MALDI-TOF MS Evaluation

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MalDI-TOF MS

- November 2011

- Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS) is a fast and reliable method for the classification and identification of microorganisms. The BioTyper™ MALDI-TOF MS system (Bruker) would allow rapid identification of bacteria, yeasts and fungi.
Aim of the Evaluation

- To introduce the MALDI-TOF as a means of routine organism identification from clinical specimens

- Detailed evaluation performed to ensure accurate and reproducible results were obtained.
Scope of the evaluation

- Precision and reproducibility study (inter-laboratory and intra-laboratory)
  - Comparison of type of extraction/specimen preparation
  - Comparison between reusable versus disposable targets
  - Effect of media/incubation time and temperature

- Comparison of MALDI organism identification with results from current laboratory identification methods and known identification of ATCC and QAP strains
Acceptance Criteria

- Correct species identification > 2.0 ✓
- Correct genus identification > 1.7 ✓
- Wrong species or genus identification is unacceptable ✗
Timeline

November 2011
Delivery, installation and training

December 2011
Precision and reproducibility study & QC/QAP isolates

January to March 2012
Parallel testing and direct blood culture testing
Precision and reproducibility study

- 20 replicates each:
  - E.coli (ATCC 25922)
  - S.aureus (ATCC 29213)
  - C.albicans (ATCC 14053)

- With/without 1ul 70% formic acid
- Reusable versus disposable targets (St Vincent’s Pathology)
## E.coli precision & reproducibility results

<table>
<thead>
<tr>
<th>Organism</th>
<th>Formic acid</th>
<th>Target type</th>
<th>CV % Scientist 1</th>
<th>CV % Scientist 2</th>
<th>CV % Scientist 3</th>
<th>Total no. of replicates</th>
<th>No. with no peaks</th>
<th>CV % combined</th>
<th>Correct ID to species</th>
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<tbody>
<tr>
<td>E.coli ATCC 25922</td>
<td>No</td>
<td>R</td>
<td>2.84</td>
<td>2.90</td>
<td>1.91</td>
<td>60</td>
<td>0</td>
<td>2.62</td>
<td>100%</td>
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<tr>
<td>E.coli ATCC 25922</td>
<td>Yes</td>
<td>R</td>
<td>1.43</td>
<td>2.27</td>
<td>1.64</td>
<td>60</td>
<td>2</td>
<td>2.09</td>
<td>97%</td>
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<tr>
<td>E.coli ATCC 25922</td>
<td>No</td>
<td>D</td>
<td>3.03</td>
<td>2.58</td>
<td>2.27</td>
<td>60</td>
<td>1</td>
<td>2.73</td>
<td>98%</td>
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<tr>
<td>E.coli ATCC 25922</td>
<td>Yes</td>
<td>D</td>
<td>5.00</td>
<td>2.29</td>
<td>2.61</td>
<td>60</td>
<td>3</td>
<td>4.09</td>
<td>95%</td>
</tr>
<tr>
<td>BTS</td>
<td>No</td>
<td>R</td>
<td>1.50</td>
<td>2.32</td>
<td>1.11</td>
<td>60</td>
<td>1</td>
<td>2.74%</td>
<td>98%</td>
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### S. aureus precision & reproducibility results

<table>
<thead>
<tr>
<th>Organism</th>
<th>Formic acid</th>
<th>Target type</th>
<th>CV % Sci 1</th>
<th>CV % Sci 2</th>
<th>CV % Sci 3</th>
<th>Total no. replicates</th>
<th>No. with no peaks</th>
<th>CV % combined</th>
<th>Correct ID to species</th>
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<tbody>
<tr>
<td>S. aureus ATCC 29213</td>
<td>No</td>
<td>R</td>
<td>4.23</td>
<td>9.86</td>
<td>1.99</td>
<td>60</td>
<td>1</td>
<td>6.42</td>
<td>98%</td>
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<tr>
<td>S. aureus ATCC 29213</td>
<td>Yes</td>
<td>R</td>
<td>2.25</td>
<td>1.89</td>
<td>1.62</td>
<td>60</td>
<td>0</td>
<td>1.93</td>
<td>100%</td>
</tr>
<tr>
<td>S. aureus ATCC 29213</td>
<td>No</td>
<td>D</td>
<td>5.39</td>
<td></td>
<td></td>
<td>20</td>
<td>0</td>
<td>5.39</td>
<td>100%</td>
</tr>
<tr>
<td>S. aureus ATCC 29213</td>
<td>Yes</td>
<td>D</td>
<td>2.93</td>
<td></td>
<td></td>
<td>20</td>
<td>0</td>
<td>2.93</td>
<td>100%</td>
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C. albicans precision & reproducibility results

