Comparison of a Commercial Multiplex Real-Time PCR to the Cell Cytotoxicity Neutralization Assay for Diagnosis of *Clostridium difficile* Infections

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A commercial multiplex real-time PCR assay (Cepheid Xpert *C. difficile* assay) for the diagnosis of *Clostridium difficile* infection was evaluated. The sensitivity and specificity of the Cepheid assay were 97.1% and 93.0% for fresh stools, using the cell cytotoxicity neutralization assay as the reference. Using PCR ribotyping as the reference for ribotype 027 strains, the corresponding figures were 100% and 98.1%, respectively.

*Clostridium difficile* infection (CDI) has increased in frequency and severity in North America and Europe over the last 5 years, largely due to the emergence of the epidemic PCR ribotype 027 strain (10, 11). The diagnosis of CDI is usually based on a clinical history of recent antimicrobial usage and diarrhea in combination with laboratory tests (9). Therefore, rapid and accurate microbiological diagnosis is urgently needed. The Cepheid Xpert *C. difficile* assay (Sunnyvale, CA) is a real-time multiplex PCR assay performed on the Cepheid GeneXpert Dx system. Proprietary primers specific for the toxin B gene (*tcdB*), binary toxin genes (*cdaA* and *cdbB*), and *tcdC* gene single-base deletion at nucleotide 117 were designed to detect toxigenic *C. difficile* and the presumptive PCR ribotype 027 strain. The purpose of this study was to evaluate the Cepheid Xpert *C. difficile* multiplex real-time PCR assay for the detection of toxigenic *C. difficile* strains and the presumptive ribotype 027.

There were four serial investigations in the present study. In investigation 1, 205 frozen *C. difficile* strains collected during 2007 and 2008 were analyzed. In investigation 2, 195 frozen stool specimens belonging to different categories were selected based on direct cell cytotoxicity neutralization assay (CCNA) and toxigenic anaerobic culture results. Because PCR ribotype 027 is uncommon in Sweden, 40 frozen stool specimens collected in the United States were also analyzed. In investigation 3, 30 pairs of fresh-frozen stool specimens were analyzed. The fresh stool was analyzed within 24 h of collection, and then the leftover was stored at −20°C for 3 days and retested. In investigation 4, 220 consecutive fresh, unfrozen stool specimens (Bristol Stool Chart grade 5 to 7) from patients older than 2 years were analyzed within 24 h of collection. Eligible participants were those symptomatic patients who had a stool sample submitted to the Karolinska University Hospital for routine *C. difficile* testing.

Unrepeated strains and stools were determined for *C. difficile* test by CCNA with a commercial *C. difficile* toxin/antitoxin kit (TechLab, Blacksburg, VA). For the stool specimens, anaerobic cultures on selective taurocholate cycloserine-cefoxitin-fructose agar plates were also performed (13). All isolates were typed by PCR ribotyping (19).

Concurrently, the Cepheid Xpert *C. difficile* assay was performed according to protocols provided by the manufacturer. Each kit contained single-use disposable cartridges with integrated reaction chambers and reagents. A sterile Copan swab was dipped into the stool specimen or used to pick one fresh *C. difficile* colony from the blood agar plate, and the stool sample or colony was resuspended in sample buffer and then transferred to the cartridge. The cartridge was placed in the GeneXpert Dx module and run. Every PCR run included a sample-processing control and a probe check control. Each day, a positive control (*C. difficile* ATCC 9689 or the ribotype 027 strain) and a negative control (diluted *C. difficile*-negative stool sample) provided by Cepheid were tested. Results were automatically interpreted by the software as follows: “*C. difficile* positive,” “*C. difficile* 027 NAP1 presumptive positive,” “*C. difficile* negative,” “invalid,” “error,” or “no result.” If any of the test results was “invalid,” “error,” or “no result,” the sample was retested.

CCNA and strain typing of the isolates were used as the reference standards for all of the investigations. If discrepant results for stool specimens were obtained with the Cepheid assay and CCNA, the results of CCNA for recovered isolates were taken into consideration. Sensitivity, specificity, positive and negative predictive values, and 95% confidence interval (CI) were calculated using SAS.

The agreement between the Cepheid assay and CCNA for 205 isolates, 235 frozen stool specimens, and 220 fresh stools was 99.5%, 89.4%, and 93.6%, respectively (Table 1). Mixed populations of isolates with different toxin profiles (toxin B negative and toxin B positive) and different PCR ribotypes were found in four fecal samples. The real-time PCR cycle threshold values for frozen-thawed samples matched those for fresh samples (data not shown). In investigation 4, the initial evaluation of the stool specimens yielded 95.7% sensitivity and...
87.3% specificity, respectively. The discrepancies between the assays are listed in Table 2. Upon reevaluation by toxigenic anaerobic culture, the sensitivity and specificity increased to 97.1% and 93.0%, respectively.

