Utility of PCR and electro-spray ionization-mass spectrometry with different types of clinical specimens

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Introduction

Detection and identification of bacterial and fungal DNA directly from clinical specimens is a useful aid to diagnosis, particularly when conventional cultures are negative1. Typically, conserved sequences, such as the bacterial 16S rRNA gene and 18S-23S ITS region for fungi, are amplified by PCR and then sequenced. The total procedure can be both labour and time intensive.

Electro-spray ionization-mass spectrometry (ESI-MS) can be used as a semi automated method of identifying organisms from the base composition of a combination of ionised PCR products calculated from their mass. The Bacteria, Antibiotic Resistance and Candida (BAC) assay® (Ibis Biosciences, Abbott, Carlsbad, CA, USA) uses this technology to identify bacteria and fungi directly from specimens and simultaneously provides information on the presence of mecA, vanA, vanB and kpc genes 2.

The BAC assay results of different specimen types originating from sterile sites were compared with the microbiological culture reports.

Materials and Methods

243 specimens were selected from material remaining after diagnostic Microbiology testing (Table 1).

- Samples were allocated a study number and frozen for analysis until all testing was complete.
- Microscopy data was used to ensure positive samples were included. Otherwise specimen collection and analysis was naïve of the culture results.
- 16S rDNA sequence analysis had been performed on some samples during the routine work up as previously described3.
- DNA from specimens were extracted and processed using Abbott equipment, reagents and protocols.
- DNA samples were tested using the high sensitivity version of the BAC assay and analysed with the BAC detection 2.0 whole blood IUO assay protocol.
- Results were compared with all available laboratory data.

Table 1. Summary of site of origin

<table>
<thead>
<tr>
<th>Site</th>
<th>Fluid</th>
<th>Pu</th>
<th>Tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central nervous system</td>
<td>13</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Bone native joints</td>
<td>95</td>
<td>3</td>
<td>17</td>
</tr>
<tr>
<td>Assoc with Prosthetic joints</td>
<td>14</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Chest cavity</td>
<td>27</td>
<td>-</td>
<td>6</td>
</tr>
<tr>
<td>Abdomen / pelvic cavity</td>
<td>28</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Other sites</td>
<td>2</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>179</td>
<td>25</td>
<td>39</td>
</tr>
</tbody>
</table>

Results and Discussion

Table 2. Comparison of PCR/ESI with culture

<table>
<thead>
<tr>
<th>Culture result</th>
<th>PCR/ESI result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Not detected</td>
</tr>
<tr>
<td>Positive</td>
<td>37</td>
</tr>
<tr>
<td>No growth</td>
<td>46</td>
</tr>
</tbody>
</table>

- 24 tests had results that were not valid due to non detection of the extraction control (19) or failure of detection by ESI-MS (5)

- There were 10 culture negative specimens with low levels of contamination detected (i.e. low levels of bacteria detected in both sample and accompanying negative control).

- No growth on culture, BAC assay POSITIVE:
  - The BAC assay detected bacterial/Candida spp DNA in 23.5% (46/196) of the culture negative specimens
  - Same organism grown from other specimens (18)
  - Confirmed by PCR or other tests, includes fastidious organisms (8)
  - Consistent with diagnosis/antibiotics (4)
  - Information not available to support result (13)
  - Identification of bacterium differed from 16S-PCR identification (3)

- Culture positive, BAC assay NEGATIVE
  - The BAC assay did not report the detection of any bacterial / Candida spp DNA in 4 culture positive samples
  - Organism not in the BAC database (1)
  - Grown from enrichment broth only (2)
  - Very few colonies grown on culture (1)

Conclusions

The BAC assay:
- Is more sensitive than culture
- Is fast and easier to perform than 16S rDNA sequencing
- Can be applied to a sample types from most sterile body sites
- Can detect fastidious bacteria including Ureaplasma urealyticus and Chlamydia trachomatis

This assay provides a useful adjunct to culture where the laboratory diagnosis is unsuccessful.

References and Acknowledgements


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