Clostridium difficile is the leading cause of antibiotic-associated diarrhea and pseudomembranous colitis in health care settings (6). Several laboratory-developed PCR assays for detection of C. difficile infection (CDI) have been described, and they have demonstrated favorable performances in comparative studies (3, 7, 10, 14). We previously described a real-time PCR assay using a LightCycler (Roche Diagnostics, Indianapolis, IN), which we refer to herein as the LC-CDTX assay (11). We compared two commercial FDA-approved real-time PCR assays, the BD GeneOhm Cdiff assay (San Diego, CA) and the Prodesse ProGastro CD assay (Waukesha, WI), to our laboratory-developed assay.

The study was approved by the Institutional Review Board, Mayo Clinic, Rochester, MN. Three hundred forty-six soft or liquid stool specimens submitted to the Clinical Microbiology Laboratory, Mayo Clinic, from different patients for laboratory-developed assay.

For LC-CDTX assay extraction, swabs were inserted into the stool sample and transferred into 1 ml of sterile water. After the sediment settled, 200 μl of supernatant was extracted using a total nucleic acid isolation kit (Roche Diagnostics) with a MagNA Pure system (Roche Diagnostics) and eluted into 100 μl of elution buffer. For sample lysis with the BD GeneOhm Cdiff assay, swabs were dipped into the stool sample, broken into 1 ml of sample buffer, and vortexed. Ten microliters of sample was transferred into a lysis tube containing 40 μl of sample buffer, vortexed for 5 min, centrifuged, heated at 95°C for 7 min, and placed on ice. For ProGastro CD assay extraction, approximately 100 μl of stool was added to 400 μl of stool transport and recovery buffer (S.T.A.R. buffer; Roche Diagnostics), vortexed, and centrifuged for 1 min at 13,000 × g. Twenty microliters of the cleared stool supernatant and 10 μl of the ProGastro CD assay internal control (IC) were extracted using bioMérieux easyMAG (bioMérieux, Durham, NC) and eluted into 110 μl of elution buffer.

The LC-CDTX assay detects tcdC, a downregulator of toxin production present in toxigenic C. difficile, and also, via melting curve analysis, detects 18- and 39-bp deletions in tcdC, which have previously been associated with increased toxin production. The assay was run as previously described (11), by using a LightCycler 2.0 real-time PCR system with software to detect the fluorescence resonance energy transfer (FRET) probes labeled with LC Red 640. The BD GeneOhm Cdiff assay targets tcdB of C. difficile and the IC, which was incorporated into the master mix. The assay was run with the SmartCycler instrument (Cepheid, Sunnyvale, CA) according to the manufacturer’s instructions, with Dx software to detect the probes labeled with 6-carboxyfluorescein (FAM) for detection of tcdB and TET (tetrachloro-6-carboxyfluorescein) for detection of the IC. The samples were run using the established BD GeneOhm amplification protocol. The ProGastro CD assay targets tcdB and the IC, which was incorporated into the master mix during the extraction. The assay was run using a SmartCycler instrument according to the manufacturer’s instructions, with Dx software to detect probes labeled with FAM for detection of tcdB and Cy5 for detection of the IC.

Testing of any specimen found to have a discrepant result by any of the assays was repeated with all three assays. Anaerobic culture was performed with discrepant specimens using taurocholate-cycloserine-cefoxitin-fructose agar (TCCFA). TCCFA plates were inoculated with fresh stool and examined after anaerobic incubation for 2 days at 35°C. Presumptive C. difficile colonies were identified by morphology. Several isolated colonies were inoculated into anaerobic broth. PCR by all three assays was repeated by processing the broth as a stool specimen (toxigenic culture).

By the LC-CDTX assay, 36 samples were positive and 310 were negative. By the ProGastro CD assay, 37 samples were positive and 309 were negative. By the BD GeneOhm Cdiff assay, 33 samples were positive and 313 were negative. By all methods, 304 of 346 samples were negative and 29 of 346 were positive. There was agreement among all methods in 333 of 346 cases (96%). The remaining 13 samples were discrepant by
one or more methods (Table 1). Of the 13 discrepant samples, 8 were considered true positives due to positive toxigenic cul-
tures, and 5 were considered true negatives due to lack of
growth in culture. Therefore, the total number of true positives
was 37 of 346, and the total number of true negatives was 309
of 346.

