milliliter



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*

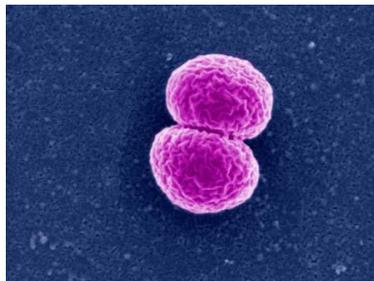


- Either CAP or hospital-acquired pneumonia
 - ***Staphylococcus aureus*** (MRSA or MSSA)
 - ↑ mortality; must be recognized quickly
 - *Moraxella catarrhalis* (most often CAP)

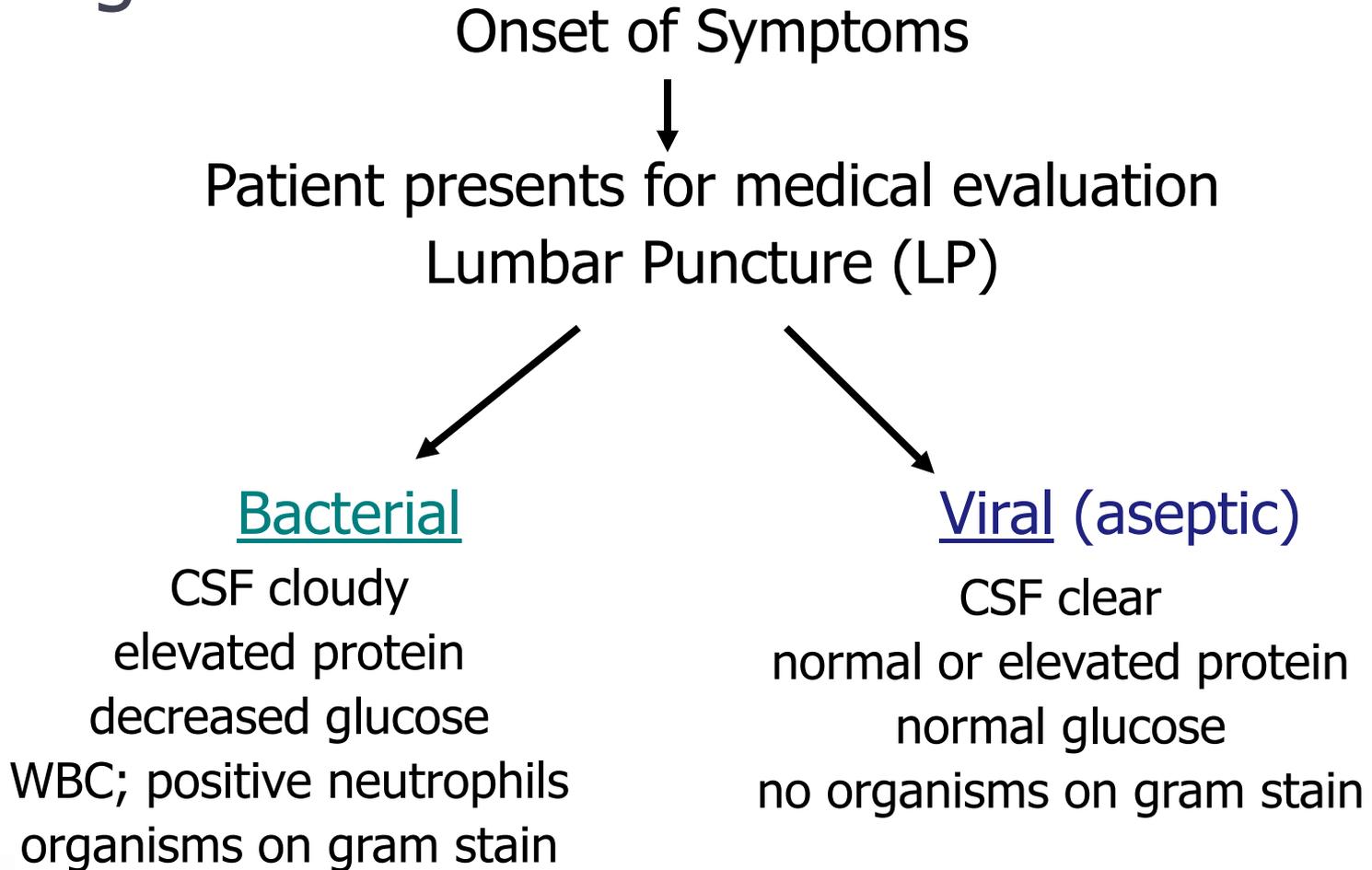
Note: Yeast is NOT usually an infecting organism for pneumonia or other lower respiratory tract infections. May be if constitutes >70% of organisms in specimen and specimen not contaminated with oral flora

