Time is of the Essence: Incorporating 2015 CLSI Updates and Rapid Diagnostics into Stewardship

Special thanks to CLSI, SIDP, and members of ACCP ID PRN

- Planning Committee
  - Christopher M. Bland, Pharm.D., BCPS, FIDSA
  - P. Brandon Bookstaver, PharmD, FCCP, BCPS (AQ-ID), AAHIVP
  - Kristi Kuper, PharmD, BCPS
  - Monica V. Golik Mahoney, PharmD, BCPS-AQ ID
  - Patrick McGinn

It is the policy of ProCE, Inc. to ensure balance, independence, objectivity, and scientific rigor in all of its continuing education activities. Faculty must disclose to participants the existence of any significant financial interest or any other relationship with the manufacturer of any commercial product(s) discussed in an educational presentation. Dr. Nagel has no relevant commercial and/or financial relationships to disclose. Dr. Abbott has no relevant commercial and/or financial relationships to disclose.

Please note: The opinions expressed in this activity should not be construed as those of the CME/CE provider. The information and ideas expressed by the faculty through clinical practice and knowledge of the professional literature. Portions of this activity may include unlabeled indications. Use of drugs and devices outside of labeling should be considered experimental and participants are advised to consult prescribing information and professional literature.
Time is of the Essence: Incorporating 2015 CLSI Updates and Rapid Diagnostics into Stewardship

April Abbott, Ph.D., D(ABMM)
Manager, Technical Operations
Director, Microbiology
Deaconess Health System
April.Abbott@Deaconess.com

Disclosures

• No conflicts of interest or significant disclosures
Objectives ("the Lab" spin)

- Discuss changes and updates in CLSI standards for antimicrobial susceptibility testing introduced in 2015
- Identify caveats in microbiology susceptibility testing that can influence results
- Review the commonly used rapid microbiology testing methods utilized in healthcare
- Discuss how to incorporate rapid diagnostic testing results into an antimicrobial stewardship program

CLSI Standards Update

- M100 – performance standards for AST
  - Breakpoints for commonly isolated bacteria
  - Suggestions for appropriate agents to test
  - Recommendations for reporting

- Other important documents
  - Developing breakpoints (M23)
  - Cumulative antibiogram (M39)
  - Fastidious bacteria (M45)
  - Many other with several in development
**ENTEROBACTERIACEAE VS CEFAZOLIN**

---

### Enterobacteriaceae vs Cefazolin

- Cefazolin breakpoints for *Enterobacteriaceae* revised in 2010
- Cefazolin became the recommended surrogate marker for oral cephalosporins (uncomplicated UTI) in 2014

<table>
<thead>
<tr>
<th>Test/Report Group</th>
<th>Agent</th>
<th>MIC Breakpoint (µg/ml)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Susc</td>
<td>Int</td>
</tr>
<tr>
<td><strong>Cephems (Parenteral)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Cefazolin</td>
<td>≤2</td>
<td>4</td>
</tr>
<tr>
<td><strong>Cephems (Oral)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U</td>
<td>Cefazolin*</td>
<td>≤16</td>
<td>-</td>
</tr>
</tbody>
</table>

*Surrogate for oral cephalosporins (cefaclor, cefdinir, cefpodoxime, cefprozil, cefuroxime, cephalexin, and loracarbef)
### Issue 1: Revised Breakpoints

<table>
<thead>
<tr>
<th>MIC Breakpoint (µg/ml)</th>
<th>Susc</th>
<th>Int</th>
<th>Res</th>
</tr>
</thead>
<tbody>
<tr>
<td>OLD Breakpoints</td>
<td>≤8</td>
<td>16</td>
<td>≥32</td>
</tr>
<tr>
<td>Current Breakpoints</td>
<td>≤2</td>
<td>4</td>
<td>≥8</td>
</tr>
</tbody>
</table>

- Commercial AST manufacturers were slow to make this adjustment leaving labs relatively unable to change
  - Manufacturers now have panels available to accommodate lowered interpretive criteria
  - What does this mean for the lab?
  - What does this mean for you?

### Issue 1:

**≤ 2 = S**

At the expense of which drugs?
Time is of the Essence: Incorporating 2015 CLSI Updates and Rapid Diagnostics into Stewardship

**Issue 2: Urine Isolates**

<table>
<thead>
<tr>
<th>Test/Report Group</th>
<th>Agent</th>
<th>MIC Breakpoint (µg/ml)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Susc</td>
<td>Int</td>
</tr>
<tr>
<td>Cephalosporins (Oral)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>U Cefazolin*</td>
<td>≤16</td>
<td>-</td>
<td>≥32</td>
</tr>
</tbody>
</table>

- Issue 2: How to report?
  - Case: 65 year old man presents with *E. coli* UTI

<table>
<thead>
<tr>
<th>Isolate 1: Urine</th>
<th>MIC</th>
<th>Interp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefazolin</td>
<td>8</td>
<td>S</td>
</tr>
</tbody>
</table>

The following day, blood cultures are positive

<table>
<thead>
<tr>
<th>Isolate 2: Blood</th>
<th>MIC</th>
<th>Interp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefazolin</td>
<td>8</td>
<td>R</td>
</tr>
</tbody>
</table>

Same MIC, different interpretation?? So can I keep the patient on cefazolin???

