Culture of Blood, CSF, and Other Sterile Body Fluids

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January 14, 2009

Objectives

- List the culture media and incubation conditions used for blood, CSF and other sterile body fluids.
- Discuss which organisms are considered to be pathogens vs. contaminants in a blood, CSF, or sterile body fluid culture.
- Determine when susceptibility testing is necessary on a blood, CSF, or sterile body fluid isolate.

Disclosure

Dr. Ledeboer serves as a consultant to Pfizer and Arcxis Biotechnologies and has performed numerous clinical studies for Remel, BD, and bioMerieux for which he has received research support.

Speaker will discuss off-label uses of various products

Blood Stream Infection (BSI)

- Accounts for 15-20% of all healthcare-acquired infections
  - Morbidity 250,000-350,000 patients/year in the U.S.
  - Mortality approximately 17-35% = 10th leading cause of death in the U.S.
- One real BSI adds ~ $40K to the hospitalization cost of a surviving patient
  - Note: the cost of a contaminated blood culture adds about $4,000 in terms of unnecessary patient care

Types of BSI

- Transient
  - cellulitis, dental procedures, endoscopy, diabetic foot ulcers, etc.
- Intermittent
  - Undrained abcesses, infected catheter
- Continuous (easiest to diagnose).
  - Infective endocarditis
- "Breakthrough"
  - BSI in the presence of concomitant and appropriate antimicrobial therapy

Sources of primary BSI (i.e., not catheter-related).

- Genitourinary tract 25%
- Respiratory tract 20%
- Abscesses 10%
- Surgical wounds 5%
- Biliary tract 5%
- Other known sites 10%
- Unknown sites 25%

Trends over time

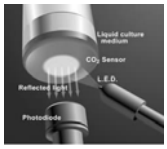
- BSI rates have increased in the past 20 years due to:
  - invasive equipment and transcutaneous devices
  - chemotherapy
  - immunosuppression
  - life extending therapies
- Spectrum of Agents
  - Before 1990, Gram positive accounted for <25%
  - Currently, gram-positive organisms predominate but several studies indicate that the trend is once again reversing
  - Fungemia with *Candida* species is on the rise

Common agents of BSI

- 1975 to 1977
  - *E. coli*
  - *S. aureus*
  - *S. pneumonia*
  - *K. pneumoniae*
  - *P. aeruginosa*
  - *B. fragilis*
  - *Enterococcus spp.*
  - *S. pyogenes*
  - *C. albicans*
  - *P. mirabilis*
- 1992 to 1993
  - *S. aureus*
  - *E. coli*
  - *CoNS*
  - *K. pneumoniae*
  - *Enterococcus spp.*
  - *P. aeruginosa*
  - *S. pneumoniae*
  - *Viridans group strep*
  - *C. albicans*
  - *E. cloacae*
- 1995 to 2002
  - *CoNS*
  - *S. aureus*
  - *Enterococcus spp.*
  - *Candida spp.*
  - *E. coli*
  - *Klebsiella spp.*
  - *P. aeruginosa*
  - *Enterobacter spp.*
  - *Serratia spp.*
  - *Acinetobacter spp.*

Adapted from Reimer, LG et al. Clin Micro Reviews 1997;10(3):444-465 and Wisplinghoff, H et al., CID 2004:39;309-317

While molecular diagnostics have blossomed, blood culture technology has remained relatively static



Culture Media



Choosing Culture Media and Incubation

- Include both aerobic and anaerobic media
  - Due to decrease in anaerobic BSIs and studies from 1990's, many hospitals have replaced aerobic/anaerobic set with 2 aerobic bottles
  - Newer data suggest that FAN/resin results in optimal sensitivity and specificity when used in aerobic/anaerobic combination
- Incubate cultures for 5 days
  - Data from Mayo Clinic suggests 100% of endocarditis BSIs and 99.5% of non-endocarditis BSIs will be detected in 5 days
- Culture for *Mycobacteria* and filamentous fungi should be performed using isolator/manual methods

Transport of Cultures to Laboratory

- Timing – ASAP
  - Most modern blood culture media will allow for propagation with extended delays, but longer than 2 hour delays may result in delay in detection
  - Sterile body fluids – ASAP
    - A patient's life can depend on the STAT Gram stain from a CSF specimen
- Temperature – Never refrigerate blood
  - Transport sterile body fluids at room temp, refrigerate or freeze in laboratory for extended storage
- Employ safe transport techniques
  - Use carriers in tube systems

