I Feel the Need…the Need for Speed
Rapid Identification Methods for Positive Blood Cultures

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Disclosures

• No financial disclosures
• Off label use of Vitek ID/AST system
• Cat and parrot mommy
Objectives

• Discuss the need for rapid identification of organisms from blood cultures
• Describe available commercial methods for the rapid identification of *Staphylococcus*, *Streptococcus*, Gram negative bacilli and yeast from positive blood cultures
• Describe the clinical impact that rapid identification methods have on patient care
Why do we need rapid identification?

Sepsis... It Kills!

- Effects >1,000,000 people annually
- Mortality - up to 50%
  - MRSA/VRE bacteremia - increased mortality
    - Delays in appropriate therapy
  - MRSA - persistent bacteremia
  - Yeast – disproportionate morbidity and mortality
Why do we need rapid identification?

- Sepsis... It Costs!
- Most expensive condition treated in US hospitals
  - Costs > $20 billion in 2011
  - Costs increasing by 11.9% annually

AHRQ. 2011. National Inpatient Hospital Costs: The Most Expensive Conditions by Payer
Why do we need rapid identification?

**Sepsis... It Costs!**

- Increased hospital charges and LOS
  - **HA-MRSA**
    - $27,083/case
    - 12 days additional LOS
  - **Candida**
    - $130,000/case
    - 30 days additional LOS
  - **VRE**
    - $27,190/case
    - 18.1 days additional LOS

Abramson et al. 1999. *Infect Control Hosp Epidemiol* 20:408-411
Stosor et al. 1998. *Arch Intern Med* 158:522-527
Why do we need rapid identification?

Sepsis... It Costs!

- Inappropriate antibiotic usage
  - Empiric use of vancomycin
    - Inferior to nafcillin for MSSA bacteremia
  - Empiric use of caspofungin
    - Antifungal susceptibility is species associated
  - Empiric use of broad spectrum antibiotics for Gram negative bacilli

What is the most critical factor for sepsis survival?

Timely administration of APPROPRIATE antimicrobial therapy
Each hour of delay in antimicrobial administration associated with an average decrease in survival of 7.6%

Kumar et al. 2006. *Crit Care Med* 34:1589-1596
Why do we need rapid identification?

- Potential pathogen... or not?
  - CNS commonly isolated from blood but only ~20% represent true infection
    - UF Health Jacksonville 2013
    - 32% of positive blood cultures
    - Coagulase negative *Staphylococcus*

- 71% Coagulase negative *Staphylococcus* Contaminant
Why do we need rapid identification?

Conventional methods are way too slow...

• Culture-based identification methods may require 1-3 days
• Culture-based susceptibility methods require at least 2 days
### Traditional Positive Blood Culture Workflow

<table>
<thead>
<tr>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture positive</td>
<td>Organism identified as <em>Enterococcus</em></td>
<td><em>Enterococcus</em> AST = VRE</td>
<td>Enterococcus AST = VRE</td>
</tr>
<tr>
<td>Blood culture positive</td>
<td>Organism identified as <em>Staph aureus</em></td>
<td><em>Staph aureus</em> AST = MRSA</td>
<td>Staph aureus AST = MRSA</td>
</tr>
</tbody>
</table>
An Example...

• 25 year old AA male with a PMH of HIV/AIDS presented to the ED with a chief complaint of right lower extremity cellulitis/abscess secondary to a previously treated wound of the right calf
  ▫ The patient was admitted and started on vancomycin and piperacillin/tazobactam empirically and underwent I&D on Day 2
• Day 6 – wound specimens obtained during I&D grew MSSA
  ▫ Vancomycin and piperacillin/tazobactam discontinued, and nafcillin initiated
  ▫ ID consulted for persistent fever and neutropenia – patient changed to broad spectrum antibiotics (vancomycin and piperacillin/tazobactam)
• Day 13
  ▫ Blood cultures were collected @ 1330 for persistent fever; continued on vancomycin and piperacillin/tazobactam

• Day 14
  ▫ Blood cultures were positive @ 1350 and Gram stain showed Gram positive cocci suggestive of *Streptococcus*; bottle subcultured

• Day 15 @ 1000
  ▫ Organism isolated from bottle subculture identified as *Enterococcus* spp. and antimicrobial susceptibility testing performed

• Day 16 @1220
  ▫ Organism reported as vancomycin resistant *Enterococcus* based on conventional susceptibility testing results

  **Total time to result – 46.5 hours**
Commercially Available Rapid Identification Systems

PNA FISH
Xpert MRSA/SA BC
Verigene BC-GP
Verigene BC-GN
BioFire BCID
PNA FISH (AdvанDx)

