

Evolution of Testing Algorithms at a University Hospital for Detection of *Clostridium difficile* Infections

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We present the evolution of testing algorithms at our institution in which the C. Diff Quik Chek Complete immunochromatographic cartridge assay determines the presence of both glutamate dehydrogenase and *Clostridium difficile* toxins A and B as a primary screen for *C. difficile* infection and indeterminate results (glutamate dehydrogenase positive, toxin A and B negative) are confirmed by the GeneXpert *C. difficile* PCR assay. This two-step algorithm is a cost-effective method for highly sensitive detection of toxigenic *C. difficile*.

The initial observation by Ticehurst and colleagues, which was confirmed by others, showing that toxin A/B immunoassays are insensitive resulted in a reexamination of the manner in which the laboratory diagnosis of *Clostridium difficile* infection is done (6, 8, 24). Two approaches are gaining favor as effective means to diagnose *C. difficile* infection (CDI). One is an algorithm in which detection of *C. difficile*-specific glutamate dehydrogenase (GDH), a cell wall antigen, is used as a screening test to be followed by the demonstration of the presence of toxigenic *C. difficile* in GDH-positive stool specimens (6, 8, 16, 18, 19, 20, 26). The presence of toxigenic *C. difficile* can be determined by toxin enzyme immunoassay (insensitive), cytotoxin neutralization (CTN; relatively sensitive), DNA amplification (most sensitive but perhaps less specific), or toxigenic culture (most sensitive but quite slow, which makes it impractical for diagnostic purposes) (1, 2, 17, 26). The second approach is to directly test stool samples for the presence of toxigenic *C. difficile* by using any of four commercially available, FDA-approved nucleic acid amplification techniques (NAATs) (3, 5, 9, 10, 11, 12, 13, 14, 22, 23, 26).

In this report, we describe the evolution of testing algorithms for toxigenic *C. difficile* in our laboratory. Prior to the implementation of algorithmic screening for *C. difficile* testing, our laboratory performed a toxin A/B immunoassay daily. The initial *C. difficile* screening algorithm used in our laboratory consisted of a screening test, the C. Diff Quik Chek Complete (TechLab, Blacksburg, VA) GDH immunochromatographic cartridge assay (ICA), that detects GDH only, followed by confirmatory testing with a CTN assay to confirm the presence of *C. difficile* toxin in feces (8). Although a negative result from the initial GDH screening could be provided quickly by using the GDH ICA, the turnaround time of the CTN assay is 24 to 48 h. GDH-positive specimens had a 24-h longer turnaround time than the toxin A/B immunoassay that was previously performed. Subsequent assay development has shown that that TechLab C. Diff Quik Chek Complete, which detects GDH and toxins A and B in a single ICA (GDH-toxin A/B ICA), is a reliable method to replace the GDH ICA in the initial step of our algorithm because of the high positive predictive value of the toxin A/B portion of the assay (18, 19). However, the GDH-toxin A/B ICA cannot be used as a stand-alone test because of the poor sensitivity of the toxin A/B portion of the assay (18, 19). We wanted to determine if a NAAT, the GeneXpert *C. difficile* (Cepheid, Sunnyvale, CA) PCR assay, could reliably replace our CTN

confirmatory test and thus improve our turnaround time. Several studies have shown that using a NAAT is a superior diagnostic approach to using CTN, compared with toxigenic *C. difficile* culture (16, 19, 22, 23). Finally we wanted to determine the economic and clinical impact of this evolved algorithm at our institution.

Consecutive soft or liquid stool specimens from patients at University of North Carolina Hospitals were submitted for detection of CDI. Specimens were refrigerated at 4°C and assayed within 24 h of receipt in the laboratory. This study was approved by the Biomedical Institutional Review Board of the University of North Carolina at Chapel Hill.

All specimens were initially screened by GDH-toxin A/B ICA. Specimens that were negative for GDH were not tested further because of the reported high negative predictive value of that assay (10, 20). Confirmatory tests (CTN, GeneXpert PCR) of all GDH-positive specimens ($n = 114$) were performed. The CTN was performed as previously described (8). The GeneXpert *C. difficile* PCR assay was performed according to the manufacturer's instructions. Specimens that were positive or negative by both PCR and CTN were considered true positive or negative. Discrepant results were resolved on the basis of the results of toxigenic *C. difficile* culture, which was the reference method.

