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Comparison of a Commercial Real-Time PCR Assay for *tcdB* Detection to a Cell Culture Cytotoxicity Assay and Toxigenic Culture for Direct Detection of Toxin-Producing *Clostridium difficile* in Clinical Samples[▽]

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Rapid detection of toxin-producing strains of *Clostridium difficile* is essential for optimal management of patients with *C. difficile* infection. The BD GeneOhm (San Diego, CA) Cdiff assay, a real-time PCR assay that amplifies *tcdB*, was compared to a cell culture neutralization assay (Wampole *C. difficile* Toxin B [TOX-B] test; TechLab, Blacksburg, VA) and to toxigenic culture. Using liquid ($n = 273$) and soft ($n = 131$) stool specimens from 377 symptomatic patients, all testing was performed on the same day by independent laboratory staff according to the manufacturers' protocols. Toxigenic bacterial culture was performed as follows. A 0.5-ml aliquot of stool was heated to 80°C for 10 min, followed by inoculation onto modified cycloserine cefoxitin fructose agar with and without horse blood (Remel, Lenexa, KS) and into prereduced chopped-meat broth. Of the 404 stool specimens tested, 340 were negative and 40 were positive (10.0% prevalence) both by PCR for *tcdB* and by cytotoxin production. The overall agreement between the BD GeneOhm Cdiff assay and the TOX-B test was 94.8% (380/401). When the TOX-B test was used as the reference method, the initial sensitivity, specificity, and positive and negative predictive values of the BD GeneOhm Cdiff assay were 90.9% (40/44), 95.2% (340/357), 70.2% (40/57), and 98.8% (340/344), respectively. When toxigenic culture was used as the "gold standard," the sensitivity, specificity, and positive and negative predictive values of the BD GeneOhm Cdiff assay were 83.6%, 98.2%, 89.5%, and 97.1%, respectively, and those of the TOX-B test were 67.2%, 99.1%, 93.2%, and 94.4%, respectively. PCRs for three samples were inhibited upon initial testing; one sample was resolved upon retesting. One sample produced nonspecific cytotoxin results. The BD GeneOhm Cdiff assay performed well compared to a standard cell culture neutralization assay and to toxigenic culture for the detection of toxigenic *C. difficile* directly from fecal specimens.

Infection with *Clostridium difficile* can cause asymptomatic colonization or a spectrum of clinical manifestations ranging from mild diarrhea to severe colitis, the latter often resulting in life-threatening complications such as pseudomembrane formation, toxic megacolon, and sepsis (13, 14, 16, 20). The epidemiology of *Clostridium difficile* infection has changed over the past decade (2, 8, 14, 16). Hypervirulent *C. difficile* epidemic strains (BI/NAP1/027) cause severe illness, which often requires colectomy for control and results in increased mortality (14, 16). These strains are more likely to spread in the hospital environment due to antimicrobial resistance and enhanced spore formation (1, 2, 14). *C. difficile* infection is being recognized more frequently in the community as well (4, 6).

Early recognition of *C. difficile* infection has a profound effect on proper disease management (12). A rapid yet sensitive and specific diagnostic assay would be advantageous to clinicians for the early recognition of disease and to infection control practitioners for swift implementation of control measures.

A variety of diagnostic methods exist for the detection of *C. difficile* in stool samples. A cell culture cytotoxicity neutralization assay (CCNA) is generally considered the optimal individual standard for the detection of toxigenic *C. difficile*. However, CCNA testing is labor-intensive, subjective, and time-consuming and therefore is not an ideal standard. Traditional enzyme-linked immunosorbent assays and lateral-flow devices for the detection of toxins A and B are relatively quick but lack sensitivity when used alone (10) and often lack specificity (15, 18, 19, 26, 27). Algorithms combining a sensitive antigen test for glutamate dehydrogenase (GDH) as a screening tool with a specific toxin confirmatory test have been utilized to enhance the detection of *C. difficile* infection (9, 10, 23, 27, 28). However, performance of a two-step algorithm often delays detection, has been reported to be variable in some institutions (9, 10), and may be impacted by staffing and financial constraints.

Anaerobic culture for *C. difficile* using a selective medium is the most sensitive method when one tests the recovered isolates for cytotoxin production (5, 7, 22, 23, 25, 26), but several days are required to complete all testing. Molecular assays that detect toxin A or B, or both, show promise, but to date no commercial diagnostic PCR platforms available in the United States have been extensively evaluated in a routine clinical

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laboratory for direct detection of toxigenic *C. difficile* in stool specimens (3, 11, 22, 26, 30–32). The BD GeneOhm (San Diego, CA) Cdiff assay is a real-time multiplex PCR assay performed on the Cepheid (Sunnyvale, CA) SmartCycler. Proprietary primers specific to the toxin B gene (*tcdB*) and an internal control (IC) amplify the *tcdB* target sequence, which is detected using proprietary molecular beacons.

