2012 SEACM Meeting Abstracts

Bloodstream Infections-Back to Basics

Lisa L Steed, Ph.D.,D(ABMM)
Director, Diagnostic Microbiology
MUSC, Charleston, SC

Back to Basics: Why are BC (Blood cultures) Obtained

- Diagnostic Importance: establishes or confirms an infectious etiology for a patient's illness
  - Identification of an infectious agents allows susceptibility testing and optimization of antibiotic therapy
  - Inappropriate antibiotic therapy is independent risk factor for mortality
- Prognostic importance: proof of a BSI (bloodstream infection) indicates failure of the host's defenses to contain an infection at a primary location or failure to remove, drain or eradicate that focus of infection.

Central-line Associated BSI (CLABSIs)

- Definition-CDC
  - CLABSI=1 positive BC with pathogen not from skin or $\geq$ 2 positive BC with pathogen from skin;unrelated to any other infection, must not have been present/incubating when patient was admitted-*MMWR, 2011, 60:243*

Types of BSI

Transient	**Intermittent**	**Continuous**
Manipulation of abscesses, furuncles, cellulitis	Undrained intraabdominal abscesses	Endocarditis & endovascular infections
Instrumentation of contaminated mucosal surfaces	Pelvic,perinephric, hepatic, prostatic abscesses	Early typhoid fever or brucellosis
Surgery of contaminated areas	"FUO"	
Meningitis, pneumonia, septic arthritis, osteomyelitis		

When to draw BC

- "In individuals 18 years of age and older, the timing of collection of blood specimens for culture can be predicated on convenience. The emphasis should be on obtaining specimens of adequate volume (8-10 ml/bottle) the performance of suitable numbers of blood cultures and the use of strict aseptic technique"
- Draw BC at intervals when:
  - Endocarditis or endovascular infection
  - When pace of illness is not acute

Bloodstream Infections-Back to Basics cont.

Pediatric BSI

- Single small volume blood cultures are OK
  - 1ml, *Dunne et al, 1994, PIDJ 13:203*
  - 0.5 ml *: Jawaheer et al, 1997, Arch Dis Child, 76-F57*
  - 0.2 ml provided 95% sensitivity compared with 2 ml for infants from birth-12 mos, *Soloranzo-Santos et al, 1998, Scand JID 30:481*
  - 1-3 ml for 3-36 mo.:*Lee and Harper, 1998, Arch Pediatr Adolesc Med 152:624*
- 25-26% of pediatric patients admitted to ICU with sepsis had positive blood cultures
  - *Jacobs et al, 1990, PIDJ 9:196, Saez-Llorens et al, 1995, PIDJ 14:557*

Pediatric BSI cont.

- Why Culture small volumes
  - Small blood volumes of young children
  - Difficulties in drawing blood from young children
  - Desire to avoid need for transfusion due to iatrogenic blood loss
  - Start antibiotics without delay
  - Bacterial concentrations are greater/far greater in blood of children than adults
- Low-level bacteremia $\leq$ 10 cfu/ml

Line Blood Culture Basics

- Higher contamination from ports/lines
- Disinfect ports/lines in same manner as skin
- ALWAYS send a venipuncture BC with line BC
  - A 2nd line draw is not equivalent to a venipuncture
- Why can we use the initial blood draw for BC when we can't use it for other lab tests
  - Bacteria have adapted to the heparin
- Maximize the volume of blood in the bottles
  - For short draw, maximize AEROBIC bottle first
    - Most bacteremias are caused by aerobic/facultative bacteria which will be recovered better from aerobic cultures
    - Yeasts are aerobes and grow almost exclusively in aerobic bottles

How long to Hold BC bottles

- Current recommendations are 5 days
- Bourbeau et al: 97% of clinically significant bacteria/fungi detected within 3 days in BTA system
- Consider geographical differences in epidemiology, different technologies with BC bottles, factors within individual HC settings (transport to lab, processing within lab, ordering problems of MDs)
  Bourbeau&Pohlman,2001,JCM 39:2079, Bourbeau &Foltzer, 2005, JCM 43:2506

Bloodstream Infections-Back to Basics cont.

How Long to Hold BC bottles cont.

