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BRIEF COMMUNICATION

# Cost-effectiveness of a modified two-step algorithm using a combined glutamate dehydrogenase/toxin enzyme immunoassay and real-time PCR for the diagnosis of *Clostridium difficile* infection

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The analytical performance and cost-effectiveness of the Wampole Toxin A/B EIA, the C. Diff. Quik Chek Complete (CdQCC) (a combined glutamate dehydrogenase antigen/toxin enzyme immunoassay), two RT-PCR assays (Progastro Cd and BD GeneOhm) and a modified two-step algorithm using the CdQCC reflexed to RT-PCR for indeterminate results were compared. The sensitivity of the Wampole Toxin A/B EIA, CdQCC (GDH antigen), BD GeneOhm and Progastro Cd RT-PCR were 85.4%, 95.8%, 100% and 93.8%, respectively. The algorithm provided rapid results for 86% of specimens and the remaining indeterminate results were resolved by RT-PCR, offering the best balance of sensitivity and cost savings per test (algorithm ~US$13.50/test versus upfront RT-PCR ~US$26.00/test).


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## Introduction

Diagnostic testing for *Clostridium difficile* infection (CDI) has undergone a paradigm shift in recent years. While most clinical laboratories in Asia, Europe and the USA still utilize rapid and inexpensive toxin A/B enzyme immunoassays (EIAs), it is increasingly recognized that these tests have poor sensitivity, ranging from 32% to 73%.[1] Nucleic acid amplification tests (NAATs) have emerged as highly sensitive tests but costs and instrumentation pose challenges for widespread implementation. EIAs using the *C. difficile* common antigen, glutamate dehydrogenase (GDH Ag), represent a cheaper and rapid alternative for detection of *C. difficile*, with reported sensitivity of >90%,[2,3] but have to be used as part of an algorithm using a second confirmatory test that detects toxin.[4]

## Methods

The aim of our study was to evaluate a modified two-step algorithm consisting of an initial rapid combined GDH Ag/toxin EIA test [C. Diff Quik Chek Complete (CdQCC), Techlab, Blacksburg, VA, USA] followed by RT-PCR for indeterminate results in comparison to upfront RT-PCR and our current methodology (Wampole Toxin A/B EIA, Techlab) for the diagnosis of CDI in our institution. For RT-PCR, we evaluated two assays that detect the *tcdB* gene (encoding toxin B) of *C. difficile*: the Progastro Cd Assay (Gen-Probe Prodesse, Waukesha, WI, USA) and the BD GeneOhm C. diff Assay (BD Diagnostics, GeneOhm, San Diego, CA, USA). A total of 192 stool samples (54 positive and 138 negative by Wampole Toxin A/B EIA) from patients with suspected CDI submitted to the Rush University Medical Center (RUMC) clinical microbiology laboratory from January 2009 to March 2010 were included in the study. Specimens were stored at 4°C and processed within 24 hours for the Wampole Toxin A/B EIA. Testing with the other methodologies was carried out on the same day if possible; if not, specimens were frozen at −70°C, and thawed once for testing. We previously validated the detection of *C. difficile* toxin in stool specimens stored at −70°C (data not shown). All tests were performed according to the manufacturers' instructions by trained personnel blinded to results from the other assays.

Specimens were considered positive for the CdQCC test if both GDH Ag and toxin EIA were positive (Ag+/Tox+), negative if both GDH Ag and toxin EIA were negative (Ag−/Tox−) and indeterminate if only either the GDH Ag or toxin EIA was positive (Ag−/Tox+ or Ag+/Tox−). Overall, specimens were considered true positives if both RT-PCR assays were positive. Discrepant RT-PCR results were resolved using toxigenic stool culture as previously described.[5] The performance characteristics were calculated using concordant RT-PCR results or toxigenic stool culture as the gold standard. The McNemar test was performed using SPSS version 16.0. Values of $p < 0.05$ were considered significant. This study was approved with exempt status by the Institutional Review Board of RUMC.

