MALDI-TOF MS: Translating Microbiology Laboratory Alphabet Soup to Optimize Antibiotic Therapy

September 8, 2017

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ASCENSION TEXAS

Learning Objectives
1. Describe conventional methods for processing blood cultures in the microbiology lab.
2. Identify advantages and disadvantages to organism identification (ID) with Matrix-Assisted Laser Desorption Ionization - Time of Flight Mass Spectrometry (MALDI-TOF MS).
3. Discuss strategies for implementation of MALDI-TOF MS in conjunction with an antimicrobial stewardship program (ASP).

Antimicrobial Stewardship (AMS)

Minimize consequences of antibiotics

Maximize patient outcomes

2016 IDSA Guidelines for Implementing an Antibiotic Stewardship Program

"We suggest rapid diagnostic testing in addition to conventional culture and routine reporting on blood specimens if combined with ASP support and interpretation." (weak recommendation, moderate-quality evidence)

"Availability of rapid diagnostic tests is expected to increase; thus, ASPs must develop processes and interventions to assist clinicians in interpreting and responding appropriately to results."

Conventional Processing of Microbiology Sample

Poll

The average time from blood culture collection to organism identification is:

a. 12 hours
b. 24 hours

c. 48-72 hours

IDSA = Infectious Diseases Society of America
ASP = antibiotic stewardship program
**Comparison of Testing Methods**

<table>
<thead>
<tr>
<th></th>
<th>Conventional</th>
<th>Rapid Diagnostic Testing (RDT)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Basis</strong></td>
<td>Phenotypic</td>
<td>Genotypic/Molecular</td>
</tr>
<tr>
<td><strong>Time to Organism ID</strong></td>
<td>48-72 hours</td>
<td>Varies*</td>
</tr>
<tr>
<td><strong>Advantages</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Gold standard</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Experience and familiarity with interpretation of results</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td></td>
<td></td>
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<tr>
<td>- Requires in vitro growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- May require multiple subcultures</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Highly dependent upon incubation period between culture positivity and testing.*

**Methods of Rapid Diagnostic Testing**

- **Peptide Nucleic Acid Fluorescent In Situ Hybridization (PNA FISH)**
- **Multiplex PCR**
- **Nanoparticle Probe Technology**
- **Matrix-Assisted Laser Desorption/ Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS)**

**MALDI-TOF MS Technology**

1. Sample culture
2. Matrix
3. MALDI-TOF/MS sample plate

**MALDI-TOF MS Processing of Microbiology Sample**

- Specimen Collection
- Incubation in Blood Culture Bottle
- Gram Stain
- Subculture for In Vitro Growth
- Organism Identification (ID)
- Antibiotic Susceptibility

**Summary of MALDI-TOF MS**

- **Sample Preparation**: Only FDA cleared for organism identification direct from colonies on plates.
  - Other strategies:
    - Direct from positive blood culture bottle
    - Early growth on plate (prior to distinct colony formation)
- **Turnaround time**: <1 hour
- **Interpretation**: Mass/charge ratio provides spectra (“fingerprint”) for the organism which is compared to a database of known spectra.
- **Susceptibility Testing**: No
Knowledge Checkpoint

Results from RDT using MALDI-TOF MS include antibiotic susceptibilities.

a. True
b. False

Does shorter time to organism identification translate to improved clinical outcomes in bacteremia?

Knowledge Checkpoint

Does shorter time to organism identification translate to improved clinical outcomes in bacteremia?

a. True
b. False

Definitions

**Time to Effective Therapy (TTET)**
- Time from blood culture draw to time of administration of the first antimicrobial with known susceptibility

**Time to Optimal Therapy (TTOT)**
- Time from blood culture draw to the time the patient received appropriate antimicrobial therapy
- Inclusive of de-escalation, antibiotic allergies or intolerances, discontinuation of antimicrobials if pathogen was a contaminant, and discontinuation of unnecessary antibiotic coverage


- Pre-post quasiexperimental study
- Single center
- Academic
- Adult patients with bacteremia
- MALDI-TOF MS with real-time AMS review from 6:00 am – 11:30 pm