This study was an industry-sponsored Food and Drug Administration clinical trial [510(k)] to evaluate the performance of the BD GeneOhm Cdiff assay in detecting toxin B-producing *C. difficile* in stool specimens by using the Wampole *C. difficile* Toxin B (TOX-B) test (TechLab, Blacksburg, VA) as the reference method. In a separate analysis, both assays were compared to toxigenic anaerobic culture as the "gold standard."

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MATERIALS AND METHODS

Patients and specimens. This study was performed in a 900-bed tertiary-care university medical center with several intensive care units and active oncology, solid-organ transplantation, and bone marrow transplantation programs. Eligible participants were those symptomatic adult patients who had a stool sample submitted to the clinical microbiology laboratory of the Johns Hopkins Hospital for routine *C. difficile* testing. Liquid or soft stool specimens were submitted in sterile containers. Participants could contribute a second sample provided that the samples were collected more than 5 days apart. This study was approved by the Johns Hopkins University School of Medicine Institutional Review Board.

Specimen processing. After completion of the standard-of-care clinical testing, stool specimens were held at 4°C until processing for PCR, CCNA, and toxigenic culture. Specimen processing and medium inoculation were performed in a biological safety cabinet. Prior to processing, samples were well mixed and then split using a sterile disposable graduated pipette. A 0.5-ml aliquot of the liquid or soft stool specimen was transferred from the original collection container to a sterile polystyrene culture tube (12 by 75 mm) for further PCR and cytotoxin processing, and a 0.5- to 1-ml aliquot of stool sample was transferred to a sterile glass test tube for culture.

BD GeneOhm Cdiff assay. A sterile Dacron swab was dipped into the stool specimen and then broken off into the sample buffer tube containing the Tris-EDTA sample preparation buffer that was provided by the manufacturer. The suspension in the sample buffer was vortexed at high speed for 1 min. In order to dilute the specimen, 40 μl of uninoculated sample buffer was added to a lysis tube with glass beads before transfer of 10 μl of sample buffer containing the stool sample suspension. The lysis tube was vortexed for 5 min at high speed and received a 10-s spin in the Labnet Spectrafuge minicentrifuge (relative centrifuge force, ≈6,000 rpm, or 2,000 × *g*) to bring the contents to the bottom of the tube before inactivation at 95°C in a dry block for 5 min. The lysed, inactivated sample was kept on a cooling block at 3 to 5°C until testing was performed, within 4 h. Sample tube and reagent manipulations were performed in a dead-air box, and tubes were kept on cold blocks at 3 to 5°C. Each sample from the lysis tube (3 μl) was added to a SmartCycler tube containing 25 μl of the reconstituted master mix. Included in the master mix was an IC, a 333-bp DNA fragment of which only 55 bp shares homology with *C. difficile*, to detect inhibition of the PCR. Every PCR run included a PCR-positive control (reconstituted DNA from the manufactured kit). An uninoculated sample buffer was used as a negative control. On each day of BD GeneOhm Cdiff assay processing, a positive control, *C. difficile* ATCC 9689, was included to serve as an external extraction control for the PCR. A negative control, *Escherichia coli* ATCC 25922, was also added to test for potential contamination. Following centrifugation for 5 to 10 s using a Cepheid microcentrifuge especially adapted to fit the SmartCycler tubes, the reaction tubes were placed in the SmartCycler I-CORE module (Cepheid, Sunnyvale, CA) and run using Cepheid SmartCycler software with the BD GeneOhm Cdiff assay amplification protocol. Results were automatically interpreted by the software as follows: "negative," no *tcdB* gene was detected; "positive," the *tcdB* gene was detected; "unresolved," either the IC was inhibited or there was reagent failure; "invalid assay run," the PCR control (positive or negative) failed; "not determined," there was an I-CORE module malfunction. Samples with unresolved results were retested from the frozen lysate on the next day of testing. The remaining PCR sample buffer and lysis tubes were frozen at −20°C.

The analytical sensitivity of the BD GeneOhm Cdiff assay was determined as follows. Serial dilutions of *C. difficile* ATCC 9689 were made in the sample buffer provided by the manufacturer in the test kit. Each dilution was tested in triplicate by the PCR assay. Dilutions were plated to CDC anaerobic blood agar plates (BBL, BD Diagnostics, Sparks, MD) for culture and were read at 48 h to determine colony counts.

***C. difficile* cytotoxin method.** The *C. difficile* TOX-B test (TechLab, Blacksburg, VA), a CCNA, was performed using the manufacturer's protocol. In short, a plastic pipette was used to transfer 0.2 ml of evenly suspended specimen in diluent (1:10 dilution), which was vortexed for 10 s and then centrifuged. The supernatant was filtered (membrane pore size, 0.45 μm) and inoculated into tissue culture plates containing human foreskin fibroblasts (Diagnostic Hybrids, Athens, OH). Each sample required two wells: a control well (to contain the sample and antitoxin) and a specimen test well (containing the sample in phosphate-buffered saline). The final dilution of the fecal filtrate in each well was 1/50. Plates were incubated at 37°C ± 2°C and were reviewed at 24 h and again at 48 h. Rounding indicated the presence of a cytotoxic effect; the sample was considered positive if at least 50% of the cells were rounded (cytotoxic). The presence of *C. difficile* toxin B was confirmed if the cytotoxic activity was neutralized in the control well containing the antitoxin. In addition to the positive and negative controls provided by the manufacturer, cultures of *C. difficile* ATCC 9689 and *E. coli* ATCC 25922 were included on every day of CCNA testing to serve as external controls.

