Validation and Implementation of MALDI-TOF MS in a Public Health Laboratory

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MALDI-TOF MS: Goodbye Biochemicals, Hello Lasers
The WC Bacteriology Lab of 2012

- 6,000 isolates/year cultured
- Biochemical tests, strips, fatty acid analysis
- ~10,000 real-time PCR tests/year
- ~1,000 sequencing reactions/year
Timeline

- **May 2011**: ASM 2011 General Meeting
- **Aug.-Dec. 2011**: Sales Representatives visits, evaluation of technology
- **Feb. 2012**: Received first MALDI-TOF MS submissions for our NYS CLEP; Decision to evaluate Bruker Daltonics MALDI Biotyper
- **May 2012**: On site demo of instrument, validation of performance in numerous areas
- **Feb. 2013**: Validation application submitted to CLEP, approval granted for identification of bacterial species at the Wadsworth Center
How does MALDI-TOF MS work?

- Individual colony from an overnight culture is smeared (spotted) onto a target plate

*Extended direct smear*
MALDI-TOF MS

Matrix-assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectrometry

Pulsed Laser irradiation of the sample

Flight tube

Sample ionization forms charged ions

Detector

Matrix desorption/evaporation

Protein ions of different masses
MALD-TOF MS in action
MALDI-TOF MS

Protein Profile

The result is a mass-spectrum of mainly ribosomal proteins that are species-specific for a large number of microorganisms. These spectra are compared to a reference database.
Bruker Daltonik MALDI Biotyper Classification Results

Project Info:
Project Name: MD013013
Project Description: Administrator@FLEX-PC
Project Owner: 2013-01-30T11:00:44.187
Project Creation Date/Time: Development
Project Analyte Count: not present
Project Type: Validation
Analysis Position: Validation Position:

Result Overview:

<table>
<thead>
<tr>
<th>AnalyteName</th>
<th>AnalyteID</th>
<th>Organism(best match)</th>
<th>ScoreValue</th>
<th>Organism(second best match)</th>
<th>ScoreValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1 (-) (C)</td>
<td>BTS</td>
<td>no peaks found</td>
<td>0.0</td>
<td>no peaks found</td>
<td>0.0</td>
</tr>
<tr>
<td>A2 (+++) (A)</td>
<td>IDR13-2899</td>
<td>Enterococcus faecalis</td>
<td>2.382</td>
<td>Enterococcus faecalis</td>
<td>2.382</td>
</tr>
<tr>
<td>A3 (+++) (A)</td>
<td>IDR13-2899</td>
<td>Enterococcus faecalis</td>
<td>3.406</td>
<td>Enterococcus faecalis</td>
<td>2.213</td>
</tr>
<tr>
<td>A4 (+++) (A)</td>
<td>IDR13-2899</td>
<td>Enterococcus faecalis</td>
<td>2.114</td>
<td>Enterococcus faecalis</td>
<td>1.422</td>
</tr>
<tr>
<td>A5 (+++) (A)</td>
<td>IDR13-2899</td>
<td>Enterococcus faecalis</td>
<td>3.123</td>
<td>Enterococcus faecalis</td>
<td>2.897</td>
</tr>
</tbody>
</table>

Matching Hints:

Pseudomonas mucoides LMG 22237 HAM
is a member of Pseudomonas fluorescens group

Meaning of Score Values:

<table>
<thead>
<tr>
<th>Range</th>
<th>Description</th>
<th>Symbols</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.300 .. 3.000</td>
<td>highly probable species identification</td>
<td>(+++)</td>
<td>green</td>
</tr>
<tr>
<td>2.000 .. 2.299</td>
<td>secure genus identification, probable species identification</td>
<td>(+)</td>
<td>green</td>
</tr>
<tr>
<td>1.700 .. 1.999</td>
<td>probable genus identification</td>
<td>(+)</td>
<td>yellow</td>
</tr>
<tr>
<td>0.000 .. 1.699</td>
<td>not reliable identification</td>
<td>(-)</td>
<td>red</td>
</tr>
</tbody>
</table>

Meaning of Consistency Categories (A - C):