Treatment-Related Outcomes

<table>
<thead>
<tr>
<th>Mean Hours to:</th>
<th>Pre-Intervention</th>
<th>Intervention</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram stain</td>
<td>30.1</td>
<td>32.5</td>
<td>0.621</td>
</tr>
<tr>
<td>Organism ID</td>
<td>84.0</td>
<td>55.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Susceptibilities</td>
<td>87.3</td>
<td>76.9</td>
<td>0.051</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Pre-Intervention (N=256)</th>
<th>Intervention (N=245)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean TTET (hours)</td>
<td>30.1</td>
<td>20.4</td>
<td>0.021</td>
</tr>
<tr>
<td>Mean TTOT (hours)</td>
<td>90.3</td>
<td>47.3</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
AMS Interventions by Type and Time Point

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Gram stain</th>
<th>Organism ID</th>
<th>Susceptibility</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Narrowed coverage</td>
<td>2</td>
<td>22</td>
<td>48</td>
<td>72 (34.3)</td>
</tr>
<tr>
<td>Discontinued therapy</td>
<td>5</td>
<td>44</td>
<td>19</td>
<td>68 (32.4)</td>
</tr>
<tr>
<td>Initiated or broadened therapy</td>
<td>39</td>
<td>5</td>
<td>9</td>
<td>53 (25.2)</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
<td>4</td>
<td>5</td>
<td>17 (8.1)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>54 (25.7)</td>
<td>75 (35.7)</td>
<td>81 (38.6)</td>
<td>210 (100)</td>
</tr>
</tbody>
</table>

Intervention Acceptance Rate = 90%

Secondary Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Pre-Intervention (N=256)</th>
<th>Intervention (N=245)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 day all-cause mortality, n (%)</td>
<td>52 (20.3)</td>
<td>31 (12.7)</td>
<td>0.021</td>
</tr>
<tr>
<td>Mean hospital length of stay (LOS) days</td>
<td>14.2</td>
<td>11.4</td>
<td>0.066</td>
</tr>
<tr>
<td>Mean intensive care unit (ICU) LOS (days)</td>
<td>14.9</td>
<td>8.3</td>
<td>0.014</td>
</tr>
<tr>
<td>Mean days to culture clearance</td>
<td>3.3</td>
<td>3.3</td>
<td>0.928</td>
</tr>
<tr>
<td>Recurrence of bacteremia, n (%)</td>
<td>15 (5.9)</td>
<td>5 (2.0)</td>
<td>0.038</td>
</tr>
<tr>
<td>30 day readmission with bacteremia, n (%)</td>
<td>9 (3.5)</td>
<td>4 (1.6)</td>
<td>0.262</td>
</tr>
</tbody>
</table>

Conclusions

- Majority (74%) of interventions to initiate or broaden therapy occurred in response to Gram stain.
- MALDI-TOF MS with AMS decreased both TTET and TTOT which lead to reductions in mortality, ICU LOS, and recurrence of bacteremia.


**Primary Outcome**

**Mean TTOT**

- Overall
- Gram Positive
- Gram Positive Contaminant
- Gram Negative

* p<0.05

**Secondary Outcomes**

- 30 day all-cause mortality
- Mean hospital length of stay (LOS)
- Mean intensive care unit (ICU) LOS (days)
- Mean days to culture clearance
- Recurrence of bacteremia
- 30 day readmission with bacteremia

**Conclusions**

- Majority (74%) of interventions to initiate or broaden therapy occurred in response to Gram stain.
- MALDI-TOF MS with AMS decreased both TTET and TTOT which lead to reductions in mortality, ICU LOS, and recurrence of bacteremia.
 AMS Interventions by Type

**Intervention Acceptance Rate = 88%**

- Narrowed Coverage (n=25): 15%
- Discontinued Therapy (n=24): 33%
- Initiated Therapy or Broadened Coverage (n=15): 20%
- Other (n=11): 32%