**Possible Solution**

- Report both results on urine cultures
- Change name of cefazolin when used as a surrogate test to better reflect its intended purpose
- For example:

<table>
<thead>
<tr>
<th>Isolate 1: Urine</th>
<th>MIC</th>
<th>Interp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral cephs</td>
<td>8</td>
<td>S</td>
</tr>
<tr>
<td>(uncomplicated UTI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefazolin (parenteral)</td>
<td>8</td>
<td>R</td>
</tr>
</tbody>
</table>

- Requires education
- Requires IT change in reporting
Cefazolin Reporting

- Understand the limitations of AST in your lab
- Be wary of what you don’t see
  - If your lab hides cefazolin on blood isolates because the AST panel does not have a low enough dilution to determine susceptibility, you may not realize how often you get cefazolin MICs as susceptible in urine, but would be resistant if any other source
- Collaborate to make decisions about what to test and how to report
- Verify that common dosing structure in your hospital matches that used to establish breakpoints
- Only applies to *E. coli*, *K. pneumoniae*, and *P. mirabilis*

**SALMONELLA VS QUINOLONES**
**Salmonella AST**

- Fluoroquinolone susceptibility testing for *Salmonella enterica* is imperfect
- No single screening test will detect all possible resistance mechanisms
- Ciprofloxacin, levofloxacin, or ofloxacin MIC testing is recommended by CLSI
  - Most commercial panels do not have dilutions low enough to separate fully susceptible strains from those with low level resistance

<table>
<thead>
<tr>
<th>Agent</th>
<th>S</th>
<th>I</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>≤ 0.06</td>
<td>0.12-0.5</td>
<td>≥ 1</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>≤ 0.12</td>
<td>0.25-1</td>
<td>≥ 2</td>
</tr>
</tbody>
</table>

**Fluoroquinolone Resistance**

- Many laboratories rely on nalidixic acid disks to assist in detection of fluoroquinolone resistance
- If MIC ≤ 1, test nalidixic acid
- Report as fluoroquinolone resistant based on nalidixic acid resistance

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>FQ (Cipro) MIC</th>
<th>Nal MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>gyrA mutation</td>
<td>0.12-0.5 (I)</td>
<td>≥ 32 (R)</td>
</tr>
<tr>
<td>gyrB, parC/E, plasmid quinolone</td>
<td>0.12-0.5 (I)</td>
<td>4-32 (S-R)</td>
</tr>
<tr>
<td>resistance determinants (qnr)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- This approach overcalls susceptibility

Deak E, et. al., JCM. 2015 (pre-print)
Testing Options and Considerations

- Pefloxacin disk diffusion recommended as surrogate for fluoroquinolone resistance (caveat: not available in the U.S.)
- Alternative MIC-based method (e.g. Etest) for ciprofloxacin or levofloxacin is well established (caveat: not FDA-approved)
- Ciprofloxacin or levofloxacin disk diffusion (caveats: only CLSI breakpoints for ciprofloxacin (none for levofloxacin); breakpoints are based primarily on Salmonella enterica ser. Typhi and Paratyphi, and disk diffusion performance is variable)

CARBAPENEMASE TESTING
Carbapenemase Testing

- 72 year old female who presents from SNF with urinary tract infection (fever, dysuria, pyuria). Cultures grow >10⁵ Enterobacter cloacae. Susceptibility testing reveals the following:

<table>
<thead>
<tr>
<th>Agent:</th>
<th>MIC, Interp</th>
</tr>
</thead>
<tbody>
<tr>
<td>cefazolin (UTI)</td>
<td>&gt; 16 R</td>
</tr>
<tr>
<td>cefepime</td>
<td>2 S</td>
</tr>
<tr>
<td>ceftriaxone</td>
<td>&gt; 32 R</td>
</tr>
<tr>
<td>ciprofloxacin</td>
<td>≤1 S</td>
</tr>
<tr>
<td>ertapenem</td>
<td>&gt;2 R</td>
</tr>
<tr>
<td>gentamicin</td>
<td>≤4 S</td>
</tr>
<tr>
<td>meropenem</td>
<td>&gt;4 R</td>
</tr>
<tr>
<td>pip-tazobactam</td>
<td>&gt; 64/4 R</td>
</tr>
<tr>
<td>trimeth-sulfa</td>
<td>&gt; 4/76 R</td>
</tr>
</tbody>
</table>

