Objectives

• Describe rationale for the utilization of rapid diagnostic technology within antimicrobial stewardship programs

• Evaluate the different rapid diagnostic tests available for interdisciplinary stewardship teams

• Understand ways to measure the impact of rapid diagnostic testing on patient outcomes
Rapid Diagnostics: Current State

• Recent explosion of FDA-approved rapid diagnostic testing (RDT) methodologies for infectious diseases as the anti-infective pipeline remains stagnant

• Major focus on pathogens associated with increased morbidity, mortality, and excessive healthcare costs
### Combating Antibiotic-Resistant Bacteria

<table>
<thead>
<tr>
<th>Threat Category</th>
<th>Organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HAZARD LEVEL</strong></td>
<td><strong>URGENT</strong></td>
</tr>
</tbody>
</table>
| • Severe consequences   | *Clostridium difficile*  
| • Urgent public attention to identify infections and prevent transmission | Carbapenem-resistant Enterobacteriaceae (CRE)  
|                          | Drug-resistant *Neisseria gonorrhoeae*                                    |
| **HAZARD LEVEL**        | **SERIOUS**                                                               |
| • Low or declining incidence | Multi-drug resistant *Acinetobacter*; Drug-resistant *Campylobacter*; Fluconazole resistant *Candida*; ESBL-producing Enterobacteriaceae; Vancomycin-resistant *Enterococcus* (VRE); Multidrug-resistant *Pseudomonas aeruginosa*; Drug-resistant Non-typhoidal Salmonella; Drug-resistant *Salmonella Typhi*; Drug-resistant *Shigella*; Methicillin-resistant *Staphylococcus aureus*; Drug-resistant *Streptococcus pneumoniae*; Drug-resistant tuberculosis |
| • Therapeutic agents available |                                                                 |
| • Require public health monitoring and prevention |                                                                 |
| **HAZARD LEVEL**        | **CONCERNING**                                                            |
| • Low threat            | Vancomycin-resistant *Staphylococcus aureus*                              |
| • Multiple therapeutic options | Erythromycin-resistant Group A *Streptococcus*                           |
| • Requires monitoring for outbreaks | Clindamycin-resistant Group B *Streptococcus*                              |

### NATIONAL STRATEGY FOR COMBATING ANTIBIOTIC-RESISTANT BACTERIA

### GOAL 3:
Advance Development and Use of Rapid and Innovative Diagnostic Tests for Identification and Characterization of Resistant Bacteria

• Role of rapid diagnostics and biomarkers in antimicrobial stewardship is recognized as a key recommendation by the IDSA
• New”-ish” niche to collaborate for stewardship teams
• What can molecular microbiology bring to the table?
  – Pathogen and resistance identification without conventional culture
  – Improved clinical outcomes
    • Tailor therapy sooner
    • Reconcile conflicts associated with empiric therapy
    • Avoid antibiotic agents causing collateral damage

The Ideal Treatment Scenario

- Must be **prompt**: delays in initiating effective antibiotics lethal
- **Appropriate**: must cover the offending pathogen(s)
- Administered at **adequate** dose and intervals consistent with pharmacokinetic/pharmacodynamic parameters
- Timely **streamlining** based on clinical response and microbiological data
- Prompt **discontinuation** when therapy complete

All contingent on accurate determination of the pathogen’s identification and antimicrobial susceptibility

Organism Identification and the Initiation of Targeted Antimicrobial Therapy

**Traditional Identification & Testing Methods:**

- Blood drawn
- Gram stain
- Empiric and broad-spectrum antimicrobial therapy
- Standard organism identification and susceptibility
- Targeted antimicrobial therapy

**Rapid Molecular Identification Methods:**

- Blood drawn
- Gram stain
- Rapid molecular identification
- Empiric antimicrobial therapy
- Targeted antimicrobial therapy

*This is an illustration of general differences between the two methods. These timelines are hypothetical and may not occur in clinical practice.*
**Polymerase Chain Reaction (PCR)-Based Testing**

## Real-time PCR; Multiplex PCR

General method: Detection and amplification of a piece of target DNA using fluorescently labeled probes with primers