Toxigenic culture and toxin testing of recovered isolates. Every specimen was processed for anaerobic bacterial culture, and culture isolates were characterized further by cytotoxin testing using the TOX-B CCNA and the BD GeneOhm Cdiff PCR. Anaerobic culture was performed as follows. A spore enrichment step was carried out by heating the stool specimen in a glass tube for 10 min on a dry heat block at 80°C and then cooling to room temperature for 5 min before inoculation onto the culture medium. Using a sterile graduated pipette, approximately 0.1 g (2 to 3 drops) of stool was placed on the first quadrant of a modified cycloserine-cefoxitin-fructose agar (CCFA) plate (catalog no. R01268; Remel, Lenexa, KS) and a plate containing modified CCFA with horse blood (CCFA-HB) (catalog no. R01266; Remel, Lenexa, KS), and the plates were streaked for isolation. The remaining sample was added to a prereduced chopped-meat glucose (CMG) broth (catalog no. 297307; BBL, BD Diagnostics, Sparks, MD). Plates were incubated anaerobically at 35°C in a GasPak EZ anaerobe pouch system. After 48 h, both the modified CCFA and the modified CCFA-HB plates were examined. Colonies morphologically resembling *C. difficile* (circular yellow colonies on CCFA plates or gray-white colonies with raised centers and irregular filamentous or opaque edges on CCFA-HB plates) were Gram stained and, if suspicious for a *Clostridium* sp., were subcultured to prereduced brucella blood agar plates (Anaerobe Systems, Morgan Hill, CA) with a vancomycin disk (5 μg) in the primary streak area and were also tested for aerotolerance on a chocolate agar plate (BBL, BD Diagnostics, Sparks, MD). *C. difficile* was identified by susceptibility to vancomycin, no growth at 35°C after 24 h of incubation under 5% CO_2, a positive result on the Pro-disk test (L-proline), yellow-green fluorescence with the Wood's lamp (254 nm), and a characteristic "horse barn" odor. CMG broth cultures incubated at 35°C were subcultured onto CCFA-HB only if the plated media were negative at 4 days and the Cdiff PCR and/or TOX B assay was positive. Isolates from the CMG broth subcultures were recovered and identified as described above. According to the manufacturer's specifications, quality control testing of culture media and reagents was performed weekly and with each new lot of media by using *C. difficile* ATCC 9689 and *Clostridium perfringens* ATCC 13124. Isolates of *C. difficile* were tested for the presence of the *tcdB* gene and for cytotoxin B production by being grown for 48 h in CMG. For PCR testing, a sterile Dacron swab was dipped into the CMG culture, broken off into the sample buffer tube containing the Tris-EDTA sample, and then processed the same as a liquid stool culture. For isolate testing by the TOX-B test, 0.2 ml of evenly suspended CMG culture in diluent (1:10 dilution) was vortexed for 10 s and then inoculated into tissue culture plates containing human foreskin fibroblasts. The results were read and interpreted as described above for direct specimen testing.

Data analysis. Our initial analysis compared the BD GeneOhm Cdiff assay to the TOX-B test as the reference method, because few laboratories offer toxigenic culture. A secondary analysis was done comparing both the BD GeneOhm Cdiff assay and the TOX-B test to toxigenic culture as the "gold standard." For the secondary analysis, a true positive was defined as anaerobic culture positive for toxigenic *C. difficile*. As appropriate, descriptive statistics and tests of strength of association were calculated using Stata 9 (Stata Corporation, TX). PCR retesting of the initial sample lysates using the BD GeneOhm Cdiff assay and PCR testing

TABLE 1. Direct comparison of the BD GeneOhm Cdiff assay to the Wampole TOX-B test[a]

TOX-B test result (cytotoxic activity)	No. of samples with the following result by the BD GeneOhm Cdiff assay:	
	Positive	Negative
Positive	40	4
Negative	17	340
Total ($n = 401$)[b]	57	344

[a] The performance characteristics of the BD GeneOhm Cdiff assay when the TOX-B test was used as a reference were as follows: sensitivity, 40/44 (90.9% [95% confidence interval, 82.4 to 99.4%]); specificity, 340/357 (95.2% [95% confidence interval, 93.0 to 97.5%]); positive predictive value, 40/57 (70.2% [95% confidence interval, 58.3 to 82.1%]); negative predictive value, 340/344 (98.8% [95% confidence interval, 97.7 to 100%]).
[b] Three samples were excluded from analysis (two samples due to inhibited PCR and one sample due to the presence of a nonspecific cytotoxin).

of the recovered *C. difficile* isolates on the samples with discrepant results were provided only as general information, and the results were not used in either analysis.