Carbapenemase Testing

- First question, does any additional testing need to occur?
- Carbapenem breakpoints revised in 2010

<table>
<thead>
<tr>
<th>Meropenem (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Old</td>
</tr>
<tr>
<td>S</td>
</tr>
<tr>
<td>≤4</td>
</tr>
</tbody>
</table>

- Many laboratories are using “old” breakpoints
- When imipenem or meropenem MICs of 2-4 µg/ml or ertapenem MIC of 2 µg/ml, test for carbapenemase production
Carbapenemase Testing

- Phenotypic
  - Modified Hodge test (MHT)
  - Combined-disc tests
  - CarbaNP test
- Molecular assays
Carbapenemase Reporting

CRE ≠ CPO
Carbapenem resistant Enterobacteriaceae ≠ Carbapenemase-producing organism

- Initial susceptibility result
  - Multi-drug resistant isolate or carbapenem resistant isolate
  - Infectious Disease consult recommended
  - Further susceptibility testing to follow
- If further carbapenemase testing follows, update the report
  - E.g. Carbapenemase-producing organism
  - Provide mechanism? (KPC, OXA-48, NDM, etc.)
- Determine additional agents to test in advance

EPIDEMIOLOGICAL CUTOFF VALUE
Introduction of Concept: ECV

- Epidemiological Cutoff Value (ECV)
  - Differs from standard CLSI breakpoint or interpretive criteria
  - **Breakpoints**:
    - Based upon MIC distributions
    - Pk/Pd modeling
    - Animal studies
    - Outcome data
    - Clinical testing reliability
    - **GOAL**: allowing one to predict whether a bug/drug combination is likely to work


- Epidemiological Cutoff Value (ECV)
  - Differs from standard CLSI breakpoint or interpretive criteria
  - **ECV**
    - MIC values that separate wild type from non-wild-type
    - Based on *in vitro* data only
    - Used to help signal the emergence of a non-wild-type strain
    - Do NOT allow one to determine if a bug/drug combination is likely to be successful

Example ECV

- Case: 45 year old male presents for revision of shoulder arthroplasty. Cultures of specimens obtained during the surgery grow *P. acnes*. Susceptibility testing is performed with the following results:

<table>
<thead>
<tr>
<th>Agent</th>
<th>MIC (μg/ml)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>0.06</td>
<td>S</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.5</td>
<td>S</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>8</td>
<td>-</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>&gt;32</td>
<td>R</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>0.125</td>
<td>S</td>
</tr>
</tbody>
</table>

Using ECVs (and Other Resources)

- *P. acnes* v vancomycin: what does a MIC of 8 mean?
  - No CLSI interpretive criteria for *P. acnes* vs vancomycin
  - Is it real?
    - What is the method?
    - Can it be replicated?
    - Has this been reported previously?
    - Does this make sense clinically?
Using ECVs (and Other Resources)

**P. acnes**

<table>
<thead>
<tr>
<th>Agent</th>
<th>WT</th>
<th>NWT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>≤2</td>
<td>≥4</td>
</tr>
</tbody>
</table>

WT = wild type, NWT = Not wild type

- Therefore, MIC of 8 would not be wild-type but this does not (necessarily) mean that the isolate is resistant
- Use of ECV allows for laboratories to provide information in the absence of set breakpoints

**P. acnes review:** Achermann Y et. al, Clin Microbiol Rev. 2014 July; 27(3): 419–440
Summary Questions

- Enterobacteriaceae vs cefazolin
  - Does the laboratory possess the ability to routinely test for cefazolin susceptibility on commercial panels?
  - How is the laboratory handling oral cephalosporin surrogate testing on urine isolates?
- Salmonella vs quinolones
  - Could we be overcalling susceptibility?
- Carbapenemase testing and reporting
  - What breakpoints are being used to assess carbapenem resistance?
  - What method is used to detect carbapenemases?
  - Do physicians understand the reporting language?
- Epidemiological cutoff value
  - Do I know where to find ECVs and ECOFFs?

