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 Clostrid-
ium 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 (cdtA and cdtB), and tcdC gene single-base deletion at nucleotide 117 were designed
to detect toxigenic C. difficile and the presumptive PCR ri-
botype 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 col-
lected in the United States were also analyzed. In investigation 3, 30 pairs of fresh-frozen stool specimens 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 inves-
tigation 4, 220 consecutive fresh, unformed stool specimens
(Bristol Stool Chart grade 5 to 7) from patients older than 2
years were analyzed within 24 h of collection. Eligible partic-
ipants 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. diffi-
cile test by CCNA with a commercial C. difficile toxin/antitoxin
kit (TechLab, Blacksburg, VA). For the stool specimens, ana-
erobic cultures on selective taurocholate cycloserine-cefox-
itin-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 inte-
grated 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
was resuspended in sample buffer and then transferred to the
cartridge. The cartridge was placed in the Gene-
Xpert 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 auto-
matically 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 sam-
ple 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
TABLE 1. Comparison between the Cepheid Xpert \textit{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 \textit{C. difficile} assay result</th>
<th>Performance [% (95% CI)] of Cepheid Xpert \textit{C. difficile} assay with CCNA$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>94.4 (83.9–105.3)</td>
</tr>
<tr>
<td>1 (205)</td>
<td>Positive</td>
<td>187</td>
<td>94.4 (90.9–98.0)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>1</td>
<td>97.1 (91.6–102.7)</td>
</tr>
<tr>
<td>2 (235)</td>
<td>Positive</td>
<td>152</td>
<td>95.5 (91.9–98.1)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>16</td>
<td>97.7 (94.6–100.0)</td>
</tr>
<tr>
<td>4 (220)</td>
<td>Positive</td>
<td>34</td>
<td>97.8 (95.3–100.3)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>13</td>
<td>98.1 (95.7–100.5)</td>
</tr>
</tbody>
</table>

$^a$ PPV, positive predictive value; NPV, negative predictive value.

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 28 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 \textit{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, 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 \textit{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 \textit{C. difficile} assay offers sensitivity and specificity for toxin B detection that are comparable to those of the CCNA reference method. With the

TABLE 2. Characterization of specimens with initial discrepant results in the Cepheid Xpert \textit{C. difficile} assay versus CCNA in investigation 4$^a$

<table>
<thead>
<tr>
<th>Initial testing</th>
<th>Isolate testing</th>
<th>Final C. difficile assignment$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cepheid assay</td>
<td>Culture</td>
<td>Cepheid assay</td>
</tr>
<tr>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>Neg</td>
<td>ND</td>
<td>Neg</td>
</tr>
</tbody>
</table>

$^a$ Neg, negative; Pos, positive; ND, not done (the isolate could not be recovered).

$^b$ Cepheid Xpert \textit{C. difficile} assay for toxin B.

$^c$ The final \textit{C. difficile} assignment was based on the combination of CCNA results for stool specimens and isolates.
results available within 1 h, it provides prompt and precise laboratory diagnosis.

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We declare no conflict of interest.

REFERENCES