RESULTS

In 15 weeks of enrollment, 404 specimens were collected from 377 participants (5.7% repeats). No participant contributed more than two samples, and all second stool samples were submitted more than 7 days after the initial specimen. The mean and median times from sample collection to testing by the BD GeneOhm Cdiff assay were 18.1 h and 19.4 h, respectively. The mean and median times from sample collection to testing by the TOX-B test were 19.6 h and 20.9 h, respectively. During the study, the prevalence of toxigenic *C. difficile* (10%) reflected the prevalence determined by routine clinical diagnostic testing in the Johns Hopkins Hospital Clinical Microbiology Laboratory.

Overall agreement between the BD GeneOhm Cdiff assay and the TOX-B test was 94.8% (380/401). There were 40 concordantly positive results, and 340 samples were negative by both tests (Table 1). The PCRs for three samples were inhibited upon initial testing; one of these samples was resolved upon retesting and was included in the data analysis. The remaining two samples with inhibited PCR tests were negative both by the TOX-B test and by toxigenic culture but were excluded from analysis. One sample produced a nonspecific-cytotoxin-positive result; this sample was negative by the BD GeneOhm Cdiff assay and by culture, and it was excluded from the analysis. The performance characteristics of the BD GeneOhm Cdiff assay compared to the TOX-B test as a reference are given with Table 1.

Table 2 lists the discrepancies between the BD GeneOhm Cdiff assay and the TOX-B test and includes the final assignment (e.g., true positive, true negative) based on toxigenic culture. Four samples tested negative by the BD GeneOhm Cdiff PCR assay but cytotoxin positive by the TOX-B test (Table 2). The lysis buffers were retested by the BD GeneOhm Cdiff assay, and the repeat PCR was again negative for all four samples. Toxigenic anaerobic culture was positive for one of the four samples (no *C. difficile* was isolated from the other three). This isolate also tested positive by the BD-GeneOhm Cdiff PCR assay. In this case, the BD GeneOhm PCR result was considered falsely negative. The other three samples were considered false positives by the TOX-B test (Table 2).

TABLE 2. Characterization of specimens with discrepant results by the BD GeneOhm Cdiff assay versus the Wampole TOX-B test[a]

Specimen no.	Result of:						Final *C. difficile* assignment[c]
	Initial testing		Repeat Cdiff PCR	Culture[b]	Isolate testing		
	Cdiff PCR	TOX-B			Cdiff PCR	TOX-B	
112	Neg	Pos	Neg	Neg			TN
271	Neg	Pos	Neg	Neg			TN
275	Neg	Pos	Neg	Neg			TN
313	Neg	Pos	Neg	Pos	Pos	Pos	TP
19	Pos	Neg	Pos	Pos	Pos	Pos	TP
20	Pos	Neg	Pos	Neg			TN
32	Pos	Neg	Pos	Pos	Pos	Pos	TP
47	Pos	Neg	Pos	Neg			TN
86	Pos	Neg	Pos	Pos	Pos	Pos	TP
97	Pos	Neg	Pos	Pos	Pos	Pos	TP
106	Pos	Neg	Pos	Pos	Neg	Neg	TN
118	Pos	Neg	Pos	Pos	Pos	Pos	TP
175	Pos	Neg	Pos	Pos	Pos	Pos	TP
274	Pos	Neg	Neg	Neg			TN
289	Pos	Neg	Pos	Neg			TN
316	Pos	Neg	Neg	Pos	Neg	Neg	TN
322	Pos	Neg	Pos	Pos	Pos	Pos	TP
359	Pos	Neg	Pos	Pos	Pos	Pos	TP
389	Pos	Neg	Pos	Pos	Pos	Pos	TP
402	Pos	Neg	Pos	Pos	Pos	Pos	TP
403	Pos	Neg	Neg	Pos	Pos	Pos	TP

[a] Cdiff, BD GeneOhm Cdiff assay; TOX-B, *Clostridium difficile* toxin B test; Pos, positive; Neg, negative; TN, true negative; TP, true positive.
[b] A positive result for culture means that *C. difficile* was isolated.
[c] Based on toxigenic culture results.

