Microbiology Applications of Mass Spectrometry
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Disclosures

**Research Contracts:** Abbott Molecular, AdvanDx, Argylla, bioMérieux, Becton Dickenson, Bio-Rad, Cepheid, Great Basin, Ibis Biosciences, Luminex, MDC, MicroPhage, NorDiag, Qiagen, Zeus

**Speaker:** Abbott Molecular, AdVanDx, Becton Dickenson, BioRad, Cepheid, Eragen, Qiagen

**Consultant:** Abbott Molecular, Cepheid, Accelr8
Objectives

1. Discuss principles of matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/MS) related to infectious disease diagnostics
2. Compare and contrast performance of commercial mass spectrometer systems that use MALDI-TOF/MS
3. Describe typical workflow and operations of MALDI-TOF/MS in a clinical microbiology laboratory
4. Provide case histories and examples of effective implementation milestones and improvement to patient care at the University of Arizona
The Pre-quel: Thinking differently about pathogen identification?
UAMC Microbiology Drives Antibiotic De-escalation

Rapid Testing

Results to ID Pharmacy

± Rx Intervention
Pre-quel: Summary of UAMC Impact for PNA FISH Intervention

• Patient Outcomes Improved
  • Higher survival rates, especially in ICU
    • 34.6 to 15.6% for GPCPC (19% improvement)
    • 41.7 to 5.9% for yeast (35.8% improvement)

• Laboratory: Faster TAT (> 3days)

• Improved Healthcare Utilization
  • > $1.2 millions saved per year
    (after ~$32,000 costs for reagents removed)

• Antibiotic Decisions Optimized Earlier escalation or de-escalation

Gamage et al, 2011 ICAAC Abstract D-1302b
Other Reports of Reduced Mortality

PNA FISH vs. Conventional Methods

1. Enterococci (ICU) -17%
2. Candida (ICU) -36%
3. Staphylococci (All) 12%
4. S. aureus (ICU) -38%
5. E. faecium (ICU) -19%

Control Group PNA FISH

1,2) University of Arizona Medical Center: Poster D-1302b. ICAAC 2011. Chicago, Illinois, USA.
3) Orlando Regional Medical Center: Poster 1023. IDSA 2010. San Diego, California, USA.
Thinking about possibilities...
Matrix Assisted Laser Desorption Ionization (MALDI)

Bruker Daltonics MALDI BioTyper (TM)
bioMérieux = Vitek MS

Measure and compare high abundance proteins
MALDI-TOF Functional units

- **Specimen Ionization source/chamber (MALDI)**
  - Laser-based vaporization of specimen

- **Analyser:** Time of Flight (TOF) analyzer
  - Separates ions according to mass-to-charge ratios (m/z)

- **Particle Detector**
  - Detects separated ions and identifies relative abundance

- **Data System**
  - Signals sent and formatted in m/z spectrum
Step 1) Sample preparation/Direct Transfer

- **Cell Disruption** bacteria/yeast colony
  
  - Mechanical for rigid cells (e.g. Sonication or boiling)
  
  - Organic solvent extraction (improves quality for difficult microbes, yeast and fungi)

  - Strong organic acid (formic or trifluoroacetic acetic acid [TFAA]) before/during matrix addition
Review of Example Full Sample Prep

- Mix Sample w/ 70% ethanol
- Centrifuge
- Dry pellet
- Add formic acid and vortex
- Add acetonitrile and vortex
- Centrifuge
- Add 1ul to metal plate
- Add matrix and dry
- Place into chamber and apply laser
2) Matrix added

- Pre-treated/untreated samples mixed/overlaid with matrix and dried

- **Matrix**: 1 μl UV absorbing sln.
  - α – cyano – 4 – hydroxy - cinnamic acid (CHCA)
    - Preferred for proteins
    - CHCA in 50% acetonitrile and 2.5% TFAA (tri-fluoroacetic acid); gives strong absorbance at UV laser wavelength 337nm
3) Laser Applied