Laboratory Rejection Criteria

- Improperly labeled specimens
  - Sterile fluids can be a problem as these are difficult (and sometimes painful) to collect again
- Leaking/cracked blood culture bottles
- Specimen submitted in improper container for tests ordered
  - e.g., collection of blood cultures in purple-top EDTA
- Expired blood culture bottles
- Incorrect volumes
- Single cultures?
  - Note on report, but process anyway

Predictive value of blood cultures

- Predictions based on mathematical model
- Pretest parameters
  - bacteremia rate
  - contamination rate
  - detection rate
  - colonization rate
  - risk ratio
- Coag-neg Staph/Central vascular line

# cultures	# cultures positive	PPV (%)
1	1	55.4
2	1	19.9
3	1	4.7

Adapted from Tokars, JI. Clinical Infectious Diseases 2004;39:333-341

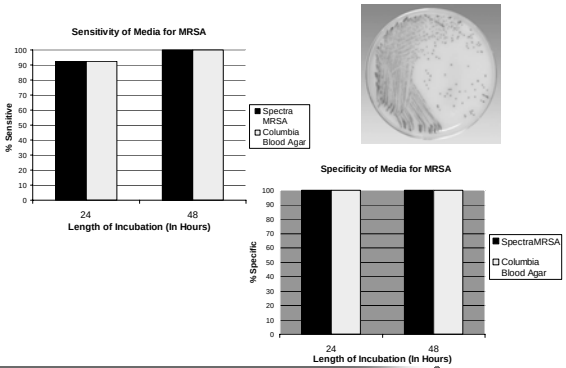
Smears

- The smear is the single most important test to perform on any positive blood culture or sterile fluid culture
  - Call following smear will result in MD action in 90% of instances
- Use descriptive terminology to describe the gram stain
  - Don't report more than you know
- Acridine orange
  - Use on signal-positive, Gram-stain-negative cultures
  - Can allow visualization of poor staining organisms such as *Mycoplasma, Brucella*, or *Campylobacter*

Culture Media – Blood cultures

- Minimum selection of media should include:
  - 5% sheep blood agar
  - Chocolate agar
- Growth in anaerobic bottle or morphology suggestive of anaerobes:
  - Vitamin K and hemin supplemented media
    - Brucella Blood agar
    - Anaerobic blood
- Add based on Gram stain:
  - MacConkey (Gram-negative)
  - Colistin-nalidixic acid agar (CNA) (Gram-positive)
  - Chromogenic agar (Gram-positive (MRSA))
  - Mycologic agar (yeast and molds)

SpectraMRSA on Positive Blood Cultures



Culture Media – Blood cultures

- Inoculate all media with a few drops of well mixed blood culture media
- Streak for isolation
- Incubate at 35-37ºC in 5% CO_2 for at least 48 hours
  - Examine all plates daily and work-up appropriately
- Incubate anaerobic plates under anaerobic atmosphere for 48 to 72 hours

The Real McCoy vs Contaminants

- Can minimize contaminants through strict skin antisepsis
- Organisms commonly associated with contaminated cultures are also suitable pathogens
  - CoNS
  - Diphtheroids (*Corynebacterium)*
  - *Bacillis spp.*
  - *Propionibacterium spp.*
  - Viridans Group *Streptococci*
- If laboratory reports identification and susceptibility, physician may be forced to initiate therapy

How to make the best use of culture?

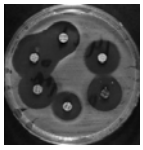
- Separate contaminant from pathogen
  - Compare blood culture sets
  - Avoid reporting confusing data (antimicrobial susceptibility testing on contaminant)
  - Assess time necessary for growth
- Educate the Clinician
- Decrease Turnaround Time

Separating pathogen from contaminant

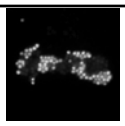
- Obtain at least two culture specimens
  - If true contaminant, likely see only one positive (unlike endocarditis)
  - Assume baseline blood culture contamination rate is 3%. Probability of recovering same organisms in two culture sets is 0.03 x 0.03 = 0.0009

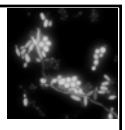
Susceptibilities

- Report antimicrobial agents appropriate for organism and per hospital formulary
  - eg do not report oxacillin on *Streptococcus* or *Enterococcus*
- Susceptibilities unnecessary for *N. meningitidis, S. pyogenes* (GAS)*, S. agalactae* (GBS), *H. influenzae*
  - May perform beta-lactamase
- Perform susceptibilities on single positive cultures with potential contaminants by request, only.