**Peptide Nucleic Acid Fluorescence In Situ Hybridization**

Fluorescent labeled peptide NA probes target species specific rRNA

- Smears from positive blood culture bottles
- PNA FISH 90 min, *QuickFISH* 20 min

**Gram Positive**

- *S. aureus/CNS*
- *E. faecalis/other Enterococcus*
- *mecA (ExpressFISH)*

**Yeast**

- *C. albicans/C. glabrata*
- Yeast Traffic Light

**Gram Negative**

- *E. coli/K. pneumoniae/P. aeruginosa*
PNA FISH

Sensitivity – >98%, Specificity – >99%
Non-charged backbone of PNA probes allow for tighter and more specific hybridization to nucleic acid targets.
PNA FISH

Gram Stain
## Pros and Cons of PNA FISH

<table>
<thead>
<tr>
<th>PROS</th>
<th>CONS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Familiar staining and microscopy (“Easy”)</td>
<td>Limited menu of organisms</td>
</tr>
<tr>
<td>Retains cellular morphology</td>
<td>Batch testing</td>
</tr>
<tr>
<td>Does not require molecular instrumentation</td>
<td>Very limited menu of resistance markers</td>
</tr>
<tr>
<td>Multi-color fluorescence</td>
<td></td>
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<tr>
<td>Species specific and rapid</td>
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</table>
GeneOhm StaphSR (BD)

- Multiplex real time PCR assay
- Simultaneous detection and differentiation of MRSA and *S. aureus* in positive blood cultures
  - MRSA: sequence near the insertion site of the SCCmec
  - *S. aureus*: another *S. aureus* specific sequence, unattached to the SCCmec cassette
- Results in < 2 hours
Pros and Cons of GeneOhm StaphSR

<table>
<thead>
<tr>
<th>PROS</th>
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<tr>
<td>• High sensitivity and specificity</td>
<td>• Batch testing</td>
</tr>
<tr>
<td>• Rapid</td>
<td>• Requires molecular instrumentation/expertise</td>
</tr>
<tr>
<td>• Same assay can be used to test nares and wounds</td>
<td>• Only detects/differentiates MSSA and MRSA</td>
</tr>
<tr>
<td></td>
<td>• Cost</td>
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Xpert MRSA/SA BC (Cepheid)

- Simultaneous detection and differentiation of MRSA and MSSA in positive blood cultures
  - *S. aureus* protein A (*spa*)
  - *mecA* gene
  - Proprietary sequence – confirms presence of SCCmec cassette inserted at chromosomal *attB* site
- 2 independent reactions (Assay time ~ 50 min)
  - 1st – *spa, meca, IC*-----preliminary determination of *S. aureus* and MRSA
  - 2nd – if 1st is +------confirm MRSA (*mecA* integrated within the SCCmec cassette)
- Results in 1 hour
Xpert MRSA/SA BC
Xpert MRSA/SA BC

1. Transfer one drop positive blood culture into elution reagent
2. Vortex and dispense Sample into port S
3. Dispense Reagent 1 into port 1
4. Dispense Reagent 2 into port 2

Total hands-on time = 2 minutes

5. Insert cartridge and start test
### Pros and Cons of Xpert MRSA/SA BC

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<td>• Very little hands on time</td>
<td>• Requires specific molecular instrumentation (GeneXpert)</td>
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<tr>
<td>• Does not require highly trained personnel</td>
<td>• Only detects/differentiates MSSA and MRSA</td>
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Verigene BC-GP and BC-GN (Nanosphere)

- Automated nucleic acid microarray for the identification of genus, species, and genetic resistance determinants
- Results in 2 - 2.5 hours
Verigene BC-GP

- *Staphylococcus* spp.
- *S. aureus*
- *S. epidermidis*
- *Staphylococcus lugdunensis*
- *meca*

- *Listeria* spp.

- *Streptococcus* spp.
- *Streptococcus pneumoniae*
- *Streptococcus anginosus Group*
- *Streptococcus agalactiae*
- *Streptococcus pyogenes*
- *Enterococcus faecalis*
- *Enterococcus faecium*
- *vanA/vanB*
Verigene BC-GN

Species
- *E. coli*
- *Klebsiella pneumoniae*
- *K. oxytoca*
- *Pseudomonas aeruginosa*

Genus
- *Acinetobacter* spp.
- *Citrobacter* spp.
- *Enterobacter* spp.
- *Proteus* spp.

