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Loop-Mediated Isothermal Amplification Compared to Real-Time PCR and Enzyme Immunoassay for Toxigenic *Clostridium difficile* Detection

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***Clostridium difficile* infection is the primary cause of health care-associated diarrhea. While most laboratories have been using rapid antigen tests for detecting *C. difficile* toxins, they have poor sensitivity; newer molecular methods offer rapid results with high test sensitivity and specificity. This study was designed to compare the performances of two molecular assays (Meridian *illumigene* and BD GeneOhm) and two antigen assays (Wampole Quik Chek Complete and TechLab Tox A/B II) to detect toxigenic *C. difficile*. Fecal specimens from hospitalized patients ($n = 139$) suspected of having *C. difficile* infection were tested by the four assays. Nine specimens were positive and 109 were negative by all four methods. After discrepant analysis by toxigenic culture ($n = 21$), the total numbers of stool specimens classified as positive and negative for toxigenic *C. difficile* were 21 (15%) and 118 (85%), respectively. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were as follows: GeneOhm (95.2%, 100%, 100%, and 99.2%), *illumigene* (95.2%, 96.6%, 83.3%, and 99.2%), Tox A/B II (52.4%, 97.5%, 78.6%, and 92.4%), and Quik Chek Complete (47.6%, 100%, 100%, and 91.9%). The *illumigene* assay performed comparably to the GeneOhm assay with a slight decrease in test specificity; the sensitivities of both far exceeded those of the antigen assays. The clinical characteristics of the concordant and discrepant study patients were similar, including stool consistency and frequency. In the era of rapid molecular-based tests for toxigenic *C. difficile*, toxin enzyme immunoassays (EIAs) should no longer be considered the standard of care.**

Clostridium difficile infection is the primary cause of health care-associated diarrhea, with a prevalence rate of 13.1 per 1,000 inpatients in the United States and a disease spectrum ranging from mild diarrhea to fulminant colitis and death (7, 10). There has been an increase in the incidence and severity of *C. difficile* infection over the last 10 years, partly due to the emergence of the BI/NAP1/027 clone (2). Further, there are high rates of disease recurrence and treatment failure, and attributable health care costs are estimated to be in excess of $1 billion annually (2). Accurate and rapid diagnostic testing for *C. difficile* infection is essential for patient management and the timely implementation of infection control measures.

Toxigenic culture (TC) and cell culture cytotoxicity neutralization assay (CCNA) are considered reference standards but are not routinely used, as they are labor-intensive and have prolonged turnaround times (2). Currently, enzyme immunoassays (EIAs) dominate the diagnostic testing arena. They can detect glutamate dehydrogenase (GDH) (so-called common antigen) and/or major toxins A and B and are inexpensive, rapid, and easy to perform. A drawback of EIA toxin tests is a lack of sensitivity (8, 9, 13, 15, 16, 20, 22, 27, 30, 32). Conversely, EIA GDH tests have good sensitivity but lack specificity, as they cannot distinguish toxigenic from nontoxigenic *C. difficile* (8, 13, 15, 23–25, 30, 32). As such, GDH is a good screening test, but GDH-positive specimens must be subjected to another test that detects toxin A and/or B or the toxin gene(s) (13, 15, 23–25, 27, 30, 32). Molecular assays directed at one or more of five genes residing within the pathogenicity locus (PaLoc) have fostered much interest. Commercial kit-based real-time PCR tests targeting the *tcdB* gene have recently become available—the BD GeneOhm Cdiff assay (Becton Dickinson), proGastro Cd (Gen-Probe/Prodesse), and Xpert *C. difficile* (Cepheid)—with exceptional performance and relative ease of use documented (5, 8, 9, 13, 28–31). FDA clearance was recently granted for the Meridian *illumigene C. difficile* assay, which uses loop-mediated isothermal amplification (LAMP) to detect the toxin A gene (*tcdA*) within the PaLoc. The goal of this study was to compare the performance of the *illumigene* assay to those of another molecular test (BD GeneOhm Cdiff) and two EIAs (Wampole C. Diff Quik Chek Complete and TechLab *C. difficile* Tox A/B II) targeting toxins A and B with or without GDH antigen. It has been suggested that clinical presentation is important when interpreting *C. difficile* diagnostic assays (6). Therefore, patient clinical characteristics were analyzed to determine their influence on test performance.