TABLE 3. Comparison of the BD GeneOhm Cdiff assay (PCR) and the Wampole TOX-B test (CCNA) to toxigenic anaerobic culture

Assay and result	No. of samples with the following result by toxigenic anaerobic culture[a]:		Performance[b] of the indicated assay compared with toxigenic anaerobic culture (95% confidence interval)			
	Positive	Negative	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
BD GeneOhm Cdiff PCR			83.6 (74.3–92.9)	98.2 (96.8–99.6)	89.5 (81.5–97.4)	97.1 (95.3–98.9)
Positive	51	6				
Negative	10	334				
Total (n = 401)[c]	61	340				
TechLab Wampole TOX-B CCNA			67.2 (55.4–79.0)	99.1 (98.1–100)	93.2 (85.7–99.9)	94.4 (92.0–96.8)
Positive	41	3				
Negative	20	337				
Total (n = 401)[c]	61	340				

[a] Approximately 1 ml of stool sample (n = 401) was heated at 80°C for 10 min before toxigenic anaerobic culture was performed using CCFA, CCFA-HB, and prereduced chopped meat broth. *C. difficile* isolates were tested for toxin B production by the TOX-B test and for the presence of the *tcdB* gene by the Cdiff assay.
[b] PPV, positive predictive value; NPV, negative predictive value.
[c] Three samples were excluded from analysis (two samples due to inhibited PCR, one sample due to the presence of a nonspecific cytotoxin).

Seventeen samples initially tested positive by the BD GeneOhm Cdiff PCR assay but were negative for direct toxin testing by the TOX-B test. Anaerobic culture yielded 13 *C. difficile* isolates, 11 of which were cytotoxin producing by the TOX-B test (Table 2) and also PCR positive for the presence of *tcdB* by the BD GeneOhm Cdiff assay. Two isolates were neither TOX-B test positive nor Cdiff PCR positive. All 17 lysis buffers were retested by the BD GeneOhm Cdiff assay, and 14 matched the original results. No *C. difficile* was recovered from four specimens that tested BD GeneOhm Cdiff assay positive and TOX-B test negative.

Table 3 shows the performance characteristics of each assay compared to anaerobic toxigenic culture as the "gold standard." The sensitivity, specificity, and positive and negative predictive values of the BD GeneOhm Cdiff assay were 83.6%, 98.2%, 89.5%, and 97.1%, respectively, and those of the TOX-B test were 67.2%, 99.1%, 93.2%, and 94.4%, respectively.

The analytical sensitivity was determined by us to be 1 to 10 CFU per reaction (data not shown). This is similar to the manufacturer's claim of 4 CFU per reaction (Keith Chiasson, BD GeneOhm, personal communication).

DISCUSSION

Upon initial testing, the BD GeneOhm Cdiff assay compared favorably to the Wampole *C. difficile* Toxin B test, with a sensitivity and specificity of 90.9% and 95.2%, respectively. When both assays were compared to anaerobic toxigenic culture, the sensitivity of the BD GeneOhm Cdiff assay dropped to 83.6%, but its specificity improved to 98.2%. The sensitivity of the TOX-B test compared to toxigenic culture was 67.2%, indicating that it is an imperfect gold standard, particularly for evaluating newer, more sensitive methods such as testing for a gene target that may be less vulnerable to the vagaries of specimen handling upon test performance.

Four specimens were BD GeneOhm Cdiff assay negative but TOX-B test positive upon initial testing. One of the concerns with a method that detects the gene encoding toxin B rather than detecting functional toxin is the possibility of false negatives due to aberrant *tcdB* genes. Mehlig et al. (17) suggest that *tcdB* is not as conserved as would be expected. In our study, a toxin-producing isolate was recovered from only one of the four specimens, and the isolate tested positive by the BD GeneOhm Cdiff assay. The possibility of variant *tcdB* isolates within our population was not investigated, but none of the isolates recovered were both BD GeneOhm Cdiff PCR negative and TOX-B positive. Repeated subcultures of the CMG broths from the other three samples were negative for *C. difficile*. It is possible that these samples contained *Clostridium sordellii* toxin, a lethal toxin that is known to cross-react with *C. difficile* toxin B, but this organism was not isolated from any of the anaerobic broth cultures. We were not able to determine the clinical relevance of the three samples that were positive only by the TOX-B CCNA.

C. difficile was recovered from 13 of the 17 specimens that initially tested BD GeneOhm Cdiff PCR positive but TOX-B CCNA negative. Eleven of the 13 isolates produced toxin B. Although they were not included in the analysis, three of the four samples that failed to grow *C. difficile* retested PCR positive from the initial lysate. Furthermore, two samples that initially tested BD GeneOhm Cdiff PCR positive but TOX-B CCNA negative, and that yielded nontoxigenic *C. difficile* isolates, were retested. Both lysates from the sample were again positive by the Cdiff PCR, even though the isolates failed to test positive for *tcdB* or to produce toxin. These were all considered false-positive PCR results. It is possible that in the specimens from which only nontoxigenic *C. difficile* isolates were recovered, toxigenic *C. difficile* was overgrown by a second, non-toxin-producing strain, since it is known that multiple strains (as determined by PCR ribotyping) of *Clostridium difficile* may exist in the same sample (29). Other explanations for failure to grow an organism include the possibility of culture failure due to low numbers of bacteria, aerobic toxicity, or the

inability of a given sample's strain to survive the culture enrichment process.