## Results

A total of 192 stool isolates were tested, representing 173 unique patient encounters. Some 115 specimens were negative by all four assays. Forty-eight specimens were considered positive for toxin (true positives): 45 specimens were positive by both RT-PCR assays and three specimens were positive by the BD GeneOhm RT-PCR assay alone and confirmed by toxigenic stool culture (Table 1). The GDH Ag component of the CdQCC successfully identified 46 of the 48 true positive specimens (sensitivity 95.8%), while the toxin component alone was positive for two CdQCC specimens (both positive by RT-PCR/culture). Some 25 of 192 specimens (13%) were Ag+/Tox−; 10/25 (40%) were positive by RT-PCR. The sensitivity of the BD GeneOhm RT-PCR and Progastro Cd RT-PCR tests were 100% and 93.8%, respectively (McNemar test, $p = 0.25$; Table 2). The average cost per test was about US $13.60 (algorithm) versus US$26.00 (upfront RT-PCR; Table 3).

**Table 1** Distribution of results for four *C. difficile* assays

| Total per category ($N = 192$) | Wampole Toxin A/B EIA | C. Diff Quik Chek Complete | | BD GeneOhm RT-PCR | Progastro Cd RT-PCR |
|---|---|---|---|---|---|
| | | GDH Ag | Toxin A/B | | |
| 34 | + | + | + | + | + |
| 1 | − | + | + | + | + |
| 4 | − | + | − | + | + |
| 5 | + | + | − | + | + |
| 1 | + | − | + | + | + |
| 13 | + | − | − | − | − |
| 15 | − | + | − | − | − |
| 1[a] | − | − | − | − | Indeterminate |
| 1[b] | − | + | − | + | − |
| 1[b] | + | − | + | + | − |
| 1[b] | − | + | + | + | − |
| 115 | − | − | − | − | − |

[a] Specimen considered negative.
[b] Toxigenic stool cultures were positive for all three specimens.

**Table 2** Test performance characteristics

| Test | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|
| Wampole Tox A/B EIA | 85.4 (71.6—93.5) | 90.9 (84.8—94.9) | 75.9 (62.1—86.1) | 94.9 (89.4—97.8) |
| C. Diff Quik Chek Complete GDH Ag component | 95.8 (84.6—99.3) | 89.6 (83.1—93.8) | 75.4 (62.4—85.2) | 98.4 (94.0—99.7) |
| C. Diff Quik Chek Complete Toxin component | 79.2 (64.6—89.0) | 100 (96.8—100) | 100 (88.6—100) | 93.5 (88.1—96.7) |
| Algorithm (C. diff Quik Chek Complete + RT-PCR) reflexed to BD GeneOhm RT-PCR for discordant results | 100 (90.8—100) | 100 (96.8—100) | 100 (90.8—100) | 100 (96.8—100) |
| BD GeneOhm RT-PCR | 100 (90.8—100) | 100 (96.8—100) | 100 (90.8—100) | 100 (96.8—100) |
| Progastro Cd RT-PCR[a] | 93.8 (81.8—98.4) | 99.3 (95.6—100) | 97.8 (87.0—99.9) | 97.9 (93.6—99.5) |

[a] One specimen that was indeterminate with Prograstro Cd RT-PCR coded as a false positive (all other assays were negative).
Data are presented as mean (95% confidence interval).
EIA = enzyme immunoassay; GDH Ag = glutamate dehydrogenase antigen; NPV = negative predictive value; PPV = positive predictive value.

## Discussion

To improve the sensitivity and cost-effectiveness of CDI diagnoses, a number of alternatives to EIA toxin tests have been advocated, including an algorithm approach using GDH Ag as an initial screen followed by confirmation of toxin production using sensitive but laborious techniques such as toxigenic culture or cytotoxin assay.[4,6] There are few data for a modified step-wise approach that involves rapid and simultaneous detection of GDH Ag and *C. difficile* toxin followed by RT-PCR. We found that a modified two-step algorithm with the CdQCC led to accurate resolution of 86% of specimens within 25 minutes. This is similar to resolution rates of 92.6% and 88% reported by Swindells et al. and Sharp et al., respectively.[2,3] The GDH Ag+/Tox− specimens (14% specimens) were easily resolved by RT-PCR, which confirms that there is no need for up-front RT-PCR. We found that the algorithm approach offers the best balance of speed, sensitivity and cost savings per test (Table 3, Fig. 1).

