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Evaluation of Diagnostic Tests for *Clostridium difficile* Infection▽

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We evaluated toxigenic *Clostridium difficile* detection by a lateral flow assay for antigen and toxin, an enzyme immunoassay, and two commercial PCR methods. Compared to the cell cytotoxicity neutralization assay and toxigenic culture, both toxin detection methods lacked sensitivity. PCR following combined antigen and toxin detection provided the most useful diagnostic information.

Since the advent of enzyme immunoassay tests for the *Clostridium difficile* toxins (CDT), the promise of a rapid diagnosis of *Clostridium difficile* infection (CDI) has led to their adoption for routine testing by many laboratories (1, 14, 16). However, concerns have recently been raised regarding the reliability of many of the rapid CDT detection methods (8, 15), leading to debate over the optimal testing strategy (2, 3, 5, 9, 10, 13). We therefore sought to compare the diagnostic accuracy of four rapid tests, including two commercially available PCR methods, against that of the reference standards: the cell cytotoxicity neutralization assay (CCNA) performed on stool samples, and toxigenic culture.

During December 2008 and January 2009, 150 consecutive liquid stool specimens were evaluated from patients over 65 years of age who developed diarrhea at least 48 h after admission. Each specimen was subjected to the following four tests: VIDAS *Clostridium difficile* A & B enzyme immunoassay for CDT (VIDAS; bioMérieux, Marcy l'Etoile, France), Gene Ohm PCR (BD Diagnostics, San Diego, CA), Xpert *C. difficile* PCR (Cepheid, Sunnyvale, CA) (the Gene Ohm PCR and Xpert detect the toxin B gene, but the latter also detects the gene for binary toxin and the *tcdC* deletion, which are features of PCR ribotype 027 [7, 9]), and *C. DIFF* Quik Chek Complete (Techlab, Blacksburg, VA), which independently detects both the constitutive antigen glutamate dehydrogenase (GDH) and CDT in a lateral flow device. Each test was performed according to the manufacturer's instructions by a different operator, with the operators blind to the other results.

The stool samples were also subjected to alcohol shock and cultured on Brazier's medium (Oxoid, Cambridge, United Kingdom) for growth of *C. difficile*, with colony identification based on morphology and cell wall antigen latex agglutination according to standard methods (6). The CCNA was performed directly on each sample and positive culture isolates using African green monkey kidney "Vero" cells (ECACC product number 84113001). After incubation at 33°C for 24 h, diluted stool or culture isolates producing a cell cytopathogenic effect that was inhibited by the presence of *C. sordellii* antitoxin (Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada) were inferred to contain CDT.

All the commercial tests were performed in accordance with the instructions issued by the manufacturer. The VIDAS, *C. DIFF* Quik Chek Complete test, and stool CCNA were all undertaken within 48 h of receipt of the samples in the laboratory, and when samples were not tested immediately, the specimens were stored at 2 to 8°C to prevent degradation of their targets. Testing was performed to simulate in-use conditions, and therefore none of the tests were repeated.

Ribotyping was performed on positive culture isolates 48 h after anaerobic subculture onto Columbia blood agar, as previously described (12). The gel electrophoresis patterns of the PCR products were then compared for an identical match with that of a *C. difficile* strain (NCTC 13366) with a known PCR ribotype, 027.

C. difficile was cultured from 19 specimens, and the GDH component of the *C. DIFF* Quik Chek Complete test was positive for all these samples. Three samples were GDH positive but negative by all other tests, one of which contained a CCNA-negative isolate of *C. difficile*. The overall toxigenic *C. difficile* carriage rate was therefore determined to be 12%, as 18 of the isolates were CCNA positive. The Xpert *C. difficile* PCR was positive in each of these cases, while the Gene Ohm PCR produced one false-negative result (Table 1).

The stool CCNA was positive for 15 specimens (10%), and with this used as the reference standard, the sensitivities were 73.3% for the CDT component of the *C. DIFF* Quik Chek Complete test and 53.3% for the VIDAS (Table 2). All CCNA-positive stool specimens were positive by both of the PCR tests, but there were four stool CCNA-negative samples that were positive in the Xpert *C. difficile* PCR, three of which also produced a positive Gene Ohm PCR result. Toxigenic isolates were recovered from three of these specimens, while the final sample yielded no organism on culture despite the detection of GDH and positive results by both PCR tests (Table 1).