Direct Testing





- AdvanDx
  - Direct identification of Yeast (*Candida*), CoNS, *S. aureus*, and *E. faecalis*
  - Results available within ~3 hours
  - Does not require additional instrumentation
- Remel PBP2
  - Directly detects MRSA
  - Not FDA approved

Molecular Testing from Positive Cultures

- Cepheid GeneXpert MRSA/SA
  - 100% sensitivity, 99.6% specificity
  - Results within 1 hour
  - Allows de-escalation of therapy when organism is MSSA
- BD GeneOhm StaphSR
  - 100% sensitivity, 98% specificity
  - Results within 1 hour, but test is better batched and must be run with controls
  - Allows de-escalation of therapy when organism is MSSA
- Laboratory developed tests (ASRs and "home-brews")

Special Circumstances for CSF

- Gram stain is critical for diagnosis of bacterial meningitis
  - Gram stain on CSF should be considered a critical value and called to ordering MD
- Perform culture on CSF including chocolate agar, 5% sheep blood agar, and others as needed
  - Broth culture recommended, but susceptible to laboratory contamination
- Consider potential contaminants when interpreting CSF cultures
  - CoNS can be a pathogen when shunt is in place, otherwise likely skin contaminant
- The use of bacterial antigen testing is obsolete and should not be performed
  - Except in neonates for Group B streptococcal meningitis

Special Circumstances for CSF

- Fungal culture on CSF should include cryptococcal antigen
- Negative mycobacterial culture will not definitively rule out AFB due to poor sensitivity of culture
  - Molecular techniques exhibit worse sensitivity (20%)
- If all else fails, consider viral causes
  - Molecular is king

Special Considerations for Sterile Fluids

- Specimens can include: synovial, pleural, peritoneal fluid, pericardial fluid
- Can be submitted in blood culture bottles
  - Specimens submitted only in blood culture bottles cannot be Gram stained
- Direct plating to solid media allows quantitation of colonies
- Media
  - 5% Sheep Blood Agar
  - Chocolate Agar
  - MacConkey Agar
  - Fungal/Mycobacterial cultures
  - Broth culture (if not submitted in blood culture bottles)
- Read plates daily for minimum of 4 days

Conclusions

- Detection and identification of agents of sepsis is one of the most important functions of clinical laboratory
  - Timely and accurate diagnosis is critical
- Select blood culture media that will allow optimal propagation of all organisms
- Perform limited work-up on potential contaminants for all sterile fluids

Molecular Detection of Sepsis

Targets

- Gram-positive
  - *E. faecalis* and *faecium*
  - *S. aureus* and *epidermidis*
  - *Staphylococcus* protein A
- Gram-negative
  - *K. pneumoniae*
  - *E. coli*
  - *P. aeruginosa*
  - *E. cloacae*
- Yeast
  - *C. albicans*
- Resistance genes
  - *vanA, B,* and *C*
  - blaSHV and blaGES-2
  - *mecA*

Assay performance indices

- Sensitivity: 47.4%
- Specificity: 84.1%
- PPV 12%
- NPV 97.2%

Reason for poor sensitivity?

- Extraction volume: while 10-20 ml of blood are being cultured, only 400 µl of blood was extracted for analysis.
- Blood would have to have 2-3 cfu of bacteria/ml to even have a chance of extracting 1 organism.
- Future assays might require concentration of bacteria or bacterial targets (how?) prior to extraction.
  - Target capture or ribosomal targets
- Only 9 organisms were represented and no "universal" primers included in version 1.

Reason for poor specificity?

- Patients in the ICU receive empiric therapy for suspected or proven sepsis such that at the time of collection, blood cultures would be negative but molecular assays could be positive (and for more than one organism).
- 53% of patients with a false-positive MDx result were receiving concomitant and appropriate antimicrobial therapy for the organism that was detected by the MDx assay while 47% were receiving discordant antimicrobial therapy.
- Line contamination?

Conclusions

- Direct diagnosis of BSI by molecular means is a long way off.
- Hurdles include target capture or concentration from a large volume of blood
- Complimentary use of molecular techniques could help in certain cases but will not replace conventional culture and will add to cost of diagnosis.
- Combined with resistance profiling, MDx might be of use for signal-positive blood cultures.
- Need to better understand microbial "echoes" in samples.