Recent concerns regarding the sensitivity of GDH with different ribotypes[7] are probably because not all GDH Ag assays are equivalent in their performance characteristics, given that GDH seems to be highly conserved among *C. difficile* ribotypes.[8] Peterson et al. recently compared the performance of two different GDH Ag assays to RT-PCR, a stool cytotoxin assay and stool culture and found that only the CdQCC (besides a single commercial RT-PCR assay) had comparable sensitivity to broth-enriched toxigenic *C. difficile* culture.[9] Interestingly, culture failed to detect approximately 6.8% of what was classified as true CDI in this study,[9] which underscores the challenge of finding a reliable gold-standard test for *C. difficile*.

A limitation of our study was that toxigenic culture was not performed on all isolates; instead, a surrogate gold standard concordance of two commercially available RT-PCR

**Table 3** Comparison of testing algorithms[a]

| Test algorithm | Estimated cost (US$)[b] | | False positives/ 1000 tests | False negatives/ 1000 tests | Turnaround time |
|---|---|---|---|---|---|
| | Per test | Per 1000 tests | (95% CI) | (95% CI) | |
| Wampole Toxin A/B EIA alone | 7.89 | 7890 (only 1 EIA)<br>14,462 (1 repeat EIA for initially negative specimens)<br>20,348 (2 repeat EIAs for initially negative specimens) | 82 (46—137) | 15 (6—28) | 30 minutes, batched |
| C. Diff QuikChek GDH Ag/ToxinEIA reflexed to GeneOhm RT-PCR for discordant results | 11.50 (QuikChek alone)<br>Additional 26.95 for RT-PCR | 13,663 (using 2 step algorithm)[c] | 0 (0—29) | 0 (0—9) | 25 minutes (QuikChek) real time, random access results |
| BD GeneOhm RT-PCR alone | 26.95 | 26,950 | 0 (0—29) | 0 (0—9) | 2 hours, batched |
| Progastro Cd RT-PCR alone | 25.00 | 25,000 | 6 (0—40) | 6 (2—18) | 3 hours, batched |

[a] Assuming 10% prevalence of CDI in specimens tested (based on recent prevalence rates at RUMC from February to March 2010).
[b] Cost based on list prices.
[c] Assuming 86% of tests can be initially resolved using the C. Diff QuikChek GDH Ag/Toxin EIA.
CI = confidence interval.

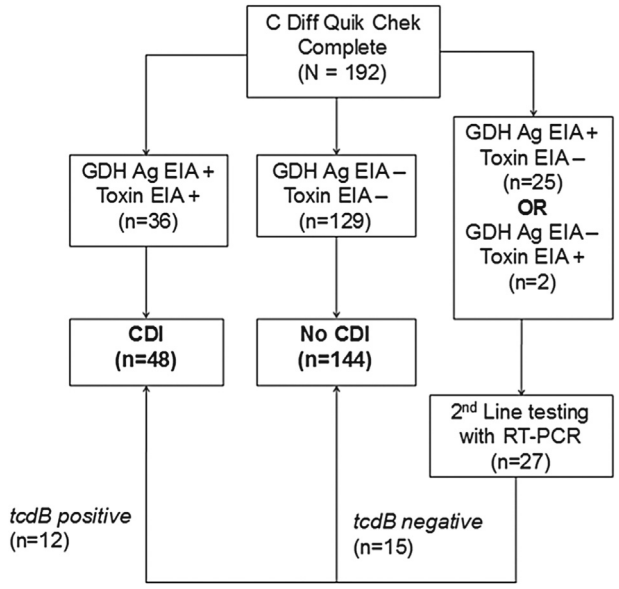


**Figure 1.** Modified two-step testing algorithm for *Clostridium difficile* infection. CDI = *Clostridium difficile* infection; EIA = enzyme immunoassay; GDH Ag = glutamate dehydrogenase antigen; *tcdB* = toxin B gene.

assays with toxigenic culture for discrepant RT-PCR assay results was used. Therefore, the sensitivity estimated for the algorithm and RT-PCR assays would be higher than expected. However, as the main aim of our study was to compare the CdQCC and the modified two-step algorithm with two commercially available RT-PCR assays for which the performance characteristics have been well described in the literature, we felt that this was a reasonable approach.

In summary, we found that a modified two-step algorithm (CdQCC followed by RT-PCR for indeterminate results) is a practical, rapid and cost-effective approach for the diagnosis of CDI. This would especially be suitable for small to medium-sized hospital laboratories for which upfront molecular testing is prohibitive.

## Conflicts of interest

All contributing authors declare no conflicts of interest.

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## Supplementary material

Supplementary material associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jmii.2012.07.008.

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