- Extended incubation for endocarditis
- Fastidious organisms (HACEK), *Brucella, Abiotrophia/Granulicatella, Francisella*
- Washington et al 1982: hold BC up to 14 days
    - Older BC systems and technology
- Endocarditis/FUO protocol: hold 3 sets of cultures for 21 days in Bactec 9240;blind subcultures at 3 days & 10 days + 4 Isolator tubes=3 clinically significant isolates from 215 BC
- 24 HACEK organisms isolated within 5 days
  Petti et al, 2006, JCM 44:257, Baron et al , 2005 CID 41:1677

BC Limitations: Fastidious Bacteria

- Signal positive/Gram stain negative BC: acridine organge, Gram stain with carbolfuschin
- *Brucella*: automated system better than lysis centrifugation or biphasic system;most isolates grow within 3 days, 95% grow in 7 days, aerobic bottles only
- *Campylobacter*:most isolates grow within 2-3 days;subcultures may appear negative @ 24 h since *C jejuni* grows slower @ 35 C than @ 42 C
- *Francisella*: variable growth rate; tiny pleomorphic rods often missed on GS
- *Helicobacter*: most isolates grow within 2-3 days but may take 5 days; optimal recovery takes 7 days & terminal subculture to enriched blood agar in H^2-enriched microaerobic atmosphere
- *Legionella*: terminal subculture @ 5 days to BCYE or lysis centrifugation to BCYE

What about Fungemias?

- Pts with AIDS, hem/onc, bone marrow/solid organ transplant or other sever immunocompromised at risk:
    - Dimorphic fungi,*Fusarium, Scedosporium, Exophiala, Rhinocladiella, Aspergillus*
    - Lysis-centrifugation (Isolator system hold for 4 weeks)

What about *Mycobacteria* & *Nocardia*

- Pts with AIDS, leukemia, multiple myeloma, solid organ malignancies, high-dose steroid therapy, chemotherapy, long-term vascular catheters at risk for:
    - Rapidly growing mycobacteria and *Nocardia*
    - Will grow in routine BC bottles
- MAC, *M. kansasii, M.genavense*
    - AFB blood culture bottles or lysis centrifugation (Isolator) system hold for 4 weeks
    - Recovery of AFB inoculated onto solid media is slower than broth/biphasic media

Interpretation of BC from short-term peripheral catheters

- $\geq$ 1 BC sets are positive and the catheter tip culture is positive ($\geq$15 CFU) for the same organisms: suggests CRBSI
- $\geq$ 1 BC sets are positive and the catheter tip is negative: inconclusive but suggestive if *S. aureus* or *Candida sp.* and no other identifiable source of infection
- Both BC sets are negative and the catheter tip is positive despite the colony count: suggest catheter colonization not CRBSI
  CLSI, M47-APrinc & Proc for BC, 2007

Bloodstream Infections-Back to Basics cont

Interpretation of BC from long-term venous catheters & venous access ports

- Venipuncture & catheter cultures obtained at same time
- Both BC sets grow same organism: suggests CRBSI if no other source of infection
- Both BC sets grow the same organisms AND the catheter BC becomes positive $\geq$ 120 min. earlier: suggestive if no other identifiable source of infection-if < 120 min. earlier, CRBSI still possible
- Lysis centrifugation: both BC sets grow the same organism and the catheter BC has $\geq$ 5-fold more cfu/ml: suggestive if no other identifiable source of infection
- Venipuncture BC set is negative and catheter BC set is positive: inconclusive of CRBSI, suggests colonization of the catheter or contaminated during collection
- Venipuncture BC set is positive and catheter BC set is negative: inconclusive but suggestive of CRBSI if *S. aureus* or *Candida sp* and no other identifiable source of infection
    - CRBSI would require positive catheter tip culture with same organism or additional positive BC sets with same organism

Hepatitis C: Latest Update on Diagnosis and Treatment

Melissa B. Miller, Ph.D., D(ABMM)
Director, Clinical Molecular Microbiology Laboratory
Associate Director, clinical Microbiology-Immunology Laboratory
UNC School of Medicine, Chapel Hill, NC

HCV Epidemiology

- 3.9 million Americans infected with HCV (1.8% of population) according to National Health and Nutrition examination Survey
- Worldwide ~ 200 million infected with HCV (3% of population)
- Chronic HCV-associated liver disease is on the rise

Hepatitis C Virus

- Enveloped RNA virus
- Highly mutable genome
    - Rapid mutation in a hypervariable region of the genome coding for the envelope protein and escapes immune surveillance by the host

HCV Laboratory Diagnosis

- The average time from exposure to antibody to HCV (anti-HCV) seroconversion is 8-9 weeks
    - Anti-HCV can be detected in > 97% of persons by 6 months after exposure
- HCV RNA can be detected in blood within 1-3 weeks after exposure

Hepatitis C: Latest Update on Diagnosis and Treatment cont.