CLSI Oldies but Goodies (M100)

- Intrinsic Resistance Table (Appendix B)
  - Provides susceptibility information about antimicrobials tested as well as those not tested in your laboratory
  - GN: Enterobacteriaceae and Non-Enterobacteriaceae
  - GP: Staphylococci and Enterococcus

![Intrinsic Resistance Table](https://example.com/intrinsic_table.png)
CLSI Oldies but Goodies (M100)

- Cumulative Antimicrobial Susceptibility Report for Anaerobic Organisms (Appendix D)
- Antibiogram for commonly isolated anaerobic bacteria, both GP and GN
- Expanded in 2015 M100

**CLSI Oldies but Goodies (M100)**

- Cumulative Antimicrobial Susceptibility Report for Anaerobic Organisms (Appendix D)
  - Antibiogram for commonly isolated anaerobic bacteria, both GP and GN
  - Expanded in 2015 M100

**COORDINATING MOLECULAR AND PHENOTYPIC RESULTS**
Detection of Methicillin Resistance

mecA gene* → PBP2a → methicillin resistance

* Others (mecC)

Abbott AN and Fang FC, Manual of Clinical Microbiology, 11th Ed. 2015

Caveats to Methicillin Resistance Testing

<table>
<thead>
<tr>
<th>Genotypic*</th>
<th>Phenotypic**</th>
<th>Result</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>S</td>
<td>S</td>
<td>Empty cassette (largely resolved)</td>
</tr>
<tr>
<td>S</td>
<td>R</td>
<td>R</td>
<td>mecC</td>
</tr>
<tr>
<td>R</td>
<td>S</td>
<td>S</td>
<td>Mixed population (MSSA + MR-coagulase negative staph)</td>
</tr>
<tr>
<td>R</td>
<td>S</td>
<td>R</td>
<td>Inducible resistance (OS-MRSA)</td>
</tr>
</tbody>
</table>

*Detection of mecA (multiple targets)

**Cefoxitin disk to test for methicillin (oxacillin) resistance
Oxacillin Susceptible-MRSA

- Increased use of molecular methods for early detection of MRSA followed by phenotypic susceptibility testing has led to recognition of discordant results
- Recent studies have demonstrated that phenotypic expression of methicillin resistance can be variable (heterogeneous population, inducible)
  - Also a phenomenon described by lack of PBP2a detection in phenotypically resistant isolates
- Troubleshooting: Induce expression of methicillin-resistant phenotype by repeat testing (PBP2a or cefoxitin disk diffusion) of growth at edge of cefoxitin disk zone

Tenover FC et. al., Clinical Microbiology Newsletter, Vo 37. No.10. May 2015

Oxacillin Susceptible-MRSA

- Frequency of occurrence and impact on the patient is largely unknown
  - Heteroresistance may occur in 1 out of every 1,000,000 cells
  - Frequency has been estimated from 1% - 25% of isolates examined (hindered by variability in methodology and effort to identify these isolates)
  - Anecdotal clinical failures have been described when such isolates are treated as MSSA, but additional work is needed
- Discrepancies between molecular and phenotypic results must be investigated and resolved
- No current system is perfect, recommended testing methods and reporting guidelines continue to evolve
Summary

- CLSI updates susceptibility testing recommendations annually to address antimicrobial therapy and resistance concerns.
- Numerous challenges exist for antimicrobial susceptibility testing and reporting, some of which were highlighted.
- Clinical laboratories must work with the antibiotic stewardship team to develop susceptibility testing and reporting protocols.
- Therapy-related comments provide the antimicrobial dosage by which breakpoints are set; these should be compared to the regimen used by practitioners.
- Results must be conveyed in the manner best able to influence appropriate prescribing practices.
- As information technology advances, so will the complexity of susceptibility testing.

Resources Available From CLSI

- **M100**—*Performance Standards for Antimicrobial Susceptibility Testing*

- **M23**—*Development of In Vitro Susceptibility Testing Criteria and Quality Control Parameters*

- **M39**—*Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data*

- **M45**—*Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria*

Visit [shop.clsi.org/microbiology](http://shop.clsi.org/microbiology) to learn more about these documents.
About CLSI

- CLSI is a not-for-profit membership organization that sets and upholds the clinical laboratory testing standards that drive quality test results, enhance patient care delivery, and improve health care around the world.

- By using CLSI standards, laboratorians can improve process quality, speed the development of standard operating procedures, and implement safer practices with greater ease and efficiency.

Learn more at www.clsi.org.
Objectives & Disclosure

- Discuss changes and updates in CLSI standards for antimicrobial susceptibility testing introduced in 2015
- Review the commonly used rapid microbiology testing methods utilized in healthcare
- Identify caveats in microbiology testing that can influence results
- Discuss how to incorporate rapid diagnostic testing results into an antimicrobial stewardship program

I have no conflicts of interest to disclose

Timeline for Organism Identification and Susceptibility Results

<table>
<thead>
<tr>
<th>Blood culture bottle</th>
<th>Gram stain</th>
<th>Automated Testing Set-Up</th>
<th>Automated Organism ID</th>
<th>Automated Susceptibilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>0h</td>
<td>12-48h</td>
<td>18-24h</td>
<td>8-48h</td>
<td>Patient care team sees ID and susceptibility results</td>
</tr>
</tbody>
</table>