<table>
<thead>
<tr>
<th>Organism</th>
<th>Formic acid</th>
<th>Target type</th>
<th>CV % Sci1</th>
<th>CV % Sci 2</th>
<th>CV % Sci 3</th>
<th>Total no. replicates</th>
<th>No. with no peaks</th>
<th>CV % Combined</th>
<th>Correct ID to species</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans ATCC 14053</td>
<td>No</td>
<td>R</td>
<td>8.37</td>
<td>7.04</td>
<td>6.45</td>
<td>60</td>
<td>17</td>
<td>9.94</td>
<td>3%</td>
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<tr>
<td>C. albicans ATCC 14053</td>
<td>Yes</td>
<td>R</td>
<td>3.20</td>
<td>2.26</td>
<td>3.47</td>
<td>60</td>
<td>1</td>
<td>3.80</td>
<td>75%</td>
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<tr>
<td>C. albicans ATCC 14053</td>
<td>No</td>
<td>D</td>
<td>12.8</td>
<td>7.04</td>
<td></td>
<td>40</td>
<td>22</td>
<td>9.34</td>
<td>5%</td>
</tr>
<tr>
<td>C. albicans ATCC 14053</td>
<td>Yes</td>
<td>D</td>
<td>3.56</td>
<td>2.26</td>
<td></td>
<td>40</td>
<td>4</td>
<td>3.45</td>
<td>65%</td>
</tr>
</tbody>
</table>
Inter-laboratory Reproducibility

- E. coli, S. aureus and C. albicans: similar results
- C. albicans gave 100% identification to species with full extraction
- E. faecalis (ATCC 5199) & Ps. aeruginosa (ATCC 27853)
Findings

- Reusable targets ✅
- Formic acid ✅
Testing of cultures

- Testing of isolates of known identification
- Parallel testing of clinical isolates (common pathogens)
- Parallel testing of clinical isolates (less common, not tested in Vitek 2)
Testing of isolates of known identification- ATCC strains

- Aeromonas hydrophila (ATCC 35654)  
- Alcaligenes faecalis (ATCC 35655)  
- Beta-haemolytic Strep Grp B (ATCC 12386)  
- Bacillus cereus (ATCC 11778)  
- Bacillus subtilis (ATCC 11774)  
- Bacteroides fragilis (ATCC 25285)  
- Bacteroides ovatum (ATCC BAA-1296)  
- Campylobacter jejuni (ATCC 33291)  
- Capnocytophaga sputigena (ATCC 33612)  
- Clostridium difficile (ATCC 43593)  
- Clostridium perfringens (ATCC 13124)  
- Corynebacterium renale (ATCC 19412)  
- Corynebacterium striatum (ATCC BAA-1293)  
- Escherichia coli (ATCC 35218)  
- Elkenella corroderns (ATCC BAA-1152)  
- Haemophilus influenzae (ATCC 10211)  
- Klebsiella oxytoca (ATCC 700324)  
- Kocuria rosea (ATCC 00186)  
- Enterococcus faecalis VRE (ATCC 51299)  
- Enterococcus faecium VRE (ATCC 700221)  
- Neisseria gonorrhoeae (ATCC 49226)  
- P anaerobius (ATCC 27337)  
- Propionibacterium acnes (ATCC 118827)  
- Proteus mirabilis (ATCC 12453)  
- Pseudomonas aeruginosa (ATCC 27853)  
- Salmonella typhimurium (ATCC 14028)  
- Shigella flexneri (ATCC 12022)  
- Staphylococcus aureus (ATCC 29213)  
- E casseliflavus (ATCC 700327)  
- Yersinia enterocolitica (ATCC 9610)
Testing of isolates of known identification - QAP isolates

