Biosafety Implications of New Technologies and Emerging Pathogens

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Report on the Potential Exposure to Anthrax
Centers for Disease Control and Prevention

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Concerns with MALDI-TOF

• Safety
  – Viability of organisms before and after testing
    • BLS-3 considerations
  – Creation of aerosols by instrument

• Accuracy
  – Ability of the instrument to accurately identify hazardous organisms
    • ROU vs. FDA approved libraries
    • Vitek vs. Bruker
Agents Tested

- *Brucella abortus* Strain 19
- *Bacillus anthracis* Sterne
- *Burkholderia thailandensis* ATCC 70038
- *Francisella tularensis* LVS
- *Yersinia pestis* A1122
- *Clostridium* spp.
  - *botulinum* types A, B, and E; *perfringens*
1. Smear biological material (single colony) as a thin film directly onto a cleaned MALDI target. **IMPORTANT** After the sample has dried, matrix must be added within 10 minutes.

2. Carefully overlay each sample position (= spot) with 1 μL HCCA matrix solution.

3. Allow the sample positions to dry at room temperature.
Sample Preparation – Extended Direct

1. Smear biological material (single colony) as a thin film directly onto a cleaned MALDI target. **IMPORTANT** After the sample has dried, matrix must be added within 10 minutes.

2. Cover smear with 1 μL of 70% formic acid and allow to dry completely.

3. Carefully overlay each sample position (= spot) with 1 μL HCCA matrix solution.

4. Allow the sample positions to dry at room temperature.
1. Pipet 300 μL ultra pure water into a clean Eppendorf tube. Transfer a single colony of biological material into the tube. Vortex for at least one minute.
2. Add 900 μL of pure ethanol into the tube and vortex the suspension for at least one minute.
3. Centrifuge the tube for 2 minutes at 13,000 rpm and remove the supernatant.
4. Repeat step 3. All residual ethanol should be removed.
5. Add 50 μL of 70% aqueous formic acid, mix thoroughly and let stand at least 5 minutes.
6. Add 50 μL acetonitrile to the tube and mix carefully.
7. Centrifuge the tube for 2 minutes at 13,000 rpm.
8. Pipette 1 μL of microorganism extract supernatant onto a cleaned MALDI target.
9. Allow the sample positions to dry at room temperature. IMPORTANT After the sample has dried, matrix must be added within 10 minutes.
10. Carefully overlay each sample position (= spot) with 1 μL HCCA matrix solution.
11. Allow the sample positions to dry at room temperature.
Sterile Cover Slip → Matrix → BT Agent (spot) → BHI

BT Agent (spot) → BT Agent (spot) → BHI + Matrix → BHI
# Results

<table>
<thead>
<tr>
<th>Organism</th>
<th>Direct Method</th>
<th>Extended Direct</th>
<th>Tube Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Target</td>
<td>Spot + Matrix</td>
<td>Spot</td>
</tr>
<tr>
<td>B. anthracis</td>
<td>3/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>B. thailandensis</td>
<td>0/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>1/5</td>
<td>1/5</td>
<td>3/5</td>
</tr>
<tr>
<td>F. tularensis</td>
<td>1/5</td>
<td>2/5</td>
<td>4/5</td>
</tr>
<tr>
<td>Y. pestis</td>
<td>2/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>B. abortus</td>
<td>0/4</td>
<td>3/4</td>
<td>4/4</td>
</tr>
</tbody>
</table>
Study Conclusions

• Drying may result in loss of viability
• Some viable organisms are present on the target using the direct and extended direct sample prep methods
• No viable organisms were found following the tube extraction
• Study is limited by the number and types of strains tested
Safety Considerations

• Clean target often
• Make sure matrix completely covers “spot”
• Use tube extraction AND 0.1 μm filter for “hazardous organisms”
Addressing safety

• New Equipment Purchase Checklist
  – Biosafety level

• New Test Implementation Checklist
  – Samples
  – Sample prep
  – Sample transport
  – Waste disposal
Participating Laboratories

- Michigan Department of Community Health
- Wadsworth Center, New York Department of Health
- Texas Department of State Health Services
- Wisconsin State Laboratory of Hygiene
- North Carolina State Laboratory of Public Health
- State Hygienic Laboratory at the University of Iowa
Questions