Toxigenic culture of all GDH-positive specimens ($n = 114$) was performed. Briefly, stool samples were cultured after being heat shocked at 80°C for 20 min and then inoculated onto cycloserine-cefoxitin fructose agar (CCFA) plates supplemented with horse blood and chopped meat broth (Remel Laboratories, Lenexa, KS), incubated anaerobically, and examined at 48 to 72 h for colonies consistent with *C. difficile*. For specimens that were cul-

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TABLE 1 Performance of *C. Diff* Quik Chek Complete ICA, CTN, and GeneXpert *C. difficile* PCR in the detection of toxigenic *C. difficile*

Method	No. of samples ^a				Sensitivity	Specificity	PPV ^b	NPV ^c
	TP	FP	TN	FN				
Toxin ICA	35	4	40	35	42.3	90.9	89.7	53.3
CTN	47	6	37	24	66.2	86	88.6	60.7
PCR	70	8	35	1	98.6	81.4	89.7	97.2

^a TP, true positive; FP, false positive; TN, true negative; FN, false negative.

^b PPV, positive predictive value.

^c NPV, negative predictive value.

ture negative for *C. difficile*-like organisms on the initial CCFA plates, a subculture of the chopped meat broth to CCFA was performed and the aforementioned culture process was repeated. Colonies with morphology consistent with isolates of *C. difficile* were then inoculated into chopped meat broth, incubated for 48 h anaerobically at 35°C, and centrifuged, and then the supernatant was filtered and assayed to determine the presence of *C. difficile* toxin by using CTN as previously described. *Clostridium* isolates used for toxin detection were identified by using Vitek ANC cards (bioMérieux, Durham, NC) according to the manufacturer's instructions. Where necessary, further biochemical analysis to confirm identification was also performed. Isolates that were tox-

genic by CTN and identified by Vitek as *C. difficile* were considered toxigenic *C. difficile*.

We assessed the performance of the GeneXpert *C. difficile* PCR as the confirmatory step in the *C. difficile* testing algorithm as an alternative to the CTN assay but with a different target. PCR, CTN, and toxin A/B ICA were performed on 114 GDH-positive specimens using the GDH-toxin A/B ICA. We found PCR to be the most sensitive confirmatory method of detecting toxigenic *C. difficile* in 70/71 (98.6%) specimens defined as true positives, compared to 47/71 (66.2%) using CTN and only 30/71 (42.3%) using the toxin A/B portion of the GDH-toxin A/B ICA. (Table 1). On the basis of these findings, we chose to replace our CTN confirmatory test with a PCR detecting toxigenic organisms.

We performed a prospective analysis of the two-step algorithm consisting of GDH-toxin A/B ICA, followed by GeneXpert *C. difficile* PCR, with 4,321 specimens over a 12-month period (1 May 2010 to 28 April 2011) (Fig. 1). Results for approximately 87% of the specimens could be reported after performance of the GDH-toxin A/B ICA; 13% required PCR confirmation. Quality management review of the GDH-toxin A/B ICA-PCR algorithm in our laboratory for the first quarter of 2011 (data not shown) documented a median turnaround time of approximately 8 h. All specimens met our stated turnaround time of 24 h. The median 8-h turnaround time for the GDH-toxin A/B ICA-PCR algorithm was