Diagnostic Tests for HCV

- HCV antibody
    - Used to diagnose HCV infections
    - Not useful in the acute phase as it takes at least 4 weeks after infection before antibody appears
    - Confirmatory test: recombinant immunoblot assay (RIBA)
    - OraSure Technologies-OraQuick HCV Rapid antibody test
- HCV RNA
    - Often used to diagnose HCV inrectio in the acute phase
    - Main use is in monitoring the response to antiviral therapy

Qualitative Molecular Tests for HCV RNA

- Diagnosis of HCV infection
- Measure success of therapy (sustained virologic response, SVR)
- Targets the 5'UTR of the HCV genome (most conserved region)
- FDA approved Qualitative Assays
    - Amplicor HCVv2.0 (Roche Molecular Systems)
    - Cobas Amplicor HCV v2.0 (Roche Molecular Systems)
    - Ampliscreen (Roche Molecular Systems)
    - Versant HCV RNA Qualitative Assay (Siemans Healthcare Diagnostics)
    - Procleix HIV-1/HCV Assay (Chiron Corporation)

Quantitative Molecular Test for HCV RNA

- Predicts efficacy of therapy
- Monitors response to therapy
- Moderate to good correlation between different assays
- Use of International units (IU) provides some standardization

Recommendations for Quantitative HCV RNA

- Initial determination of baseline viremia
- 4 weeks into treatment to assess rapid virologic response (RVR)
- 12 weeks into treatment to assess early virologic response (EVR)
- After 24/48 weeks of therapy to assess end of treatment response (ETR)
- 24 weeks after completion of therapy to determine sustained virologic response (SVR)

FDA Approved Quantitative HCV RNA Tests

- Amplicor HCV Monitor 2.0 (Roche Molecular)
- Versant HCV RNA 3.0 Siemans Healthcare Systems
- Cobas Ampliprep/Cobas TaqMan HCV (Roche Molecular Systems)
- Cobas TaqMan HCV 2.0 for use with High Pure System (Roche Molecular Systems)
- Abbott RealTime HCV (Abbott Molecular Diagnostics)
- All used for pretreatment quantification and determination of response to treatment

Hepatitis C: Latest Update on Diagnosis and Treatment cont.

Treatment of HCV

- Combination of Peginterferon (PegIFN) and ribavirin (RBV)
- New Directly acting antivirals (DAA) or protease inhibitors (PI)
    - Boceprevir (Victrelis)-N53/4A Protease inhibitor
    - Telaprevir (Incivek)-drug class: N53 protease inhibitor
    - Approved for use in combination with peginterferon/Ribavirin
    - New DAA/PI therapies increase SVR percentages in both treatment naïve patients and previous non/null responders
        - Nonresponders-failure to clear HCV RNA after 24 weeks of therapy
        - Null responder: failure to decrease HCV RNA by $2\log_{10}$ after 24 weeks of therapy

Ocular Infections and the Laboratory Diagnosis of Ocular infections

Larry D Gray, Ph.D., ABMM

University of Cincinnati College of Medicine

Cincinnati, Ohio

Why is the Clinical Microbiology of Ocular Specimens Different?

- The eye is delicate, unprotected, exposed
- Each structure of the eye can be infected
- Severe infections can result in loss of sight
- Eye specimens: Small volumes; no rejects; no recollects
- Routine AST (antimicrobial susceptibility testing) usually not applicable
- Ophthalmologists traditions- they inoculate their own culture plates
- Sparse literature

Ocular Infections

- Blepharitis: irritation, redness, itching, styes
- Conjunctivitis (pink eye): redness, itching, edema, secretions, crust, vision OK
- Keratitis-Medical Emergency
    - Pain, conjunctivitis, loss of transparency
    - Entry: epithelial defect (surgery, trauma, contact lens, wood/plant matter
    - Clin. Micro: Must use corneal tissue (or Dacron ^{TM} swab)
    - Gram stain, culture and AST
- Endophthalmitis
    - Severe visual impairment, pain, loss of transparency, lid/corneal/orbital edema
    - Clin. Micro: Must culture inner eye fluids and/or tissue
    - Gram stain, culture and AST imperative
    - Look for *Propionibacterium*: susceptible to penicillin, Ampcillin/Sulbactam. Pip/Tazo, Cefox, Ertapenem and imipenem
    - Look for and speciate *Bacillus subtilis/Bacillus cereus*
        - *Bacillus subtilis*: susceptible to penicillins and cephalosporins
        - *Bacillus cereus*: resistant to penicillins, ampicillin ,cephalosporins, TMP

Ocular Infections and the Laboratory Diagnosis of Ocular infections cont.

