[C-309] A New Rapid Test for Detecting *Clostridium difficile* Toxins A and B in Fecal Specimens

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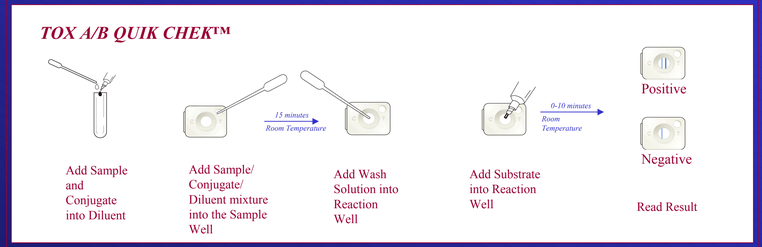
INTRODUCTION

Clostridium difficile is the leading cause of hospital-acquired antibiotic-associated diarrhea (AAD) and colitis. The two toxins of *C. difficile* are responsible for about 25% of AAD and most cases of pseudomembranous colitis. The diagnosis of *C. difficile* disease is based on clinical history such as antibiotic treatment, symptoms, and the presence of *C. difficile* toxin in stool specimens. Cytotoxicity assay using cultured cells and specific neutralization antiserum (tissue culture assay) is considered by many to be the gold standard for detecting toxin because of its superior sensitivity and specificity. However, the tissue culture (TC) assay takes 24 to 48 hours to complete and requires cell culture equipment. A sensitive rapid test will reduce the labor and turn-around time for detecting the presence of toxin in fecal specimens. In this study we evaluated a new rapid test, the *TOX A/B QUIK CHEK™*, and compared its performance with the tissue culture assay.

METHODS

- A total of 769 AAD fecal specimens, submitted for routine *C. difficile* toxin testing from AAD patients, were collected for analysis. The specimens included solid, semi-solid and liquid samples. The test results were not linked to the diagnosis of *C. difficile* disease.
- *TOX A/B QUIK CHEK™* - This test is a new rapid test from TECHLAB, Inc. Fecal specimens were prepared by a simple dilution. No filtering of specimens was required.



- Tissue culture assay – The *TOX–B TEST* from TECHLAB, Inc. was used with human foreskin cell monolayers.
- Commercial A/B ELISAs – *TOX A/B II™* (TECHLAB, Inc.) and another commercial A/B Test.

RESULTS

•Compared to the tissue culture assay, the gold standard, the sensitivity and specificity of the *TOX A/B QUIK CHEK™* was 90.1% and 99.7%, respectively. The positive and negative predictive values were 98.4% and 97.8%, respectively, and the correlation was 97.9%. The performance characteristics of the *TOX A/B QUIK CHEK™* and the *TOX A/B II™* were similar when compared to tissue culture assay.

Table 1. Comparison of antibody-based tests to Tissue Culture Assay

Test	Results	Tissue Culture Assay Pos	Tissue Culture Assay Neg	Sens	Spec	PPV	NPV	Corr
TOX A/B QUIK CHEK™ (n = 769)	Pos	127	2	90.1%	99.7%	98.4%	97.8%	97.9%
	Neg	14	626					
TOX A/B II™ (n = 546)	Pos	75	4	89.3%	99.1%	94.9%	98.1%	97.6%
	Neg	9	458					
Commercial A/B Test (n = 222)	Pos	41	7	85.4%	96.0%	85.4%	96.0%	93.7%
	Neg	7	167					

Table 2. Reaction of *C. difficile* strains in the *TOX A/B QUIK CHEK™*

The *TOX A/B QUIK CHEK™* test reacted with a typical strain (VPI strain 10463) that produces toxins A and B and with an atypical strain (CCUG 8864) that produces only an atypical toxin B. The test did not react with nontoxigenic strains.

Strain	toxin phenotype	Rx in TOX A/B QUIK CHEK™
C. difficile VPI strain 10463	A+ B+ CDT-	+
C. difficile CCUG 8864	A- B+ CDT-	+
C. difficile VPI strain 11186	A- B- CDT-	-
C. difficile strain 6088	A- B- CDT+	-

A, toxin A; B, toxin B; CDT, binary toxin related to iota toxin from *C. spiroforme* and *C. perfringens.*

A variety of normal intestinal bacteria and intestinal pathogens, including bacteria, viruses, and parasites, were checked for cross-reactivity in the *TOX A/B QUIK CHEK™*. Only toxigenic *C. sordellii*, which produces toxins HT (hemorrhagic toxin) and LT (lethal toxin) that are immunologically related to toxins A and B, reacted in the test.

DISCUSSION

•The *TOX A/B QUIK CHEK™* was comparable or slightly better than commercial A/B ELISAs when compared to the tissue culture assay, considered to be the gold standard. The sensitivity and specificity of the test was 90.1% and 99.7%, respectively, with a correlation of 97.9% with tissue culture. Of the 14 specimens that were tissue culture-positive, *TOX A/B QUIK CHEK™*-negative, 12 were negative by an A/B ELISA. Of the 2 specimens that were tissue culture-negative, *TOX A/B QUIK CHEK™*-positive, 1 was positive by an A/B ELISA.

•The *TOX A/B QUIK CHEK™* did not exhibit any cross-reactivity with members of the normal intestinal flora or with enteric pathogens including bacteria, viruses, and parasites.

•The *TOX A/B QUIK CHEK™* does not require any filtration of the fecal specimen, simplifying the preparation of the specimen. In addition, the test does not require the washing steps used with the microwell ELISAs. Thus, the procedure is easier to perform and more rapid than the ELISAs.

•The high sensitivity and high negative predictive value, along with a rapid turnaround time demonstrate that the *TOX A/B QUIK CHEK™* test is a suitable *in vitro* diagnostic test for the detection of toxins A and B in fecal specimens.

CONCLUSIONS

The *TOX A/B QUIK CHEK™* test is a new rapid test for the detection of toxins A and B in fecal specimens. The test offers clinical laboratories a suitable alternative assay that correlates well with tissue culture assay and commercial A/B ELISAs.

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