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Evaluation of the C.Diff Quik Chek Complete Assay, a New Glutamate Dehydrogenase and A/B Toxin Combination Lateral Flow Assay for Use in Rapid, Simple Diagnosis of *Clostridium difficile* Disease▽

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The diagnosis of *Clostridium difficile* infection continues to be a challenge for many clinical microbiology laboratories. A new lateral flow assay, the C.Diff Quik Chek Complete assay, which tests for the presence of both glutamate dehydrogenase (GDH) and *C. difficile* toxins A and B, was evaluated for its ability to diagnose *C. difficile* disease. The results of this assay were compared to those of both PCR and toxigenic culture. The results showed that this assay allows 88% of specimens to be accurately screened as either positive (both tests positive) or negative (both tests negative) for the presence of toxigenic *C. difficile* in less than 30 min and with minimal hands-on time. Use of a random-access PCR for the analysis of specimens with discrepant results (one test positive and the other negative) allows the easy, rapid, and highly sensitive (100%; 95% confidence interval [CI], 89.6 to 100%) and specific (99.6%; 95% CI, 97.3 to 99.9%) diagnosis of *C. difficile* disease. The use of this algorithm would save institutional costs, curtail unnecessary isolation days, reduce the nosocomial transmission of disease, and increase the quality of care for patients.

The laboratory diagnosis of *Clostridium difficile* disease has evolved significantly over the last several years, and many tests that may be used to assist with the detection of *C. difficile* infection are now available. These assays include enzyme immunoassays (EIAs), lateral flow tests, PCR assays, tissue culture cytotoxicity neutralization tests, and toxigenic culture. Many recent papers have reported on the use of different algorithms that use the tests mentioned above to allow the better diagnosis of *C. difficile* disease (4, 5, 8, 18, 19, 20, 23, 26). Many of these approaches incorporate cytotoxicity neutralization (CTN) assays or anaerobic agar culture with identification of the organism, followed by toxin testing. However, many laboratories do not have the technical expertise, facilities, or training to perform CTN assays (which are labor-intensive and somewhat subjective), and an anaerobic agar culture with toxin detection may take several days; both of these methods delay the reporting of results (14, 17, 19, 20, 21, 29). The use of PCR for the diagnosis of this disease has been shown to be very specific and sensitive but often does not allow for random-access (i.e., real-time) results and can be quite costly to perform as a stand-alone testing method (2, 6, 10, 12, 15, 17, 27, 28). There is a real need for a rapid and simple testing strategy for the accurate diagnosis of *C. difficile* disease. With this in mind, we examined a simple, rapid two-step algorithm for the laboratory diagnosis of *Clostridium difficile* disease utilizing the new C.Diff Quik Chek Complete assay, a lateral flow kit that uses a combination of glutamate dehydrogenase (GDH) antigen detection plus toxin A and B detection for initial screening and a random-access, highly sensitive Xpert *C. difficile* PCR assay which detects the toxin B gene for samples with discrepant results.

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MATERIALS AND METHODS

Patients and specimens. The study described here was prospectively conducted at the Northwest Kaiser Permanente regional laboratory in Portland, OR. The regional laboratory performs *C. difficile* diagnostic testing for two medical centers and 20 clinics. Testing was performed with nonformed stool specimens from May to November 2009. The specimens were transported and stored at 4°C until they were assayed, and testing was performed with fresh specimens daily for all assay systems. Of the 261 patients included in the study, only 21 patients provided two samples and 1 patient provided three samples for evaluation.

