[C-329] Two Rapid Tests for Detection of *Clostridium difficile* 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 of the toxin test 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 (1). A sensitive screening test, *C. DIFF CHEK*™, reduces the labor and turn-around time for reporting the negative results (2). In this study we evaluated 2 new membrane tests by comparing them to *C. DIFF CHEK*™ and TC, the gold standard. Both of these tests detect *C. difficile* glutamate dehydrogenase (GDH), also called the "common antigen" because it is expressed at a high level by all *C. difficile* strains (3). These screening tests are:

- *C. DIFF QUIK CHEK*™, a rapid membrane test using peroxidase-linked immunoglobulins for detecting *C. difficile* GDH in fecal specimens.
- *C. DIFF EZ VUE*™, a rapid membrane test using gold-labeled conjugate for detecting GDH in fecal specimens.

METHODS

Three hundred and seventy-seven fecal specimens, submitted for routine *C. difficile* toxin testing from AAD patients, were collected from hospitals and clinical laboratories. The specimens included solid, semi-solid and liquid samples. Stool samples from babies (8-months to 2 years) were not excluded from this study because only the presence of *C. difficile* were tested and the test results were not linked to the diagnosis of *C. difficile* disease.

These samples were screened for *C. difficile* using:

- *C. DIFF CHEK*™ from TECHLAB, Inc. based on the package insert. *C. DIFF CHEK*™ is an ELISA test for detection of *C. difficile* GDH in fecal specimens.
- *C. DIFF QUIK CHEK*™, a prototype rapid membrane test using antibody conjugated with HRP. The test was performed according to manufacturer's instruction.
- *C. DIFF EZ VUE*™, a prototype rapid membrane test. The test was performed by adding the sample into a diluent before loading the diluted sample into the sample well of a test device. The device was read in 10 minutes for result.

One hundred and sixty-two samples were also tested using the TC assay. The cytotoxicity assay was performed using the *C. DIFFICILE TOX-B TEST* and cultured human foreskin cells or CHO cells.

RESULTS

The *C. DIFF QUIK CHEK*™ and the *C. DIFF EZ VUE*™ test were comparable to the *C. DIFF CHEK*™ test. The correlations of the two new rapid tests with the *C. DIFF CHEK*™ test were 97.3% and 96.6%, respectively (Table 1).

The antigen tests were positive for all the samples that were TC positive. Compared to the tissue culture assay, the gold standard, the sensitivities of the *C. DIFF QUIK CHEK*™, *C. DIFF EZ VUE*™, and *C. DIFF CHEK*™ were all 100%. The negative predictive values for the screening tests were all 100%. The correlations of the tests to tissue culture assay were 85.2%, 86.4%, and 87.7%, respectively (Table 2).

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Table 1. Comparison of the Screening Tests

Test	Results	*C. DIFF CHEK*™ Positive	*C. DIFF CHEK*™ Negative	Sensitivity	Specificity	PPV	NPV	Correlation
C. DIFF QUIK CHEK™	Positive	110	10	100.0%	96.3%	91.7%	100.0%	97.3%
	Negative	0	257					
C. DIFF EZ VUE™	Positive	104	6	93.7%	97.7%	94.5%	97.4%	96.6%
	Negative	7	260					

Table 2. Comparison of the Screening Tests to Tissue Culture Assay

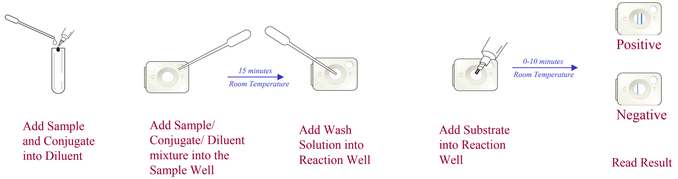
Test	Results	Tissue Culture Assay Positive	Tissue Culture Assay Negative	Sensitivity	Specificity	PPV	NPV	Correlation
C. DIFF QUIK CHEK™	Positive	30	24	100.0%	81.8%	55.6%	100.0%	85.2%
	Negative	0	108					
C. DIFF EZ VUE™	Positive	30	22	100.0%	83.3%	57.7%	100.0%	86.4%
	Negative	0	110					
C. DIFF CHEK™	Positive	30	20	100.0%	84.8%	60.0%	100.0%	87.7%
	Negative	0	112					

These screening tests detect both toxigenic and nontoxigenic strains of *C. difficile*. Thus the positive predictive value and correlation are lower. See DISCUSSION for details.

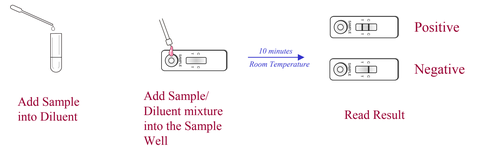
DISCUSSION

- The *C. DIFF QUIK CHEK*™ and *C. DIFF EZ VUE*™ tests were comparable to the *C. DIFF CHEK*™ test in this study. All three tests detected all 30 TC positive samples. The high sensitivity and high negative predictive value, along with a rapid turnaround time demonstrated that the *C. DIFF QUIK CHEK*™ test and *C. DIFF EZ VUE*™ test are suitable rapid screening tests for laboratories using the tissue culture assay or PCR for toxin genes. Using these tests as a screen could eliminate approximately two-thirds of the samples in less than 30 minutes from further toxin testing, which translates into cost savings on unnecessary patient isolation and extra precaution used for *C. difficile* disease patients.
- In our study, only about 55-60% of the fecal specimens positive for *C. difficile* common antigen tests were positive for toxin B by the TC assay. Although a GDH-positive/TC-negative result may indicate growth of nontoxigenic isolates of *C. difficile* in the patient, we cannot rule out the possibility that some of these specimens were true positives that contained amounts of toxin below the detection limits of the tissue culture assay. Therefore, these results should alert the physician to monitor the patient closely and to perform additional testing if necessary.
- Like other *C. difficile* antigen tests, the specificity and positive predictive value of the *C. DIFF QUIK CHEK*™ test and the *C. DIFF EZ VUE*™ test are lower compared to toxin tests because antigen tests detect both toxigenic and nontoxigenic isolates. This has been reported by other investigators (4,5). However, the high sensitivity and the high predictive negative value demonstrate the value of these tests as a screen for patients with AAD.

C. DIFF QUIK CHEK™



C. DIFF EZ VUE™



CONCLUSIONS

The *C. DIFF QUIK CHEK*™ test and *C. DIFF EZ VUE*™ test are excellent screening tests for laboratories using the tissue culture assay or toxin-PCR for detecting *C. difficile* in stool samples from patients with AAD. The tests should be followed with toxin testing, because these tests do not distinguish between toxigenic and non-toxigenic strains of *C. difficile*.

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