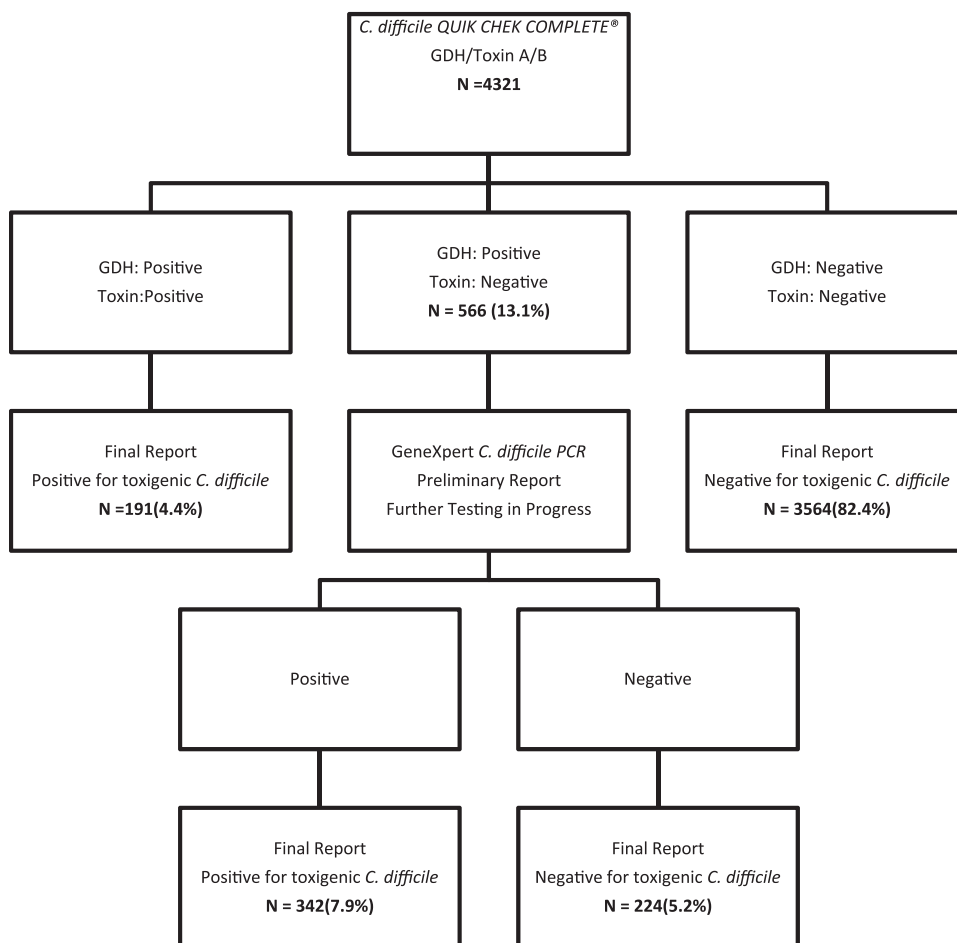


FIG 1 Results of two-step algorithm testing for *C. Diff* Quik Chek Complete ICA and GeneXpert *C. difficile* PCR.

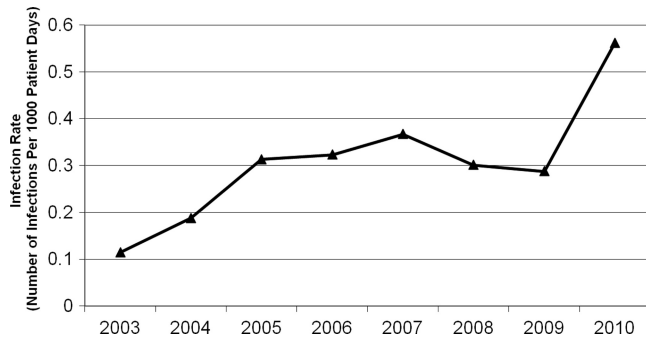


FIG 2 CDI rates prior to and after the institution of a two-step algorithm with PCR for toxigenic *C. difficile* as the confirmatory method.

due to the performance of testing three times per day, with immediate testing of screen-positive samples with the random-access GeneXpert system. This is a >1-day turnaround time improvement for GDH-positive specimens over our previously used GDH ICA-CTN algorithm, which had a stated turnaround time of 48 h in part because the CTN assay was performed and the result was reported only once per day. Specimens that were only GDH positive represented 13.1% of our work load.

Concurrent with the implementation of this new algorithm, there was an increase in the CDI rate in our institution. Infection control review (Fig. 2) showed close to a doubling of the CDI rate in 2010 after the algorithm was introduced compared to that in the years prior to its implementation (2005 to 2009). We believe that this increase may have been due, in part, to the increased detection of *C. difficile*-positive specimens with the GDH-toxin A/B ICA-PCR algorithm. We anticipated that there likely would be an increase in the number of CDI cases because of the increased detection of positive specimens by the GDH ICA-CTN algorithm observed in the developmental phase of this work.

The GDH-toxin A/B ICA-PCR algorithm not only detects more positive patients than a GDH ICA-CTN algorithm but is more economical than a PCR-only testing approach. With the cost of the GDH-toxin A/B ICA at \$11.50 and that of the GeneXpert *C. difficile* PCR at \$37, the cost of goods for the algorithmic approach described in Fig. 1 was \$70,633, while that of a PCR-only approach would be \$159,877.

This study produced four specific findings. (i) We determined, as have others, that NAAT is a more sensitive method than CTN for the detection of toxigenic *C. difficile* in stool samples (Table 1). Therefore, PCR replaced CTN in our testing algorithm (3, 11, 12, 13, 16, 17, 23). (ii) Institution of the new GDH-toxin A/B ICA-toxigenic *C. difficile* PCR algorithm resulted in an improvement of the turnaround time for the detection of toxigenic *C. difficile* from 48 h to a maximum of 24 h, with a median turnaround time of 8 h. (iii) A concordant increase in the number of cases of CDI in our institution with the change in testing suggests that the new testing algorithm is detecting an increased number of *C. difficile*-positive patients. (iv) Based on the cost of goods, an algorithmic approach to the diagnosis of CDIs saved our laboratory approximately \$80,000 during the first year of use compared to a PCR-only approach using the GeneXpert system. Additional savings due to the capability of discontinuing *C. difficile*-specific infection control measures a day earlier because of reduced testing turnaround time were likely but not evaluated.