***C. difficile* assays.** Briefly, 284 samples (from 261 patients) were received for the diagnosis of *C. difficile* infection and were tested by four assays, resulting in five results: a GDH antigen-specific EIA (GDH-EIA) that tests for glutamate dehydrogenase (C.Diff Chek-60; Techlab, Blacksburg, VA); a lateral flow assay for toxins A and B (LF-TOX assay; C.Diff Quik Chek; Techlab); the C.Diff Quik Chek Complete lateral flow assay (Techlab), which tests for both GDH (COMP-GDH assay) and toxins A and B (COMP-TOX assay); and the Xpert *C. difficile* PCR assay (Cepheid, Sunnyvale, CA), a random-access, rapid PCR assay for the detection of the toxin B gene (*tcdB*), which is associated with toxigenic *C. difficile*. All assays were performed and the results were interpreted according to the manufacturers' instructions, including the use of appropriate controls, as specified by each company. A sample was determined to be positive for *C. difficile* disease if it was positive by at least one test for GDH, at least one test for toxin, and the Xpert *C. difficile* PCR assay. Patients determined to be negative for *C. difficile* disease tested negative by all five tests.

Enriched toxigenic culture. All discrepant specimens (not determined to be positive or negative by the tests described above) were subjected to enriched toxigenic culture, and a part of the sample was frozen at −20°C on the same day. Toxigenic culture was performed as described previously (20). The specimens were first subjected to alcohol shocking by adding a small portion of the stool specimen to equal amounts of 95% ethanol for 15 min. Three to 4 drops of these treated specimens were added to chopped meat anaerobic broth (Anaerobe Systems, Morgan Hill, CA) and incubated for 24 to 48 h. The inoculated chopped meat broths (CMBs) were filtered through a 0.45-μm-pore-size filter (Fisher Scientific, Pittsburgh, PA) and tested for toxin by the LF-TOX assay after 24 h

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TABLE 1. Results for 284 specimens tested

Result by:					No. of samples:		
GDH-EIA	COMP-GDH assay	LF-TOX assay	COMP-TOX assay	PCR	With initial results	Positive by toxigenic culture	TP or TN[c]
+[a]		+[b]		+	27	NT[d]	27 TP
−	−	−	−	−	224	NT	224 TN
+	+	−	−	+	15[e]	15	15 TP
+	+	−	−	−	10	0	10 TN
+	−	−	−	−	3	0	3 TN
−	+	−	−	−	2	0	2 TN
−	+	+	+	−	1	0	1 TN
−	−	+	+	−	1	0	1 TN
−	−	−	−	+	1[a]	0	1 TN

[a] At least one positive result by GDH-EIA or COMP-GDH.
[b] At least one positive result by LF-TOX or COMP-TOX.
[c] TP, true positive; TN, true negative.
[d] NT, not tested.
[e] One of 15 and 1 of 1 specimens were from the same patient and were tested within the same week.

of incubation, and if the result was negative, they were tested again after 48 h of incubation. If the CMBs were negative by this assay, they were subcultured onto cycloserine-cefoxitin-fructose agar (CCFA) anaerobic plates (Anaerobe Systems), incubated for up to 7 days, and observed daily for growth. *C. difficile* organisms isolated from CCFA cultures were subcultured into chopped meat broth (C-CMB), incubated for 24 and 48 h, and then tested as described above. In addition, samples that were positive by the Xpert *C. difficile* PCR assay but negative by the enriched toxigenic cultures described above were sent for referral testing to the R. M. Alden Research Laboratories (Culver City, CA). Briefly, freshly frozen stool samples were thawed, placed directly into TAL broth (cycloserine-cefoxitin-mannitol broth with taurocholate and lysozyme; Anaerobe Systems), and then ethanol treated and plated directly onto CCFA-HT agar plates (cycloserine-cefoxitin-fructose agar with horse blood and taurocholate; Anaerobe Systems). If the specimens directly plated on CCFA-HT were negative for the growth of *C. difficile*, the TAL broths were subcultured onto CCFA-HT agar plates to look for organisms. If organisms grew at any time, they were directly tested for toxin production by the *C. difficile* Tox A/B II EIA (Techlab).

Freeze-thaw of specimens. Several GDH-positive specimens were frozen at −20°C immediately after they were tested (within ~30 h after collection). After the initial experimentation was concluded, these specimens were then removed from the freezer, thawed at room temperature, and retested by the two assays for GDH.

