Applications for MALDI-TOF MS in Clinical Microbiology

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Identification of Microorganisms—Historical Perspective

- Microbial identification based on the Gram-stain and metabolic activities of an isolate
  - Pure culture
  - Time for growth and metabolism
  - Requires pre-selection of assays
  - Organisms may be biochemically inert
  - Missteps early in the process can result in mis-identification or no identification

The Ideal Identification System

- Quick
- Economical
- Accurate
- Simple
- Minimize the effect of different culture/growth conditions
- Reproducible
- Applicable to most clinically relevant microbes

MALDI-TOF is not a Cocktail

- M-Matrix
- A-Assisted
- L-Laser
- D-Desorption
- I-Ionization
- T-Time
- O-Of
- F-Flight
- M-Mass
- S-Spectrometry

MALDI-TOF for Microbial Proteomics

- First reported in 1975
- Early studies focused on mass spectra of whole bacterial cells to generate patterns of mass signals
  - Reproducible
  - Patterns specific and unique to different strains
- Identification of microorganisms by analyzing their protein content
  - Primarily ribosomal proteins detected (relative abundance)
  - Protein profile/identification not directly related to metabolic function

Mass Spectrometry--Overview

- Principle:
  - Separation of molecules based on the mass to charge ratio
  - Sample is ionized, ions are separated, and then detected
  - Data “fingerprint” is compared to a mass spectra database

Sample Preparation → Ionization → Detection → Data Analysis

Commercially Available Systems

- Not yet FDA cleared

Matrix

- Small acid molecules
- Strong optical absorption in the range of laser wavelength used
- Size and intensities of the peaks detected matrix dependant
- Must analyze samples using same matrix used to make database

Matrix: HCCA
alpha-cyano-4-hydroxycinnamic acid
Proteins accelerated over a short distance
A molecular fingerprint is generated, and compared to a reference database
Mass range: 2 to 20 kDa
Primarily ribosomal proteins detected (relative abundance)
Basic proteins 2-20 kDa make up ~65% of the dry cell weight of bacteria

Targets/Slides

Bruker Biotyper
bioMerieux MS

Commercially Available Systems

Bruker Biotyper

- Bench top model
- No special electricity or temperature requirements
- No additional venting needed
- Shorter flight tube
- Re-usable or disposable targets
- Bacterial and yeast database
- Mold and Mycobacterial database
Commercially Available Systems

bioMerieux MS
- Floor model instrument (approx. the size of a vending machine)
- Tight temperature requirements
- Disposable targets
- SARAMIS database
- Vitek MS will be the IVD version
  - To have both, will need two computers for the time being
  - Myla
- Results reported as a % probability score

Bruker Biotyper

Score Based Pattern Matching
- Calculation of a “Match Score” based on:
  - Matches to peaks in the reference database
  - Peaks that do not match any known reference spectra
  - A “score” is generated

Score Based Pattern Matching

<table>
<thead>
<tr>
<th>Range</th>
<th>Description</th>
<th>Symbols</th>
<th>Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00-3.00</td>
<td>Highly probable species identification</td>
<td>++ + +</td>
<td>green</td>
</tr>
<tr>
<td>1.00-1.99</td>
<td>Probable genus identification</td>
<td>(+)</td>
<td>yellow</td>
</tr>
<tr>
<td>0.00-1.09</td>
<td>Unreliable identification</td>
<td>( - )</td>
<td>red</td>
</tr>
</tbody>
</table>
Quality Control

- Bacterial Test Standard ("BTS")
  - Extracted E. coli isolate with additional proteins spiked in to span mass range
  - Provides two levels of quality control
    - Calibrator
    - BTS must be identified as E. coli
  - Performed with each run
- User defines additional quality control

Formic Acid Extraction

- Direct smear method may not work well with all organism types
  - Yeast, mucoid organism, thick cell wall

1. Suspend colony in water
2. Add ethanol, mix (inactivation of pathogens)
3. Centrifuge, decant ethanol, remove all excess, dry pellet
4. Add 70% Formic Acid and 100% Acetonitrile.
5. Centrifuge, 1 uL of supernatant is pipetted onto the steel target.

MALDI-TOF in Clinical Use

Aerobic Gram-negative bacilli from clinical specimens (blood, tissue, urine, wound, CSF, respiratory, etc.), included commonly encountered species, supplemented with for infrequently isolated organisms.

Workflow

- Any isolate for which a score of >2.0 was not obtained after a direct colony test was repeated by direct colony testing
- If a score of >2.0 was still not achieved, the isolate was extracted and re-analyzed

Summary

- Bruker Biotyper outperformed a phenotypic identification system for Gram-negative bacteria
  - The Biotyper was especially strong for unusual/infrequently encountered isolates
- Bruker Biotyper-based identification was cost effective with a good turn around time

Dhiman et al., JCM 2011. 49:1614-1616.

What About Yeast?

Routine Yeast Identification

- Evaluation of the MALDI Biotyper (2.0) Microflex LT for identification of common and uncommon yeast species
- 1 month, blinded, prospective study
- 145 fresh yeast isolates from routine workflow
- 116 archived strains of less common species
- Analysis was performed on formic-acid extracted isolates (full extraction process)

Dhiman et al., JCM 2011. 49:1614-1616.