For 7 of 8 discrepant samples which grew in culture, all three
PCR assays were positive after inoculation of the isolates into
broth, confirming toxin production. For one of the discrepant
samples, there was growth on the plate; however, the isolate
did not grow after inoculation into broth, and toxigenicity thus
could not be definitively established. This was still considered
to be a true positive result, because two PCR assays were
positive by the original analysis and the plate culture was pos-
itive.

By using positive results by all three molecular assays and/or
a positive toxigenic culture result as the “gold standard,” the
sensitivities and specificities, respectively, of the three assays
were 94.6% and 99.7% for the LC-CDTX assay (P
value, 1.0) (Table 2). The performance characteristics of the
three assays were compared to each other using McNemar’s
test (Table 3). All P values were >0.05, indicating no statisti-
cally significant difference among the three assays in sensitivity
or specificity.

Many studies have examined testing methods and algorithms
to determine the optimal approach for detection of CDI (8,
12–13). Although some studies found that enzyme immunoass-
say (EIA) compared favorably to cytotoxicity assays (2, 9),
several subsequent studies found EIA to have low sensitivity
and specificity compared to those of both toxigenic culture and
PCR (1, 5, 11). Also, some concluded that the performance of
PCR is superior to that of other available methods (3, 7, 10–11,
14). Although commercial FDA-approved assays have been
evaluated compared to cytotoxicity assays and culture (4), our
study is the first, to our knowledge, to compare two commerci-
ally available PCR methods to each other and to a laboratory-
developed PCR assay with previously published performance
characteristics.

One limitation is that we did not perform cultures of spec-
imens that gave concordant results by all three molecular
assays. It is possible that we missed true positives that gave
false-negative results by all three molecular assays. Conversely,
it is possible that there were false positives by all three assays.
Both scenarios are unlikely, however, because the performance
of the LC-CDTX assay has previously been compared to that
of toxigenic culture, and the LC-CDTX assay demonstrated
high sensitivity and specificity (11).

### TABLE 1. Discrepant analysis

<table>
<thead>
<tr>
<th>Assay and result</th>
<th>Toxigenic culture confirmation (no. of samples)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pos</td>
</tr>
<tr>
<td>LC-CDTX</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>BD GeneOhm Cdiff</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>ProGastro CD</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>3</td>
</tr>
</tbody>
</table>

* Shown is a confirmation of the discrepant results from the three *Clostridium difficile* PCR assays by selective culture and broth confirmation of the toxin gene by PCR (toxigenic culture). Pos, positive; Neg, negative.

### TABLE 2. Comparison of molecular assay results and “gold standard” results

<table>
<thead>
<tr>
<th>Assay and result</th>
<th>“Gold standard” result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of samples:</td>
</tr>
<tr>
<td></td>
<td>Sensitivity (%)</td>
</tr>
<tr>
<td>LC-CDTX</td>
<td>Pos 35 Neg 2</td>
</tr>
<tr>
<td>BD GeneOhm Cdiff</td>
<td>Pos 31 Neg 6</td>
</tr>
<tr>
<td>ProGastro CD</td>
<td>Pos 34 Neg 3</td>
</tr>
</tbody>
</table>

* Included are results of all three positive molecular assays and/or positive toxigenic cultures. Pos, positive; Neg, negative.

### TABLE 3. Comparison of performance characteristics of three molecular assays

<table>
<thead>
<tr>
<th>Performance characteristic and assays being compared</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>LC-CDTX and BD GeneOhm Cdiff</td>
</tr>
<tr>
<td></td>
<td>LC-CDTX and ProGastro CD</td>
</tr>
<tr>
<td></td>
<td>BD GeneOhm Cdiff and ProGastro CD</td>
</tr>
<tr>
<td>Specificity</td>
<td>LC-CDTX and BD GeneOhm Cdiff</td>
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<tr>
<td></td>
<td>LC-CDTX and ProGastro CD</td>
</tr>
<tr>
<td></td>
<td>BD GeneOhm Cdiff and ProGastro CD</td>
</tr>
</tbody>
</table>

### REFERENCES

8. Eastwood, K., P. Else, A. Charlett, and M. Wilcox. 2009. Comparison of nine commercially available *Clostridium difficile* toxin detection assays, a real-time