- Staphyloccus saprophyticus
- Streptococcus pyogenes
- Eikenella corroden
d- Pleismomonas shigelloides
- Shigella boydii
- Bordetella bronchiseptica
- Neisseria gonorrhoeae
- Elizabethkingia meningoseptica
- Listeria monocytogenes
- Bacteroides fragilis group
- Proteus penneri
- Aeromonas hydrophila
- Yersinia enterocolitica
- Campylobacter coli
- Acinetobacter baumannii
- Candida albicans
- Leuconostoc lactis
- Klebsiella pneumoniae
- Serratia marcescens
- Streptococcus anginosus
- Stenotrophomonas maltophilia
- Prevotella intermedia
- Staphyloccus lugdunensis
- Rothia aeria
- Streptococcus pyogenes
- Moraxella catarrhalis
- Haemophilus influenzae
- Edwardsiella tarda
- Clostridium ramosum
- Arcanobacterium haemolyticum
- Streptococcus Group G
- Escherichia coli
<table>
<thead>
<tr>
<th>Organism</th>
<th>Source</th>
<th>MALDI ID direct colony</th>
<th>Repeat MALDI ID direct colony</th>
<th>Full extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arcanobacterium haemolyticum</td>
<td>QAP 2009</td>
<td>Correct to genus (1.972)</td>
<td>Correct to species</td>
<td></td>
</tr>
<tr>
<td>Leuconostoc lactis</td>
<td>QAP 2009</td>
<td>No peaks found</td>
<td>Correct to species</td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>QAP 2010</td>
<td>No peaks found</td>
<td>Correct to species</td>
<td></td>
</tr>
<tr>
<td>Actinomyces turicensis</td>
<td>QAP 2009</td>
<td>No reliable identification</td>
<td>No peaks found</td>
<td>Correct to species</td>
</tr>
<tr>
<td>Mycobacterium fortuitum</td>
<td>QAP 2010</td>
<td>No peaks found</td>
<td>No peaks found</td>
<td>Correct to species (1.935)</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>QAP 2009</td>
<td>No peaks found</td>
<td>No peaks found</td>
<td>Correct to species</td>
</tr>
<tr>
<td>Tsukamurella inchnonensis</td>
<td>QAP 2009</td>
<td>No peaks found</td>
<td>Arthrobacter castelli (1.999)</td>
<td>Correct to genus</td>
</tr>
<tr>
<td>Shigella boydii</td>
<td>QAP 2009</td>
<td>Identified as E.coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shigella boydii</td>
<td>QAP 2010</td>
<td>Identified as E.coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nocardia asteroides</td>
<td>QAP 2010</td>
<td>No reliable identification</td>
<td>No peaks found</td>
<td>No reliable identification</td>
</tr>
<tr>
<td>Mycobacterium chelonae</td>
<td>QAP 2009</td>
<td>No reliable identification</td>
<td>No reliable identification</td>
<td>No reliable identification</td>
</tr>
<tr>
<td>Peptontophilus asaccharolyticus</td>
<td>QAP 2010</td>
<td>No peaks found</td>
<td>No reliable identification</td>
<td>No reliable identification</td>
</tr>
<tr>
<td>Roseomonas gilardii</td>
<td>QAP 2010</td>
<td>No reliable identification</td>
<td>No peaks found</td>
<td>No reliable identification</td>
</tr>
<tr>
<td>Haemophilus ducreyi</td>
<td>QAP 2009</td>
<td>No reliable identification</td>
<td>Not performed</td>
<td>Not performed</td>
</tr>
</tbody>
</table>
## Fungal isolates

- **ATCC and QAP isolates**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Source</th>
<th>MALDI-TOF ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. tropicalis</td>
<td>ATCC 201380</td>
<td>Correct to species</td>
</tr>
<tr>
<td>C. albicans</td>
<td>ATCC 60193</td>
<td>Correct to species</td>
</tr>
<tr>
<td>C. neoformans</td>
<td>ATCC 32045</td>
<td>No reliable id</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>ATCC 22109</td>
<td>Correct to species</td>
</tr>
<tr>
<td>C. krusei</td>
<td>ATCC 6258</td>
<td>Correct to species</td>
</tr>
<tr>
<td>Trichosporon mucoides</td>
<td>ATCC 204094</td>
<td>Correct to species</td>
</tr>
<tr>
<td>C. albicans</td>
<td>ATCC 10231</td>
<td>Correct to species</td>
</tr>
<tr>
<td>C. albicans</td>
<td>ATCC 14053</td>
<td>Correct to species</td>
</tr>
</tbody>
</table>
Parallel testing of common clinical isolates

112 Gram-positive isolates

- Species identification without formic acid: 89.3%
- Species identification with formic acid: 97.3%
Parallel testing of common clinical isolates

128 Gram-negative isolates

Species identification without formic acid: 97.66%

Species identification with formic acid: 95.31%
Parallel testing of less common clinical isolates