The issue of an algorithmic approach using PCR as either the screening or confirmatory test versus the use of PCR as a stand-alone test has been a subject of debate (26). A shortcoming of this study was the inability to obtain clinical information to correlate with laboratory findings. Thus, replacement of the CTN confirmatory test with PCR may increase the detection of patients who are carrying toxigenic organisms but do not have clinical disease. Carriage of toxigenic organisms for as long as 30 days after the resolution of symptoms has been described in the literature (21, 25). Some of the patients we studied may, in fact, fit into this category, but two arguments that this number is relatively small can be made. First, the increase in the number of CDI cases after the institution of the GDH-toxin A/B ICA-PCR algorithm was based on infection prevention chart review and not strictly laboratory findings, although the latter are used as part of the CDI case definition. Our findings are consistent with those of others who observed an increase in the number of specimens positive for *C. difficile* after the initiation of molecular testing (7, 14). Second, we have strictly enforced rules in place in our laboratory that allow only the testing of diarrheic stool samples and no testing of cure stool specimens without consultation with a laboratory director (4). These clinical rules should reduce the number of PCR-positive patients who are potential toxigenic *C. difficile* carriers.

The estimated cost savings of using an algorithmic testing approach versus a PCR-only approach we report may be excessive. It can be argued that the greater sensitivity of PCR could lead to reduced testing of repeat specimens. In one study, it was estimated that 20% of tests could be eliminated (15). If we went to an all-PCR testing approach in our laboratory, even with the 20% savings, the cost of the goods used in this approach would be \$58,000 more per year than with the algorithmic approach. The test that we used in our study has the highest cost of the four FDA-approved *C. difficile* NAATs. Since the reported performance characteristics of the four NAATs are similar (3, 4, 10, 11, 12, 13, 16, 17, 22, 23), it is likely that the other three NAATs could be used in an algorithmic approach with similar results. This would have two impacts, i.e., reduction of the cost of the algorithmic approach and narrowing of the cost difference between the algorithmic and NAAT-only approaches. Two reasons why we used the GeneXpert *C. difficile* PCR are (i) its ease of use, which allows it to be performed on all shifts by personnel who do not have extensive training in molecular techniques, and (ii) the fact that the GeneXpert system is random access and does not require batching as other systems do, thus keeping the turnaround time to a minimum.

What would be the advantages and disadvantages of an all-NAAT approach? Fang et al. (26) and others (10, 16, 23) have argued that more true-positive patients would be found because of the method's enhanced sensitivity. What is less certain is what percentage of *C. difficile* NAAT-positive patients are GDH negative and thus would be missed by the algorithm presented in this study. A large study by Peterson and colleagues that carefully examined this issue showed that PCR would detect approximately 1% more positives than the GDH test but that the difference from detection by toxigenic *C. difficile* culture is not statistically significant (17). As a result, we allow physicians, by request, to bypass the first step in the algorithm and go directly to PCR. Thus far, with a small number of specimens (<10), we have found no PCR positives, but on the basis of the Peterson data, we would need to test approximately 100 specimens to find one additional positive.

In a setting where NAAT is readily available as a confirmatory

test, a GDH-toxin A/B combination ICA is likely to be of very little value, as has been argued by others (9). The value of this test lies in its speed in settings where confirmatory tests have to be sent to reference laboratories, as is the case at one of our small satellite hospitals that performs the GDH-toxin A/B ICA test and refers GDH-positive/toxin-negative specimens to our institution. Data presented here and elsewhere suggest that the toxin A/B portion of the GDH-toxin A/B ICA has a positive predictive value of 90 to 100%, allowing positives to be reported without further testing (18, 19). Results from our prospective study (Fig. 1) indicate that when the GDH-toxin A/B ICA is used, 36% of the positive results (191/533) and 94% of the negative results (3564/3788) could be reported within 30 min. Confirmation of GDH-positive/toxin-negative results would be dependent on the confirmatory test turnaround time at the reference laboratory. If a laboratory chose to perform GDH ICA-PCR instead of GDH-toxin A/B-PCR, on the basis of our data, it would cost an additional \$7,067/year.

The GDH-toxin A/B ICA-PCR algorithm not only detects more toxigenic *C. difficile*-positive patients than our initial GDH ICA-CTN algorithm but also detects them more quickly. The new algorithm influenced the detection of a larger nosocomial CDI problem in our institution than previously appreciated. With the advent of mandatory reporting of nosocomial infection rates, improved diagnostics for *C. difficile* become a double-edged sword, on the one hand improving diagnostic accuracy while on the other hand increasing detection and thus nosocomial infection rates. The hope, then, is that improved *C. difficile* diagnostics will lead to improved infection prevention interventions and eventually declining rates of infections due to this important nosocomial pathogen.

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