Resistance markers
- CTX-M (ESBL)
- IMP
- KPC
- NDM
- OXA

Carbapenem resistance
Verigene
Verigene
Pros and Cons of Verigene BC-GP/GN

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<td>instrumentation (Verigene)</td>
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<td>• High sensitivity and specificity</td>
<td>• Cost</td>
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<tr>
<td>• Ability to identify multiple organisms and resistance markers</td>
<td></td>
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<tr>
<td>• Rapid</td>
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BioFire Film Array BCID (BioFire/bioMerieux)

- Automated multiplex PCR panel for the identification of genus, species, and genetic resistance determinants
- Only multiplex system FDA approved for yeast
- Hands on time – 2 minutes
- Results in 1 hour
BioFire Film Array BCID

Gram Negative
- Acinetobacter baumannii
- Haemophilus influenzae
- Neisseria meningitidis
- Pseudomonas aeruginosa
- Enterobacteriaceae
- E. coli
- Klebsiella pneumoniae
- K. oxytoca
- Enterobacter cloacae
- Proteus spp.
- Serratia marcescens

Gram Positive
- Enterococcus
- Listeria monocytogenes
- Staphylococcus spp.
- S. aureus
- Streptococcus spp.
- S. agalactiae
- S. pyogenes
- S. pneumoniae
BioFire Film Array BCID

Yeast
- *Candida albicans*
- *C. glabrata*
- *C. krusei*
- *C. parapsilosis*
- *C. tropicalis*

Resistance markers
- *mecA*
- *vanA/B*
- KPC
### Pros and Cons of BioFire Film Array

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<td>• Very little hands on time</td>
<td>• Requires specific molecular instrumentation (Film Array)</td>
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<td>• Does not require highly trained personnel</td>
<td>• Cost</td>
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<tr>
<td>• High sensitivity and specificity</td>
<td></td>
</tr>
<tr>
<td>• Ability to identify multiple organisms, including yeast</td>
<td></td>
</tr>
<tr>
<td>• Rapid</td>
<td></td>
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</table>
MALDI-TOF

- Uses MALDI-TOF mass spectrometry for organism ID
- Does not detect resistance determinants
- Not currently FDA approved for ID from blood cultures
- Hands on time – longer for blood cultures
- Results in minutes

Rand and Deland. 2014. *DMID* 79:293-297
## Pros and Cons of MALDI-TOF

<table>
<thead>
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<tbody>
<tr>
<td>• Ability to identify multiple organisms, ever growing database</td>
<td>• Not yet FDA-approved for blood cultures</td>
</tr>
<tr>
<td>• Rapid</td>
<td>• Does not detect resistance determinants</td>
</tr>
<tr>
<td>• Cost (ID)</td>
<td>• Requires specific instrumentation</td>
</tr>
<tr>
<td></td>
<td>• Cost (instrumentation)</td>
</tr>
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</table>
Impact of Rapid Identification

PNA FISH


• Rapid differentiation of *S. aureus*/CNS
  ▫ Significant reduction in median LOS (4 days vs. 6 days)
  ▫ ↓ Vancomycin usage
  ▫ ↓ Hospital costs ($4000/pt)
  ▫ Negative PNA FISH – prevent or limit vancomycin Rx
Impact of Rapid Identification
PNA FISH


- PNA FISH (S. aureus/CNS) + rapid reporting
  - 80% reduction in ICU-related mortality
  - Median savings $19,441/patient
Impact of Rapid Identification

PCR Assays


- PCR for *mecA* + reporting to ID pharmacist
  - 25.4 hour reduction in time to optimal antimicrobial therapy

Bauer et al. 2010. *CID* 51:1074-1080

- Xpert MRSA/SA + reporting to ID pharmacist
  - Mean LOS 6.2 days
  - Time to appropriate therapy (2 vs. 3.7 days)
Impact of Rapid Identification Multiplex Assays


- Verigene BC-GP + reporting to ID pharmacist for enterococcal bacteremia
  - 23.4 hour reduction in time to optimal antimicrobial therapy
  - ↓mean LOS (13 days)
  - ↓mean hospital costs ($60,729)
Impact of Rapid Identification Multiplex Assays

Southern et al. 2015. DMID 81:96-101

- BioFire Film Array + empiric therapy recommendations
  - Empiric therapy recommendations developed by ASP for targets in panel
  - Applying ASP recommendations to reported panel results hypothetically resulted in 99.2% positive blood cultures being treated with appropriate therapy
Not quite ready for prime time...

Rapid Susceptibility Testing Systems

Accelerate Diagnostics
GeneWEAVE
Not quite ready for prime time...

- **Accelerate Diagnostics**
  - ID and next gen phenotypic AST
    - 1 hour ID using FISH
      - Sensitivity – 98%
    - 5 hour AST using time-lapse imaging and analysis of bacterial growth
      - 96% agreement with conventional AST
  - MIC determination and SIR interpretation
  - Eventually available for multiple specimen types

www.acceleratediagnostics.com
Not quite ready for prime time...