Secondary Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Pre-Intervention (N=113)</th>
<th>Intervention (N=83)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean TTET (hours)</td>
<td>16.8</td>
<td>12.15</td>
<td>0.082</td>
</tr>
<tr>
<td>In-hospital all-cause mortality, n (%)</td>
<td>12/116 (10.3)</td>
<td>15/123 (12.2)</td>
<td>0.805</td>
</tr>
<tr>
<td>Mean hospital LOS (days)</td>
<td>15.03</td>
<td>9.02</td>
<td>0.021</td>
</tr>
<tr>
<td>Mean ICU LOS (days)</td>
<td>4.30</td>
<td>1.22</td>
<td>0.053</td>
</tr>
<tr>
<td>Mean total length of antimicrobial therapy (days)</td>
<td>18.57</td>
<td>15.93</td>
<td>0.117</td>
</tr>
<tr>
<td>Mean hours to culture clearance</td>
<td>55.07</td>
<td>42.49</td>
<td>0.059</td>
</tr>
<tr>
<td>Recurrence of bacteremia, n (%)</td>
<td>4 (3.5)</td>
<td>1 (1.2)</td>
<td>0.255</td>
</tr>
<tr>
<td>Average direct costs</td>
<td>$28,677</td>
<td>$15,784</td>
<td>0.010</td>
</tr>
</tbody>
</table>

Conclusions

- MALDI-TOF MS with real-time AMS review reduced TTOT in Gram negative bacteremia and in Gram positive contaminants.
- The addition of AMS to MALDI-TOF MS decreased LOS and direct costs, but did not decrease overall mortality.

### Strengths

- Inclusion of adult and pediatric patients
- Significantly more Gram positive contaminants in the intervention group
- Designated primary outcome and power calculation
- Detailed institution guidelines for determination of contamination and treatment for bacteremia

### Limitations

- Significantly more Gram positive contaminants in the intervention group
- Communication and technology barriers between laboratories and pharmacy


- Pre-post quasiexperimental study
- Single center
- Qualaramy care, academic hospital (1000 beds)
- Adult patients with Gram negative bacteremia
- MALDI-TOF MS utilizing early positive blood cultures with real-time (24/7) review by AMS pharmacist

### Treatment-Related Outcomes

**Patients with Inactive Antibiotics**

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Culture Positivity</th>
<th>24 Hours</th>
<th>48 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Intervention (N=112)</td>
<td>19.6% (p=0.38)</td>
<td>15.5%</td>
<td>13.4% (p&lt;0.001)</td>
</tr>
<tr>
<td>Intervention (N=107)</td>
<td>19.6% (p=0.001)</td>
<td>4.7%</td>
<td>0.2%</td>
</tr>
</tbody>
</table>
Secondary Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Pre-Intervention (N=100)</th>
<th>Intervention (N=101)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average hours to therapy adjustment</td>
<td>75</td>
<td>29</td>
<td>0.004</td>
</tr>
<tr>
<td>30 day all-cause mortality, n (%)</td>
<td>6/107 (5.6)</td>
<td>12/112 (10.7)</td>
<td>0.19</td>
</tr>
<tr>
<td>Mean hospital LOS (days)</td>
<td>11.9</td>
<td>9.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean LOS after bacteremia (days)</td>
<td>3.9</td>
<td>8.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean ICU LOS (days)</td>
<td>7.3</td>
<td>6.3</td>
<td>0.005</td>
</tr>
<tr>
<td>Mean ICU LOS after bacteremia (days)</td>
<td>6.1</td>
<td>4.9</td>
<td>0.09</td>
</tr>
<tr>
<td>Mean total hospital costs</td>
<td>$45,709</td>
<td>$26,162</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Conclusions

- Analysis of early blood cultures with MALDI-TOF MS reduced time to organism ID and time to susceptibilities.
- MALDI-TOF MS with AMS decreased time to therapy adjustment, hospital LOS, and total costs in Gram negative bacteremia.