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MATERIALS AND METHODS

Ethics approval. This study was approved by the Human Investigative Committee of Beaumont Hospitals.

Setting, specimen acquisition, and testing. Fecal specimens for *C. difficile* testing at Beaumont Hospitals (Royal Oak, MI) had to be liquid or soft stool that conformed to the shape of the collection container. Specimens were held at 4 to 7°C and tested within 24 h of collection. During a 3-week period, stool specimens ($n = 145$) from hospitalized patients ($n = 139$; age range, 5 to 94 years; mean, 67 years; male, 45%; female, 55%) suspected of having *C. difficile* infection were prospectively subjected to four diagnostic tests: TechLab *C. difficile* Tox A/B II assay (a microwell EIA distributed by Alere [formerly Inverness Medical Innovations], Waltham, MA), GeneOhm Cdiff (Becton Dickinson Diagnostics, La Jolla, CA), *illumigene C. difficile* (Meridian Bioscience, Cincinnati, OH), and C. Diff Quik Chek Complete (a lateral-flow EIA card from Alere Medical Inc.). The last assay detects both GDH and toxins A and B; both components must be positive for the test to be positive for toxigenic *C. difficile*. Additionally, two 1-ml aliquots from each specimen were deidentified and frozen at −20°C for discrepant analysis. All testing was performed according to the manufacturer's instructions by two medical technologists dedicated to the study at the same physical location.

Discrepant analysis. Discrepant testing was performed without knowledge of the prior test results. Each stool specimen was thawed, followed by ethanol shock and inoculation in parallel onto selective cycloserine-cefoxitin-fructose agar (CCFA) supplemented with 0.1% taurocholate (Sigma, St. Louis, MO) and into chopped-meat broth (BD BBL, Sparks, MD) supplemented with 0.1% taurocholate, 250 μg/ml cycloserine, and 16 μg/ml cefoxitin. If visible growth was observed in the broth culture after 48 h or at 5 to 7 days (late growth), 0.1 ml was subjected to the Premier Tox A/B enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Meridian Bioscience, Cincinnati, OH). A positive result (optical density [OD] > 0.10) supported the detection of toxigenic *C. difficile.* Colonies on CCFA with typical characteristics of *C. difficile* (flat yellow colonies) were tested by PCR in an internally validated PCR assay targeting the putative toxin repressor gene *tcdC* (primers 5′-TCTAGCTAATTGGTCATAAG-3′ and 5′-AATAGCAAATT GTCTGAT-3′) and the GDH "common-antigen" gene (*gdh*) using published primers (33). Despite variability in other regions of the PaLoc, the *tcdC* primers bind conserved regions in all toxigenic strains of *C. difficile* in GenBank (evaluated in August 2005) and over 1,200 sequenced strains in a recent survey (4). All PCRs were performed with Qiagen HotStarTaq Master Mix PCR reagents (Qiagen, Valencia, CA) and $MgCl_2$ (2.5 mM Mg) on a Perkin-Elmer 2400 thermocycler using a multiplex PCR protocol consisting of 5 min at 95°C followed by 45 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 2 min. The PCR amplicons were resolved on 2% agarose gels stained with ethidium bromide. A visible band of approximately 200 bp from the *tcdC* PCR and 750 bp from the *gdh* PCR supported the detection of toxigenic *C. difficile* and *C. difficile*, respectively.

Specimen classification and statistical analysis. In order to determine performance characteristics for each test, stool specimens were classified according to the following rules. If the results for all four tests were positive, then the stool specimen was considered positive for toxigenic *C. difficile*. If the results for all four tests were negative, then the stool specimen was considered negative for toxigenic *C. difficile*. If the stool specimen yielded a positive result for 1, 2, or 3 of the 4 tests, it underwent discrepant analysis as described above. The results of discrepant analysis were deemed definitive regardless of the results of any other tests.

Clinical evaluation. Chart review was conducted on 139 patients to obtain the following clinical information: age; gender; number of stools per day, maximum white blood cell (WBC) count, and creatinine (each within 1 day of testing); prior *C. difficile* infection within 90 days; and prior use of antibiotics, proton pump inhibitor (PPI) medication, and cancer chemotherapy (each within 30 days of testing). Stool quality (liquid, mucoid, or semisolid that still conformed to the shape of the container) was recorded by the laboratory. Specimens were stratified into five groups (see Table 3). Differences in clinical parameters among the five groups were assessed by chi-square analysis or Fisher's exact test where appropriate for categorical variables and a 2-tailed *t* test for pairwise comparisons of continuous variables using Microsoft Excel.