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Species Consistency: The best match was classified as 'green' (see above). Further 'green' matches are of the same species as the first one. Further 'yellow' matches are at least of the same genus as the first one.</td>
</tr>
<tr>
<td>B</td>
<td>Genus Consistency: The best match was classified as 'green' or 'yellow' (see above). Further 'green' or 'yellow' matches have at least the same genus as the first one. The conditions of species consistency are not fulfilled.</td>
</tr>
<tr>
<td>C</td>
<td>No Consistency: Neither species nor genus consistency (Please check for synonyms of names or microbial mixture).</td>
</tr>
</tbody>
</table>
Is it that easy?
sometimes...
But there is a back-up plan
Testing Algorithm

Isolate sub-cultured for purity and fresh growth

Extended Direct Smear

Score ≥2.0
Report result*

Score <2.0
Reflex to FA-ACN Extraction

Reflex to FA-ACN Extraction

Score ≥2.0
Report result*

Score <2.0
Reflex to 16S sequencing analysis

Suspect aerobic actinomycete or non-tuberculous mycobacteria

Modified FA-ACN Extraction

Score ≥2.0
Report result**

Score <2.0
Reflex to rpoB or 16S sequencing when appropriate, biochemical workup
Three extraction methods routinely run according to a testing algorithm:

1. Extended direct smear (direct smear with 70% Formic Acid overlay)

2. Formic Acid-Acetonitrile Extraction (FA-ACN)

3. Modified FA-ACN with beads
### Reporting Algorithm

<table>
<thead>
<tr>
<th>Score Value</th>
<th>Consistency Category</th>
<th>Extraction Method</th>
<th>Next closest species match</th>
<th>Result/Report/Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥2.2</td>
<td>A</td>
<td>Extended Direct Smear or Formic Acid/Acetonitrile Extraction</td>
<td>Not applicable</td>
<td>Report ID of top match</td>
</tr>
<tr>
<td>≥2.0 and &lt;2.2</td>
<td>A</td>
<td>Extended Direct Smear or Formic Acid/Acetonitrile Extraction</td>
<td>&gt;10% lower than top score</td>
<td>Report ID of top match</td>
</tr>
<tr>
<td>≥2.0 and &lt;2.2</td>
<td>A</td>
<td>Extended Direct Smear or Formic Acid/Acetonitrile Extraction</td>
<td>Within 10% of top score</td>
<td>Report as Genus. Additional testing may be required</td>
</tr>
<tr>
<td>≥2.0</td>
<td>B or C</td>
<td>Extended Direct Smear or Formic Acid/Acetonitrile Extraction</td>
<td>&gt;10% lower than top score</td>
<td>Report ID of top match</td>
</tr>
<tr>
<td>≥2.0</td>
<td>B or C</td>
<td>Extended Direct Smear or Formic Acid/Acetonitrile Extraction</td>
<td>Within 10% of top score</td>
<td>Report as Genus. Additional testing may be required</td>
</tr>
<tr>
<td>&lt;2.0</td>
<td>B or C</td>
<td>Extended Direct Smear</td>
<td>Not applicable</td>
<td>Repeat using Formic Acid/Acetonitrile Extraction method.</td>
</tr>
<tr>
<td>≥1.9 and &lt;2.0</td>
<td>B or C</td>
<td>Formic Acid/Acetonitrile Extraction</td>
<td>&gt;10% lower than top score</td>
<td>Report as Genus. Include note: “Most closely resembles Genus species” of top match.</td>
</tr>
<tr>
<td>≥1.9 and &lt;2.0</td>
<td>B or C</td>
<td>Formic Acid/Acetonitrile Extraction</td>
<td>Within 10% of top score</td>
<td>Report as Genus. Additional testing may be required</td>
</tr>
<tr>
<td>≥1.7 and &lt;1.9</td>
<td>B or C</td>
<td>Formic Acid/Acetonitrile Extraction</td>
<td>Not applicable</td>
<td>Report as Genus.</td>
</tr>
<tr>
<td>&lt;1.7</td>
<td>C</td>
<td>Formic Acid/Acetonitrile Extraction</td>
<td>Not applicable</td>
<td>Additional testing is required. Reflex to appropriate lab as determined by specimen source and</td>
</tr>
</tbody>
</table>

