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Comparison of TaqMan PCR Assays for Detection of the Melioidosis Agent Burkholderia pseudomallei in Clinical Specimens

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Melioidosis is an emerging infectious disease caused by the soil bacterium Burkholderia pseudomallei. In diagnostic and forensic settings, molecular detection assays need not only high sensitivity with low limits of detection but also high specificity. In a direct comparison of published and newly developed TaqMan PCR assays, we found the TTS1-orf2 assay to be superior in detecting B. pseudomallei directly from clinical specimens. The YLF/BTFC multiplex assay (targeting the Yersinia-like fimbrial/Burkholderia thailandensis-like flagellum and chemotaxis region) also showed high diagnostic sensitivity and provides additional information on possible geographic origin.
TABLE 1

<table>
<thead>
<tr>
<th>Assay (size in bp)</th>
<th>Reference</th>
<th>Forward primer (5’—3’)</th>
<th>Reverse primer (5’—3’)</th>
<th>Probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTS1-orf2 (115)</td>
<td>22</td>
<td>CGTCTCTATACTGTCGAGCAATCG</td>
<td>CGTGCACACCGGTCAGTATC</td>
<td>FAM-CCGGAATCTGGATCACCACCACTTTCC-BHQ</td>
</tr>
<tr>
<td>lpxO (8653)</td>
<td>29</td>
<td>ATCGCCAAATGCCGGGTTTCC</td>
<td>CAAATGGCCATCGTGATGTTC</td>
<td>FAM-TCGGCGAACGCGATTTGATCGTTC-TAMRA</td>
</tr>
<tr>
<td>orf11 (91)</td>
<td>27</td>
<td>TTGTTTCGCCTATGCGTTCTC</td>
<td>CCACTCGCGCTTGAGGAT</td>
<td>FAM-ACGTGCCGAACACGCCGTATATCG-BHQ</td>
</tr>
<tr>
<td>mprA (199)</td>
<td>21</td>
<td>ACTGCTTCGTTCAAGGCGACCGT</td>
<td>TGACGGCCTGAACGTCCGC</td>
<td>FAM-CAACTTGACGATCAACTGA-MGB</td>
</tr>
<tr>
<td>B. pseudomallei</td>
<td>266152</td>
<td>E. P. Price et al., unpublished data</td>
<td></td>
<td></td>
</tr>
<tr>
<td>YLF (54)</td>
<td>204</td>
<td>TGTGGCTGCCTGGATGAGG</td>
<td>GACGCCAAGACGCCGTATATCG</td>
<td>FAM-CGCCGCAAGACGCCATCGTTCAT-TAMRA</td>
</tr>
<tr>
<td>BTFC (96)</td>
<td>160</td>
<td>GGCAGCGTCGAACTGTTCTAG</td>
<td>CGAATCAATTCGTTTCCCTTGT</td>
<td>VIC-TTCGGCTGCGAAACA-MGBNFQ</td>
</tr>
<tr>
<td>266152 (68)</td>
<td>8653</td>
<td>ATCGAATCAGGGCGTTCAAG</td>
<td>CATTCGGTGACGACACGACC</td>
<td>FAM-CGCCGCAAGACGCCATCGTTCAT-TAMRA</td>
</tr>
</tbody>
</table>

C(4,6) was defined as the lowest possible template concentration detectable with 95% probability with at least 61/64 positive replicates (4, 6). Specificity was evaluated by screening 365 B. pseudomallei strains and 115 non-B. pseudomallei strains (with 71 Burkholderia spp. and 44 bacterial species of differential diagnostic importance [see full list in the supplemental material]). A threshold cycle (C_T) value of 40 was the declared cutoff for a positive result.

Clinical evaluation was performed by screening each assay across 50 clinical specimens (22 sputum, 20 blood, and 8 urine samples) from 22 patients with acute melioidosis, 59% of whom presented with pneumonia and 18% with genitourinary infection, with 55% overall being bacteremic. These samples were part of a study comparing DNA extraction techniques for molecular detection of B. pseudomallei in clinical specimens (24); while all samples were from culture-confirmed melioidosis cases, not every specimen from each patient was cultured for B. pseudomallei. Blood samples from 22 healthy volunteers were used as negative controls. DNA was extracted using the QIAamp DNA minikit (Qiagen, Australia) and PureGene blood core kit B (Qiagen). PCR conditions were as previously described (24), using the 1X Applied Biosystems environmental master mix. Samples were declared positive if 2/2 duplicates had C_T values of <40.