<table>
<thead>
<tr>
<th>Overall Advantages</th>
<th>Overall Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Rapid results</td>
<td>• Susceptible to contamination</td>
</tr>
<tr>
<td>• Low detection limits</td>
<td>• Require dedicated lab space for instruments</td>
</tr>
<tr>
<td>• Specific organism detection or subtyping</td>
<td>• Sensitive to inhibitors present in many clinical specimens</td>
</tr>
<tr>
<td>• Does not require growth on media</td>
<td>• Dependent on quality of nucleic axis primers and probes</td>
</tr>
<tr>
<td>• High throughput</td>
<td>• Most require initiation from positive cultures/single colonies</td>
</tr>
<tr>
<td></td>
<td>• Can not indicate viability of pathogen detected</td>
</tr>
<tr>
<td></td>
<td>• Practical limitations can affect turnaround time</td>
</tr>
</tbody>
</table>

## PCR-Based RDTs Available

<table>
<thead>
<tr>
<th>Organisms/ Antibiotic Resistance Targets</th>
<th>Detection Time, hours</th>
<th>Manufacturer</th>
<th>Test Commercial Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Real-time PCR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRSA</td>
<td>2</td>
<td>Roche Diagnostics</td>
<td>Light Cycler MRSA</td>
</tr>
<tr>
<td><em>C. difficile</em></td>
<td>2</td>
<td>BD GeneOhm</td>
<td>BD GeneOhm Cdiff Assay</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Gen-Probe Prodesse</td>
<td>ProGastro Cd Assay</td>
</tr>
<tr>
<td><strong>Multiplex PCR</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSSA, MRSA, CoNS</td>
<td>2</td>
<td>BD GeneOhm</td>
<td>BD GeneOhm Staph SR</td>
</tr>
<tr>
<td>MSSA, MRSA</td>
<td>1</td>
<td>Cepheid</td>
<td>Xpert MRSA/SA BC</td>
</tr>
<tr>
<td><em>C. difficile</em></td>
<td>0.5</td>
<td>Cepheid</td>
<td>Xpert C. difficile</td>
</tr>
<tr>
<td></td>
<td>0.75</td>
<td>Cepheid</td>
<td>Xpert C. difficile/Epi</td>
</tr>
<tr>
<td></td>
<td>&lt;2</td>
<td>Nanosphere</td>
<td>Verigene: Gram-negative blood culture</td>
</tr>
<tr>
<td>Multiple bacterial, fungal, viral pathogens, and mecA, vanA/B, carbapenem resistance</td>
<td>1</td>
<td>BioFire Diagnostics</td>
<td>FilmArray System &amp; panels</td>
</tr>
</tbody>
</table>
PCR-Based Testing – Clinical Outcomes

Rapid *S. aureus* Identification and Targeted Antimicrobial Therapy in Patients with *S. aureus* Bacteremia

Single-center, Non-equivalent, Comparative Study [2008, 2009]

PCR results were communicated to an ID pharmacist who recommended effective, targeted antimicrobial therapy and an ID consult

PCR-Based Testing – Clinical Outcomes

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Peptide Nucleic Acid Fluorescent In Situ Hybridization (PNA FISH)

- PNA FISH technology employs synthetic oligonucleotide fluorescence labeled probes to target species specific rRNA sequences.
- Enables visualization of target pathogen via fluorescence microscopy.

![Yeast Traffic Light® PNA FISH® diagram]
## Peptide Nucleic Acid Fluorescent In Situ Hybridization (PNA FISH)