Eight samples were identified by the Xpert *C. difficile* PCR as containing all three genetic targets. One of these samples was culture negative, but an isolate belonging to PCR ribotype 027 was recovered from the remaining seven. The 11 specimens lacking these molecular features all yielded *C. difficile* isolates belonging to other PCR ribotypes. In addition, 7/8 of the samples with all three Xpert *C. difficile* PCR targets produced positive toxin results in the *C. DIFF* Quik Chek Com-

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TABLE 1. Comparison of results obtained with *C. DIFF* Quik Chek Complete, VIDAS, Xpert *C. difficile* PCR, Gene Ohm PCR, stool CCNA, *C. difficile* culture, and CCNA performed on culture isolates

Interpretation	No. of specimens	Test result[b] by:							
		C. DIFF Quik Chek Complete for:		VIDAS	Xpert *C. difficile* PCR	Gene Ohm PCR	Stool CCNA	*C. difficile* culture	Culture CCNA
		GDH	CDT						
C. difficile infection	8	+	+	+	+	+	+	+	+
	3	+	+	−[a]	+	+	+	+	+
	4	+	−[a]	−[a]	+	+	+	+	+
Toxigenic *C. difficile* carriage	2	+	−	−	+	+	−	+	+
	1	+	−	−	+	−[a]	−	+	+
	1	+	−	−	+	+	−	−[a]	NA
Nontoxigenic carriage	1	+	−	−	−	−	−	+	−
False-positive GDH result	2	+	−	−	−	−	−	−	NA
Negative	128	−	−	−	−	−	−	−	NA
Total	150	22	11	8	19	18	15	19	18

[a] Interpreted as a false-negative result.
[b] +, positive result; −, negative result; NA, not applicable.

plete test compared to only 4/11 specimens lacking these molecular features ($P = 0.037$, Fisher's exact test).

We found that the GDH component of the *C. DIFF* Quik Chek Complete test and both PCR methods were highly sensitive for the detection of toxigenic *C. difficile* organisms in stool specimens. These tests can therefore be relied upon for the exclusion of *C. difficile* carriage or infection and are ideally suited to screening large numbers of specimens, as the results are quickly available. The PCR methods offer greater specificity, although their cost is greater and there is a risk that mutations in the toxin B gene may reduce their sensitivity in the future, which may go undetected if PCR is used alone.

The stool CCNA remains a suitable reference standard for detection of CDT itself in specimens (3, 5, 9, 13, 14), and by comparison, both rapid tests for CDT lacked the required sensitivity to warrant their use in isolation. Previous studies have reached similar conclusions for different enzyme immunoassays (3, 5, 13, 17), while concerns regarding poor positive predictive values of these tests have also been raised (8, 15).

As in the present study, other authors have observed good agreement between PCR methods and stool CCNA testing (4, 9, 11, 14). We also found that there was even better concordance between PCR and toxigenic culture, which has been suggested to be a more sensitive method for diagnosing CDI (1). Additionally, our data suggest that the Xpert *C. difficile* PCR was able to indicate the presence of PCR ribotype 027, which could aid in the epidemiological assessment of clusters and has been proposed as a potential risk factor for metronidazole treatment failure (7). Our observation that toxin detection by the *C. DIFF* Quik Chek Complete test was significantly more likely in these samples is consistent with the fact that the *tcdC* deletion leads to loss of suppression of CDT production (7, 9), and suggests that the relative burden of this ribotype may be overestimated if CDT detection is relied upon without utilizing the enhanced sensitivity of PCR.