- Campylobacter species
- Salmonella species
- Neisseria gonorrhoeae
- Corynebacterium species
- Anaerobes
- Beta-haemolytic Streptococci (A,B,G)
- Viridans Streptococci
- Haemophilus influenzae
- Moraxella catarrhalis
- Enteric pathogens
### Neisseria gonorrhoeae

<table>
<thead>
<tr>
<th></th>
<th>Vitek NH</th>
<th>Phadebact</th>
<th>Maldi ex Choc 24hrs</th>
<th>Maldi ex choc 48hrs</th>
<th>Maldi full extraction</th>
<th>PCR</th>
<th>MDU</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Not done</td>
<td>Positive</td>
<td>No peaks</td>
<td>2.282A</td>
<td>2.356A</td>
<td>Positive</td>
<td>Confirmed</td>
</tr>
<tr>
<td>2</td>
<td>98% N. cinerea</td>
<td>Positive</td>
<td>No peaks</td>
<td>2.248B</td>
<td>2.366A</td>
<td>Positive</td>
<td>Confirmed</td>
</tr>
<tr>
<td>3</td>
<td>93% N. cinerea</td>
<td>Positive</td>
<td>No peaks</td>
<td>2.16B</td>
<td>2.231A</td>
<td>Positive</td>
<td>Confirmed</td>
</tr>
<tr>
<td>4</td>
<td>99% N. gonorrhoeae</td>
<td>Positive</td>
<td>No peaks</td>
<td>2.322A</td>
<td>2.392A</td>
<td>Positive</td>
<td>Confirmed</td>
</tr>
<tr>
<td>5</td>
<td>97% N. gonorrhoeae</td>
<td>Positive</td>
<td>No peaks</td>
<td>2.179B</td>
<td>2.404A</td>
<td>Positive</td>
<td>Confirmed</td>
</tr>
<tr>
<td>6</td>
<td>Low discrimination</td>
<td>Positive</td>
<td>No peaks</td>
<td>2.271A</td>
<td>2.362A</td>
<td>Positive</td>
<td>Confirmed</td>
</tr>
<tr>
<td>7</td>
<td>93% N gonorrhoeae</td>
<td>Not done</td>
<td>No peaks</td>
<td>2.265A</td>
<td>2.437A</td>
<td>Positive</td>
<td>Confirmed</td>
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<td>8</td>
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<td>No peaks</td>
<td>2.187B</td>
<td>2.284B</td>
<td>Not done</td>
<td>Confirmed</td>
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</tbody>
</table>
Corynebacterium species

Identification

- species: 67%
- genus id: 21%
- none: 12%
Results and Discussion

- Highly reproducible inter-laboratory and intra-laboratory results
- Better performance when 70% formic acid is used in the direct colony method
- Excellent correlation with conventional tests and Vitek2
- Correctly identified a wide range of QC organisms and well characterised QAP organisms

**However:**
- 7/263 isolates (2.7%) required full extraction for identification. This included mucoid Klebsiella species and some unusual fastidious organisms
- 5/263 isolates (1.9%) remained unidentified even with full extraction
- No reproducible errors with the exception of the misidentification of Shigella as E.coli
Implementation

- Staged introduction
- Direct Blood culture testing
- Campylobacter from TPV and Salmonella from CSA
- Enteric pathogen screening
- Routine use for Staphylococci, Enterococci, Enterobacteriaceae and Pseudomonas
- Routine use for Corynebacteria, Neisseria gonorrhoeae and anaerobes
- Routine use for respiratory pathogens
- Evaluation for yeast and fungi
NATA
Verification of MALDI-TOF

- NATA Technical Note 17 - October 2013; Guidelines for the validation and verification of quantitative and qualitative test methods
- Objective evidence that a method is fit for purpose
- “It is the responsibility of the facility to choose the validation or verification procedure and protocol most suitable for the desired outcome”
- Verification versus Validation
- Verification of a previously validated method
Method Verification

- Verification under conditions of use is demonstrated by:
  - Meeting established system suitability specifications
  - Meeting accuracy and precision parameters

Method performance may be demonstrated by:
- *Blanks, or un-inoculated media to assess contamination;*
- *Laboratory control samples to assess accuracy;*
- *Duplicates to assess precision;*
- *Calibration check standards analysed periodically in the analytical batch for quantitative analyses;*
- *Monitoring quality control samples; and*
- *Participation in a performance testing program provided that the tested material is representative of the method in terms of matrix, analytical parameters, concentration level(s), etc*
Thanks!