Comparison of five methods for recovery of Mycobacterium tuberculosis DNA from stool samples

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INTRODUCTION

Laboratory diagnosis of pulmonary tuberculosis is spuim based in patients who cannot produce sputum (i.e., children) invasive procedures, such as gastric aspirates, may be used to detect or diagnose TB.

Stool and other non-invasive samples could be an alternative for diagnosis because is possible to find TB cells, however the presence of specimen inhibitors is a challenge for molecular testing.

In this work, we compared five different DNA extraction kits to determine which method maximizes the DNA recovery while minimizing the presence of PCR inhibitors. All this will be assessed by real time PCR.

1. QIAamp DNA Stool Mini Kit.
2. QIAamp DNA Stool Mini Kit with Microsens TB-Beads.
4. Akonni Biosystems automated TruTip Kit.
5. Akonni TruTip with Microsens TB-Beads.

RESULT

As part of a larger study evaluating new tools for diagnosis of tuberculosis in children, we set out to select an optimal DNA extraction method for Mycobacterium tuberculosis from stool samples.

REFERENCES


MATERIALS AND METHODS

Stool samples from five healthy Peruvian adult volunteers, divided into two aliquots each.

DNA Extraction

Controls for each method:
- Positive: Mtb cells
- Negative: Only Stool
- System: Only Water

RESULTS

Table 1. Total number of extractions performed

<table>
<thead>
<tr>
<th>Extraction Method</th>
<th>Number of samples</th>
<th>Conc. concentration</th>
<th># of controls</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qiagen</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>Qiagen + beads</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>TruTip</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>TruTip + beads</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>PowerFecal</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>13</td>
</tr>
<tr>
<td>TOTAL</td>
<td>20</td>
<td>25</td>
<td>21</td>
<td>71</td>
</tr>
</tbody>
</table>

Fig 7. Amplification curves obtained from the LightCycler 480

Fig 8. Scatter plot for Cp values obtained from High (A) and Low (B) concentration samples.

Table 2. Summary Real Time PCR results: Mean Cp values and DNA yield

<table>
<thead>
<tr>
<th>Extraction method</th>
<th>Cp Value</th>
<th>Concentration (pg/ul)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qiagen</td>
<td>33.3</td>
<td>0.013</td>
<td>0.015</td>
</tr>
<tr>
<td>Qiagen + beads</td>
<td>33.8</td>
<td>0.008</td>
<td>0.015</td>
</tr>
<tr>
<td>TruTip</td>
<td>33.8</td>
<td>0.009</td>
<td>0.032</td>
</tr>
<tr>
<td>TruTip + beads</td>
<td>33.7</td>
<td>0.010</td>
<td>0.025</td>
</tr>
<tr>
<td>PowerFecal</td>
<td>31.3</td>
<td>0.041</td>
<td>0.073</td>
</tr>
</tbody>
</table>

DISCUSSION AND CONCLUSIONS

Results show that the MoBio PowerFecal DNA Isolation Kit (mean Cp values 30.7 [high concentration] and 31.3 [low concentration]), followed by Akonni TruTip (Cp value 31.7 [high concentration] and 33.8 [low concentration]), are the most optimal methods for the recovery of DNA of Mycobacterium tuberculosis from stool samples inoculated with mycobacterial suspensions.

These methods should be tested with clinical samples to determine real levels of inhibition and which method would be the most effective to enhance the diagnosis of Mycobacterium tuberculosis in children.