RESULTS

During the study period, 145 stool specimens were collected from 139 hospitalized patients clinically suspected of having *C. difficile* infection. Six patients had two stool specimens analyzed, and both were negative by all four tests. Thus, of the 139 unique patient specimens included in the final data set, 9 were positive and 109 were negative by all four tests and were classified as positive and negative, respectively, for toxigenic *C. difficile*. Twenty-one specimens required discrepant analysis, with the following results: toxigenic *C. difficile* detected ($n = 12$); nontoxigenic *C. difficile* detected ($n = 3$); no *C. difficile* detected ($n = 6$). Therefore, the total numbers of stool specimens classified as positive and negative for toxigenic *C. difficile* were 21 (15%) and 118 (85%), respectively.

Five stool specimens initially yielded an invalid test result: 1 with GeneOhm and 4 with *illumigene*. Upon repeat testing, a valid result was obtained for each specimen. Table 1 summarizes the results of each test and discrepant analysis. The positivity rates for GeneOhm, *illumigene*, Tox A/B II, and Quik Chek Complete were 14.4%, 14.4%, 7.9%, and 7.2%, respectively. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for each test (Table 2) were as follows: GeneOhm (95.2%, 100%, 100%, and 99.2%), *illumigene* (95.2%, 96.6%, 83.3%, and 99.1%), Tox A/B II (52.4%, 97.5%, 78.6%, and 92.0%), and Quik Chek Complete (47.6%, 100%, 100%, and 91.5%). The differences in sensitivity and NPV for the combined molecular tests versus the combined EIAs were both significant ($P < 0.001$; Fisher). There was no significant difference in the PPVs of the GeneOhm and *illumigene* assays ($P = 0.11$; Fisher).

During discrepant analysis, all TC-positive samples had growth in broth, and all but one specimen had extensive growth on CCFA within 72 h. The one specimen was negative by the GeneOhm PCR and GDH EIAs. There was no significant difference in *in vitro* toxin expression from the cultured isolates using the Meridian Premier Tox A/B EIA, again with one exception. This specimen was positive by three amplified methods (*illumigene*, GeneOhm, and *tcdC* PCR from culture); however, it was negative by initial stool EIA toxin testing (Tox A/B II and Quik Chek Complete EIA) and had a negative to low-positive reading from a cultured isolate using the Meridian Premier Tox A/B EIA, but when a pure culture was retested with a different EIA, Tox A/B II, a strong positive reading was obtained.

With patients categorized as concordant or discordant based on the results of their *C. difficile* laboratory testing (Table 3), there were no significant differences in the numbers of loose bowel movements (BMs) per day, prior use of antibiotic or chemotherapeutic agents, or renal insufficiency. However, those patients with stool specimens testing positive for toxigenic *C. difficile* were more likely to have had prior *C. difficile* infections (range, 7 to 90 days; $P < 0.001$; Fisher) and elevated WBC counts ($P < 0.001$; Fisher) and tended to be older than patients with negative test results ($P = 0.05$; *t* test). The three patients with nontoxigenic *C. difficile* were significantly younger than all other patients in the study ($P = 0.03$; *t* test) and tended to have fewer BMs. Overall, 31% of patients with known numbers of BMs ($n = 112$) had less than 3 BMs per day and potentially should not have been tested, as

TABLE 1 Summary of individual testing modalities to detect toxigenic *C. difficile*[a]

	Test result summary[b]							Final classification[c]				
				Wampole Quik Chek Complete		Discrepancy analysis					Wampole Quik Chek Complete	
n	BD GeneOhm	Meridian *illumigene*	TechLab Tox A/B II	GDH	Tox A/B	*C. difficile* Present	Toxigenic *C. difficile* Present	BD GeneOhm	Meridian *illumigene*	TechLab Tox A/B II	GDH	Tox A/B
109	−	−	−	−	−	Not performed	Not performed	TN	TN	TN	TN	TN
9	+	+	+	+	+	Not performed	Not performed	TP	TP	TP	TP	TP
7	+	+	−	+	−	Yes	Yes	TP	TP	FN	TP	FN
3	−	−	−	+	−	Yes	No	TN	TN	TN	TP	TN
3	−	+	−	−	−	No	No	TN	FP	TN	TN	TN
2	−	−	+	−	−	No	No	TN	TN	FP	TN	TN
2	+	+	+	+	−	Yes	Yes	TP	TP	TP	TP	FN
1	−	+	−	−	−	Yes	Yes	FN	TP	FN	FN	FN
1	+	+	−	+	+	Yes	Yes	TP	TP	FN	TP	TP
1	+	−	−	+	−	Yes	Yes	TP	FN	FN	TP	FN
1	−	+	+	−	−	No	No	TN	FP	FP	TN	TN