RESULTS

Two hundred eighty-four stool specimens were tested by all five tests. Twenty-seven specimens (9.5%) tested positive by at least one GDH test, at least one toxin test, and the Xpert *C. difficile* PCR assay (25 of these 27 specimens were positive by all five tests); and these specimens were considered positive without further testing. In addition, 224 (78.9%) specimens were negative by all five tests and were considered negative without further testing (Table 1). Thirty-three samples (11.6%) that did not meet the criteria mentioned above, including samples positive only for the GDH antigen, were considered discrepant and were subjected to in-house toxigenic culture. On the basis of toxigenic culture testing, 15 of these discrepant specimens were resolved to be positive, 12 of these samples were resolved by in-house toxigenic culture testing, and 3 were resolved by referral toxigenic culture testing (Table 1). A total of 18 specimens were resolved to be negative: 15 by in-house toxigenic culture and 3 by referral toxigenic culture testing (no nontoxigenic *C. difficile* isolates were found) (Table 1). Thus, of the 284 samples tested, 42 were considered positive (prevalence, 14.8%) and 242 were considered negative for toxigenic *C. difficile*. On the basis of these data, the assays tested had the following sensitivities and specificities: GDH-EIA, 100% and 94.2%, respectively; LF-TOX assay, 59.5% and 99.2%, respectively; COMP-GDH assay, 97.6% and 94.6%, respectively; COMP-TOX assay, 61.9% and 99.2%, respectively; and the Xpert *C. difficile* PCR assay, 100% and 99.6%, respectively. When the C.Diff Quik Chek Complete assay was used and two results which were deemed to be indeterminant (GDH negative and toxin positive [these two specimens were PCR negative]) were discarded, as recommended by the manufacturer, the sensitivity and the specificity were 60.0% and 99.6%, respectively. Use of the C.Diff Quik Chek Complete assay with reflex to the Xpert *C. difficile* PCR assay to test any discrepant samples (GDH positive/toxin negative or GDH negative/toxin positive) (Fig. 1) demonstrated a sensitivity and a specificity of 100% and 99.6%, respectively, for the diagnosis of toxigenic *C. difficile* infection (Table 2).

DISCUSSION

As reported by others, we found that both toxin assays (the LF-TOX and COMP-TOX assays) had poor sensitivities (59.5% and 61.9%, respectively), although they were both highly specific (1, 3, 4, 5, 16). However, both GDH assays proved to be highly sensitive in our study, having sensitivities of from 97.6 to 100%. This agrees with the findings of other studies that concluded that the test for GDH is excellent for

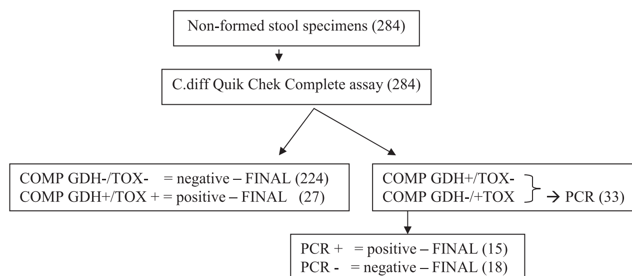

FIG. 1. Easy, rapid two-step algorithm for the diagnosis of *C. difficile* disease.

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TABLE 2. Sensitivity, specificity, hands-on time, material costs, and reimbursements for *C. difficile* assays

Assay	% sensitivity (95% CI[a])	% specificity (95% CI)	Hands-on time (min)	Turnaround time (min)	Material cost ($)/test	Medicare/Medicaid reimbursement ($)
PCR[b]	100 (89.6–100)	99.6 (97.3–99.9)	5[c]	60[c]	33.38	50.27
GDH	100 (89.6–100)	94.2 (90.3–96.7)	18[d]	70[d]	7.35	17.18
LF-TOX	59.5 (43.3–74.0)	99.2 (96.7–99.9)	9[c]	34[c]	5.50	17.18
C.Diff Complete	60.0 (43.4–74.7)	99.6 (97.3–99.9)	9[c]	34[c]	11.50	$34.36
Algorithm[e]	100 (89.6–100)	99.6 (97.3–99.9)	14[c]	94[c]	11.50, 44.88[f]	34.36, 50.27[f,g]

