A real time PCR (RT-PCR) assay based on \(tcdB\) gene detection has been developed by BD geneOhm™. We performed a prospective study to compare the performances of this assay to the cytotoxicity assay and the toxigenic culture for the diagnosis of CDI.

### Methods I

- **Inclusion and exclusion criteria**
  - 300 consecutive fresh diarrheal stools (stools taking the shape of the container) from patients suspected of having CDI were included.
  - Formed stools and stools collected for more than 36h were excluded.

- **Stool cytotoxicity assay (CTA):**
  - Initial dilution of stools 1:10 and filtration 0.22µ
  - A cytotoxic effect neutralized by C. sordellii antiserum is reported as a positive result.

- **Culture**
  - On selective medium (taurocholate, cycloserine, cefoxitin, agar containing 5% horse blood)
  - Incubation 48 hours in anaerobic atmosphere at 37°C

- **Toxigenic culture**
  - In case of toxin-negative and culture-positive stools, C. difficile isolates were incubated in Brain Heart Infusion broth for 5 days and the supernatant was tested using the cytotoxicity assay.

### Methods II

- RT-PCR (BD GeneOhm™ C. diff assay, BD geneOhm)
  - A dry swab was dipped into liquid stools or soft stool samples and resuspended in sample preparation buffer.
  - Genomic DNA was extracted using a lysis buffer combining chemical and physical actions.
  - Amplifications were performed using the Smart Cycler™ (Cepheid) with primers specific for \(tcdB\) and for an internal control.
  - Discrimination of amplicons was done using two beacons with different fluorimetric properties (TET, FAM).
  - In case of unresolved (internal control invalid due to the presence of inhibitory specimen or failure reagent) or invalid results (positive or negative control invalid) or in case of discordant results between the RT-PCR and the cytotoxicity assay and/or the toxigenic culture, the different tests were repeated.
  - The results of RT-PCR were not known by the technicians performing culture or cytotoxicity assay (blind test).

### Results

**Prevalence of toxigenic culture:** 11.4%

**Comparison of RT PCR vs. CTA**

<table>
<thead>
<tr>
<th>RT-PCR</th>
<th>CTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LNR</td>
<td>0</td>
</tr>
</tbody>
</table>

- Sensitivity: 96.1%
- Specificity: 95.1%
- NPV: 94.3%
- PPV: 96.7%
- Prevalence: 8.6%

*The stool presented with a positive toxigenic culture.*

**Comparison of RT PCR vs. Toxigenic culture**

<table>
<thead>
<tr>
<th>RT-PCR</th>
<th>Toxigenic culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LNR</td>
<td>0</td>
</tr>
</tbody>
</table>

- Sensitivity: 93.0%
- Specificity: 97.1%
- NPV: 93.0%
- PPV: 99.2%
- Prevalence: 11.4%

**% Unresolved results**

- 1st determination = 7.33%, 2nd determination = 3.33%

### Conclusions

The BD GeneOhm™ Cdiff Assay is a rapid and sensitive method for the detection of toxigenic C. difficile directly from stool samples. Performance is comparable to cytotoxicity and toxigenic culture with results available in about one hour.

### References