[C-018] Evaluation of a Screening Test for Detection of *Clostridium difficile* in Fecal Specimens

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INTRODUCTION

Clostridium difficile is the leading cause of nosocomial antibiotic-associated diarrhea (AAD) and colitis. The diagnosis of *C. difficile* disease is based on clinical history (antibiotic treatment), symptoms, and the presence of toxin in fecal specimens from the patient. The tissue culture assay detects the cytotoxic activity of toxin B using cultured mammalian cells. The test is very sensitive, detecting as little as 1 picogram of toxin B. It is highly specific when neutralization of the toxin activity is observed using specific *C. difficile* antitoxin. Thus tissue culture is considered by many to be the gold standard for *C. difficile* toxin testing. However, tissue culture is time consuming (24 to 72 hours before a sample can be ruled negative), tedious, and less cost-effective than enzyme immunoassays (for a review, see 1). Here we evaluate the use of a *C. difficile* common antigen EIA, *C. DIFF CHEK*™, that detects glutamate dehydrogenase (GDH), as a screening test to identify samples for further toxin testing.

METHODS

•One hundred and seventy-nine AAD fecal specimens were collected from hospitals and clinical laboratories. The specimens included solid, semi-solid and liquid samples.

•*C. difficile* common antigen GDH was analyzed at TechLab using the TechLab *C. DIFF CHEK*™ test in 2 formats, the 30-minute format (20-minute incubation plus 10-minute substrate development) and the 60-minute format (50-minute incubation plus 10-minute color development).

•*C. difficile* toxin B was analyzed at TechLab by tissue culture using the TechLab *C. DIFFICILE TOX-B TEST* (TC).

•Fecal DNA was extracted using the Qiagen QIAamp DNA Stool Mini Kit and analyzed for *C. difficile* GDH gene and toxin A and toxin B genes by polymerase chain reaction amplification, followed by electrophoretic identification of the amplicons. Primers were based on previously published sequences (2).

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RESULTS

•Both formats of *C. DIFF CHEK*™ gave the same results. The test detected all 13 of the TC-positive specimens, thus exhibiting a sensitivity and predictive negative value of 100% (Table 1).

•Seventeen samples that were positive in the *C. DIFF CHEK*™ were negative by TC assay. Sixteen of these 17 were confirmed positive for GDH gene by PCR, demonstrating a high specificity (99%) of the *C. DIFF CHEK*™ test for *C. difficile* (Table 2).

•Of the 16 samples that were positive for the GDH gene but negative by TC assay, 10 were carrying the *toxA* and *toxB* genes as determined by PCR (Table 3). Six samples were negative for *C. difficile toxA* or *toxB* gene by PCR.

Table 1. Comparison of *C. DIFF CHEK*™ to Tissue Culture Assay

N = 179	Tissue Culture Positive	Tissue Culture Negative
C. DIFF CHEK™ Positive	13	17
C. DIFF CHEK™ Negative	0	149

Sensitivity = 100%
Specificity =90%
Predictive Positive Value (PPV) = 43%
Predictive Negative Value (PNV) = 100%
Correlation = 91%

Note: PPV is lower in our study here (see DISCUSSION for possible causes). In other studies when fresher fecal specimens were tested, the reported PPV ranged from 53% to 69% (3, 4). This is because the antigen tests detect both toxigenic and nontoxigenic *C. difficile*.

Table 2. Comparison of *C. DIFF CHEK*™ to GDH Gene Detection by PCR

N = 179	GDH PCR Positive	GDH PCR Negative
C. DIFF CHEK™ Positive	29	1
C. DIFF CHEK™ Negative	2	147

Sensitivity = 94%
Specificity = 99%
Predictive Positive Value (PPV) = 97%
Predictive Negative Value (PNV) = 99%
Correlation = 98%

Note: These 2 PCR positive/*C. DIFF CHEK*™ negative samples were negative by TC and PCR for *toxA* or *toxB*.

Table 3. Comparison of *C. DIFF CHEK*™ to *toxA*/*toxB* Gene Detection by PCR

N = 179	*toxA*/*toxB* PCR Positive	*toxA*/*toxB* PCR Negative
C. DIFF CHEK™ Positive	24	6
C. DIFF CHEK™ Negative	0	149

Sensitivity = 100%
Specificity = 96%
Predictive Positive Value (PPV) = 80%
Predictive Negative Value (PNV) = 100%
Correlation = 97%

Note: PPV was lower because the *C. DIFF CHEK*™ test detects both toxigenic and nontoxigenic isolates of *C. difficile*.

DISCUSSION

•The newly developed *C. DIFF CHEK*™ test detected all 13 TC positive samples in this study. The high sensitivity and high negative predictive value, along with a rapid turnaround time demonstrate that the *C. DIFF CHEK*™ test is a suitable cost-effective screening test for laboratories using the tissue culture assay. Using *C. DIFF CHEK*™ as a screen could eliminate approximately 80% (83% in our study) of the negative samples in an hour or less from further toxin testing.

•*C. difficile* GDH is produced by both toxigenic and nontoxigenic isolates. Five of 29 (17%) of the GDH gene positive samples did not carry *toxA* or *toxB* gene, indicating that these specimens were from patients carrying nontoxigenic *C. difficile*. For this reason, we recommend that additional toxin testing be performed to confirm the presence of toxin in all *C. DIFF CHEK*™ positive samples.

•Eleven of 24 fecal specimens were positive for *C. difficile* common antigen by *C. DIFF CHEK*™ test and for toxin genes by PCR, but negative for toxin B by TC assay. This may result from low level of expression of the toxin genes and/or loss of the biological activity of the toxin during specimen storage.

CONCLUSIONS

Like other *C. difficile* antigen tests, the specificity and predictive positive value of the *C. DIFF CHEK*™ test are lower compared to toxin tests because antigen tests detect both toxigenic and nontoxigenic isolates. However, the high sensitivity and the high predictive negative value demonstrate the value of this test as a screen for patients with AAD.

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