Time from blood draw till microbiology results

<table>
<thead>
<tr>
<th>Test</th>
<th>Time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>30.1</td>
</tr>
<tr>
<td>Organism identification</td>
<td>84.0</td>
</tr>
<tr>
<td>Organism susceptibilities</td>
<td>87.3</td>
</tr>
</tbody>
</table>

Huang, Clin Infect Dis. 2014
Time is of the Essence: Incorporating 2015 CLSI Updates and Rapid Diagnostics into Stewardship

Importance of Appropriate Initial Therapy in Patients with Bacteremia

- Leibovici et al. 1998
- Micek et al. 1998

Impact of Delayed Effective Antibiotic Therapy in Septic Shock

- Kumar et al. 2006

References:
Advances in Clinical Microbiology

- Mass spectrometry
  - MALDI-TOF
- Nucleic acid hybridization
  - PNA-FISH™
- Nucleic acid amplification
  - Real-time PCR, Multiplex arrays
- Magnetic resonance imaging
  - T2 Biosystems™
- Next generation whole genome sequencing

Rapid Molecular Diagnostics for Infectious Diseases

- Microarray, PCR, PNA-FISH, MR, WGS
- Positive blood culture bottle
  - Incubation
  - 0h
- Gram stain
  - Incubation
  - 12-24h
- Automated Testing Set-Up
  - Incubation
  - 12-24h
- MALDI-TOF
- Automated Organism ID
- Automated Susceptibilities
  - Incubation
  - 12-48h
- Targeted Organism ID & Detection of Genes Linked to Resistance
- Organism ID
  - Positive blood culture bottle
- Patient care team sees ID and susceptibility results
MALDI-TOF

- **Matrix-Assisted Laser Desorption/Ionization-Time Of Flight**
  - Utilizes mass spectrometry
  - Identifies bacteria based on unique protein sequences
  - Process for organism identification takes ~1 hour
  - Widely used in Europe, gaining popularity in the US

---

**Isolation of bacterial colony and dilution**

**Extraction and addition of matrix**

**Ionization, vaporization and travel up TOF tube**

---

Time is of the Essence: Incorporating 2015 CLSI Updates and Rapid Diagnostics into Stewardship

MALDI-TOF

Sample currently being testing

MALDI-TOF

Closest match

<table>
<thead>
<tr>
<th>Rank (Quality)</th>
<th>Matched Pattern</th>
<th>Score Value</th>
<th>NCBI Identifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (+++)</td>
<td><em>Escherichia coli</em> 3rd VMMA</td>
<td>3.543</td>
<td>562</td>
</tr>
<tr>
<td>2 (+++)</td>
<td><em>Escherichia coli</em> MG1655 1_CERB</td>
<td>2.194</td>
<td>562</td>
</tr>
<tr>
<td>3 (+++)</td>
<td><em>Escherichia coli</em> DSM 30033_VFL</td>
<td>2.344</td>
<td>562</td>
</tr>
<tr>
<td>4 (+++)</td>
<td><em>Escherichia coli</em> DSM 30033_HAM</td>
<td>2.094</td>
<td>562</td>
</tr>
<tr>
<td>5 (+)</td>
<td><em>Escherichia coli</em> 152242_1</td>
<td>2.082</td>
<td>562</td>
</tr>
<tr>
<td>6 (+)</td>
<td><em>Escherichia coli</em> 35215_1</td>
<td>2.305</td>
<td>562</td>
</tr>
<tr>
<td>7 (+)</td>
<td><em>Escherichia coli</em> 25222_CERB</td>
<td>1.954</td>
<td>562</td>
</tr>
<tr>
<td>8 (+)</td>
<td><em>Escherichia coli</em> 25222_TUL</td>
<td>1.977</td>
<td>562</td>
</tr>
<tr>
<td>9 (+)</td>
<td><em>Escherichia coli</em> E302 E303 RS3 15097_CERB</td>
<td>1.777</td>
<td>562</td>
</tr>
<tr>
<td>10 (+)</td>
<td><em>Escherichia fergusonii</em> DSM 16698_HAM</td>
<td>1.752</td>
<td>564</td>
</tr>
</tbody>
</table>

MALDI-TOF Laser irradiation

Red spectra: sample to be identified

Blue spectra: match from library

www.ProCE.com
**Timeline for Organism Identification with MALDI-TOF compared with Automated Testing**

- **Positive blood culture bottle**
- **Grain stain**
- **Incubation**
- **12-24h**
- **MALDI-TOF**
- **Automated Testing Set-Up**
- **12-24h**
- **MALDI-TOF**
- **Automated Susceptibilities**
- **12-48h**
- **Patient care team sees ID and susceptibility results**