Our results with the BD GeneOhm Cdiff assay and the TOX-B test are very similar to those of other investigators who have compared real-time PCR assays and cytotoxin or enzyme immunoassay (EIA) testing to anaerobic toxigenic culture (3, 22, 26, 30, 32). These studies are discussed in detail below. Bélanger et al. (3) were among the first to describe a comprehensive analytical study using a fluorescence-based multiplex PCR assay targeting conserved regions of *tcdA* and *tcdB* that was performed on the SmartCycler. The assay described had a limit of detection of 10 genome copies for nine *C. difficile* strains tested. There was no cross-reactivity with 10 non-*C. difficile Clostridium* species, including *Clostridium innocuum* and *C. sordellii*. The authors, however, performed limited testing directly on stool samples, but results were concordant for 55/56 samples tested (3). In a prospective multicenter evaluation using 367 fecal samples from 300 patients, van den Berg et al. (30) evaluated a real-time PCR assay that amplified a 177-bp region of the nonrepeat region of *tcdB* on the iCycler detection system. Resolution of the results using toxigenic culture demonstrated the superiority of the PCR method (88% sensitivity) to a cell culture cytotoxicity test (70% sensitivity) and an immunochromatographic assay (79% sensitivity) (30). In that study, the limit of detection of the PCR assay compared to that of toxigenic culture was 1 log unit higher (thus showing less sensitivity), likely accounting for some of the false negatives (30). In a subsequent study performed by the same authors, the real-time PCR assay showed the greatest concordance with toxigenic culture compared to the VIDAS instrument and the Premier Toxin A and B test (Meridian Diagnostics, Inc.) (32). Two recent studies (22, 26), both using the LightCycler (LC) technology, confirm the superiority of real-time PCR to EIAs and/or cell culture cytotoxicity assays. In the study by Peterson et al. (22), the authors used clinical criteria as part of the reference standard for a positive test result supporting a diagnosis of *C. difficile* infection. This in-house-developed assay amplified a highly conserved region of *tcdB*. The sensitivity and specificity of the PCR method were 93.3% and 97.4%, respectively. The in-house-developed LC assay described by Sloan et al. (26) detects the presence of *tcdC* as well as the 18-bp deletion within this gene associated with the epidemic strain. Toxigenic culture served as the "gold standard," and the PCR was compared to three EIAs that detect toxins A and B and an EIA that detects GDH. The sensitivity and specificity of the LC real-time PCR assay were 86% and 97%, respectively, whereas the sensitivity and specificity of the EIAs were 48% and 84 to 99%, respectively (26). In both studies, toxigenic culture was the most sensitive method for *C. difficile* infection diagnosis. The results of these studies are similar to our findings using the commercially developed assay manufactured by BD GeneOhm.

While real-time PCR methods have been shown to be clinically useful and superior to conventional test methods, both in our study and in those summarized above, some theoretical concerns will need to be assessed over time. One concern is that the broad use of a PCR method in place of an assay that measures functional toxin in the stool could lead to overtreatment of patients who are colonized with toxin-producing strains but who have diarrhea caused by some other mechanism. However, the concept that a less sensitive test for the detection of toxigenic strains of *C. difficile* in stool is more specific for the clinical diagnosis of *C. difficile* infection is not evidence based. Also, it will be important, going forward, to periodically use toxigenic anaerobic culture to monitor the emergence of new genotypes, such as the possible appearance of a virulent *tcdA*-positive, *tcdB*-negative *C. difficile* strain, which could impact the clinical performance of *tcdB*-based assays (21, 24).

The procedure for the BD GeneOhm Cdiff assay took less than 3 h from specimen processing in the laboratory to reporting of the results, as opposed to 24 to 48 h for the TOX-B test and about 5 days for completion of toxigenic culture. The BD GeneOhm Cdiff assay was no more technically difficult to perform than the TOX-B test. Such a quick turnaround time combined with such strong performance characteristics and ease of use can greatly facilitate patient management. Also, infection control activities will be aided by more rapid identification of infected patients.

Cost considerations are often cited as reasons for failure to perform more-sensitive tests in some clinical laboratory environments. While the BD GeneOhm assay is not yet available, the approximate cost per test is estimated to be between $40 and $50. Costs for other methods (from list prices) are as follows: standard microtiter plate format EIA, $8.00 per test; lateral-flow single-use EIA format, $17.00 per test; two-step algorithm using a GDH microtiter EIA format, $17.00 plus an additional $13.00 for toxin tests for GDH-positive samples; anaerobic toxigenic culture as performed in our laboratory, $22.

In summary, the BD GeneOhm Cdiff assay is more rapid, more sensitive, and as specific as cell culture cytotoxin testing directly from patient samples for the diagnosis of *C. difficile* infection.