[a] *n* (number of specimens) = 139.
[b] −, negative; +, positive.
[c] TN, true negative; TP, true positive; FN, false negative; FP, false positive.

they did not fulfill 1 definition of diarrhea (21); this number is reduced to 21% if the definition of diarrhea includes liquid stool (data not shown). However, these patients without significant diarrhea were fairly evenly distributed among all diagnostic categories (Table 3). Interestingly, all patients in this study had high rates of PPI utilization, except those determined to have "false-positive" test results for *C. difficile* ($P = 0.003$; Fisher).

DISCUSSION

This study compares methods for detecting the genes and respective antigens for *C. difficile* toxins, incorporating clinical information and the results of toxigenic culture. During the latter analysis, it was shown that all strains with toxin genes actually expressed toxin protein *in vitro*, as was observed in testing of nearly 600 isolates (P. Riska, unpublished data) and in some published reports (24, 25), but not others (6, 8, 26). Notably, one isolate in our present study carried toxin genes, as demonstrated by multiple assays, yet failed to produce detectable toxin by the Meridian Premier toxin EIA while producing a very high toxin yield when retested by the Inverness Toxin A/B EIA. This suggests that toxin was expressed but the epitopes of the toxin were not readily detected by one of the toxin A/B assays.

Meridian *illumigene C. difficile*. The *illumigene* assay is based upon LAMP (12, 17, 18), in which primers qualitatively amplify a 204-bp region of the conserved 5′ sequence of the *tcdA* gene within the PaLoc of toxigenic *C. difficile* via continuous isothermal amplification. Magnesium pyrophosphate is an amplification byproduct that forms a white precipitate that is detected via turbidimetric measurement. The test is relatively simple to perform, requires minimal hands-on time, and can be completed in about 1 h, the shortest turnaround time of all the amplified assays. In our study, the *illumigene* assay performed exceptionally well, with a sensitivity and a specificity of 95.2% and 96.6%, respectively, comparable to the manufacturer's performance claims (with 95% confidence intervals) of 95.2% (89.2 to 97.9%) and 95.3% (92.3 to 96.7%), respectively. Our results are also congruent with those of Noren et al. (19), Dubberke et al. (6), and Lalande et al. (14), who demonstrated excellent sensitivity and specificity compared to TC and CCNA, respectively. Invalid results (2.8% overall; near the manufacturer's claim of 2.9%) occurred early in assay implementation, with semisolid stools too heavily inoculated on the collection brush. With experience, invalid results were minimized.

Four false-positive results and one false-negative result were obtained with the *illumigene* assay. Three of the false-positive stool specimens yielded negative results with the other three tests, as well as TC (discrepant analysis). The fourth false-positive result was also falsely positive with the Tox A/B II assay (OD = 0.176; cutoff > 0.08) and negative with all other assays, including TC. Specimen-to-specimen cross-contamination was excluded for the four false-positive results. Notably, these four patients (i) had no history of *C. difficile* infection within the previous 90 days, (ii) did not develop laboratory-confirmed *C. difficile* infection over the next 6 months, and (iii) had not received PPI therapy.