Ocular and Ear Specimens

Group I (lesion)	Group II (normally sterile)
Eye swabs	All corneal swabs
Lid swabs	All cornea specimens
Conjunctiva swabs	All inner eye and ear fluids
Sclera swabs	All scrapings
	All cornea, lens, iris & retina
	All AC, PC, vitreous fluids
	All surgical specimens, biopsies and tissue
	Foreign body
	Optisol™ and irrigation fluid

Collection of Ocular specimens

Who: Physicians only at chairside

How:

- Dacron swabs (Thio or TSB; no BHI;no VCTM
- Kimura/platinum spatula
- Bent-tip needle
- No calcium alginate swab, no cotton swab
- Flocked swabs not evaluated;suspect very good

Slide: prepare at chairside

Media: Inoculate at chairside

Transportation and Storage of Ocular Specimens

- Send specimen to Lab ASAP
- Keep specimens at room temperature (RT) and in original container
- No transport devices
- No store for end-of-day or next day pick-up
- Group I: should be received within 3 hours
- Group II should be received within ½ hour

Storage

- Non-virus: RT $\leq$24 hours
- Virus in VTM: cold $\leq$ 3 days

Ocular Infections and the Laboratory Diagnosis of Ocular infections cont.

Suggested Culture Media

- By Group
  - Group I: Blood agar and chocolate agar
  - Group II: Blood agar, Chocolate, SABHI, Thio
- By Organism
  - Bacterial: Blood agar, Chocolate, TSB, Thio MAC
  - Anaerobic: Thio, CDC ANA
  - Fungal: BHI+/- Blood, SABHI+/- Blood, IMA, PDA, PF
  - AFB: Lowenstein Jensen, Middlebrook 7H11
  - Viral: Cell lines for HSV, VZV and adeno
- By Disease
  - Virtually all bacterial and fungal infections
    - Blood agar, Chocolate, Fungal media and thio

Suggested Incubation and Examination of routine Ocular Cultures

- Conditions: 5-7% CO_2, 35-37 ^{0}C
- Length:
  - Solid media: $\geq$ 3 days
  - Broths: $\geq$ 5 days
- Examination of Cultures: Daily
- Unclear broths
  - Gram stain, subculture and exam for *Bacillus* and *Propionibacterium*

Interpretation of Ocular Cultures and Extent of Identification-Part 1

- Group I specimens
  - Treat as lesions
  - Example: conjunctiva-------skin lesion
- Group II specimens
  - Treat as normally sterile specimens
  - Example: corneal scraping-----CSF

Interpretation of Ocular and Extent of Identification Part 2

- All specimens
  - ID all isolates from ocular cultures (even if only GS and morphology), and report all isolates separately. Do not report isolates as “Normal Ocular (skin) flora” or similar
- Group I specimens
  - Limited ID (CNS, Alpha hemolytic strep, alpha hemolytic GPC, GNR and diphtheroids)
- Group II specimens, Corneas and Preinoculated Media
  - Full ID and AST of isolates

Ocular Infections and the Laboratory Diagnosis of Ocular infections cont.

Interpretation of Ocular Cultures and Extent of Identification-Part 3

- *Pseudomonas aeruginosa*
  - Keratitis---corneal perforation in < 48 hours
  - **Contact physician immediately and perform full ID and AST**
- Acantamoeba keratitis
  - Culture (usually reference Labs)
    - Sensitive, easy works well
  - Specimens
    - Lens, case, lens fluid and corneal tissue
    - Contact testing lab for specifics
  - Microscopy
    - Direct stains: calcofluor white
    - Biopsies: H&E

- **Antimicrobial Susceptibility Testing**
  - AST panels should include classes of agents which are commercially available in or can be compounded into ophthalmic formulations (compounding pharmacists are invaluable)
    - Cephalosporins-cefazolin
    - Sulfonamides-trimethoprim/Sulfa
    - Macrolides-azithromicin,erythromicin
    - Aminoglycosides-gentamicin, tobramycin
    - Fluoroquinolones-ciprofloxacin, moxifloxacin, gatifloxacin,levofloxacin