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REFERENCES

1. **Akerlund, T., I. Persson, M. Unemo, T. Norén, B. Svenungsson, M. Wullt, and L. G. Burman.** 2008. Increased sporulation rate of epidemic *Clostridium difficile* type 027/NAP1. J. Clin. Microbiol. **46:**1530–1533.
2. **Bartlett, J. G.** 2006. Narrative review: the new epidemic of *Clostridium difficile*-associated enteric disease. Ann. Intern. Med. **145:**758–764.
3. **Bélanger, S. D., M. Boissinot, N. Clairoux, F. J. Picard, and M. G. Bergeron.** 2003. Rapid detection of *Clostridium difficile* in feces by real-time PCR. J. Clin. Microbiol. **41:**730–734.
4. **Benson, L., X. Song, J. Campos, and N. Singh.** 2007. Changing epidemiology of *Clostridium difficile*-associated disease in children. Infect. Control Hosp. Epidemiol. **28:**1233–1235.
5. **Bouza, E., T. Peláez, R. Alonso, P. Catalán, P. Muñoz, and M. R. Créixems.** 2001. "Second-look" cytotoxicity: an evaluation of culture plus cytotoxin assay of *Clostridium difficile* isolates in the laboratory diagnosis of CDAD. J. Hosp. Infect. **48:**233–237.
6. **Centers for Disease Control and Prevention (CDC).** 2008. Surveillance for community-associated *Clostridium difficile*—Connecticut, 2006. MMWR Morb. Mortal. Wkly. Rep. **57:**340–343.
7. **Delmée, M., J. Van Broeck, A. Simon, M. Janssens, and V. Avesani.** 2005. Laboratory diagnosis of *Clostridium difficile*-associated diarrhoea: a plea for culture. J. Med. Microbiol. **54:**187–191.
8. **Drudy, D., N. Harnedy, S. Fanning, M. Hannan, and L. Kyne.** 2007. Emergence and control of fluoroquinolone-resistant, toxin A-negative, toxin B-positive *Clostridium difficile*. Infect. Control Hosp. Epidemiol. **28:**932–940.