Of 41 study patients not using PPIs, 12% were false positive by *illumigene* ($n = 4$) or Tox A/B EIA ($n = 1$) versus 7% ($n = 3$)

TABLE 2 Performance characteristics of individual testing modalities to detect toxigenic *C. difficile*[a]

	Value				
				Wampole Quik Chek Complete[b]	
Parameter	BD GeneOhm	Meridian *illumigene*	TechLab Tox A/B II	GDH	Tox A/B
True positive (no.)	20	20	11	23	10
True negative (no.)	118	114	115	115	118
False positive (no.)	0	4	3	0	0
False negative (no.)	1	1	10	1	11
Sensitivity (%)	95.2	95.2	52.4	95.8	47.6
Specificity (%)	100	96.6	97.5	100	100
PPV (%)	100	83.3	78.6	100	100
NPV (%)	99.2	99.1	92.0	99.1	91.5

[a] *n* = 139.
[b] A positive test result requires that both the GDH and Tox A/B components be positive. The sensitivity, specificity, PPV, and NPV of the Wampole Quik Chek Complete test were identical to the data listed for the Tox A/B component. The GDH component was assessed for the ability to detect any (toxigenic or nontoxigenic) *C. difficile*.

TABLE 3 Summary of patient clinical parameters

Parameter	Value				
	Concordant positives[b]	Concordant negatives[c]	Discordant false positives[d]	Discordant false negatives[e]	Nontoxigenic[f]
n[a]	9	109	6	12	3
Mean age (yr)	75.8	66.5	68.7	68.3	45
Gender (% female)	55.5	54.1	50	66.7	33.3
Mean no. of stools/day[g] (range)	4.1 (2–7)	4.1 (1–13)	3.9 (1–10)	3.8 (1–7)	2.3 (2–3)
No. (%) with $<$3 stools/day[g]	3 (33)	26 (30)	2 (40)	2 (22)	2 (66)
Median WBC count (range, 4.0–10.1 billion/liter)	12.25	7.95	10.7	11.2	7.4
% WBC count $>$ 10.1	88	27	50	58	0
Median creatinine (range, 0.6–1.40 mg/dl)	0.91	0.94	1.01	0.905	0.5
Prior *C. difficile* infection (7–90 days) [no. (%)]	5 (56)	4 (4)	0 (0)	4 (33)	0
Antibiotic use (last 30 days) [no. (%)]	8 (89)	89 (82)	5 (83)	9 (75)	2 (66)
PPI use (last 30 days) [no. (%)]	7 (78)	75 (69)	1 (17)	11 (92)	1 (33)
Chemotherapy use (last 30 days) [no. (%)]	2 (22)	16 (15)	2 (33)	3 (25)	1 (33)

[a] *n* (number of specimens) = 139.
[b] Concordant positives, positive test results were obtained by all four initial testing modalities.
[c] Concordant negatives, negative test results were obtained by all four initial testing modalities.
[d] Discordant false positives, false-positive test results (final classification) were obtained for any of the initial four testing modalities following discrepant analysis.
[e] Discordant false negative, false-negative test results (final classification) were obtained for any of the initial four testing modalities following discrepant analysis.
[f] Specimens (n = 3) originally considered false positive by the GDH component of the Wampole Quik Chek Complete Test. Discrepancy analysis yielded nontoxigenic *C. difficile* in all three specimens.
[g] Only 112 specimens had the number of stools per day recorded in the medical chart.

confirmed positive. Of 95 patients using PPIs, only 1% ($n = 1$) had a false-positive *C. difficile* test (by Tox A/B II), while 19% ($n = 18$) were confirmed positive. A mechanism for this apparent association between nonuse of PPI and false-positive tests for *C. difficile* ($P < 0.01$; Fisher) remains unclear. This finding may be due to a confounding effect of PPIs on the *C. difficile* infection risk: PPI nonusers could be considered a low-prevalence population and thus more likely to test false positive. However, the prevalence of *C. difficile* infection was not statistically different ($P = 0.12$; Fisher) between PPI users (19%) and nonusers (7%). It is possible that the *illumigene* assay was more sensitive than TC, as suggested by others (12, 19); however, this is unlikely, given the multiple and extended culture protocols used during discrepant analysis and the extended clinical follow-up on these patients.

The one false-negative result with the *illumigene* assay was positive by GeneOhm, the GDH component of the Quik Chek Complete, and TC (discrepant analysis) and negative by both toxin assays. In theory, this specimen may have contained a *tcdA*-negative *tcdB*$^{+}$ strain of toxigenic *C. difficile.* While certain *tcdA*-negative strains in toxinotypes VIII and X (1, 3, 11) are still detected by *illumigene* (manufacturer's package insert; 3), there may be other primer-binding site mutations or deletions that are not recognized.