Sometimes it is that easy, 10 min of prep and 10 min run.
Our Plan

- **Step 1- Evaluation (4 months)**
  - Isolates sent for testing
  - Age of culture
  - Preliminary testing

- **Step 2- Validation (6months)**
  - 16S
  - General Bacteriology
  - Anaerobic Bacteriology
  - Enteric Bacteriology

- **Step 3- Implementation (11 months)**
Overview of Implementation of MALDI-TOF MS

In the 11 months since implementation:

- >1700 clinical isolates from >1400 unique specimens
- This testing has resulted in the identification of >100 genera comprising >200 species.
### Reportable score (>2.0) by Extraction Method

<table>
<thead>
<tr>
<th>Extended Direct Smear</th>
<th>FA-ACN Extraction</th>
<th>No Reliable Identification</th>
</tr>
</thead>
<tbody>
<tr>
<td>76%</td>
<td>15%</td>
<td>9%</td>
</tr>
</tbody>
</table>

### Testing Required for Final Identification

<table>
<thead>
<tr>
<th>MALDI-TOF MS only</th>
<th>MALDI-TOF MS + Conventional Biochemical Analysis</th>
<th>MALDI-TOF MS + 16S rDNA sequence analysis</th>
<th>MALDI-TOF MS + Conventional Biochemical Analysis + 16S rDNA sequence analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>37%</td>
<td>1%</td>
<td>12%</td>
</tr>
</tbody>
</table>
NTM testing

<table>
<thead>
<tr>
<th>Final ID by <em>rpoB/16S</em> sequencing</th>
<th>Total isolates received</th>
<th>Reportable with score ≥ 2.0</th>
<th>Score ≥ 1.8 and &lt; 2.0</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. gordonae</em></td>
<td>24</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td><em>M. abscessus</em></td>
<td>11</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td><em>M. fortuitum</em></td>
<td>16</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td><em>M. chelonae</em></td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td><em>M. xenopi</em></td>
<td>9</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><em>M. marinum</em></td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>M. mageritense</em></td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>M. gastri</em></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>M. neoaurum</em></td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>M. paragordonae</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. peregrinum</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. salmonophilum</em>#</td>
<td>1</td>
<td>0</td>
<td>1#</td>
</tr>
<tr>
<td><em>M. septicum</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. triviale</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. arupense</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. parafinicum</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. terrae complex</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>M. phocaicum</em></td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td><em>M. porcinum</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Mycobacteria spp.</em></td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>total</strong></td>
<td><strong>89</strong></td>
<td><strong>39 (44%)</strong></td>
<td><strong>14 (16%)</strong>**</td>
</tr>
</tbody>
</table>
Results

• MALDI-TOF MS implementation has resulted in a significant in testing volume
  • 46 % reduction in the # of biochemical tests needed
  • 52 % reduction in 16S rDNA sequence analysis
  • 72 % reduction in Campylobacter species real-time PCR

• TAT to final identification has 13.2 net work days pre-implementation to 8.2 net work days post-implementation, a time savings of 5 full days on average

• Average TAT by genus also for nearly every genus analyzed
  • 2-4 days for Campylobacter, Clostridium, Corynebacterium, and Enterococcus
  • 6-9 days for Actinomyces, Burkholderia, Nocardia and Pseudomonas
  • 18-24 days for Achromobacter, Moraxella and Staphylococcus
  • 32-37 days for Acinetobacter, Enterobacter and Streptococcus (excluding S. pneumoniae, S. pyogenes and S. agalactiae)
Decrease in 16S and Real-time PCR testing volume due to implementation of MALDI-TOF MS
Decrease in biochemical testing volume due to implementation of MALDI-TOF MS
Decrease in TAT by month as a result of implementation of MALDI-TOF MS
The average TAT remained the same or decreased, by as much as 36 days.