[a] CI, confidence interval.
[b] The FDA-cleared sensitivity of the Xpert *C. difficile* PCR assay is 93.5% compared to the results of broth-enriched toxigenic culture for *C. difficile*. The 100% sensitivity used in this table is based on our results and reasonable assumptions that were made, as discussed in the Materials and Methods and the Discussion sections.
[c] Hands-on time and turnaround time are based on testing of a single specimen.
[d] Hands-on time and turnaround time are based on batch testing of 20 specimens.
[e] The algorithm comprised the C.Diff Quik Chek Complete assay and testing of samples with discrepant results by PCR.
[f] Data are for 88%, 12% of specimens.
[g] Reimbursement for samples with discrepant results by the C.Diff Quik Chek Complete assay that are then tested by PCR consists of only that for the PCR component.

screening (5, 17, 18, 20) but is in contrast to the findings of others who have reported that GDH assays have sensitivities of from 70 to 88% compared to the results of toxigenic culture and/or PCR (1, 3, 8, 10, 12, 16, 21). These different sensitivities might possibly be due to regional/geographical differences in strain ribotypes that affect the GDH assays (indicating the possibility of a limited number of strains in our area) or may be due to the freezing and thawing of the stool samples prior to testing by the GDH assay. Some of the previously cited studies, which exhibited lower sensitivities for the GHD assay, performed this testing after the stool specimens had been frozen and then thawed (8, 12). In order to gain some insight into whether a freeze-thaw cycle might possibly account for the differences in the sensitivities of GDH assays that have been published, we thawed and tested 27 GDH-positive specimens that had been frozen within a few hours after they tested and that were positive by both the GDH-EIA and the COMP-GDH assay. For the GDH-EIA, 26 of the 27 specimens again tested positive, for a loss of 3.7% positivity; for the COMP-GDH assay, 24 of the 27 specimens tested positive, with 3 specimens testing negative (a loss of 11.1% positivity). Although these results are based on the results for a small number of retested samples, if a laboratory is using fresh stool specimens, either assay can be used; however, if frozen samples are to be used for testing for GDH, the GDH-EIA appears to be preferred.

Of the Xpert *C. difficile* PCR assay-positive discrepant samples in our study, 15/16 were toxigenic culture positive. The one sample with discrepant results by the Xpert *C. difficile* PCR assay that was not positive by toxigenic culture was from a patient from whom an additional sample was collected on the same day and which did resolve to a positive result by toxigenic culture. In all likelihood, this was also a truly positive sample but was considered to have a negative result for this study. These results might be because the patient received therapy for *C. difficile* infection, because of sampling discrepancies, or perhaps because of the less than 100% sensitivity of toxigenic culture. Even with this one false-positive result compared to the result of toxigenic culture, this Xpert *C. difficile* PCR assay could certainly be used as a stand-alone assay for the detection of *C. difficile* disease, as it has a sensitivity and a specificity of 100% and 99.6%, respectively. All patients in this study would have received the appropriate diagnosis from the results of the Xpert *C. difficile* PCR assay alone, and the results would have been available in a much more timely fashion compared to current toxigenic culture methods. The results of the Xpert *C. difficile* PCR assay would allow the more rapid institution of therapy as well as the implementation of the appropriate isolation procedures for patients who test positive, decreasing the spread of disease through nosocomial transmission. These data coincide with those from previously published studies regarding the performance of PCR assays for the detection of *C. difficile* infections (3, 7, 8, 10, 12, 21, 25).

The two-step algorithm used in this study, which utilizes a highly sensitive lateral flow GDH assay in combination with a highly specific lateral flow toxin assay (the C.Diff Quik Chek Complete assay), allows the detection of virtually all *C. difficile*-negative specimens within 30 min. Those specimens with discrepant results (GDH positive/toxin negative or GDH negative/toxin positive) would reflex to Xpert *C. difficile* PCR testing, and the results would be available within an additional 1 h. The C.Diff Quik Chek Complete assay is very simple to perform and permitted the very rapid reporting of final results for up to 88% of our specimens. For those samples with discrepant results that would reflex in this work flow to PCR testing, the Xpert *C. difficile* PCR assay is a simple and quick, random-access PCR method that provides confirmatory results in an expedient manner. These data show that although the Xpert *C. difficile* PCR assay method could be used as a stand-alone testing procedure, the price per test would add significant cost to the laboratory, and an initial capital expenditure is necessary for purchase of the instrument. Use of the C.Diff Quik Chek Complete assay as the first step in this algorithm to eliminate the need for secondary testing for up to 88% of specimens would vastly decrease the cost associated with confirmatory testing by PCR.