---

**MALDI-TOF**

- **Advantages**
  - Reduced time to organism ID by 24-36 hours
  - Can replace conventional or automated systems for organism identification for most organisms
  - Very low reagent cost

- **Disadvantages**
  - Costly initial investment
  - Currently unable to detect resistance mechanisms
  - Minor discrepancies in organism identification at species level
    - Streptococci, Shigella, Propionibacterium
Time is of the Essence: Incorporating 2015 CLSI Updates and Rapid Diagnostics into Stewardship

**PNA-FISH™, Quick-FISH™, Xpress-FISH™**

**FISH (Fluorescent In Situ Hybridization)**

1. Genes
2. DNA probe (preparation)
3. Hybridization (73°C)
4. Derivation (on slides)
5. Hybridization (on slides)
6. Fixation (slides)
7. Immunostaining (reagents)
8. Fluorescent labeling
9. The gene is located

**PNA-FISH™, Quick-FISH™, Xpress-FISH™**

![Image of PNA-FISH, Quick-FISH, Xpress-FISH](image_url)
Time is of the Essence: Incorporating 2015 CLSI Updates and Rapid Diagnostics into Stewardship

PNA-FISH™, Quick-FISH™, Xpress-FISH™

- **Products:**
  - Staphylococcus (*S. aureus* vs. Coag-neg Staph)
  - Enterococcus (*E. faecalis* vs. *E. faecium* vs. other Enterococcus)
  - GNR (Pseudomonas, Klebsiella, *E. coli* vs. other GNR)
  - Candida (*C. albicans*, *C. glabrata* vs. *C. parapsilosis*)
  - MRSA (*MecA* probe)

Timeline for Organism Identification with FISH Technology

- **Testing process takes 20 min-2 hours**
PNA-FISH™, Quick-FISH™, Xpress-FISH™

- **Advantages**
  - Extremely quick testing process (Quick-FISH™)
  - Excellent clinical experience with the product
  - Proven clinical benefits

- **Disadvantages**
  - Current products are limited to three targets
  - Limited ability to detect resistance mechanisms
  - Moderately complex methodology for micro technicians

Nucleic Acid Amplification

- **Nanosphere Verigene™**
- **Biofire Filmarray™**
  - Multiplex pcr product for GNR, GPC and Yeast
### Nanosphere Verigene GNR™

<table>
<thead>
<tr>
<th>Organism</th>
<th>Resistance genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>CTX-M (ESBL)</td>
</tr>
<tr>
<td><em>K. oxytoca</em></td>
<td>IMP (Carbapenemase)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>KPC (Carbapenemase)</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>NDM (Carbapenemase)</td>
</tr>
<tr>
<td><em>S. marcenses</em></td>
<td>OXA (Carbapenemase)</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>VIM (Carbapenemase)</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td></td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td></td>
</tr>
<tr>
<td>Citrobacter spp.</td>
<td></td>
</tr>
</tbody>
</table>

### Nanosphere Verigene GPC™

<table>
<thead>
<tr>
<th>Organism</th>
<th>Resistance genes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staph. aureus</em></td>
<td>MecA (MRSA)</td>
</tr>
<tr>
<td><em>Staph. epidermidis</em></td>
<td></td>
</tr>
<tr>
<td><em>Staph. lugdunensis</em></td>
<td></td>
</tr>
<tr>
<td>Strep. anginosis group</td>
<td></td>
</tr>
<tr>
<td>Strep. agalactiae</td>
<td></td>
</tr>
<tr>
<td>Strep. pneumoniae</td>
<td></td>
</tr>
<tr>
<td>Strep. pyogenes</td>
<td>VanA, VanB, (VRE)</td>
</tr>
<tr>
<td><em>E. faecalis</em></td>
<td></td>
</tr>
<tr>
<td><em>E. faecium</em></td>
<td>VanA, VanB, (VRE)</td>
</tr>
</tbody>
</table>

Nanosphereus.com: Jan 2015
Time is of the Essence: Incorporating 2015 CLSI Updates and Rapid Diagnostics into Stewardship

Timeline for Organism Identification with Nucleic Acid Amplification Technology

- Testing process takes about 1-2 hours

Nucleic Acid Amplification (Verigene™)

- Advantages
  - Multiplex technology: organism ID and select resistance genes
  - Can escalate and de-escalate therapy for Staphylococcus and Enterococcus

- Disadvantages
  - GNR panel allow for escalation of therapy only
  - Product is an add-on, and doesn’t replace current technology for organism ID or susceptibility
  - Diminished sensitivity and specificity directly from specimen
Time is of the Essence: Incorporating 2015 CLSI Updates and Rapid Diagnostics into Stewardship

