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Comparison of the *TOX A/B QUIK CHEK*™ to tissue culture assay and to a commercial A+B ELISA for the detection of *Clostridium difficile* toxins in fecal specimens

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Abstract

Clostridium difficile causes about 25% of the cases of antibiotic-associated diarrhea and most cases of pseudomembranous colitis. The disease results from the production of toxins A and B by *C. difficile* in the colon once the normal flora has been altered, most typically by antibiotics. The *TOX A/B QUIK CHEK*™ is a new rapid membrane test for the detection of toxins A and B in fecal specimens from patients suspected of having *C. difficile* disease. In the following study, we compared the *TOX A/B QUIK CHEK*™ to our in-house tissue culture assay and to the *C. DIFFICILE TOX A/B II*™, a commercial ELISA for toxins A and B of *Clostridium difficile*. The results are shown in the tables. Our results show that this new rapid test, which gives results in 25 minutes or less, correlates well (>98%) with both of these tests. The sensitivity for the *TOX A/B QUIK CHEK*™ compared to tissue culture assay was 89.1% with a specificity of 99.7%.

Introduction

Clostridium difficile is the causative agent of pseudomembranous colitis. The syndrome is most often associated with antibiotic use. The organism produces two main toxins which are associated with the disease. Toxin A, a potent enterotoxin with minimal cytotoxic capabilities, involves the erosion of the intestinal mucosa and then a fluid response in the intestine. The second, cytotoxin B, is a heat labile toxin that causes a decrease in protein synthesis, disorganization of actin filaments and loss of intracellular potassium.

The cytotoxin B assay has been the "gold standard" for the determination of *Clostridium difficile* disease. However many hospitals elect not to perform the assay. This choice is often made since the test is technically difficult to perform, is difficult to transport due to its sensitivity to heat, and the time required to detect a negative sample. For these reasons, many laboratories have elected to assay for toxin. Toxin assays utilize a same day enzyme immunoassay. The purpose of this study was to evaluate a new rapid test for the simultaneous detection of both Toxin A and B.

Materials and Methods

Specimens: Samples: A total of 400 stool samples were included in the study. Stool samples were included in the study when a *Clostridium difficile* test was ordered by a physician. Samples were analyzed fresh or were stored at 4 C for fewer than 24 hours prior to testing.

Assays: *TOX A/B QUIK CHEK*™ (TECHLAB , Blacksburg, VA) is a rapid membrane-filter immunoassay for detecting both toxin A and B. The assay can easily be completed in 30 minutes. All reagents are contained in the kit ready for use (Figure 1). One drop of conjugate is added to 500 ml of diluent, and then 25 ml of specimen is added and mixed thoroughly. The membrane device is removed from a foil pack, and 400 ml of the diluted specimen is added into the *Sample Well*.

The device is then incubated at room temperature for 15 minutes. Following incubation, 300 ml of *Wash Buffer* is added to the *Reaction Window* and allowed to filter through completely. Two drops of *Substrate* are then added to the *Reaction Window* and allowed to incubate at room temperature for 10 minutes before evaluation. The *Reaction Window* contains a control line and a test line (see Figure 2).

C. DIFFICILE TOX A/B II ™ (TECHLAB , Blacksburg, VA) is a rapid immunoassay for detection of both toxin A & B. It is a 96-well format assay, and wells may be broken off so that only the required number of wells are used for each run. This assay may be performed in either 1 hour, or 30 minutes using the rapid format. Results may be evaluated visually, or with a spectrophotometer.

Cytotoxin Assay – The cytotoxin assay was performed by standard methods using MRC-5 cells. All positives were confirmed by toxin-neutralization, and cultures were held for 48 hours before reporting as negative.

Figure 1

Figure 2

Results

Fifty-five (13.8%) of samples were determined to be positive indicating *Clostridium difficile* disease, 49 were positive by *TOX A/B QUIK CHEK*™ and 55 were positive by cell culture. One sample was positive by EIA and negative by cell culture, while 6 were positive by cell culture and negative by EIA. The sensitivity, specificity and positive and negative predictive values compared to the cytotoxin assay were 89.1%, 99.7%, 98%, 98.3%, respectively.

TOX A/B QUIK CHEK™ VERSUS TISSUE CULTURE ASSAY

N = 400	Tissue Culture Pos	Tissue Culture Neg
A/B QUIK CHEK Pos	49	1
A/B QUIK CHEK Neg	6	344
Sensitivity	89.1%	
Specificity	99.7%	
Predictive Pos Value	98.0%	
Predictive Neg Value	98.3%	
Correlation	98.3%	

TOX A/B QUIK CHEK™ VERSUS *C. DIFFICILE TOX A/B II*™

N = 400	A/B II Pos	A/B II Neg
A/B QUIK CHEK Pos	46	1
A/B QUIK CHEK Neg	2	351
Sensitivity	95.8%	
Specificity	99.7%	
Predictive Pos Value	97.9%	
Predictive Neg Value	99.4%	
Correlation	99.3%	

Conclusions

The rapid immunoassay performed well as to sensitivity and specificity. The *TOX A/B QUIK CHEK*™ was simple to perform, provided rapid results, and did not require additional equipment, such as an incubator, plate shaker, or spectrophotometer. It is well suited for settings where rapid results on low test volumes are required.