The turnaround time from specimen collection to the reporting of results for our current routine testing (batch testing by the GDH-EIA once per day and, if the result is positive, reflexing to the LF-TOX test and then, finally, reflexing to toxigenic broth culture if the LF-TOX test is negative) would range from a minimum of ~30 h to up to ~3 days. Our institution implements contact isolation upon the order of a diagnostic test for *C. difficile*, and deterring the costs associated with *C. difficile* disease has become a significant issue for a majority of medical institutions (9, 11, 13, 22). Implementation

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of a new testing algorithm by use of the C.Diff Quik Chek Complete assay with reflex to the Xpert PCR for discrepant results would allow patient results to be available within 3 h after the specimen reaches the laboratory and would save approximately 85% of annual isolation costs in our medical center (as only 15% of patients for whom the test is ordered truly have *C. difficile* disease). Adoption of this new algorithm not only will result in an up to 3-day reduction in the turnaround time for the reporting of results but also will increase our ability to detect *C. difficile*-positive patients, as it is more sensitive than our current method (20).

A limitation of this study is that only discrepant samples were further analyzed by the toxigenic culture assay, with assumptions being made that if samples are positive for the GDH antigen, for the toxin A and B antigens, and by the Xpert *C. difficile* PCR, they would also be positive by toxigenic culture; and those that were negative by all five assays would have been negative by toxigenic culture. This is a reasonable assumption, based on the known performance characteristics of the each of these assays (17, 24), and similar assumptions have been made in a previously published study (5); however, this is still a limitation of our study that must be taken into account. Our study was strengthened by use of a respected referral laboratory for testing of the Xpert *C. difficile* PCR assay-positive samples that were negative by in-house toxigenic culture, which resulted in three additional samples being identified as true positives. The referral laboratory had the added advantage of using culture directly onto CCFA plates, in addition to anaerobic broth culture. Another limitation to our study could relate to the relatively small sample size of 284 specimens. However, although the studies of both Quinn et al. (17) and Swindells et al. (24) (discussed below) did test all specimens by comparison with a known "gold standard," our sample included more than 100 additional specimens compared to the numbers used in those two studies.

Our results agree with those of Quinn and colleagues (17) and Swindells and colleagues (24), who both compared the C.Diff Quik Chek Complete assay to PCR. Both groups found that the C.Diff Quik Chek Complete assay had sensitivities of 78.3% and from 61.1 to 73.3%, respectively, whereas our study found a sensitivity of 60%. This difference could be due to the number of specimens in each evaluation, as we tested additional specimens, or again, it could be due to geographical differences associated with the different ribotypes of the organisms. In addition, during our study we found that one specimen tested toxin A/B positive and GDH negative by the C.Diff Quik Chek Complete assay, but such results were not seen by Quinn et al. (17) or Swindells et al. (24), which, again, is likely associated with the additional specimens that we evaluated. Our findings also agree with the results of the studies of Quinn et al. (17) and Swindells et al. (24) on the specificity of the C.Diff Quik Chek Complete assay, for which our data also showed a specificity of >99%. Quinn and colleagues concluded that for a reasonable expense, an FDA-cleared, user-friendly product is not currently available for confirmatory testing of *C. difficile* disease (17). In contrast, we found that the Xpert *C. difficile* PCR assay, when it was used with the C.Diff Quik Chek Complete assay, is a product that will allow the real-time, confirmatory testing of discrepant results, finally revolutionizing laboratory testing for *C. difficile* infection, as also discussed by Swindells and colleagues (24). This algorithm will also allow timely decisions for the appropriate placement of patients into isolation to be made.