Nanoparticle-Magnetic Resonance Imaging


Timeline for Organism Identification with MRI compared with Automated Testing

- Testing process takes 3-5 hours

T2Biosystems.com: Jan 2015
Nanoparticle-Magnetic Resonance Imaging

- **Advantages**
  - Recently FDA-approved product identifies Candida
    - Sensitivity 91.1%, Specificity 99.4% for candida
    - Can detect candida at low concentration: down to 1 CFU/ml
  - Developing hand-held device
  - Developing test for bacteria
  - Developing test for genetic targets (resistance mechanisms)

- **Disadvantages**
  - Potential high ratio of tests per positive result for candida
  - Lack of data from clinical microbiology labs
  - Lack of clinical outcomes data

Next Generation Whole Genome Sequencing

- Likely the future of microbiology, but currently not routinely used in clinical microbiology labs
- Able to detect pathogens and resistance mechanisms directly from specimen
- Two major limitation:
  - Technology to efficiently and cost effectively run a large number of samples
  - Information overload: need for bioinformatics
Time is of the Essence: Incorporating 2015 CLSI Updates and Rapid Diagnostics into Stewardship

### When Does the Prescriber Review Results?

- **Positive blood culture bottle**
  - Incubation
  - 0h
- **Gram stain & biochemical tests**
  - Incubation
  - 12-24h
- **Automated Testing Set-Up**
  - MALDI-TOF
  - 12-24h
- **Automated Organism ID**
  - 12-48h
- **Automated Susceptibilities**
- **Patient care team sees ID and susceptibility results**

#### Targeted Organism ID & Detection of Genes Linked to Resistance:

- **Microarray, PCR, PNA-FISH, WGS, MRI**
- **Targeted Organism**
  - **ID**
  - **Detection of Genes Linked to Resistance**

### It’s NOT Enough to Simply Report Results

<table>
<thead>
<tr>
<th>Study</th>
<th>RDT/pathogen(s)</th>
<th>Study Design</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forrest, 2006</td>
<td>PNA-FISH Candida spp.</td>
<td>Pre/post-intervention: RDT + AST</td>
<td>ID of C. albicans 3 days earlier (9.5h vs 44h), ↓ antifungal costs by $1,978/patient</td>
</tr>
<tr>
<td>Forrest, 2008</td>
<td>PNA-FISH Enterococcus spp.</td>
<td>Pre/post-intervention: RDT + AST</td>
<td>↓ mortality (45% vs 35%), ↓ time to appropriate abx (1.3 vs 3.1 days)</td>
</tr>
<tr>
<td>Ly, 2008</td>
<td>PNA-FISH S. aureus vs GPCs</td>
<td>RDT and pre/post AST</td>
<td>↓ mortality (17% vs 8%), ↓ inappropriate abx use by 2.3 days, trend towards ↓ LOS and cost</td>
</tr>
<tr>
<td>Carver, 2008</td>
<td>RT-PCR mecA (MRSA)</td>
<td>mecA gene reporting and pre/post AST</td>
<td>↓ time to optimal abx (64.7h vs 39.9h), ↓ duration of S. aureus BSI</td>
</tr>
<tr>
<td>Wong, 2010</td>
<td>rPCR S. aureus</td>
<td>Pre/post intervention: RDT + AST</td>
<td>↓ LOS (21.5d vs 15.3d)</td>
</tr>
<tr>
<td>Perez, 2013</td>
<td>MALDI-TOF GNRs</td>
<td>Pre/post intervention: RDT + AST</td>
<td>↓ LOS (11.9d vs 9.3d), Trend towards ↓ mortality (10.7 vs 5.6%)</td>
</tr>
<tr>
<td>Huang, 2013</td>
<td>MALDI-TOF All Pathogens</td>
<td>Pre/post intervention: RDT + AST</td>
<td>↓ 30d mortality (20.3 vs 12.7%), ↓ LOS (21 vs 18.7)</td>
</tr>
</tbody>
</table>
Rapid Organism Identification plus Real-Time Stewardship Team Review & Intervention

- Implemented initiative to analyze differences in outcomes in patients with blood stream infections using rapid organism identification via MALDI-TOF with AST intervention compared to historical pre-intervention group
- AST member received real-time notification (page and email) of positive Gram stain, species identification and susceptibility data and provided antimicrobial recommendations to prescribers
- Pre-Intervention group: Sept 1 – Nov 30, 2011
  Intervention group: Sept 1 – Nov 30, 2012

Algorithm for Antibiotic Selection

Huang, CID 2013

Huang, CID 2013
Outcomes: 30-day All-cause Mortality

<table>
<thead>
<tr>
<th>Mortality</th>
<th>Pre-intervention</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.3%</td>
<td></td>
<td>12.7%</td>
</tr>
</tbody>
</table>