All assays showed high analytical specificity, with the TTS1-orf2, 8653, mprA and 266152 assays being 100% specific for both B. pseudomallei and nontarget strains (Table 2). The lpxO assay showed reduced specificity as it also amplified 14/23 Burkholderia mallei DNA targets. The TTS1-orf2 and YLF/BTFC assays had the lowest LoD of 5 genome equivalents (GE) per reaction (Table 2). Variations from previously reported LoD were apparent from our data and are likely due to the strict LoD definition we used to determine the lowest possible template concentration detectable with 95% probability (4, 6). The low LoD of TTS1-orf2 and YLF/BTFC assays was also reflected in their high diagnostic sensitivity. Of the 43 clinical samples that tested positive for B. pseudomallei by one or more assays, 42 were positive by several assays and one only by TTS1-orf2. The mprA assay performed least well in the clinical evaluation and had a significantly lower detection rate than the TTS1-orf2, YLF/BTFC, and lpxO assays (McNemar’s test for paired samples, P < 0.001 for all, 2-tailed) (Table 2).

These data support the TTS1-orf2 assay as the best-performing assay to date for direct detection of B. pseudomallei in clinical specimens. The YLF/BTFC multiplex assay also performed well and in addition to B. pseudomallei detection provides information on the potential geographic origin of the tested isolate, with BTFC being common in Australia (88%) but rare in Thailand (2%) (31). As the YLF locus was also found in some close relatives of B. pseudomallei as being common in Australia (88%) but rare in Thailand (2%) (31). As the YLF locus was also found in some close relatives of B. thailandensis and B. pseudomallei strains and 115 non-B. pseudomallei strains (with 71 Burkholderia spp. and 44 bacterial species of differential diagnostic importance [see full list in the supplemental material]). A threshold cycle (C_T) value of 40 was the declared cutoff for a positive result.

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As an additional informative assay, we included a dual-probe assay discriminating between B. pseudomallei and B. thailandensis. This assay was designed for screening culture isolates and showed high specificity. No differences in clinical detection rates were found when including both probes or only the B. pseudomallei-specific probe. Due to cross-hybridization, this assay should be used with caution on environmental samples where potentially both B. pseudomallei and B. thailandensis could be present.

Although the probe chemistry was tested as in the original publications, the PCR conditions were not adjusted to the original optimized conditions for each assay; we used an adaptation of the
Table 2: List of validation results from real-time PCR assays

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result by assay</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analytical specificity, % (no. positive/total)</strong></td>
<td></td>
</tr>
<tr>
<td>B. pseudomallei strains</td>
<td>100 (365/365)</td>
</tr>
<tr>
<td>Non-target strains</td>
<td>100 (0/115)</td>
</tr>
</tbody>
</table>

**LoD (GE/reaction)** = 5

**Diagnostic sensitivity, % (no. positive/total)**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result by assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTS1-orf2</td>
<td>100 (365/365)</td>
</tr>
<tr>
<td>TTS1-orf11</td>
<td>100 (365/365)</td>
</tr>
<tr>
<td>lpxO</td>
<td>57.8 (14/245)</td>
</tr>
<tr>
<td>mprA</td>
<td>100 (0/115)</td>
</tr>
<tr>
<td>YLF/BTFC</td>
<td>99.7 (364/365)</td>
</tr>
</tbody>
</table>

TaqMan universal PCR protocol (Life Technologies), with the same conditions for all assays. The inferior performance of the mprA assay in the clinical evaluation may reflect that the original assay was not probe based and had an annealing temperature of 68°C, which differs from the conditions we used.

In summary, we have shown that the TTS1-orf2 assay provides the best available molecular target to date for B. pseudomallei detection directly from clinical samples. Furthermore, the YLF/BTFC multiplex assay, which provides additional information on the possible geographic origin of B. pseudomallei isolate, also showed high diagnostic sensitivity.

Acknowledgments

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References