In summary, use of the combination of the C.Diff Quik Chek Complete assay with reflex to Xpert *C. difficile* PCR testing for discrepant results provides a rapid, easy, and cost-effective means of accurately diagnosing *C. difficile* disease. Use of a test to screen for both GDH and toxins A and B will allow the laboratory to detect more samples with true-positive and true-negative results (>88%) without having to test these specimens by the more expensive PCR assay. Use of only a test for GDH and testing of all GDH-positive samples by PCR, as well as use of only a test for toxins A and B and testing of all toxin A/B-negative samples, would unnecessarily increase the number of PCR tests. The material costs per test for each of these assays are $11.50 (reimbursement cost, $34.36) for the C.Diff Quik Chek Complete assay (hands-on time, ~9 min per specimen) and $33.38 (reimbursement cost, $50.27) for the Xpert *C. difficile* PCR assay (hands-on time ~5 min per specimen). Thus, use of the C.Diff Quik Chek Complete assay as a screen, followed by use of the Xpert *C. difficile* PCR assay if the C.Diff Quik Chek Complete assay results are discrepant, would keep the expense for diagnosis much lower than the use of PCR alone and would be more accurate than the use of testing for GDH and/or toxins A and B alone (Table 2). Use of a GDH-EIA format and, if the result of that assay is positive, reflexing to the LF-TOX assay and then reflexing to the Xpert *C. difficile* PCR assay, if necessary, would give similar findings. Choosing the appropriate algorithm should be based on the specimen volume, work flow needs, and the clinical impact. One must keep in mind that if any assay is used in the batch mode, the turnaround time will be lengthened. Rapid reporting of results for *C. difficile* toxin will assist infection control with the isolation of patients with active disease, save the institution the costs associated with isolation, as well as save patients from unnecessarily being placed into isolation.

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REFERENCES

1. **Alcala, L., L. Sanchez-Cambronero, M. P. Catalan, M. Sanchez-Somolinos, M. T. Pelaez, M. Marin, and E. Bouza.** 2008. Comparison of three commercial methods for rapid detection of *Clostridium difficile* toxins A and B from fecal specimens. J. Clin. Microbiol. **46:**3833–3835.
2. **Belanger, 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.
3. **Eastwood, K., P. Else, A. Charlett, and M. Wilcox.** 2009. Comparison of nine commercially available *Clostridium difficile* toxin detection assays, a real-time PCR assay for *C. difficile tcdB*, and a glutamate dehydrogenase detection assay to cytotoxin testing and cytotoxigenic culture methods. J. Clin. Microbiol. **47:**3211–3217.
4. **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.
5. **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.
6. **Guilbault, C., A. C. Labbe, L. Poirier, L. Busque, C. Beliveau, and M. Laverdiere.** 2002. Development and evaluation of a PCR method for detec-