Cerebrospinal Fluid (CSF) Bacteria

- Source: often upper respiratory flora
- Meningitis due to gram negative rods or *Staphylococcus* usually with predisposing factors such as trauma
- Adult, most common: *Strep pneumo* (gram positive cocci in pairs)
 - generates increased WBC response
- Meningococemia: 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 (≤ 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

Common contaminants

- Coag neg staph
- 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

Common Pathogens of Superficial Surgical Site Infections (SSI)

- Not usually anaerobes
- Generally skin flora, but not necessarily
- Can be gram negative rods (GNR)



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
 - GNRs

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.

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

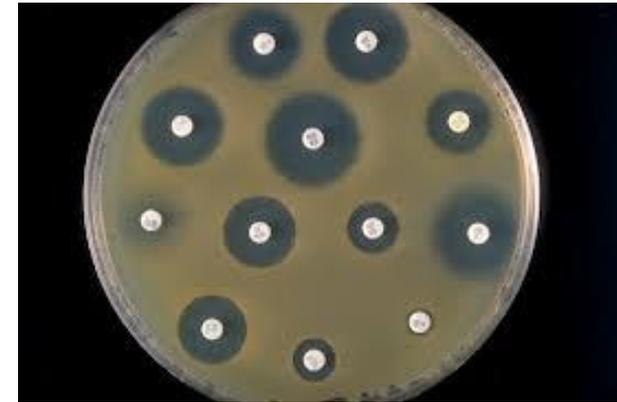


Table 2.

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 ~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
- Carbapenemase-resistant Enterobacteriaceae (CRE) beginning to emerge, leaving few treatment options
 - Seen in 42 states by Feb 2013
- New Delhi metallo-beta-lactamase 1 (ndm-1) CRE detected in 2008; susceptible only to polymyxins and tigecycline

See 2013 CDC guidance for management of CRE infected patients at www.cdc.gov/hai/organisms/cre



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



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



Hepatitis **B** Viral Markers

Especially important
in women of
childbearing years

Hepatitis B Virus (HBV)

- Hb**s**Ag – current or chronic HBV
- Hb**s**Ab – recovery or immunity to HBV
- Anti-Hbc – current or previous HBV infection
- Anti-Hb**c** IgM – recent acute infection
 - If also HbsAg ⊕ - acute infection
 - Distinguishes acute from chronic infection
- Hb**e**AG – highly infectious
 - Guidelines exist for HCWs who are HbeAG positive

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



Ag = antigen
Ab = antibody

c = core
s = surface



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

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
 - Confirmatory test of antibodies to the virus
 - Demonstrates if HCV was true positive (present or past is unanswered)
- Recommended that all 'boomers' be tested for HCV; 5x more likely to be infected



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
- Direct antigen testing for group A strep, influenza, RSV – easy, cheap, not always sensitive
- Polymerase chain reaction (PCR) assays amplify gene segments specific to organism of interest; available for a number of bacterial and viral pathogens
 - Highly sensitive; may not indicate viability of organism
 - Expensive but getting cheaper, more rapid

Laboratory Tests of Interest to IP - continued

- Serology testing to look for antibodies (e.g., IgM, IgG) that demonstrate exposure/infection to viruses HBV, measles, chickenpox
 - Indicates patient immunity or not
 - 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

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

