Introduction

- Annually in U.S., approximately 750,000 patients develop bacteremia or fungemia with an associated mortality up to 34%

- Three automated blood culture systems available in U.S.
  - BACTEC and BacT/Alert 3D systems monitor increases in CO₂ levels
  - VersaTREK instruments measure changes in gas pressure in bottle head space

- Variable rates of blood culture positivity based on patient diagnoses
  - Most patients with untreated meningitis have positive blood cultures
  - However, overall only 50% of patients with septic shock have positive blood cultures
  - Reasons for low rate of positive cultures unclear
  - Sensitivity of culture techniques, biology of infectious process or both?
✧ Techniques to diagnose catheter-associated blood stream infections
✧ Old and new approaches to monitoring blood culture volumes
✧ Blood culture Gram stains – What is this organism?
✧ Molecular and non-molecular methods to directly identify microorganisms in blood cultures
Techniques Used to Diagnose Catheter – Related Infections

- Multiple methods to determine if bacteremia is catheter-associated, all have potential disadvantages
- Semi-quantitative catheter tip culture
- Paired quantitative blood cultures
- Semi-quantitative superficial cultures of skin entry site and catheter hubs
- Differential time to positivity
Catheters Commonly Used for Intravenous Access

- Midline peripheral catheter
- Peripherally inserted central venous catheter
- Positions of Groshong valve
- Tunneled central venous catheter
- Implantable subcutaneous central-catheter port
- Access to ports
- Silver-impregnated collagen cuff
Semi – Quantitative Catheter Tip Cultures

- **Most common method**
- **Maki roll plate - greater than 15 colonies considered significant**
  - Intraluminal organisms missed
  - Procedure modifications with sonication or vortexing of catheter tip not shown superior

- **Possible pitfalls**
  - Catheter must be removed
  - Interpretation requires accompanying peripheral blood culture
    - Patient may have catheter colonization without bloodstream involvement
    - What is your lab policy?
  - Defined length of catheter tip (5 cm) to culture
    - Do you assess length of catheter tip prior to culture?
  - Antibiotic-coated catheter may give false negative results
Paired Quantitative Blood Cultures

- Not routinely performed
- Blood collected from catheter and peripheral site at same time
- Use lysis-centrifugation (Isolator) or pour plate method
- Recovery of 5-fold or greater organisms in catheter culture suggests CR-BSI
Semi-quantitative superficial cultures of skin insertion site and catheter hubs

- Performed in non-neutropenic ICU patients prior to catheter removal
- Cultures considered positive if $\geq 15$ CFUs recovered
- Skin cultures demonstrated high NPV
- Hub cultures little benefit compared to skin cultures
- Authors recommended combining insertion site cultures with differential time to positivity cultures; avoid catheter removal and increase sensitivity
Differential Time to Positivity

- Blood drawn from catheter and peripheral site at same time
- Not routinely performed
- If same organisms recovered from both blood cultures, and catheter culture positive at least 120 minutes before peripheral blood culture, highly suggestive of CR-BSI
Example 1

<table>
<thead>
<tr>
<th>Blood Cultures</th>
<th>Collection Time</th>
<th>Receipt Time</th>
<th>Detection Time</th>
<th>Time to Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lt. PICC</td>
<td>0600</td>
<td>0630</td>
<td>1200</td>
<td>6 hr.</td>
</tr>
<tr>
<td>Rt. Arm</td>
<td>0610</td>
<td>0630</td>
<td>1610</td>
<td>10 hr.</td>
</tr>
</tbody>
</table>

- Gram positive cocci identified as *S. epidermidis* recovered from both blood cultures
- PICC line culture positive 4 hr before peripheral blood culture
- Suggests CR-BSI because catheter culture detected as positive at least 120 minutes before peripheral blood culture
Example 2

<table>
<thead>
<tr>
<th>Blood Cultures</th>
<th>Collection Time</th>
<th>Receipt Time</th>
<th>Detection Time</th>
<th>Time to Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>LT PICC</td>
<td>0700</td>
<td>0900</td>
<td>1800</td>
<td>11 hr.</td>
</tr>
<tr>
<td>Rt. Arm</td>
<td>0610</td>
<td>0630</td>
<td>1610</td>
<td>10 hr.</td>
</tr>
</tbody>
</table>

✧ Same patient with *S. epidermidis* recovered from both blood cultures but different results

✧ Peripheral blood culture positive before catheter culture - Why?
Potential Problems

- Failure to coordinate blood draws
- Variability in transport to the lab
- Influence of antiseptic-coated catheters
- Possible impact of systemic antibiotic administration through lines
- Selection of infected catheter in patient with multiple lines.
Monitoring Blood Culture Volumes

- CAP requirement indicates laboratories should periodically monitor blood volumes and provide feedback to clinical staff

- Belgian study with 4 participating hospitals recorded blood culture volumes
  
  - Bottles with greater than 2 ml. above or below manufacture’s recommendations were tallied
  
  - More than one-third of bottles incorrectly filled
    
    - Overfills ranged from 7.6% to 12.8%
    
    - Under filled bottles ranged from 26.3% to 30%; pediatric cultures were excluded from study
Why Are Bottles Incorrectly Filled?