Strengths
- Utilized early blood cultures directly after Gram stain

Limitations
- Difficult to discern impact of MALDI-TOF MS and AMS independently
- No description of AMS program during pre-intervention period

Review

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Types of Bacteraemia</td>
<td>MALDI-TOF MS with real-time AMS vs. conventional methods with AMS</td>
<td>MALDI-TOF MS with real-time AMS vs. MALDI-TOF MS</td>
<td>MALDI-TOF MS using early positive blood cultures with AMS vs. conventional methods</td>
</tr>
<tr>
<td>Setting</td>
<td>Single center; academic</td>
<td>Single center; teaching facility</td>
<td>Single center; academic</td>
</tr>
</tbody>
</table>

Time to Result

- Huang, et al: 30.1 vs. 32.5, p=0.621
- Beganovic, et al: NR
- Perez, et al: 15.1 vs. 16.2, p=0.30

Organism ID

- Huang, et al: 30.3 vs. 35.9, p=0.001
- Beganovic, et al: NR
- Perez, et al: 36.6 vs. 11.0, p=0.001

Susceptibilities

- Huang, et al: 87.3 vs. 76.9, p=0.051
- Beganovic, et al: NR
- Perez, et al: 47.1 vs. 24.4, p=0.001

Clinical Outcomes

<table>
<thead>
<tr>
<th></th>
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</tr>
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<tbody>
<tr>
<td>TTOT</td>
<td></td>
<td>Time to therapy adjustment:</td>
</tr>
<tr>
<td>TTET</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mortality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LOS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICU LOS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrence</td>
<td></td>
<td>NR</td>
</tr>
<tr>
<td>Readmission</td>
<td></td>
<td>NR</td>
</tr>
<tr>
<td>Costs</td>
<td></td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&lt;</td>
</tr>
</tbody>
</table>

NR = not reported
Poll

Does shorter time to organism identification translate to improved clinical outcomes in bacteremia?

a. Yes
b. No
c. It depends

Factors Influencing Clinical Impact

<table>
<thead>
<tr>
<th>Institution</th>
<th>RDT</th>
</tr>
</thead>
</table>
| • Resistance rates  
• Prescribing patterns  
• Antimicrobial stewardship program  
• Patient population | • Technology chosen  
• Specimen processing  
• Central vs. on-site  
• Laboratory workflow  
• Batching vs. on-demand  
• Frequency and method for reporting results |

Implementation of RDT

Diagnosis & Treatment

Patient

Antimicrobial Stewardship
• Right interpretation  
• Right antimicrobial  
• Right time

Diagnostic Stewardship
• Right test  
• Right patient  
• Right time

RDT result

Microbiology Laboratory

RDT ordered

Overall Conclusions

• MALDI-TOF MS reduces time to organism ID by ≥1 day.

• Improvements in clinical outcomes with MALDI-TOF MS and AMS may lead to significant cost savings.

• RDT with AMS should be considered a best practice for management of patients with bacteremia.

• Additional research to optimize workflow and utilization of RDT is warranted.

Acknowledgements

Dusten Rose, Pharm.D., BCPS (AQ-ID), AAHIVP
Theresa Jaso, Pharm.D., BCPS (AQ-ID)
Kathryn Givens Merkel, Pharm.D., BCPS (AQ-ID), BCPPS

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Appendices

Appendix A: Abbreviations
Appendix B: Comparison of Testing Methods
Appendix C: MALDI-TOF MS Technology
Appendix D: MALDI-TOF MS Technology
Appendix E: Beganovic, et al. Primary Outcome
Appendix F: Beganovic, et al. AMS Interventions by Type
Appendix G: Perez, et al. Treatment-Related Outcomes
Appendix H: Time to Result
Appendix I: Clinical Outcomes
Appendix J: Implementation of RDT
Appendix A: Abbreviations

AMS: antimicrobial stewardship
ASP: antibiotic stewardship program
FDA: Food and Drug Administration
ICU: intensive care unit
IDSA: Infectious Diseases Society of America
LOS: length of stay
MALDI-TOF MS: matrix-assisted laser desorption ionization - time of flight mass spectrometry
NR: not reported
NS: not statistically significant
Organism ID: organism identification
RDT: rapid diagnostic testing
TTET: time to effective therapy
TTOT: time to optimal therapy
## Comparison of Testing Methods

