The UW and bioMerieux protocols were significantly effective.

Add 10 µl of 100% acetonitrile to 250 µl of lyophilized biomass.
Add 900 µL of 100% ethanol
Add 10 µl of 70% formic acid
All extracts tested on Bruker MicroFlex and bioMerieux VITEK MS.
Aspirate off as much ethanol
Transfer the supernatant to a screw top Eppendorf tube
Centrifuge for 2 min at 14,000x g.
Let sit at room temperature for 15 min.
Transfer 1 µL biomass into a biosafety cabinet
Centrifuge for 2 min at 14,000x g.
Aspirate off as much ethanol
Centrifuge for 2 min at 10,000x g.
10.Use 1 µl of supernatant for MALDI-ToF MS.

Table 1: Species tested using both protocols and scores on the MALDI Observation.

<table>
<thead>
<tr>
<th>Species</th>
<th>N tested</th>
<th>Min library</th>
<th>Max library</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. avium ssp. hominis</td>
<td>17</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>M. avium ssp. voror</td>
<td>14</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>M. caudiverum</td>
<td>14</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>M. fortuitum</td>
<td>15</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>M. chelonae</td>
<td>14</td>
<td>10</td>
<td>16</td>
</tr>
<tr>
<td>M. simiae</td>
<td>13</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>M. abscessus ssp. abscessus</td>
<td>12</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>M. immunogenum</td>
<td>12</td>
<td>6</td>
<td>18</td>
</tr>
</tbody>
</table>

Methods

Protein Extraction Protocols

UW protocol
1. Transfer 1 µl biomass into a screw top Eppendorf tube with 300 µl of desorbed water and approximately 200 µl of 1 mm silica beads.
2. Heat to 95ºC for 3 min.
3. Add 500 µl of 100% ethanol and vortex in a horizontal position for 10 min.
4. Transfer the supernatant to a new tube using a pipette.
5. Centrifuge for 2 min at 10,000 x g.
6. Aspirate off as much ethanol as possible.
7. Add 10 µl of 70% formic acid and mix by repeat aspiration/expulsion.
8. Add 10 µl of 100% acetonitrile and vortex for 20 sec.
9. Centrifuge for 1 min at 10,000 x g.
10.Use 1 µl of supernatant for MALDI-ToF MS.

bioMerieux protocol
1. Transfer 1 µl biomass into a screw top Eppendorf tube with 300 µl of 70% ethanol and approximately 200 µl of 0.5 mm silica beads.
2. Vortex in a horizontal position for 13 min.
3. Let sit at room temperature for 10 minutes.
4. Transfer the supernatant to a new tube using a pipette.
5. Centrifuge for 2 min at 10,000 x g.
6. Aspirate off as much ethanol as possible.
7. Add 10 µl of 70% formic acid and mix by repeat aspiration/expulsion and let sit 15 min.
8. Add 10 µl of 100% acetonitrile and vortex for 20 sec.
9. Centrifuge for 2 min at 10,000 x g.
10.Use 1 µl of supernatant for MALDI-ToF MS.

Results

Both protocols produce adequate spectra on the Microflex and VITEK mass spectrometers.

Comparison of the Bruker Biotyper MS and bioMerieux protocol.

Table 2: Percent of isolates identified to the species level by the Saramis, Biotyper and/or spectra identified using reference spectra.

<table>
<thead>
<tr>
<th>Species</th>
<th>UW Protocol</th>
<th>bioMerieux Protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. xenopi</td>
<td>55%</td>
<td>59%</td>
</tr>
<tr>
<td>M. abscessus</td>
<td>60%</td>
<td>64%</td>
</tr>
<tr>
<td>M. kansas</td>
<td>30%</td>
<td>34%</td>
</tr>
<tr>
<td>M. fortuitum</td>
<td>60%</td>
<td>64%</td>
</tr>
<tr>
<td>M. simiae</td>
<td>30%</td>
<td>34%</td>
</tr>
<tr>
<td>M. avium</td>
<td>60%</td>
<td>64%</td>
</tr>
<tr>
<td>M. chelonae</td>
<td>30%</td>
<td>34%</td>
</tr>
</tbody>
</table>

Discussion

The UW and bioMerieux protocols were significantly simpler than previously published protocols.

Both protocols successfully identified isolates representing 18 clinically relevant Mycobacterium species.

The Saramis library outperformed the Biotyper library using either protocol when isolates are identified according to manufacturer specifications.

The Biotyper library could be used to successfully identify 99.5% of isolates using the UW protocol if a score threshold of 1.9 was accepted. This did not result in false positive identifications.

Internal library creation allowed for successful mycobacterial identification on the Microflex MS platform using either protocol.

References


Disclosure

None.