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Comparison of Two Commercial Molecular Assays to a Laboratory-Developed Molecular Assay for Diagnosis of *Clostridium difficile* Infection▽

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We compared two commercial PCR assays, the Prodesse ProGastro CD assay and the BD GeneOhm Cdiff assay, with a laboratory-developed *Clostridium difficile* toxin PCR assay with previously established performance characteristics. Results of all methods were in agreement for 333 (96%) of 346 stool specimens. No significant difference in performance among the assays was found (*P* values, >0.05).

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 *C. difficile* testing by PCR assay (LC-CDTX assay) were used to compare three molecular assays.

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

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TABLE 1. Discrepant analysis[a]

Assay and result	Toxigenic culture confirmation (no. of samples)	
	Pos	Neg
LC-CDTX		
Pos	6	1
Neg	2	4
BD GeneOhm Cdiff		
Pos	2	2
Neg	6	3
Prodesse ProGastro CD		
Pos	5	3
Neg	3	2

[a] 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.

one or more methods (Table 1). Of the 13 discrepant samples, 8 were considered true positives due to positive toxigenic cultures, 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 positive.

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, 0.56), 83.8% and 99.4% for the BD GeneOhm Cdiff assay (*P* value, 0.16), and 91.9% and 99.0% for the ProGastro CD 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 statistically significant difference among the three assays in sensitivity or specificity.

TABLE 2. Comparison of molecular assay results and "gold standard" results[a]

Assay and result	"Gold standard" result			
	No. of samples:		Sensitivity (%)	Specificity (%)
	Pos	Neg		
LC-CDTX				
Pos	35	1	94.6	99.7
Neg	2	308		
BD GeneOhm Cdiff				
Pos	31	2	83.8	99.4
Neg	6	307		
ProGastro CD				
Pos	34	3	91.9	99.0
Neg	3	306		

[a] 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

Performance characteristic and assays being compared	*P* value
Sensitivity	
LC-CDTX and BD GeneOhm Cdiff	0.10
LC-CDTX and ProGastro CD	0.65
BD GeneOhm Cdiff and ProGastro CD	0.18
Specificity	
LC-CDTX and BD GeneOhm Cdiff	0.32
LC-CDTX and ProGastro CD	0.32
BD GeneOhm Cdiff and ProGastro CD	0.65

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 immunoassay (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 commercially 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 specimens 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).

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