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<tr>
<td><strong>Advantages</strong></td>
<td>Gold standard</td>
<td>Rapid turnaround time</td>
</tr>
<tr>
<td></td>
<td>Experience and familiarity with interpretation of results</td>
<td>Automated</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May not require subculture</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>Requires <em>in vitro</em> growth</td>
<td>Technically complex</td>
</tr>
<tr>
<td></td>
<td>May require multiple subcultures</td>
<td>Limited to validated organisms</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced accuracy in polymicrobial infections</td>
</tr>
<tr>
<td></td>
<td></td>
<td>May be limited to certain sample types</td>
</tr>
</tbody>
</table>

*Highly dependent upon incubation period between culture positivity and testing

---

MALDI-TOF MS Technology

1. Sample culture
2. Matrix
3. MALDI-TOF/MS sample plate

MALDI-TOF MS Technology

MS Detector (Mass to charge (m/z) ratio)

Neutral field (Drift)

Electrostatic field (Acceleration)

Laser

Protein and Matrix

MS Profile (match to database)
Primary Outcome

Mean TTOT

* p<0.05

AMS Interventions by Type

Intervention Acceptance Rate = 88%

- Narrowed Coverage (n=25) - 33%
- Discontinued Therapy (n=24) - 15%
- Initiated Therapy or Broadened Coverage (n=15) - 20%
- Other (n=11) - 20%

Treatment-Related Outcomes

Patients with Inactive Antibiotics

- Culture Positivity: 19.6% (p=0.38) for Pre-Intervention (N=112) and 19.6% (p=0.001) for Intervention (N=107).
- 24 Hours: 15.0% for Pre-Intervention and 4.7% for Intervention.
- 48 Hours: 13.4% (p<0.001) for Intervention.

**Time to Result**

**Culture Positivity/Gram Stain**
- Huang, et al: 30.1 vs. 32.5, \( p=0.621 \)
- Beganovic, et al: NR
- Perez, et al: 15.1 vs. 16.2, \( p=0.30 \)

**Susceptibilities**
- Huang, et al: 87.3 vs. 76.9, \( p=0.051 \)
- Beganovic, et al: NR
- Perez, et al: 47.1 vs. 24.4, \( p<0.001 \)

**Organism ID**
- Huang, et al: 84.0 vs. 55.9, \( p=0.001 \)
- Beganovic, et al: NR
- Perez, et al: 36.6 vs. 11.0, \( p<0.001 \)

**Time to Adjusted Therapy**
- Huang, et al: 90.3 vs. 47.3, \( p=0.021 \)
- Beganovic, et al: 75.17 vs. 43.06, \( p<0.001 \)
- Perez, et al: 75 vs. 29, \( p=0.004 \)
## Clinical Outcomes

<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>TTOT</td>
<td>![Down Arrow]</td>
<td>![Down Arrow]</td>
<td>Time to therapy adjustment: ![Down Arrow]</td>
</tr>
<tr>
<td>TTET</td>
<td>![Down Arrow]</td>
<td>![Left and Right Arrows]</td>
<td>![Left and Right Arrows]</td>
</tr>
<tr>
<td>Mortality</td>
<td>![Down Arrow]</td>
<td>![Left and Right Arrows]</td>
<td>![Left and Right Arrows]</td>
</tr>
<tr>
<td>LOS</td>
<td>![Left and Right Arrows]</td>
<td>![Down Arrow]</td>
<td>![Down Arrow]</td>
</tr>
<tr>
<td>ICU LOS</td>
<td>![Down Arrow]</td>
<td>![Left and Right Arrows]</td>
<td>![Left and Right Arrows]</td>
</tr>
<tr>
<td>Recurrence</td>
<td>![Down Arrow]</td>
<td>![Left and Right Arrows]</td>
<td>NR</td>
</tr>
<tr>
<td>Readmission</td>
<td>![Left and Right Arrows]</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Costs</td>
<td>NR</td>
<td>![Down Arrow]</td>
<td>![Down Arrow]</td>
</tr>
</tbody>
</table>

NR = not reported
Implementation of RDT

Antimicrobial Stewardship
- Right interpretation
- Right antimicrobial
- Right time

Diagnostic Stewardship
- Right test
- Right patient
- Right time

Patient

Microbiology Laboratory

Diagnosis & treatment

Clinical evaluation

RDT ordered

RDT result