P = 0.021

Huang. CID 2013

Outcomes

<table>
<thead>
<tr>
<th>Therapy-Related Outcome</th>
<th>Pre-Interv (n=256)</th>
<th>Interv (n=245)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to Effective Therapy (hrs)</td>
<td>30.06</td>
<td>20.35</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time to Optimal Therapy (hrs)</td>
<td>90.34</td>
<td>47.25</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical Outcome</th>
<th>Pre-Interv (n=256)</th>
<th>Interv (n=245)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to clinical response (days)</td>
<td>3.97</td>
<td>2.5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time to microbiological cure (days)</td>
<td>3.32</td>
<td>3.27</td>
<td>0.928</td>
</tr>
<tr>
<td>Length of hospitalization (days)</td>
<td>21.03</td>
<td>16.73</td>
<td>&lt;0.054</td>
</tr>
<tr>
<td>Length of ICU stay (days)</td>
<td>16.58</td>
<td>9.15</td>
<td>0.012</td>
</tr>
<tr>
<td>Recurrence of same BSI (%)</td>
<td>15 (5.9)</td>
<td>5 (2.0)</td>
<td>0.038</td>
</tr>
<tr>
<td>30-day Readmission with same BSI (%)</td>
<td>9 (3.5)</td>
<td>4 (1.6)</td>
<td>0.262</td>
</tr>
</tbody>
</table>

Huang. CID 2013
Time is of the Essence: Incorporating 2015 CLSI Updates and Rapid Diagnostics into Stewardship

**Total Cost per Bacteremic Episode**

<table>
<thead>
<tr>
<th>Cost</th>
<th>Pre-Intervention</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$90,000</td>
<td>$19,253</td>
</tr>
<tr>
<td></td>
<td>$80,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$70,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$60,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$50,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$40,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$30,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$20,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$10,000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>$0</td>
<td></td>
</tr>
</tbody>
</table>

Total Cost Saving for 3-month Intervention Period: $4.8 million

Huang. CID 2013

**Reduction in Total Hospital Costs with Rapid Diagnostic Testing plus Real-time Culture Review**

<table>
<thead>
<tr>
<th>Cost Savings per Bacteremia Episode</th>
<th>Perez MALDI-TOF GNR</th>
<th>Wong PNA-FISH S. aureus</th>
<th>Huang MALDI-TOF GNR, GPC &amp; yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$19,583</td>
<td>$21,387</td>
<td>$19,253</td>
</tr>
<tr>
<td></td>
<td>$15,000</td>
<td>$17,000</td>
<td>$16,000</td>
</tr>
<tr>
<td></td>
<td>$10,000</td>
<td>$12,000</td>
<td>$11,000</td>
</tr>
<tr>
<td></td>
<td>$5,000</td>
<td>$7,000</td>
<td>$6,000</td>
</tr>
<tr>
<td></td>
<td>$0</td>
<td>$2,000</td>
<td>$1,000</td>
</tr>
</tbody>
</table>

Perez Wong Huang

MALDI-TOF GNR, GPC & yeast

76

ProCE, Inc.
www.ProCE.com

38
Implementation of Bacteremia Review
(without rapid diagnostic testing)

- Antimicrobial stewardship group received real-time notifications for gram-negative bacteremia M-F, 8am-5pm.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Stewardship intervention</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to appropriate therapy</td>
<td>14 hours</td>
<td>8 hours</td>
<td>0.014</td>
</tr>
<tr>
<td>Infection related mortality</td>
<td>14%</td>
<td>11%</td>
<td>0.42</td>
</tr>
<tr>
<td>Length of hospitalization</td>
<td>8 days</td>
<td>7 days</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Pogue J. ICHE, 2014

Conclusions

- Incorporating new molecular diagnostics can play an essential role in improving patient outcomes

- Implementing rapid molecular diagnostic testing should also involve a rapid communication process
  - Pharmacists can play a huge role in improving time to appropriate antibiotic therapy and clinical outcomes

- Optimizing patient outcomes often involves a multifactorial process, and collaboration with other healthcare professionals is essential
Time is of the Essence: Incorporating 2015 CLSI Updates and Rapid Diagnostics into Stewardship

To Receive Pharmacist CE Credit

- Return to the CE activity page and click the Post-Test/Evaluation link to connect to the ProCE CE Center
- Complete the Post-Test and Evaluation
- Score of ≥ 70% is required to receive credit
- Your CE credit will be sent to NABP/CPE Monitor

Click Here
To return to CE activity page

To Receive Laboratory PACE Credit

- Return to the CE activity page and click the link to email CLSI and request PACE credit for this activity.

Thank You!