- **Energy Transfer: Matrix to analyte**

- Pulsed laser causes vibrational excitation of CHCA, which transfers protons to proteins

- Responsible for sublimation (transition of analyte from solid to gas phase, without intermediate liquid phase); analyte desorbed
4) MALDI-TOF Fragments Proteins

- High-energy electron beam breaks molecule apart
- Fragment’s mass and relative abundance reveal information ~
  - Structure
  - Composition of the molecule

To TOF MS

Pulsed Laser Beam, N₂ ~ 337nm

Sample

Ions
5) Spectra generated directly from organisms
   Compare to reference database of known organisms.

   Report results based on confidence of the spectral match.

*Kaleta E., Wolk D. Clin Lab News.*
May 2012: Volume 38, Number 5
Variability of Spectra Based on Genus and Species

- Aspergillus fumigatus
- Bacillus subtilis
- Candida albicans ATCC 10231
- Escherichia coli DH5alpha

Intens. [a.u.] vs. m/z
Score based pattern matching to a reference database

- Unknown microorganism is compared against reference library of spectra from culture collection strains

- ‘Goodness of fit’ is ranked and a threshold is applied for identification
Thresholds Set

- Match profile of unknown organism to reference database/library
- Ranking according to matching score (scale 0-3) for Bruker (% scores used for Vitek)
- Threshold for correct identification 2.0 (1.8, etc.)

<table>
<thead>
<tr>
<th>Range</th>
<th>Description</th>
<th>Symbols</th>
<th>Color</th>
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</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>no reliable identification</td>
<td>(-)</td>
<td>red</td>
</tr>
</tbody>
</table>
Commercial Systems

MALDI-TOF Systems Aimed at Clinical Use

Bruker Daltonics MALDI BioTyper (TM)
bioMérieux/Shimadzu = Vitek MS
Bruker MALDI Biotyper (TM) system

MALDI-TOF-MS
MALDI BioTyper™ system

• Measures high-abundance proteins, including many ribosomal proteins
  • ID based on characteristic spectrum of protein expression patterns

• Circa 2006, IVD-CE Mark 2009, RUO in US

• For Identification/classification
  • Gram positive and negative aerobic/anaerobic bacteria
  • Yeasts and multi-cellular fungi
Bruker Ultraflex III Data Acquisition window
MBT: Client Server Architecture

Links to Phoenix and Kiestra

LIMS Integration

MBT-Software (Server)

microflex

MBT-Satellite Software

MBT-Client / Interactive Validation

MBT-Satellite on Tablet PC
NEW: Control the MBT workflow with multiple Tablet PC s and iPhone/SmartPhone

- Links target/specimen/isolate
- Wireless connectivity to MBT

MALDI Biotyper 3.0
Tablet PC Project setup

New Orleans, 05/22/2011
bioMérieux Vitek MS®

MALDI-TOF-MS
bioMérieux/Shimadzu

- Shimadzu (Kyoto, Japan) & bioMérieux (Marcy l’Etoile, France)

- Microbial database acquired from AnagnosTec, advanced further by bioMérieux (for IVD)

- Soon: Fully integrated with Vitek antibiotic susceptibility testing (AST) via MYLA software
Vitek MS Data Acquisition window
High level quality results (ready to be sent):
- Perfect quality control (acquisition and identification).
- Very good match with the knowledge base (single species call).
- Isolate results consolidation for duplicate deposit.

Medium quality results (decision to be made by user):
- Perfect quality control (acquisition and identification).
- Low discrimination.
- Discrepant Isolate results for duplicate deposit.