BD GeneOhm Cdiff. The GeneOhm assay performed exceptionally well, with sensitivity of 95.2% and specificity of 100%, minimally exceeding the performance of the *illumigene* assay ($P >$ 0.05 for the comparison of all parameters). Our findings are compatible with those of other evaluators of the GeneOhm assay who used CCNA and/or TC as a reference standard (13, 23, 30, 31). Our invalid-test rate (0.7%) is below the manufacturer's claims (mean, 4.6%; range 0.8% to 8.5%) and comparable to the 1.1% reported by Eastwood et al. (8) One false-negative result was obtained with the GeneOhm assay. For this specimen, toxigenic *C. difficile* was detected by the *illumigene* assay and TC (growth only in broth) but not with the Tox A/B II and Quik Chek Complete assays. This likely reflects a concentration of toxigenic *C. difficile* at or below the limit of detection of the GeneOhm assay.

TechLab Tox A/B II. The Tox A/B II assay demonstrated poor sensitivity (52.4%), near the lower end of the ranges reported by others compared to TC (66 to 88.6%) (6, 8, 13, 20, 27) or CCNA (38 to 90.7%) (8, 13, 20, 32). The high specificity of the Tox A/B II assay of 97.5% is congruent with the work of others (95.7 to 100%) (6, 8, 13, 20, 27, 32).

Wampole Quik Chek Complete. The Wampole Quik Chek Complete alternative EIA approach, targeting GDH antigen and toxin A/B together, performed similarly to the Techlab Tox A/B II. The toxin A/B component of the Quik Chek Complete assay had poor sensitivity (47.6%) and high specificity (100%) (Table 2). However, considering only the GDH component of the Quik Chek Complete to detect any *C. difficile* in this study, the calculated sensitivity, specificity, positive predictive value, and negative predictive value were 95.8%, 100%, 100%, and 99.1%, respectively (Table 2). One recent suggestion was that using fresh rather than frozen stool specimens increased the sensitivity of the assay (25); of note, we tested only fresh samples in our study. Interestingly, only three nontoxigenic *C. difficile* isolates were detected overall in our study versus 21 toxigenic isolates for a "false-positive" rate of 12.5%. However, the reported rate of false-positive GDH EIAs approaches 50% (8, 13, 15, 23–25, 30, 32), suggesting that the specificity and PPV of a GDH assay alone could be much lower in other settings. Nonetheless, the high sensitivity reported with this GDH EIA format supports the use of GDH assays as an initial screen, followed by a more specific 2nd test, such as a CCNA, a TC, or a DNA amplification assay, to confirm the presence of toxin A and/or B or their respective genes (23, 25, 30). The net benefit (i.e., the expense of personnel and supplies versus reimbursement) of performing multiple tests compared to DNA amplification alone remains to be determined, with stand-alone real-time PCR demonstrating equivalent to superior sensitivity and specificity (13, 15, 24, 32).

The primary limitation of this study was failure to perform TC on all specimens, potentially overestimating the sensitivity of each testing modality. However, recent analyses of the GDH EIA and its performance here support its high sensitivity as a screening tool, thus minimizing the likelihood of finding additional *C. difficile*-positive stools by culture-based methods (23, 25, 30). The strengths of this study are that all testing, except discrepant analysis, was performed by two dedicated medical technologists in the same physical location, avoiding test result bias due to variations in technical expertise or the testing environment (e.g., temperature or humidity). Additionally, chart reviews performed by an infectious-diseases fellow and a staff pathologist without knowledge of the test results confirmed that most patients had clinically significant diarrhea, as judged by stool quality and/or frequency. While the number of documented BMs per day is most likely underreported by nursing staff records, a majority of subjects still had documented diarrhea. Other clinical features were analyzed and were distributed evenly across the categories of patients. A larger sample size may have revealed other clinical predictors of false-negative and false-positive test results for *C. difficile*.

In summary, these results support the use of the *illumigene C. difficile* assay as a rapid, cost-effective, and technically simple test. Minor concerns are raised about the slightly higher false-positive and initial invalid-test rates of the *illumigene* assay; however, the latter was resolved with practice in obtaining the correct stool inoculum. The association of false-positive results with lack of PPI use needs further investigation. The theoretical risk of missing *tcdA*-negative *tcdB*$^+$ strains of toxigenic *C. difficile* other than those belonging to toxinotype VIII or X should also be monitored. This study further confirms that toxin EIAs should no longer be the standard of care for detecting toxigenic *C. difficile* in the United States.

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