- **Under fills usually due to poor peripheral access**
  - Study showed increased contamination rate associated with lower blood volumes
  - Poor peripheral access may decrease ability to maintain sterile technique during blood draw

- **Overfills**
  - Lack of correlation between vacuum in bottles and optimal blood volume, regardless of manufacturer
  - Vacuum may exceed that needed for optimal blood fill volumes
    - BACTEC aerobic media vacuum draws increase during shelf life to as high as 30 ml.
  - Excess vacuum avoids recoil of blood culture medium toward patient’s bloodstream and minimizes collection time
Why Isn’t More Blood Better?

- **Correct ratio of blood to media** prevents clotting and prevents inhibitory substances in the blood from interfering with growth of microorganisms.

- **Excess blood** can cause extremely high rates of background signals:
  - Interpreted as false positives
  - **OR** Mask rate changes associated with growth, potentially causing false negatives

- **Red blood cells** compete with organisms for nutrients and oxygen; detection of obligate aerobic organisms can be inhibited.
Automatic Monitoring of Blood Culture Volumes

- BD Diagnostic has introduced blood volume monitoring for BACTEC™ FX instrument in conjunction with Epicenter software
- Only assesses blood volumes in the BACTEC Plus Aerobic/F bottle
- Not available for 9240 or 9140 instruments
BLOOD VOLUME DISTRIBUTION HISTOGRAM: HOSPITAL SERVICE
BACTEC FX Instrument Version

![Graphs showing blood volume distribution for different areas: NORTH, EAST, ER, and ICU. Each graph represents the number of bottles containing blood volume in mL categories.](image-url)
Manual Monitoring of Blood Culture Volumes

- BacTAlert
  - Currently recommends weighing blood culture bottles
  - Plans for new instrument and software in 2014

- VersaTREK also recommends manual monitoring of blood culture volumes visually measuring blood draw against 10 ml seeded bottle or weighing bottle

- Children’s Medical Center of Dallas
  - Label and weigh subset of bottles from each new lot or shipment
  - Record weights and distribute bottles to floors
  - Weigh bottles after incubation on blood culture instrument
  - Track average monthly blood volumes by hospital location
Renal transplant patient with intermittent vomiting and subjective fever and chills for past 2 days

*Paracoccus yeei* recovered from 1 of 2 blood culture sets
Paracoccus yeei

✧ Formerly CDC group EO-2
✧ Doughnut-shaped morphology due to vacuolated or peripherally stained cells
✧ Nonfermentative, oxidase-positive Gram negative rod that grows on MAC and produces mucoid, pale yellow colonies on BAP
✧ Natural habitat soil and brine
✧ Uncertain clinical significance in patient; rare human pathogen
Febrile HIV-positive patient presented to the ED

Bacteria, fungus or alien from another planet?

*Fusobacterium mortiferum* – highly pleomorphic anaerobic Gram-negative rod residing in oral mucosa
HIV-positive patient with fever and cellulitis on lower extremities

Helicobacter cinaedi
Helicobacter cinaedi

✧ Enterohepatic *Helicobacter* species that causes recurrent bacteremia and cellulitis primarily in immunocompromised hosts

✧ Propose transmission from animals (hamsters, dogs, cats, rats and foxes) to humans

✧ Optimal growth in microaerophilic environment containing 5-10% H₂ at 35°C

✧ Oxidase-positive, curved Gram negative rod that forms thin film on agar plates
28 y.o. female presented with 2 months of nausea, vomiting, night sweats and 20 lb weight loss

*Brucella suis*

Patient lived on farm and had contact with horses, cattle, dogs and pigs. She had helped with birth of piglets

Case courtesy of Dallas VA Microbiology lab
Characteristic appearance on Gram stain

- Brucella species

- Previous descriptions of gram variable bacteria resisting decolorization
- Misidentified as coryneform bacillus or Gram positive cocci
48 y.o. unemployed male construction worker presented to ED with fever, muscle pain, diarrhea and general malaise during the last two weeks and a 30 lb. weight loss over one month

- History of asthma and newly diagnosed but untreated HIV

- Presented with nonproductive cough and SOB; chest X-ray revealed emphysema and calcified granulomas in left lower lobe

- Physical exam revealed cervical lymphadenopathy and probable oral candidiasis

- Yeast detected in routine blood cultures after 4 days
2-4 μm yeast staining Gram negative
Candida glabrata, 2-5 μm yeast staining Gram positive
Histoplasma capsulatum grew in fungal blood culture at 9 Days incubated at 30°C
Direct Identification of Microorganisms in Blood Cultures

