Comparison of BD GeneOhm Cdiff and Seegene Seeplex ACE PCR assays using toxigenic Clostridium difficile culture for direct detection of tcdB from stool specimens

Bo-Moon Shin, 1,2  Se Jin Mun, 1  Soo Jin Yoo, 1  Eun Young Kuak 1

Department of Laboratory Medicine, Sanggye Paik Hospital, Inje University, Seoul, South Korea, 1  Office of Infection Control, Sanggye Paik Hospital, Inje University, Seoul, South Korea, 2

Running Title : PCRs for direct detection of tcdB from stool

Corresponding Author ;
Bo-Moon Shin M.D., PhD.

Department of Laboratory Medicine and Office of Infection Control, Sanggye Paik Hospital, Inje University, Seoul, South Korea

Tel: +82-2-950-1227
Fax:+82-2-950-1244
Email: bmshin@unitel.co.kr
Abstract

We evaluated the performances of the 2 PCR assays (BD GeneOhm and Seegene ACE) for direct detection of \( \text{tcdB} \) from stool specimens. The concordance rate between BD and Seegene was 96.3%. The sensitivity, specificity, PPV, and NPV of BD and Seegene were 95.7%/96.5%/91.8%/98.2% and 90.0%/97.1%/92.6%/96.0%, respectively.

Key words: \textit{Clostridium difficile}, toxin B, culture, PCR
Nowadays, many laboratories have implemented PCR assays for the direct detection of toxigenic *Clostridium difficile* in stool specimens (1, 2, 5, 6). PCR assays have some limitations as a practical method because they require a special DNA extraction procedure to eliminate PCR inhibitors from stool specimens and PCR assays are more expensive to perform than enzyme immunoassay (EIA), culture, or cytotoxicity neutralization assay (CCNA) (6, 15). However, the increasing mortality and morbidity rates and the increasing number of recurrence associated with *C. difficile* infection (CDI) demand the need of a rapid and reliable method for direct detection of toxigenic *C. difficile* in stool specimens (10). The BD GeneOhm Cdiff assay (Becton Dickinson Diagnostics, San Diego, CA, USA; BD) is a real-time PCR assay for *tcdB*, which was approved by the Food and Drug Administration (FDA). The Seeplex Diarrhea-B1 ACE Detection assay (Seegene Diagnostics, Seoul, Korea; Seegene) is a newly developed multiplex PCR assay using proprietary primers specific to *tcdB* and other enteropathogens (*Salmonella* spp., *Shigella* spp., *Vibrio* spp., and *Campylobacter* spp.). In this study, we focused only on *tcdB* detection. Seegene contains 5 pairs of primers adopting a dual priming oligonucleotide (DPO) system. Each primer consists of 2 separate priming regions and a polydeoxyinosine linker. A shorter 3’-segment of primer is designed to block non-specific annealing and the other 5’-segment initiates stable annealing. The linker contributes to the lowering of melting temperature in spite of the length of primer with more than 35 bases. The aim of this study was to evaluate the performance of these 2 commercial PCR assays (BD and Seegene) for *tcdB*, and to compare the results with toxigenic culture for direct detection of *tcdB* in stool specimens. This is the first study to evaluate the performance of the Seeplex Diarrhea-B1 ACE Detection assay in detecting *tcdB* in clinical specimens. A total of 243 fresh stool specimens were collected from patients with clinical signs compatible with CDI who were hospitalized to 3 teaching hospitals in Seoul City during a 3-month period between October and December of 2010. Toxigenic culture was performed as previously...
Semi-quantitative culture for *C. difficile* was performed and the extent of growth was rated as follows: grade 1, <10 colonies; grade 2, 10-50 colonies; grade 3, 51-100 colonies; and grade 4, >100 colonies. All 86 culture positive isolates were tested using a laboratory developed multiplex PCR assay detecting *tcdA*, *tcdB* and triose phosphate isomerase (*tpi*) as described previously (9). BD GeneOhm assay was performed according to the manufacturer’s instructions as described previously (7, 17). Seeplex Diarrhea-B1 ACE detection assay was performed according to the manufacturer’s instructions. After manual DNA extraction via the QIAamp DNA stool minikit (Qiagen, Crawley, UK), amplification was performed on the GeneAmp PCR system 9700 (AB Applied Biosystems, CA, USA). The PCR products were resolved by capillary electrophoresis on the ScreenTape System (Lab 901 Tape station, UK). Results were reported after about 4 hr.

Of the 243 stool specimens, the culture positivity rate was 35.4% (86/243). Of the 86 *C. difficile* isolates, 65 (75.6%), 5 (5.8%), and 16 (18.6%) were *tcdA*⁺*tcdB*⁺, *tcdA*⁻*tcdB*⁺ and *tcdA*⁻*tcdB*⁻ strains, respectively, and all of them were *tpi* positive. We placed the stool specimens containing *tcdA*⁺*tcdB*⁺ and *tcdA*⁻*tcdB*⁺ positive isolates in a hypothetical *C. difficile* positive (*tcdB*⁺) group, and the stool specimens containing *tcdA*⁻*tcdB*⁻ isolates and culture-negative results in a hypothetical *C. difficile* negative (*tcdB*⁻) group. On the basis of these results, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of BD were 95.7% (67/70), 96.5% (167/173), 91.8% (67/73) and 98.2% (167/170), respectively, and those of the Seegene were 90.0% (63/70), 97.1% (168/175), 92.6% (38/43) and 96.0% (168/175), respectively. There were no significant differences between BD and Seegene in sensitivity (*P* = 0.325) and specificity (*P* = 0.683).