Only one PCR ribotype 027 presumptive positive strain was detected by the Cepheid assay in investigation 1; this result was verified by PCR ribotyping. In investigation 2, the presumptive ribotype 027 strain was detected in 24 of the 235 samples by the Cepheid assay; 24 were verified by PCR ribotyping. The sensitivity, specificity, and positive and negative predictive values of the Cepheid assay were 100%, 98.1% (95% CI, 96.3 to 99.9%), 85.7% (95% CI, 72.8 to 98.7%), and 100%, respectively. No ribotype 027 strain was detected in investigations 3 and 4.

The difficulty of choosing an optimal test for the diagnosis of CDI has long been known. Toxigenic C. difficile detection by CCNA is considered to be the “gold standard” but is time consuming (24 to 48 h) and requires cell culture facilities. Anaerobic culture has sensitivity approaching 100%, but the false-positive rate exceeds 10% because of the high rate of asymptomatic carriage (8, 15). Toxin enzyme immunoassays are more rapid but are associated with widely varying sensitivities (69% to 99%) and specificities (92% to 100%), making their reliability questionable if used as stand-alone assays (1, 2, 14). A two-stage testing strategy with an initial rapid screening test to identify positive samples that were then confirmed by a reference method was suggested, but it still delays detection and has been reported to have varying results in some institutions (5, 6, 16).

Several reports of the use of real-time PCR for the detection of toxigenic C. difficile have appeared over the past few years. In the present study, the overall agreement between the results of the Cepheid assay and CCNA was similar to the results of previous in-house or commercial real-time PCR evaluations (3, 17–18, 21–22). The discrepancies between Cepheid assay and CCNA results may represent false positives or false negatives. This could be due to the fact that the Cepheid assay detects the gene encoding toxin B rather than the functional toxin. Hence, false-negative results may be due to aberrant tcdB genes (12) and the number of nontoxigenic strains being higher than the number of toxigenic strains, since multiple strains (determined by PCR ribotyping) may coexist simultaneously in the stool (20). False-positive results may be due to antibiotic treatment prior to receiving a sample for analysis; in this case, the DNA might still be present but toxin and/or culture might be negative. Finally, it has been reported that toxin is not uniformly distributed in stool samples (4).

One of the significant advantages of the Cepheid assay is predicting the presence of the ribotype 027 strain. It can greatly facilitate the tracing of outbreaks, and with the recognition of ribotype 027 in an institution, quinolone restriction may be an important component of the infection control strategy (7). In investigation 2, the Cepheid assay was 100% sensitive and 98.1% specific for the diagnosis of ribotype 027, using PCR ribotyping as the reference. However, in investigations 3 and 4 in which fresh fecal samples from Swedish patients were analyzed, no ribotype 027 strain was found. More ribotype 027-positive fresh samples are needed for further evaluation.

Another important advantage of the Cepheid assay in the clinical microbiology field is the rapidity that it offers. The procedure takes 1 h from specimen processing in the laboratory to reporting the results and is easier to perform than CCNA or conventional PCR.

In conclusion, the Cepheid Xpert C. difficile assay offers sensitivity and specificity for toxin B detection that are comparable to those of the CCNA reference method. With the

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### TABLE 1. Comparison between the Cepheid Xpert C. difficile assay and CCNA

<table>
<thead>
<tr>
<th>Investigation (no. of samples)</th>
<th>CCNA result</th>
<th>No. of samples with indicated Cepheid Xpert C. difficile assay result</th>
<th>Performance [% (95% CI)] of Cepheid Xpert C. difficile assay with CCNA&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>1 (205)</td>
<td>Positive</td>
<td>187</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>2 (235)</td>
<td>Positive</td>
<td>152</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>16</td>
<td>58</td>
</tr>
<tr>
<td>4 (220)</td>
<td>Positive</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>13</td>
<td>172</td>
</tr>
</tbody>
</table>

<sup>a</sup> PPV, positive predictive value; NPV, negative predictive value.

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### Table 2. Characterization of specimens with initial discrepant results in the Cepheid Xpert C. difficile assay versus CCNA in investigation 4

<table>
<thead>
<tr>
<th>No. of samples</th>
<th>Initial testing</th>
<th>Isolate testing</th>
<th>Final C. difficile assignment&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cepheid assay&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Culture</td>
<td>Cepheid assay</td>
</tr>
<tr>
<td>1</td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
</tr>
<tr>
<td>12</td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>12</td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>1</td>
<td>Pos</td>
<td>Neg</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> Neg, negative; Pos, positive; ND, not done (the isolate could not be recovered).

<sup>b</sup> Cepheid Xpert C. difficile assay for toxin B.
results available within 1 h, it provides prompt and precise laboratory diagnosis.

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REFERENCES