9. **Fenner, L., A. F. Widmer, G. Goy, S. Rudin, and R. Frei.** 2008. Rapid and reliable diagnostic algorithm for detection of *Clostridium difficile*. J. Clin. Microbiol. **46:**328–330.
10. **Gilligan, P. H.** 2008. Is a two-step glutamate dehydrogenase antigen-cytotoxicity neutralization assay algorithm superior to the premier toxin A and B enzyme immunoassay for laboratory detection of *Clostridium difficile*? J. Clin. Microbiol. **46:**1523–1525.
11. **Guilbault, C., A. C. Labbé, L. Poirier, L. Busque, C. Béliveau, and M. Laverdière.** 2002. Development and evaluation of a PCR method for detection of the *Clostridium difficile* toxin B gene in stool specimens. J. Clin. Microbiol. **40:**2288–2290.
12. **Hubert, B., V. G. Loo, A. M. Bourgault, L. Poirier, A. Dascal, E. Fortin, M. Dionne, and M. Lorange.** 2007. A portrait of the geographic dissemination of the *Clostridium difficile* North American pulsed-field type 1 strain and the epidemiology of *C. difficile*-associated disease in Québec. Clin. Infect. Dis. **44:**238–244.
13. **Kyne, L., M. B. Hamel, R. Polavaram, and C. P. Kelly.** 2002. Health care costs and mortality associated with nosocomial diarrhea due to *Clostridium difficile*. Clin. Infect. Dis. **34:**346–353.
14. **Loo, V. G., L. Poirier, M. A. Miller, M. Oughton, M. D. Libman, S. Michaud, A. M. Bourgault, T. Nguyen, C. Frenette, M. Kelly, A. Vibien, P. Brassard, S. Fenn, K. Dewar, T. J. Hudson, R. Horn, P. René, Y. Monczak, and A. Dascal.** 2006. 2005. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*-associated diarrhea with high morbidity and mortality. N. Engl. J. Med. **353:**2442–2449. (Erratum, **354:**2200, 2006.)
15. **Lyerly, D. M., L. M. Neville, D. T. Evans, J. Fill, S. Allen, W. Greene, R. Sautter, P. Hnatuck, D. J. Torpey, and R. Schwalbe.** 1998. Multicenter evaluation of the *Clostridium difficile* TOX A/B test. J. Clin. Microbiol. **36:**184–190.
16. **McDonald, L. C., G. E. Killgore, A. Thompson, R. C. Owens, Jr., S. V. Kazakova, S. P. Sambol, S. Jonson, and D. N. Gerding.** 2005. An epidemic, toxin gene-variant strain of *Clostridium difficile*. N. Engl. J. Med. **353:**2433–2441.
17. **Mehlig, M., M. Moos, V. Braun, B. Kalt, D. E. Mahony, and C. von Eichel-Streiber.** 2001. Variant toxin B and a functional toxin A produced by *Clostridium difficile* C34. FEMS Microbiol. Lett. **198:**171–176.
18. **Merz, C. S., C. Kramer, M. Forman, L. Gluck, K. Mills, K. Senft, I. Steiman, N. Wallace, and P. Charache.** 1994. Comparison of four commercially available rapid enzyme immunoassays with cytotoxin assay for detection of *Clostridium difficile* toxin(s) from stool specimens. J. Clin. Microbiol. **32:**1142–1147.
19. **Musher, D. M., A. Manhas, P. Jain, F. Nuila, A. Waqar, N. Logan, B. Marino, and E. A. Graviss.** 2007. Detection of *Clostridium difficile* toxin: comparison of enzyme immunoassay results with results obtained by cytotoxicity assay. J. Clin. Microbiol. **45:**2737–2739.
20. **O'Brien, J. A., B. J. Lahue, J. J. Caro, and D. M. Davidson.** 2007. The emerging infectious challenge of *Clostridium difficile*-associated disease in Massachusetts hospitals: clinical and economic consequences. Infect. Control Hosp. Epidemiol. **28:**1219–1227.
21. **Perelle, S., M. Gibert, P. Bourlioux, G. Corthier, and M. R. Popoff.** 1997. Production of a complete binary toxin (actin-specific ADP-ribosyltransferase) by *Clostridium difficile* CD196. Infect. Immun. **65:**1402–1407.
22. **Peterson, L. R., R. U. Manson, S. M. Paule, D. M. Hacek, A. Robicsek, R. B. Thomson, Jr., and K. L. Kaul.** 2007. Detection of toxigenic *Clostridium difficile* in stool samples by real-time polymerase chain reaction for the diagnosis of *C. difficile*-associated diarrhea. Clin. Infect. Dis. **45:**1152–1160.
23. **Reller, M. E., C. A. Lema, T. M. Perl, M. Cai, T. L. Ross, K. A. Speck, and K. C. Carroll.** 2007. Yield of stool culture with isolate toxin testing versus a two-step algorithm including stool toxin testing for detection of toxigenic *Clostridium difficile*. J. Clin. Microbiol. **45:**3601–3605.
24. **Rupnik, M.** 2008. Heterogeneity of large clostridial toxins: importance of *Clostridium difficile* toxinotypes. FEMS Microbiol. Rev. **32:**541–555.
25. **Shanholtzer, C. J., K. E. Willard, J. J. Holter, M. M. Olson, D. N. Gerding, and L. R. Peterson.** 1992. Comparison of the VIDAS *Clostridium difficile* toxin A immunoassay with *C. difficile* culture and cytotoxin and latex tests. J. Clin. Microbiol. **30:**1837–1840.
26. **Sloan, L. M., B. J. Duresko, D. R. Gustafson, and J. E. Rosenblatt.** 2008. Comparison of real-time PCR for detection of the *tcdC* gene with four toxin immunoassays and culture in diagnosis of *Clostridium difficile* infection. J. Clin. Microbiol. **46:**1996–2001.
27. **Snell, H., M. Ramos, S. Longo, M. John, and Z. Hussain.** 2004. Performance of the TechLab *C. DIFF* CHEK-60 enzyme immunoassay (EIA) in combination with the *C. difficile* Tox A/B II EIA kit, the Triage *C. difficile* panel immunoassay, and a cytotoxin assay for diagnosis of *Clostridium difficile*-associated diarrhea. J. Clin. Microbiol. **42:**4863–4865.
28. **Ticehurst, J. R., D. Z. Aird, L. M. Dam, A. P. Borek, J. T. Hargrove, and K. C. Carroll.** 2006. Effective detection of toxigenic *Clostridium difficile* by a two-step algorithm including tests for antigen and cytotoxin. J. Clin. Microbiol. **44:**1145–1149.
29. **van den Berg, R. J., H. A. Ameen, T. Furusawa, E. C. Claas, E. R. van der Vorm, and E. J. Kuijper.** 2005. Coexistence of multiple PCR-ribotype strains of *Clostridium difficile* in faecal samples limits epidemiological studies. J. Med. Microbiol. **54:**173–179.
30. **van den Berg, R. J., L. S. Bruijnesteijn van Coppenraet, H. J. Gerritsen, H. P. Endtz, E. R. van der Vorm, and E. J. Kuijper.** 2005. Prospective multicenter evaluation of a new immunoassay and real-time PCR for rapid diagnosis of *Clostridium difficile*-associated diarrhea in hospitalized patients. J. Clin. Microbiol. **43:**5338–5340.
31. **van den Berg, R. J., E. J. Kuijper, L. E. van Coppenraet, and E. C. Claas.** 2006. Rapid diagnosis of toxinogenic *Clostridium difficile* in faecal samples with internally controlled real-time PCR. Clin. Microbiol. Infect. **12:**184–186.
32. **van den Berg, R. J., N. Vaessen, H. P. Endtz, T. Schülin, E. R. van der Vorm, and E. J. Kuijper.** 2007. Evaluation of real-time PCR and conventional diagnostic methods for the detection of *Clostridium difficile*-associated diarrhoea in a prospective multicentre study. J. Med. Microbiol. **56:**36–42.