<table>
<thead>
<tr>
<th>Overall Advantages</th>
<th>Overall Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Easy to perform</td>
<td>• Labor intensive</td>
</tr>
<tr>
<td>• Does not require expensive infrastructure</td>
<td>• Amount of setup time necessary usually requires batching</td>
</tr>
<tr>
<td>• Rapid procedure</td>
<td>• Limited menu of test organisms</td>
</tr>
<tr>
<td>• Useful method for detection of fastidious organisms</td>
<td>• Cost of fluorescence microscope</td>
</tr>
<tr>
<td>• Can detect multiple species simultaneously using 2 or more specific probes, each labeled with a unique fluorescent dye</td>
<td>• Requires skilled and experienced technicians to interpret results</td>
</tr>
<tr>
<td>• Less likely affected by contamination</td>
<td></td>
</tr>
<tr>
<td>• Can detect bacteria directly in specimens or after enrichment culture</td>
<td></td>
</tr>
<tr>
<td>• PNA probes have higher specificity, increased stability</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organisms/ Antimicrobial Resistance Targets</th>
<th>Detection Time, hours</th>
<th>Manufacturer</th>
<th>Test Commercial Name</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em>, CoNS</td>
<td>0.3</td>
<td>AdvanDx</td>
<td>S. aureus/ CoNS PNA QuickFISH</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em>, <em>Enterococcus faecium</em></td>
<td>0.5</td>
<td></td>
<td>Enterococcus faecalis/OE PNA QuickFISH</td>
</tr>
<tr>
<td><em>E. coli</em>, <em>Pseudomonas aeruginosa</em>, <em>Klebsiella pneumoniae</em></td>
<td>0.5</td>
<td></td>
<td>GNR Traffic Light PNA QuickFISH</td>
</tr>
<tr>
<td><em>C. albicans</em>, <em>C. parapsilosis</em>, <em>C. tropicalis</em>, <em>C. glabrata</em>, <em>C. krusei</em></td>
<td>1.5</td>
<td></td>
<td>Yeast Traffic Light PNA Fish</td>
</tr>
</tbody>
</table>
Impact upon clinical outcomes of translations of PNA FISH-generated laboratory data from the clinical microbiology bench to bedside in real time

Prospective, Single-center, Randomized, Controlled Study [2006]

PNA FISH – Clinical Outcomes

PNA FISH for hospital-acquired enterococcal bacteremia: delivering earlier effective antimicrobial therapy

- Quasiexperimental study performed from 2005 - 2006
  - Comparison of rapid identification method with traditional methods
  - Differentiate E. faecalis from E. faecium in + blood cultures in 90 minutes

- Results
  - Species identification 2.3 days sooner (p<0.001)
  - Decrease in time to effective therapy from 3.1 days to 1.3 days (p<0.001)
  - Reduced 30-day mortality (26% vs. 45%, p=0.4)


• Rapid identification of bacteria, yeast and fungi
  – Minimum of 24-96 h reduced turnaround time compared to conventional culture-based methods.
  – Even more dramatic results for certain organisms (i.e. *Legionella*).

• Test directly from positive blood cultures
  – Bypasses time-consuming subculture steps

### MALDI TOF

#### Overall Advantages

- Rapid (turnaround time ≤1-2 hours)
- Automated, high throughput, batching not required
- Can be used to analyze uncultured bacteria
- Minimal consumables, relatively inexpensive reagent costs (~$0.35 /test)
- No prior knowledge of organism type necessary, can identify wide range of organisms
- Accurate in the identification of rare or fastidious bacteria

#### Overall Disadvantages

- Accurate identification requires well-curated, regularly updated, comprehensive reference database, current database are proprietary
- Does not provide antimicrobial susceptibility results
- Requires isolated colony; small mucoid colonies not easily identifiable
- Growth on some media may be associated with no/lower identification certainty
- High instrument cost
- Some closely related organisms not differentiated; poor identification with polymicrobial samples
- Laboratory errors can affect identification
- Gram negative organisms seem to be easier to detect than Gram-positives
## MALDI-TOF MS Platforms
Currently FDA-Approved

<table>
<thead>
<tr>
<th>Organism</th>
<th>Detection Time, hours</th>
<th>Manufacturer</th>
<th>Test Commercial Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple bacterial and fungal pathogens</td>
<td>0.2</td>
<td>Bruker Corporation</td>
<td>MALDI Biotyper CA</td>
</tr>
<tr>
<td></td>
<td>0.25-1</td>
<td>bioMerieux</td>
<td>VITEK MS</td>
</tr>
</tbody>
</table>
MALDI-TOF MS – Clinical Outcomes

An Antimicrobial Stewardship Program’s Impact with Rapid Pathogen Identification for Gram-negative BSIs

• **Objective:** Determine the clinical and economic impact of MALDI-TOF MS and ID Pharmacists’ interventions on patients with Gram-negative bacteremia.