- Non-molecular methods

- Molecular methods without DNA amplification

- Molecular tests that amplify specific bacterial or fungal gene sequences

- Broad-based PCR assays that target universal genes
Non-Molecular Methods

- Conventional tests including coagulase and thermonuclease

- MALDI-TOF MS
  ✷ Microflex LT Biotyper (Bruker Daltonics) and Vitek MS IVD (biomérieux)
  ✷ Variety of blood culture extraction methods
  ✷ MALDI Sepsityper kit for Bruker Biotyper
    ◇ Includes extraction reagents and protocol for identifying yeast, Gram-positive and Gram-negative bacteria from positive blood cultures
    ◇ Multi-step extraction including four centrifugation steps
    ◇ Requires additional software module due to proteins present in blood cultures
    ◇ May not detect all organisms in mixed cultures
  ✷ Vitek MS recently received FDA approval for microbial identification
Molecular Methods without DNA Amplification

- **PNA-FISH® Rapid Pathogen ID (AdvanDX)**
  - Employs fluorescent-labeled peptide nucleic acid probes to target species-specific rRNA sequences using *in situ* hybridization
  - TAT approximately 1.5 hrs
  - Yeast Traffic Light® with *C. glabrata / C. krusei*, *C. albicans / C. parapsilosis* and *C. tropicalis* probes
  - Bacterial PNA-FISH® probes include *S. aureus / CNS*, *E. coli / P. aeruginosa* and *E. faecalis / E. faecium* and other enterococci
Molecular Methods without DNA Amplification

- **Nanosphere Assays**
  - Extracted bacterial DNA hybridizes to complementary oligonucleotides on a microarray
  - Mediator oligonucleotide added with 2 domains, one complementary to bacterial DNA and second complementary to oligonucleotide with signal-generating gold nanoparticle
  - TAT approx. 2.5 hr
Nanosphere Assays

- **Verigene® Gram-Positive Blood Culture Nucleic Acid Test (FDA-approved)**
  - Detects and identifies the following bacterial genera and species
    - *Staphylococcus* spp.
    - *S. aureus*
    - *S. epidermidis*
    - *S. lugdunensis*
    - *Streptococcus* spp.
    - *S. pneumoniae*
    - *S. pyogenes*
    - *S. agalactiae*
    - *S. anginosus* grp
  - Detects resistance markers
    - *mecA*, *vanA* and *vanB*

- **Verigene® Gram-Negative Blood Culture Nucleic Acid Test (RUO)**
  - Detects and identifies the following genera & species
    - *Escherichia coli*
    - *Klebsiella pneumoniae*
    - *Klebsiella oxytoca*
    - *Pseudomonas aeruginosa*
    - *Serratia marcescens*
    - *Enterobacter* spp.
    - *Acinetobacter* spp.
    - *Proteus* spp.
  - Resistance markers include
    - CTX-M, KPC, NDM, VIM, IMP AND OXA
Blood culture collected from febrile diabetic patient receiving dialysis

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<thead>
<tr>
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<th>GPCL</th>
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<tbody>
<tr>
<td>Nanosphere result</td>
<td><em>Staphylococcus</em> species</td>
</tr>
<tr>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td></td>
<td><em>mecA</em></td>
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**Actions taken**
- Notified clinician, pharmacy and infection control
- Discontinued Gram-negative coverage
- Patient placed in contact isolation

**Culture results**
- **MRSA**
Blood culture collected from oncology patient with presumed mediport infection

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<td><em>S. aureus</em></td>
</tr>
<tr>
<td>Actions taken</td>
<td>Discontinued vancomycin</td>
</tr>
<tr>
<td></td>
<td>Changed therapy to nafcillin</td>
</tr>
<tr>
<td></td>
<td>(more effective treatment for MSSA infections)</td>
</tr>
<tr>
<td>Culture result</td>
<td>MSSA</td>
</tr>
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</table>
Blood culture collected from patient admitted with abdominal pain and recent history of abdominal surgery

Gram stain: GPCH

Nanosphere result: *Streptococcus* species

Actions: Unchanged by Nanosphere result

Culture results: *Streptococcus bovis* group
*Enterococcus faecalis* (missed)

- Nanosphere acknowledges that mixed cultures are a limitation
- However, mixed cultures may not always be detected in GS
- *E. faecalis* below limit of detection for BC-GP assay
- Resistance markers (*vanA* or *vanB*) only released if *E. faecalis* or *E. faecium* reported
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</table>
| Culture result    | MRSA (missed *S. aureus, mecA*)  
                  | MR-S. *simulans* |

✧ **Assume CNS in greater concentration than *S. aureus***
✧ **Since *S. aureus* was not detected, *meca* not released**
Molecular Tests that Amplify Specific Bacterial or Fungal Gene Sequences

- **BD Gene Ohm™ StaphSR assay**
- **Cepheid Xpert® MRSA /SA BC**
- **Biofire Film Array® Blood Culture Identification Panel**
Biofire Film Array® Blood Culture Panel

- FDA approval on June 25, 2013
- Single panel with 27 targets including Gram-negative and Gram-positive bacteria, meca, vanA/vanB and KPC resistance markers and yeasts
- Minimal manipulation and approx. 1 hour TAT
- Panel more expensive than single Nanosphere test, but can identify multiple groups of organisms
- **Additional targets** include *Haemophilus influenzae*, *Neisseria meningitidis* and five species of *Candida* (*C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*)
- **Excluded targets** include those for coagulase-negative staphyloccocci and some markers for carbapenemase resistance
Thank you for your attention