OUTLINE

• Introduction to Basic Clinical Microbiology
  • Didactic
• Plate Rounds
  • Practical
• Tech Demo
  • Practical
OBJECTIVES

• Match a specimen and/or specimen associated rules to the appropriate work-up on the bacteriology bench.
• Identify the common media, assays and algorithms used to provide diagnostic microbiology support to patient in austere environments.
• Identify the common pathogenic microorganisms associated with each organ system infection to include the most common bacterial, fungal, viral, and parasitologic agents causing disease.
Practicing Clinical Microbiology in Austere Environments

Insights from adapting clinical microbiology testing to war zones could prove helpful in other harsh settings

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In 2002, the United States Army began sending clinical microbiologists to active war zones, where they were tasked to do infectious disease diagnostic testing for sick and hospitalized troops and associated personnel. Practicing clinical microbiology in such circumstances proves challenging, but also critical for maintaining the fitness of those troops even when they encounter a wide variety of infectious diseases.

Clinical microbiologists in war zones sometimes confront unique challenges. However, many of their experiences track with those of other clinical microbiologists working in similarly austere environments throughout the developing world. Indeed, a variety of U.S. organizations, including ASM, are working with those in other countries, the World Health Organization (WHO), and similar international entities to improve the infrastructure needed for doing diagnostic testing as a way of responding more effectively to emerging, endemic, and man-made infectious disease outbreaks and requirements to support patient care.

Although such efforts first focus on immediate responses, experts soon realize the value of sustaining clinical care capabilities and in supporting ongoing efforts to mitigate infectious disease threats. For example, the ASM International Laboratory Capacity Program (LabCap), which was established in 2005, aims to build a sustainable infrastructure for clinical microbiology in resource-limited countries, where clinicians typically were forced to rely mainly on syndromic diagnosis because they had little confidence in the accuracy and quality of laboratory test results. Information on LabCap can be obtained at https://www.asmcap.org/index.cfm. We believe that the U.S. Army experience of adapting clinical microbiology procedures for use in war zones can be leveraged and adapted to other austere environments—improving patient care and public health.

Summary

- The US Army in 2002 began deploying special microbiology test packages as well as highly trained clinical microbiologists to Afghanistan and Iraq.
- Practicing clinical microbiology near and in war zones, where resources are limited and conditions austere, proves challenging.
- Despite hardships, deploying clinical microbiologists to work in combat support hospitals speeds efforts to identify and diagnose infectious diseases, including nosocomial outbreaks.

The Basics of Diagnostic Testing in Austere Environments

Before the 20th century, disease and nonbattle injuries (HNBI) caused much more casualties among troops than did combat itself (see box, p. 298). Deployed troops are often naïve to local endemic diseases, and combat stresses may further predispose them to illness. Further, war leads to public health instability that fosters the spread of diseases such as plague, yellow fever, typhus, dengue fever, malaria, diphtheria, cholera, influenza, and typhoid, all of which have played major roles in past wars. Some of these diseases are endemic;
Austere lab environment challenges

- Mission
- Facilities and Equipment
- Logistics
- Personnel
- Test menu
- Workload
- Regulatory
Test Menu

- Blood (Septi-Chek)
- Urine (bi-plates)
- Wounds/fluids (aerobic/anaerobic)
- Respiratory
- Stools (TCBS, EIA’s)
- AFB (Kinyuon)
- Mycology (direct exam and culture)
- Parasitology (O&P's, EIA’s, blood parasites)
Rapid Tests (Direct)

- Group A Streptococcus
- Streptococcus pneumoniae Urinary Antigen
- Legionella pneumophila Urinary Antigen
- Influenza types A & B
- Campylobacter antigen
- Shiga Toxin (and from GN broth)
- Meningitis screen
- HIV-1
- Parasite screening panel
- Syphilis RPR
- Infectious Mononucleosis
Identification and susceptibility testing

Semi-automated

Manual
Laboratory Organization

• Clinical Microbiology
  • *Bacteriology* - ID and Susceptibility
  • Virology - Culture and/or antigen detection
  • Mycobacteriology/Mycology - ID/Susc
  • *Parasitology* - Microscopy or antigen detection

• Infectious Disease Serology
  • Hepatitis, other viral, autoimmune, Lyme, etc.