Yeast Identification

<table>
<thead>
<tr>
<th>Type of isolate (number tested)</th>
<th>Number of isolates with a Score of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1.7 n (%)</td>
</tr>
<tr>
<td>Common Isolates</td>
<td>4 (2.9)</td>
</tr>
<tr>
<td>Uncommon Isolates</td>
<td>12 (11.6)</td>
</tr>
<tr>
<td></td>
<td>1.7-1.177 n (%)</td>
</tr>
<tr>
<td>Common Isolates</td>
<td>1 (0.7)</td>
</tr>
<tr>
<td>Uncommon Isolates</td>
<td>4 (3.9)</td>
</tr>
<tr>
<td></td>
<td>1.8-1.999 n (%)</td>
</tr>
<tr>
<td>Common Isolates</td>
<td>6 (4.3)</td>
</tr>
<tr>
<td>Uncommon Isolates</td>
<td>3 (2.9)</td>
</tr>
<tr>
<td></td>
<td>&gt;2.0 n (%)</td>
</tr>
<tr>
<td>Common Isolates</td>
<td>127 (92.0)</td>
</tr>
<tr>
<td>Uncommon Isolates</td>
<td>84 (81.6)</td>
</tr>
</tbody>
</table>

**Note: Spectral scores of above 1.8 gave accurate identification with no misidentification.

Time and Cost Estimates

<table>
<thead>
<tr>
<th>Method</th>
<th>Hands on Time Per Specimen (min)</th>
<th>Total Turn Around Time per Specimen (h)</th>
<th>Cost of Materials per Specimen ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>API 20C Yeast</td>
<td>6.5</td>
<td>48-72</td>
<td>6.86</td>
</tr>
<tr>
<td>Germ Tube</td>
<td>4.4</td>
<td>2.5</td>
<td>0.8</td>
</tr>
<tr>
<td>Rapid Assimilation of Trehalose</td>
<td>6.4</td>
<td>1.5-19.5</td>
<td>0.95</td>
</tr>
<tr>
<td>Sequencing</td>
<td>8 (? )</td>
<td>3.5 (but in reality would batch test)</td>
<td>20.02 (??)</td>
</tr>
<tr>
<td>MALDI Biotyper</td>
<td>5.1</td>
<td>0.64</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Bread and Butter**—Microbial Identification

- Validation Studies
  - Organism identification
    - Gram-positive
    - Gram-negative (Enteric and Non-Fermenting/Fastidious)
    - Anaerobes
    - Yeast

Dhiman et al., JCM 2011. 49:1614-1616.
Validation Studies

- **Overview:**
  - Test 150 to 200 of each organism group
  - No more than 25 isolates of a single species
  - Minimum of 15 species represented in each set
  - Most isolates from “regular” clinical workflow
  - Supplement with unusual/rare isolates
- **Bruker Biotyper (3.0) MALDI-TOF identification compared to phenotypic identification**
- **If identification discrepant (or no identification via either method), full length 16S rRNA gene sequencing**

Each isolate tested in quadruplicate
- Direct smear technique X 2 (“Heavy” and “Light”)
- Direct smear formic acid overlay X2
- 1 µl, “modified” extraction method

**Work Flow:**
- If none of the 4 spots yield a score >2.0, repeat
- If repeat does not yield score >2.0, Perform full formic acid extraction method
- Use results to optimize method and streamline workflow
  - Avoid unnecessary “full extractions”

Complexities of Taxonomy

<table>
<thead>
<tr>
<th></th>
<th>Stenotrophomonas maltophilia</th>
<th>Pseudomonas putida group</th>
<th>Enterobacter cloacae complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possible MALDI-TOF Identification:</td>
<td>Stenotrophomonas maltophilia</td>
<td>P. putida</td>
<td>E. cloacae</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas hibiscale</td>
<td>P. fluorescens</td>
<td>E. kobei</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas betel</td>
<td>P. plecoglossicola</td>
<td>E. ludwigi</td>
</tr>
<tr>
<td></td>
<td>P. geniculata</td>
<td>P. montelii</td>
<td>E. hormaechei</td>
</tr>
<tr>
<td></td>
<td>Stenotrophomonas maltophilia</td>
<td>P. mosselii</td>
<td>E. nimipressuralis</td>
</tr>
<tr>
<td></td>
<td>P. cynxyanea</td>
<td>P. plecoglossicola</td>
<td>P. putida</td>
</tr>
</tbody>
</table>

Integration into Clinical Practice

- **Implications for antimicrobial stewardship programs, patient safety**
  - Optimization of therapy
- **Continue to improve/develop methods for direct detection from clinical specimens and antimicrobial resistance**
- **Future—resolution of polymicrobial specimens??**
- **Continue to add taxa to the database**

Summary: MALDI TOF Strengths

- Very low consumable costs
- Very little waste (“Green”)
- Only a single colony of organism is needed
- Broadly applicable to different organism types
- Rapid turn around time, high throughput
- “Early errors” don’t affect downstream accuracy
  - Gram stain, oxidase, etc.
- Potential for numerous other applications

Summary: MALDI TOF Limitations

- Can’t resolve polymicrobial specimens
  - Software developments
- Limited use for direct detection from clinical specimens
- Extraction needed for some organisms
- Databases, breadth and depth
- Single instrument—downtime
- Current format: no routine antimicrobial susceptibility data
- Not yet FDA cleared (validation required)
SUPPORT BACTERIA!

it's the only culture some people have