We therefore propose a two-step testing algorithm using the *C. DIFF* Quik Chek Complete test to screen all diarrheal samples, followed by Xpert *C. difficile* PCR testing of any that are GDH positive. Our strategy has several advantageous features: results can be reported rapidly for samples if they are GDH negative (85.3% in our study) or positive for both GDH and CDT (7.3% in our study). For samples with discordant results, PCR testing can then exclude the presence of toxigenic *C. difficile* strains in approximately one additional

TABLE 2. Performance of *C. DIFF* Quik Chek Complete, VIDAS, Xpert *C. difficile* PCR, and Gene Ohm PCR compared to those of stool CCNA and toxigenic culture[a]

Comparator test	Parameter	Performance (95% CI) by:				
		C. DIFF Quik Chek Complete for:		VIDAS	Xpert *C. difficile* PCR	Gene Ohm PCR
		GDH	CDT			
Stool CCNA	% sensitivity	100 (79.4–100)	73.3 (47.6–89.0)	53.3 (29.9–75.3)	100 (79.4–100)	100 (79.4–100)
	% specificity	94.8 (89.6–97.4)	100 (97.3–100)	100 (97.3–100)	97.0 (92.6–98.8)	97.8 (93.7–99.2)
	% PPV	68.2 (47.1–83.6)	100 (73.5–100)	100 (66.4–100)	78.9 (56.3–91.3)	83.3 (60.4–93.9)
	% NPV	100 (97.2–100)	97.1 (92.8–98.8)	95.1 (90.2–97.6)	100 (97.2–100)	100 (97.3–100)
Toxigenic culture	% sensitivity	100 (82.4–100)	61.1 (38.4–79.7)	44.4 (24.4–66.5)	100 (82.4–100)	94.4 (74.0–98.7)
	% specificity	97.0 (92.5–98.8)	100 (97.3–100)	100 (97.3–100)	99.2 (95.9–99.8)	99.2 (95.9–99.8)
	% PPV	81.8 (61.2–92.5)	100 (73.5–100)	100 (66.4–100)	94.7 (75.1–98.8)	94.4 (74.0–98.7)
	% NPV	100 (97.2–100)	95.0 (90.0–97.5)	93.0 (87.5–96.1)	100 (97.2–100)	99.2 (95.9–99.8)

[a] CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

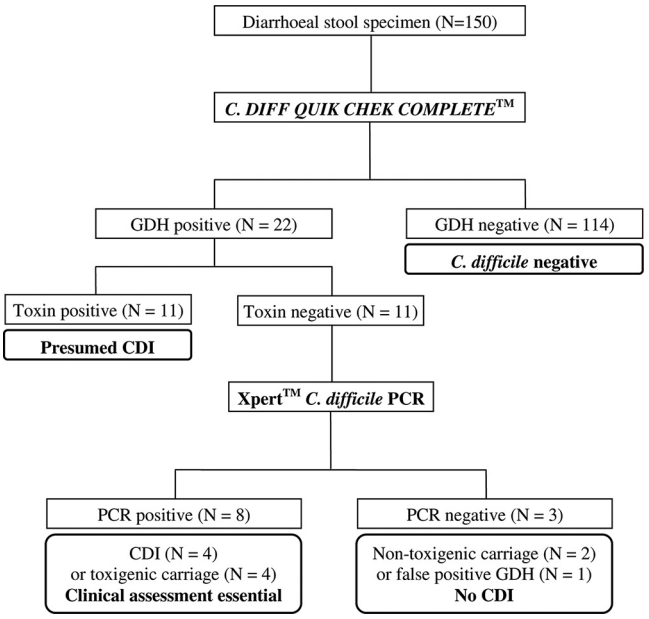

FIG. 1. Illustration of the proposed testing algorithm for detection of *C. difficile* in stool samples for our study population.

hour. Figure 1 shows how this algorithm would have performed in the present study. For samples with positive CDT or PCR results, the diagnosis of CDI will always require clinical correlation with the laboratory findings. However, it is desirable that all such patients be isolated, and even where the results are thought to represent toxigenic carriage, a higher treatment threshold may be adopted when antibiotic therapy is started for other indications.

In conclusion, to provide an optimal laboratory service that incorporates rapid turnaround times and reliable diagnostic accuracy, the rapid methods for *C. difficile* detection must all be combined. The most useful information can be obtained by screening all samples for the presence of GDH, with toxin detection performed either concurrently or subsequently on GDH-positive specimens, followed by PCR to distinguish between toxigenic and nontoxigenic strains in those samples with discordant GDH and CDT results. Our observation that CDT detection rates may vary between PCR ribotypes deserves further study.

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