**GeneWEAVE**

- **Smarticles**
  - Specifically target a species, genus, or family of bacteria
  - In the presence of antibiotics, drug-resistant bacteria targeted by Smarticles produce light (luciferase)

- Can be used to detect MDRO in direct specimens
- Can be used to perform AST from positive blood cultures
Use of “Conventional” Tests for Rapid Identification and Susceptibility Testing

Thermonuclease agar
Direct tube coagulase
Direct ID/AST
Thermonuclease Agar

Boil blood for 15 min
Incubate at 35-37°C for 2-4 hours
Direct Tube Coagulase

Add several drops of culture broth directly to tube
Incubate at 35-37°C for 4 hours
Thermonuclease Agar/Direct Tube Coagulase

TN agar
  - Sensitivity (2 hr) – 100%

dTC
  - Sensitivity 34% (2 hr), 65% (4 hr)

TN/dTC
  - TN sensitivity 85% (2 hr), dTC sensitivity 92% (2 hr)
- Sharp, McCarter et al. ASM 2005 (Abstract C-104)
  - TN sensitivity 96.9% (4 hr), dTC sensitivity 96.5% (4 hr)
# Pros and Cons of Thermonuclease Agar/Direct Tube Coagulase

<table>
<thead>
<tr>
<th>PROS</th>
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</tr>
</thead>
<tbody>
<tr>
<td>• Inexpensive</td>
<td>• Subjective</td>
</tr>
<tr>
<td>• Can be easily integrated into workflow</td>
<td>• Only differentiates <em>S. aureus</em> from CNS</td>
</tr>
<tr>
<td>• Does not require highly trained personnel</td>
<td>• Does not identify resistance determinants</td>
</tr>
<tr>
<td>• Does not require instrumentation</td>
<td>• Decreased sensitivity with charcoal-containing blood culture media</td>
</tr>
</tbody>
</table>
Direct ID/AST using Automated ID/AST Systems

- Direct inoculation of organisms from positive blood culture bottle into automated system
  - Serum separator tube
- ID and AST agreement in literature mixed
  - ? System dependent
- Can be useful adjunct to other rapid testing methods
Direct ID/AST using Automated ID/AST Systems

UF Health Jacksonville

- Monomicrobial Gram stain

- Perform Verigene BC-GP or BC-GN
  - *S. aureus*, *S. lugdunensis* ➔ Direct AST
  - *Enterococcus* ➔ Direct AST
  - Gram negative rod ➔ Direct ID/AST
Direct ID/AST using Automated ID/AST Systems

1. Add BC broth to SST and centrifuge
2. Remove supernatant
3. Swab organism pellet from top of gel for 0.5 McFarland suspension
What about....

Bypassing the blood culture altogether?

T2 Candida
T2 Candida (T2 Biosystems)

- Requires no blood culture – whole blood tested
- FDA approved for 5 most common yeast
- Hands on time – <5 minutes
- Results in 3-5 hours
- LOD – 1-3 CFU/mL
- Sensitivity 91.1%
  Specificity 99.4%
T2 Candida (T2 Biosystems)

- Utilizes target amplification, nanoparticle capture and T2 magnetic resonance signal amplification
  - No extraction or sample purification required
- Testing based on risk stratification and serial testing of high risk patients
  - Prior to development of symptoms

Mylonakis et al. 2015. *Clin Infect Dis* 60:892-899
Case Conclusion

• Day 13
  ▫ Blood cultures were drawn @ 1330...

• Day 14
  ▫ Blood cultures were positive @ 1350 and Gram stain showed Gram positive cocci suggestive of *Streptococcus*

• Day 14 @ 1645
  ▫ Organism identified as vancomycin resistant *Enterococcus* (VRE) by rapid molecular assay

• Day 14 @ 1650
  ▫ Pharmacy paged with VRE result; pharmacy notified physician of inappropriate antibiotic therapy (vancomycin)

• Day 14 @ 1653
  ▫ Daptomycin ordered by patient's physician

• Day 14 @ 2113
  ▫ Daptomycin therapy initiated

Time to initiation of appropriate therapy after positive blood culture—7.5 hr
Conclusions

• Rapid identification of organisms causing bacteremia/fungemia results in:
  ▫ Decreased mortality
  ▫ Decreased length of stay
  ▫ Decreased time to appropriate therapy
  ▫ Identification of potential contaminants

• Use of rapid methods must be coupled with antimicrobial stewardship intervention

• Think outside the Lab - The use of rapid methods can result in lower hospital costs
Questions?

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