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tion of the *Clostridium difficile* toxin B gene in stool specimens. J. Clin. Microbiol. **40:**2288–2290.
7. **Haihui, H., A. Wientraub, H. Fang, and C. E. Nord.** 2009. Comparison of a commercial multiplex real-time PCR to the cell cytotoxicity neutralization assay for diagnosis of *Clostridium difficile* infections. J. Clin. Microbiol. **47:** 3729–3731.
8. **Kvach, E. J., D. Ferguson, P. F. Riska, and M. L. Landry.** 2010. Comparison of BD GeneOhm Cdiff real-time PCR assay with a two-step algorithm and a toxin A/B enzyme-linked immunosorbent assay for diagnosis of toxigenic *Clostridium difficile* infection. J. Clin. Microbiol. **48:**109–114.
9. **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.
10. **Larson, A. M., A. M. Fung, and F. C. Fang.** 2010. Evaluation of *tcdB* real-time PCR in a three-step diagnostic algorithm for detection of toxigenic Clostridium difficile. J. Clin. Microbiol. **48:**124–130.
11. **Lawrence, S. J., L. A. Puzniak, B. N. Shadel, K. N. Gillespie, M. H. Kollef, and L. M. Mundy.** 2007. *Clostridium difficile* in the intensive care unit: epidemiology, costs, and colonization pressure. Infect. Control Hosp. Epidemiol. **28:**123–130.
12. **Novak-Weekley, S. M., E. M. Marlowe, J. M. Miller, J. Cumpio, J. H. Nomura, P. H. Vance, and A. Weissfeld.** 2010. Clostridium difficile testing in the clinical laboratory by use of multiple testing algorithms. J. Clin. Microbiol. **48:**889–893.
13. **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.
14. **Peterson, L. R., M. M. Olson, C. J. Shanholtzer, and D. N. Gerding.** 1988. Results of a prospective, 18-month clinical evaluation of culture, cytotoxin testing, and Culturette brand (CDT) latex testing in the diagnosis of *Clostridium difficile*-associated diarrhea. Diagn. Microbiol. Infect. Dis. **10:**85–91.
15. **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.
16. **Peterson, L. R., and A. Robicsek.** 2009. Does my patient have *Clostridium difficile* infection? Ann. Intern. Med. **151:**176–179.
17. **Quinn, C. D., S. E. Sefers, W. Babiker, Y. He, R. Alcabasa, C. W. Stratton, K. C. Carroll, and Y. W. Tang.** 2010. C. Diff Quik Chek Complete enzyme immunoassay provides a reliable first-line method for detection of *Clostridium difficile* in stool specimens. J. Clin. Microbiol. **48:**603–605.
18. **Reller, M. E., C. A. Lema, T. M. Perl, M. Cai, T. L. Ross, K. A. Spek, 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.
19. **Reller, M. E., R. C. Alcabasa, C. A. Lema, and K. C. Carroll.** 2010. Comparison of two rapid assays for *Clostridium difficile* common antigen and a *C. difficile* toxin A/B assay with the cell culture neutralization assay. Am. J. Clin. Pathol. **133:**107–109.
20. **Sharp, S. E., W. M. Ivie, M. R. Buckles, D. M. Coover, J. C. Pohl, and P. A. Hatcher.** 2009. A simple 3-step algorithm for improved laboratory detection of *Clostridium difficile* toxin without the need for tissue culture cytotoxicity neutralization assays. Diagn. Microbiol. Infect. Dis. **64:**344–346.
21. **Sloan, L. M., B. J. Dureski, 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.
22. **Song, X., J. G. Bartlett, K. Speck, A. Naegeli, K. Carroll, and T. M. Perl.** 2008. Rising economic impact of Clostridium difficile-associated disease in adult hospitalized patient population. Infect. Control Hosp. Epidemiol. **29:** 823–828.
23. **Stamper, P. D., R. Alcabasa, D. Aird, W. Babiker, J. Wehrlin, I. Ikpeama, and K. C. Carroll.** 2009. 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. J. Clin. Microbiol. **47:**373–378.
24. **Swindells, J., N. Brenwald, N. Reading, and B. Oppenheim.** 2010. Evaluation of diagnostic tests for Clostridium difficile infection. J. Clin. Microbiol. **48:**606–608.
25. **Terhes, G., E. Utban, J. Soki, E. Nacsa, and E. Nagy.** 2009. Comparison of a rapid molecular method, the BD GeneOhm Cdiff assay, to the most frequently used laboratory tests for detection of toxin-producing *Clostridium difficile* in diarrheal feces. J. Clin. Microbiol. **47:**3478–3481.
26. **Ticehurst, J. R., D. Z. Aird, L. M. Dam, A. P. Borek, J. R. 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.
27. **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.
28. **van den Berg, R. J., N. Vaessen, H. P. Endtz, T. Schulin, 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.
29. **Zheng, L., S. F. Keller, D. M. Lyerly, R. J. Carman, C. W. Genheimer, C. A. Gleaves, S. J. Kohlhepp, S. Young, S. Perez, and K. Ye.** 2004. Multicenter evaluation of a new screening test that detects *Clostridium difficile* in fecal specimens. J. Clin. Microbiol. **42:**3837–3840.

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