Low quality results (to be discarded by user):
- Acquisition failed (lack of matrix).
- Calibration failed.
- No identification.
Low background
Bar Codes
Integration
Traceability

4 “benches”

* For research use only-USA; IVD-planned
Thinking about results...
MALDI-TOF Applications
Review of Literature

High accuracy for most microbes
Gaps still exist
Think critically
Concordance Results of 2 MALDI-TOF IDs (n=720)

• **Bruker Biotyper**
  • High-confidence ID for 674/680 isolates, \(99.1\%\) correct

• **bioMérieux Vitek MS**
  • High-confidence ID for 635/639 isolates, \(99.4\%\) correct
    • Saramis dbase will improve with IVD

Concordance Results of 2 MALDI-TOF IDs (n=1129, 928 ID to species level)

- **Bruker Biotyper MicroFlex LT**
  - Correct species ID 92.7%

- **bioMérieux Vitek MS IVD**
  - Correct species ID 93.2%

E.g. Clinical Pathogens/high concordance

- **Non-fermenters** (*Degand et al, 2008; Mellman et al 2008, Van Veen et al, 2010; Saffett et al, 2011*)
- **HACEK** (*Courtier et al, 2011*)

- **Enterococci** (*Eigner et al. Clin Lab 2009*)
- **Streptococci** (*Friedrichs et al. J Clin Microbiol 2007*)

- **Neisseria spp** (*Ilina et al. J Mol Diagn 2009*)
- **Listeria monocytogenes** (*Barbuddhe et al. 2008*)

- **Inter-lab reproducibility 98.75% accuracy** (*Mellmann et al 2009*)
- **Comparison with genetics**: (Kaleta, E., et al 2011, Clin. Chem)
**Anaerobes**

- **Bacteroides**: Nagy et al. CMI 15: 796-802 2009
- **Misc. anaerobes**: Soki J, Nagy E, Backer S: Publication in preparation
**Mycobacteria and Nocardia**

Macheras E, JCM. 2009 and 2011; Leao SC JCM 2009; Saleeb et al, JCM, 2011; Zelazny et al JCM, 2009

*Mycobacteria and Nocardia*: Conville PS et. al JCM 2006; Zelazny AM et.al. JCM 2005

*Nocardia*: Verroken et. al. JCM 2010
Antimicrobial Resistance

Carbapenem resistance in *B. fragilis*: Wybo et al. JCM, 2011

Yeast susceptibility: Marinach et al, Proteomics, 2009, 9, 1-5
Genotyping

**MRSA:** Wolters, et al. 2011 *Int J Med Microbiol* 301:64-68

Fungi: *Candida* spp.

More preparation required for higher quality of spectra

*Candida:*


Fungi: Molds

More preparation required for higher quality of spectra


- *Fusarium spp.*: Marinach et al, Et Clin Microbiol Infect., 2010
Thinking about change...
Tips for RUO Implementation

- Save isolates for > 1 year (total n ~ > 1,000)
- Ensure diversity of bacteria and MLS staff (all shifts)
- Target sample exchange to increase rare samples
- Integrate method verification with routine training and competency
- Consider duplicate testing at first
- Phase in with common microbes
Try double spotting during training
Good Concordance: Results of MALDI-TOF ID vs. Conventional ID (n=1660)

Seng et al, *CID*, 2009

Database and **spotting quality** were biggest challenges
New Hires?
GET A MAGNIFIER!

GET A LAMP!
Set up a tracking log to avoid mix-ups...
Ergonomics: Get a “rolling chair”
Vitek MS vs. Vitek 2
UAMC Results

Aerobic/Anaerobic bacteria and Candida spp.
166 different species

n = 858 (38 Anaerobes, 63 yeast, rest were aerobic)

From ~ all body sites” normally sterile and other
Agreement for microbes, N < 5 each

- Aeromonas hydrophilia
- Eickenella corrodens
- Fusobacterium nucleatum
- Pseudomonas putida
- Beta Strep, not group A or B
- Enterococcus gallinarum
- Streptococcus intermedius
- Bacteroides ovatus
- Clostridium septicum
- Nutritional variant strep
- Prevotella disiens
- Ralstonia spp.
- Staphylococcus hominis
- Bacillus spp.
- Eickenella spp.
- Moraxella spp.
- Staphylococcus haemolyticus
- Candida krusei
- Neisseria meningitidis
- Acinetobacter ursingii
- Beta Strep, Group F
- Corynebacterium striatum
- Pasteurella canis
- Proteus vulgaris
- Raoultella planticola
- Staphylococcus intermedius
- Candida dublindiensis
- Enterococcus avium
- Prevotella bivia
- Staphylococcus simulans
- Diphteroids (Corynebacterium spp.)
- Propionibacterium acnes
- Anaerobic Gram Neg Rod
- Candida guilliermondii
- Haemophilus parainfluenzae
- Pasturella canis
- Pseudomonas stutzeri
- Salmonella
- Streptococcus maltophilia