• Molecular Microbiology
Microbiology - Bacteriology

- Specimen Collection
- Smear - Gram stain, fluorescent, direct
- Culture -
  - artificial media, incubation, isolation, ID
- Rapid detection -
  - Group A Strep (pharyngitis), Influenza (pneumonia)
- Susceptibility -
  - Predicting resistance or susceptibility
Specimen Collection

- Sterile Containers
  - urine, sputum, tissue, stool, BAL, CSF
- Blood Cultures – broth or bi-phasic bottles
- Swabs and appropriate transport media
  - TISSUE IS BEST!!
Acute pharyngitis
Rapid Detection / Screening Tests
Plating onto media
Enterococcus

GAS
Identification and susceptibility testing

Semi-automated

Manual
Antimicrobial Susceptibility Testing (AST)
Bacterial Endocarditis

- Splinter hemorrahges
- Osler’s Nodes
- Vegetation on Heart Valve
Blood Culture Bench - RULES

- Volume - > 30 mls total
- 2 different sites, ie two cultures - rule out contaminants
- Automation - continuous monitoring
- Critical values - call Gram stain result
- ID and susceptibility
Blood Culture Contamination

- Growing “bugs”
- Pseudobacteremia
- Phlebotomy
  - Skin Prep (CHG/EtOH)
  - Skin Contaminants OR
  - Endocarditis
Organisms most often associated with clinical bacteremia
- GPCs in Clusters
- GPCs in pairs and chains
- GNRs, short, fat
- GNCP
- GP, large oval, “too large to be bacteria!”

Coagulase Neg *Staphylococcus* (often skin contaminant)

*Staphylococcus aureus*

*Streptococcus*

*Enterococcus*

*E. Coli*

*Pseudomonas*

*Haemophilus*

*Yeast*
Urine Bench - RULES

• Quantitation
  • > 100,000 cfu/ml - full ID and Susceptibility
  • > 10,000 cfu/ml - partial ID and Susceptibility
  • < 10,000 cfu/ml - no work up

• MUF - mixed urogenital flora, 3 or more organisms cultured from same urine: Contamination!

• MEF - mixed enteric flora: Contamination!
E. coli, E. coli, E. coli ...
Wounds Bench

- No Rules
- TISSUE
- Skin contaminants
- ID and Susceptibility
Organisms most often associated with clinical wound infections?

- *S. aureus, S. aureus*
- Mixed anaerobes - polymicrobial
- Skin contaminants
- Nosocomial organisms
  - MRSA, VRE
  - Acinetobacter
  - Gram negative enteric bacilli (E. coli, Klebsiella sp.)
  - *Pseudomonas aeruginosa*
MRSA, GAS
S. aureus
Clostridium perfringens

- Gas gangrene
- Myonecrosis

- boxcar shape
- double zone B
- hemolysis
Respiratory
Respiratory Bench - RULES

< 10 SECs / > 25 PMNs per lpf

SPIT VS SPITUM

10X 10X 10X 10X
• Organisms most often associated with clinical respiratory infections?

• Upper Respiratory -
  • Otitis media - *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis*
  • Pharyngitis - *Streptococcus pyogenes* (GAS)
    • Viral (respiratory, EBV, Coxsackie) GrpC&G Strep, C. & M. pneumoniae, A. haemolyticum, HIV (acute)
Acute respiratory presentations
Chest X Ray
Respiratory Bench - Common Organisms - Cont.