* The average TAT remained the same or decreased, by as much as 36 days.
**Estimated Cost Savings Analysis Resulting from MALDI-TOF MS Implementation**

<table>
<thead>
<tr>
<th>Test</th>
<th>Cost of test (reagents only)</th>
<th>Estimated staff time needed to perform test</th>
<th>Hourly rate including benefits</th>
<th>Estimated cost in staff time per test</th>
<th>Total cost per test (staff and reagents)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rDNA Sequence Analysis</td>
<td>$12.00</td>
<td>3 hours</td>
<td>$73</td>
<td>$219.00</td>
<td>$231.00</td>
</tr>
<tr>
<td>Campylobacter Real-time PCR*</td>
<td>$5.00</td>
<td>0.5 hours</td>
<td>$73</td>
<td>$36.50</td>
<td>$41.50</td>
</tr>
<tr>
<td>Individual Biochemical Test</td>
<td>$1.00</td>
<td>0.15 hours</td>
<td>$73</td>
<td>$10.95</td>
<td>$11.95**</td>
</tr>
<tr>
<td>MALDI-TOF MS</td>
<td>$0.50</td>
<td>0.5 hours</td>
<td>$73</td>
<td>$36.50</td>
<td>$37.00</td>
</tr>
</tbody>
</table>

**Total cost per test calculation of four unique tests performed by the WC.**
The table above calculates the total cost per test including reagent cost and staff time of 4 tests performed for bacterial identification.

*This real-time PCR test represents one of many that are impacted by this implementation.

**This represents one biochemical test not a panel of tests utilized.
## Total Savings

<table>
<thead>
<tr>
<th>Test</th>
<th>Total cost per test (staff and reagents)</th>
<th>Pre-implementation</th>
<th>Post-implementation</th>
<th>Predicted cost savings per month</th>
<th>Estimated cost savings since implementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rDNA Sequence Analysis</td>
<td>$231.00</td>
<td>66</td>
<td>32</td>
<td>$7,392.00</td>
<td>$7,854.00</td>
</tr>
<tr>
<td>Campylobacter Real-time PCR</td>
<td>$41.50</td>
<td>47</td>
<td>13</td>
<td>$539.50</td>
<td>$1,411.00</td>
</tr>
<tr>
<td>Individual Biochemical Test</td>
<td>$11.95</td>
<td>1649</td>
<td>895</td>
<td>$10,695.25</td>
<td>$9,010.30</td>
</tr>
<tr>
<td>MALDI-TOF MS</td>
<td>$37.00</td>
<td>0</td>
<td>140</td>
<td>$5,180.00</td>
<td>-$5,180.00</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>$36,902.05</strong></td>
<td><strong>$13,095.30</strong></td>
<td><strong>$23,806.75</strong></td>
<td><strong>$183,334.20</strong></td>
<td></td>
</tr>
</tbody>
</table>
Considerations

- Importing to in-house LIMs system
- Many algorithms
  - Deciding where to implement
  - Reflex testing
  - Decision making
  - Requisition test request
- Determining which staff will run, interpret, review results
- Gram-negative rods- rule out *Brucella* spp.
- Controls, SOP cleaning plates, remote laser tuning
Future applications

- Upcoming projects involving:
  - Assessment specimen viability
  - Other bacterial genus not examined in evaluation
  - Typing, species differentiation
  - Antibiotic resistance detection
  - Identification from clinical specimens (blood culture, urine, CSF)
Conclusions

- MALDI-TOF MS is a fast, reliable, inexpensive technique for bacterial identification in our laboratory.
- Extended direct smear is adequate for most bacterial isolates.
- FA-ACN extraction is a faster, more cost effective alternative to 16S rDNA sequence analysis and biochemical analysis for a subset of isolates.
- Implementation of MALDI-TOF MS has decreased workload, TAT and costs for WC.
- We estimate at least 22 days in staff time is saved each month.
- It is estimated that use of MALDI-TOF MS has resulted in over $180,000.00 in reagent and staff savings since February 2013.
- We predict that we can save nearly $13,000 a month in reagents and staff time with MALDI-TOF MS in our testing algorithm.
The WC Bacteriology Lab of Today

- 6,000 isolates/year sub-cultured
- MALDI-TOF MS
- ½ amt. Biochemical tests
- ~4000 real-time PCR tests/year
- ~350 sequencing reactions/year
Acknowledgements

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  - Colleen Walsh

- Applied Genomic Technologies Core

- Media and Tissue Culture Core

- Evaluation Committee
  - Ron Limberger
  - Kimberlee Musser
  - Christina Egan
  - Lisa Mingle
  - Elizabeth Nazarian
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  - Mike Perry
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