> 39 species
Accuracy of Vitek MS in AZ Microbe Set
n=858 (24 Discordant)

- Family Match Only
- Discordant
- No Match
- Split Call Match
- Concordant

91%
5%
3%
1%
<1%
Shigella n=7
Gardnerella n=3
Others n=1

Discordant Samples, Vitek vs Vitek MS

- Alcaligenes faecalis
- Citrobacter sedlakii
- Corynebacterium striatum
- Enterobacter asburiae
- Klebsiella oxytoca
- Pantoea spp.
- Ralstonia mannitolyltica
- Serratia fonticola
- Brevundimonas vesicularis
- Citrobacter youngae
- Enterobacter aerogenes
- Gardnerella vaginalis
- Nocardia otitidiscavarium
- Proteus vulgaris
- Raoutella ornithina
- Shigella sonnei

Send for sequencing
### Split calls (send for sequencing)

<table>
<thead>
<tr>
<th>Vitek</th>
<th>Vitek MS</th>
</tr>
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<tbody>
<tr>
<td><strong>Enterobacter aerogenes</strong></td>
<td><strong>Enterobacter aerogenes/Klebsiella pneumoniae</strong></td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td><strong>Shigella / Escherichia coli</strong></td>
</tr>
<tr>
<td></td>
<td><strong>Escherichia coli</strong></td>
</tr>
<tr>
<td></td>
<td><em>(MS second repeat correct but only 82.3% score)</em></td>
</tr>
<tr>
<td><strong>Neisseria animalonis/N. zoodegmatis</strong></td>
<td><strong>Neisseria zoodegmatis</strong></td>
</tr>
</tbody>
</table>
E.g. Patient “Saves”

- Salmonella ID, day 1
- *S. aureus* ID, day 1 and when latex faltered
- *Methylobacterium*, day 3, same day as growth
- Cost savings vs sequencing of fastidious GNR
UA/JHH/RI Hospital Study

Sepsityper 381 samples
  JHH n=226, Bactec
  Brown n=155, TREK

34 mixed organism samples excluded

347 single-organism samples
  (45 species by conventional methods)
    230 gram-positive bacteria
    103 gram-negative bacteria
    14 yeasts

Median time from BC + to extraction was 4.3 h
Results UA/JHH/RI, n=347

- Agree: 275 (79%)
- Discordant: 9 (5%)
- No Reliable ID: 16 (3%)
- Insufficient protein: 47 (13%)
Thinking about discoveries...
Goal: Actionable Results

Microbiology Laboratory, Pharmacy, Physicians Focus on Antimicrobial De-escalation and Antimicrobial Stewardship

- Direct MALDI-TOF Improves Appropriateness of Antibiotic Treatment of Bacteremia

- 11.3% increase in the patients w/ appropriate Rx 24 hours after blood culture positive

- (75.3% vs 64.0% (p = 0.01).
Strategy for Rapid ID/Susceptibility

- Tested Gram-Negative Bacteria from Positive Blood Cultures
  - Bruker MALDI Biotyper coupled w/ rapid susceptibility testing (BD Phoenix)

Thinking about a different perspective...
Identification and typing of bacteria in the future by MALDI-TOF MS?

• Take great care with implementation

• More development of the databases is needed

• Standardization should be carried out to obtain comparable results
  • Watch for ARUP Publication (Fisher)

• Develop practice guidelines and algorithms for clinicians and pharmacists
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Questions