• **Method:** A comparative study of hospitalized patients before and after implementation of MALDI-TOF MS and real-time ASP notifications.

• **Outcomes:** Differences in time to optimal therapy, hospital length of stay (LOS), hospital costs

Results

**Pre-Intervention (PI)**
- Blood culture drawn
- Culture positivity
- Growth on solid media
- Identification (Conventional)
- Susceptibility (Conventional)
- Passive notification via EMR
- Adjust therapy

**Intervention (Int)**
- Blood culture drawn
- Culture positivity
- Identification (MALDI-TOF)
- Susceptibility (BD Phoenix)
- Adjust therapy
- Active antimicrobial stewardship

**Average hours post-time to positivity**
- 0
- 12
- 24
- 36
- 48
- 60
- 72

**Inactive/No antibiotics**
- PI: n = 22
- Int: n = 16
- \( P = 0.38 \)

**Active antimicrobial stewardship**
- PI: n = 22
- Int: n = 5
- \( P = 0.001 \)

**Post-Intervention**
- PI: n = 15
- Int: n = 0
- \( P < 0.001 \)

MALDI-TOF MS – Clinical Outcomes

• Gram-negative bacteremia:
  – Decreased length of stay from 11.9 to 9.3 days (p=0.1)
  – Decreased hospital costs by $19,547 per patient (p=0.009)
  – 21% vs 8.9% reduction in mortality in antibiotic-resistant Gram-negative bacteremia (p=0.01)

• Gram-positive, Gram-negative bacteremia, Candidemia
  – Improved time to effective therapy from 30.1 vs. 20.4 h (p=0.02)
  – Decreased length of stay by 2.8 days
  – Reduced mortality from 20.3% to 14.5% (p=0.02)
MALDI-TOF MS – Clinical Outcomes

Use of rapid diagnostics and antimicrobial stewardship in community hospital setting to improve outcomes in patients with Gram-negative bacteremia

• Quasiexperimental study design evaluating clinical and economic outcomes in adults with GN-bacteremia at either Houston Methodist (HM) Sugar Land Hospital (235 beds) and HM Willowbrook Hospital (241 beds)

• MALDI-TOF MS coupled with ASP notification effects currently being analyzed
  – To date: Workflow, centralized microbiology has been rolled out at each outlying hospital
• T2 Biosystems
  – Potential to dramatically raise the standard of care in clinical practice

• Miniaturized nuclear magnetic resonance (NMR) technology
  – Limit of detection 1 cfu/mL vs 100-1000 cfu/mL typically required for tradition PCR-based methods

• FDA Approval for *Candida* detection directly from a patient’s blood sample
  – Results in 3-6 hours compared with 5 days

• T2Candida, T2Bacteria in pipeline

• Reach out to your microbiology laboratory and find out:
  – What is currently offered? What is being sent out?
  – Can anything be consolidated?
  – Will knowing X, Y, or Z sooner allow for more timely care?
    • Time to antibiotics
    • Time to discharge
    • Time to definitive therapy
    • Time to isolation precautions ... or discontinuation of isolation

• Stewardship can be instrumental in justifying and implementing new technology
  – Purchase vs. lease
  – Laboratory space
  – High tech vs. low tech
The Ideal Molecular Test

• Key questions to ask about any new molecular test:
  – Does it provide a clinically useful result?
  – Does it cover your routine organisms of interest?
  – Does it provide useful, reproducible results?
  – Is it cost effective?
  – Does it provide good or improved turn-around-time?
Rapid Diagnostic Testing & Antimicrobial Stewardship

• Rapid diagnostic tests are “game changing” for patient care moving forward
  – Advances in RDTs provide new opportunities for stewardship programs

• Enhance function of clinical microbiology laboratories

• Yields significant improvement to patient care, increases the effectiveness of ASPs and infection control efforts
  – Represents one of the few bright spots in the changing world of escalating antimicrobial resistance and stewardship