- Lower Respiratory -
  - Viral - RSV, Influenza, Parainfluenza, Adenovirus
  - Bacterial - community acquired
    - *S. pneumoniae, M. pneumoniae, C. pneumoniae*
  - Bacterial - nosocomial
    - Pseudomonas, Gram negative enterics, Legionella
  - Bacterial - aspiration
    - Anaerobes
    - Gram negative enterics
Gram stains

*S. pneumoniae*  *H. influenzae*  *N. meningitidis*
Stool bench - 3 DAY RULE

- Inpatient - >3 days
  - On antibiotics - antibiotic associated colitis associated with *Clostridium difficile*
- Outpatient, Clinic, Inpatient < 3 days
  - Bacteria - Gram negative bacilli such as Salmonella, Shigella, Yersinia, Campylobacter, E. coli O157:H7
  - Parasites - Giardia, Cryptosporidium, *Entamoeba histolytica*; worms
Bacterial Gastroenteritis

*Campylobacter species*

- Campylobacter sp.
  - Curved GN rods
    - “gull wing”
  - Oxidase POS
- Most common cause of community acquired bacterial gastroenteritis
- Microaerophilic, grown on selective media
Bacterial Gastroenteritis

*Vibrio cholerae*
Non-fermenters (look for lack of color)
Cytotoxin mediated disease
Physically damages the intestinal epithelium. These infections are sometimes called invasive or inflammatory.

Bacteria that produce cytotoxins include:

- *Shigella* species (A-D)
- *Salmonella* species (1400 serotypes)
- *Campylobacter jejuni*
- *Yersinia enterocolitica*
- *Clostridium difficile* – cytotoxin B, this organism also produces an enterotoxin A
- *Vibrio* species, not cholera
- Enteroinvasive *E. coli* (EIEC)
- Enterohemorrhagic *E. coli* (EHEC), an example is E. coli O157:H7
- Enteropathogenic *E. coli* (EPEC)

Normal flora

Facultative anaerobes:
- Enterobacteriacea – E. coli, Klebsiella sp., Enterobacter sp., Proteus sp.
- Gram-positive cocci – S. aureus, Enterococcus, Streptococci

Anaerobes:
- Gram-negative – Bacteroides fragilis, Fusobacterium
- Gram-positive – Lactobacillus, Clostridium, Peptostreptococcus
Enterotoxin mediated disease
Physiologic change to the intestinal epithelium resulting in fluid and electrolyte secretion.

These infections are sometimes called malabsorptive or non inflammatory.

Bacteria that produce enterotoxins include:

- *Vibrio cholerae*
- Enterotoxigenic *E. coli* (ETEC)
- *Staphylococcus aureus* – toxin mediated, rapid symptoms
- *Bacillus cereus* - toxin mediated, rapid symptoms
- *Clostridium perfringens* - toxin mediated, rapid symptoms

The latter three are primarily toxin mediated not requiring growth of the organism in the host to cause disease. Other pathogens such as *Cryptosporidium parvum, Giardia lamblia*, rotavirus, calicivirus and norwalk agent (virus) also manifest with similar symptoms. *Clostridium botulinum* produces neurotoxin.
Rapid detection respiratory virus

Influenza A+B

Influenza A+B
Mycobacteriology and Mycology Bench

- Mycobacteriology - respiratory, tissue
  - *Mycobacterium tuberculosis* (M. tb)
  - *Mycobacterium other than tb* (MOTT)
  - Slow growth, slower susceptibilities
  - Smear, culture, molecular methods

- Mycology - tissue, body fluids
  - Yeast - *Candida species*, *Cryptococcus*
  - Mold - *Aspergillus*, dimorphs, dermatophytes
Smears – fluorescent and acid-fast
Tissue preparation for fungal elements

KOH digestion

Lactophenol Cotton Blue
Yeasts

- Candida spp
- Candida albicans
- Cryptococcus spp
- Cryptococcus neoformans
- Torulopsis glabrata
- Trichosporon spp
- Geotrichum spp
- Malassezia furfur
Parasitology Bench

• Blood - Malaria, Trypanosome

  RDT
  
  Microscopy

• Stool parapak - 2 preservatives

  ParaPak

  Microscopy

• PVA - small things (100x) such as protozoa seen on permanently stained slide

• Formalin - somewhat larger things (40x) such as eggs, larvae, adult worms seen on wet mount
Need Help?

• microbiology.consult@us.army.mil

• Attach pictures, clinical consult, questions

  • Skin scraping for Leishmaniasis
  • Malaria confirmation
  • Fungal elements/immunocompromised
  • Acinetobacter