The concordance rate between BD and Seegene was 96.3% (234/243) (Table 1).

Performances of various PCR assays for *tcdB* have been evaluated by several authors, who have shown a sensitivity ranging from 77.3% to 100% and a specificity ranging from 93% to 99% (1, 2, 4, 5, 11, 15-18). The sensitivities and specificities of BD have been
reported to be between 84% and 96% and between 94% and 99%, respectively, depending on comparative methods (7, 8, 14, 17). Thus, the sensitivities and specificities of BD and Seegene in our study represented reliable performances compared to previous PCR studies for tcdB from stool specimens.

For the 234 concordant cases, there were 1 culture negative case and 2 tcdB- cases in 66 BD/Seegene co-positive specimens, and 3 tcdB+ cases in 168 BD/Seegene co-negative specimens. Culture was the most sensitive method for the detection of toxigenic C. difficile, but it also missed some positive cases (3, 13). This may be the explanation for culture- /BD+/Seegene+ cases. There may be some mixed colonies of tcdB- and tcdB+ strains in a primary culture plate, but only colonies of tcdB can be picked up for toxigenic culture assay. These may explain the 2 tcdB+/BD+ /Seegene+ cases. The grades of semi-quantitative culture of these 2 cases all belonged to grade 4, and some non-toxigenic colonies appeared to be mixed among toxigenic C. difficile colonies. The 3 tcdB+ cases out of 168 BD/Seegene co-negative specimens could be explained by their low yields of colonies on each agar plate (actually less than 5 CFU by semi-quantitative culture in each case). In our study, the positivity rates of BD and Seegene correlated with those of semi-quantitative cultures (Table 2). The positive rates of BD and Seegene were 70% and 60% in grade 1, respectively, but sharply increased from grade 2 (100% in grades 2, 3 and 4 for BD; 84.2% in grade 2 and 100% in grades 3 and 4 for Seegene) compared to grade 1 (Chi-square for trend, \( P = 0.003 \) in BD and \( P < 0.001 \) in Seegene). These results suggest that although both PCR assays may be very highly sensitive, the detection rates of the PCRs may partly depend on the amount of tcdB in stools, and that a false negative PCR result may consequently be associated with a low number of toxigenic C. difficile in stools (13). The 9 discordant cases between results of BD and Seegene (4 tcdB+ cases and 3 tcdB- cases in the BD+/Seegene- specimens, and 1 culture negative case and 1 tcdB- case in the BD-/Seegene+ specimens) may also be explained by lower grades in semi-quantitative culture of cases (4 tcdB+ cases in the BD+/Seegene-
specimens were grade 1 in *C. difficile* culture) or mixed colonies on a culture plate. Storage condition of stool specimens, such as freezing/thawing between cultures and PCR assays, may be another cause of culture+/PCR assay- cases.

In conclusion, both of the 2 commercial PCR assays, BD and Seegene, allows for a rapid and reliable method for direct detection of *tcdB* in stool specimens.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0022347).

References


Table 1. Comparison of the results of BD GeneOhm PCR and Seegene Seeplex PCR for tcdB based on toxigenic C. difficile culture in stool specimens.

<table>
<thead>
<tr>
<th>BD</th>
<th>Seegene</th>
<th>Culture positive (n=86)</th>
<th>Culture negative (n=157)</th>
<th>Total (n=243)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>tcdA</td>
<td>tcdB</td>
<td>tcdA tcdB</td>
<td>tcdA tcdB</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>59</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>3</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>65</td>
<td>5</td>
<td>16</td>
</tr>
</tbody>
</table>

Concordance rate between BD GeneOhm and Seegene: 234/243= 96.3%.
Table 2. Correlation between the results of BD GeneOhm and Seegene Seeplex PCRs in semi-quantitative toxigenic *C. difficile* culture positive cases.

<table>
<thead>
<tr>
<th>Grade</th>
<th>No. of toxigenic culture (+) cases</th>
<th>BD (+) cases (%)</th>
<th>Seegene (+) cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>7 (70.0)</td>
<td>6 (60)</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>19 (100)</td>
<td>16 (84.2)</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>17 (100)</td>
<td>17 (100)</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>24 (100)</td>
<td>24 (100)</td>
</tr>
<tr>
<td>Total</td>
<td>70</td>
<td>67 (95.7)</td>
<td>63 (90.0)</td>
</tr>
</tbody>
</table>

*Extent of growth of semi-quantitative culture for *C. difficile*: grade 1 (<10 colonies), grade 2 (10-50 colonies), grade 3 (51-100 colonies), grade 4 (>100 colonies)