



Toxigenic *C. difficile*?

C-026 Comparison of the *C. difficile* TOX A/B QUIK CHEK™ with commercial *C. difficile* A+B ELISAs

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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 fecal specimens. ELISAs for the detection of toxins A and B in fecal specimens are commonly used as *in vitro* diagnostic aids for *C. difficile* disease. However, there is a need for more rapid tests for these toxins. 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™ test, and compared its performance with commercial A+B ELISAs.

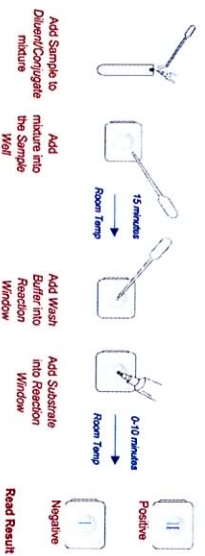
METHODS

Fecal specimens that were submitted for routine fecal testing were collected from the West Virginia University Hospital (Morgantown, WV) and the Carilion Medical Center (Roanoke, VA). The specimens included solid, semi-solid, and liquid samples. Stool specimens from babies (8-months to 2-years) were included in these studies because only the presence of *C. difficile* and its toxins were tested. Test results were not linked to the diagnosis of *C. difficile* disease. The following tests were used in the evaluation:

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.

Commercial A+B ELISAs – **C. DIFFICILE TOX A/B I/II™** (TECHLAB®, Inc.) and another commercial A+B Test.

Procedure for performing the TOX A/B QUIK CHEK™



RESULTS

Comparison of the *C. difficile* TOX A/B QUIK CHEK™ test to the *C. DIFFICILE* TOX A/B I/II™ ELISA

N=466	TOX A/B I/II™ positive	TOX A/B I/II™ negative
TOX A/B QUIK CHEK™ positive	72	1
TOX A/B QUIK CHEK™ negative	2	391

Sensitivity	97.3% (93.7 to 99.5%)	95% Confidence Intervals are in parentheses
Specificity	99.7% (98.4 to 99.9%)	
Predictive Pos Value	98.6% (91.6 to 99.9%)	
Predictive Neg Value	99.5% (98.0 to 99.9%)	
Correlation	99.4% (93.3 to 99.4%)	

Comparison of the *C. difficile* TOX A/B QUIK CHEK™ test to another commercial A+B ELISA

N=256	A+B ELISA positive	A+B ELISA negative
TOX A/B QUIK CHEK™ positive	50	2
TOX A/B QUIK CHEK™ negative	1	203

Sensitivity	98.0% (88.2 to 99.9%)	95% Confidence Intervals are in parentheses
Specificity	99.0% (96.1 to 99.8%)	
Predictive Positive Value	96.2% (85.7 to 99.3%)	
Predictive Negative Value	99.5% (96.9 to 99.9%)	
Correlation	98.4% (98.0 to 98.7%)	

RESULTS

In the first study, a total of 466 specimens were analyzed. There were 72 specimens that were positive in both the TOX A/B QUIK CHEK™ and the *C. DIFFICILE* TOX A/B I/II™ ELISA. There were 391 specimens negative in both tests. The positivity rate was 15.5%. The sensitivity and specificity were 97.3% and 99.7%, respectively, and the correlation was 99.4%.

In the second study, a total of 256 specimens were analyzed. There were 50 specimens positive in the TOX A/B QUIK CHEK™ and the other A+B ELISA. There were 203 specimens negative in both tests. The positivity rate was 19.5%. The sensitivity and specificity were 98.0% and 99.0%, respectively, and the correlation was 98.4%.

DISCUSSION

The TOX A/B QUIK CHEK™ was comparable in its performance to two commercial A+B ELISAs. In both studies, the sensitivity and specificity were >97% and 99.0% or higher, respectively. The correlation in both studies was >98%.

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 TOX A/B QUIK CHEK™ can be performed as soon as the samples reach the labs. This eliminates the waiting time for batch testing, which is routine in some labs using ELISA.

The high sensitivity and high predictive values, 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 commercial A+B ELISAs.

REFERENCES

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2. Stanek JL, Weckbach LS, Allen SD, Siders JA, Gilligan PH, Goppit G, Kraft JA, Willis DH (1998) Multicenter Evaluation of Four Methods for *Clostridium difficile* Detection: Immunocard *C. difficile* Cytoxin Assay, Culture, and Latex Agglutination. J. Clin. Microbiol. 34(11):2718-21

C-026. Comparison of the *C. difficile* TOX A/B QUIK CHEKTM with Commercial *C. difficile* A+B ELISAs

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Background: *Clostridium difficile* is the leading cause of nosocomial diarrhea and colitis. The disease typically develops in older persons following treatment with antibiotics although there are reports of community-acquired cases. Recently the organism and its disease has been associated with "strain 027" outbreaks in Europe, Canada, and the U.S. Because *C. difficile* is becoming an increasingly important healthcare problem, there is an urgent need for rapid tests that detect both toxin A and toxin B.

Methods: The *C. difficile* TOX A/B QUIK CHEKTM test is a new rapid membrane test that detects toxins A and B in fecal specimens. The test is formatted to allow mixing of the diluted specimen with conjugate, transfer of the mixture to a cassette containing immobilized anti-toxin antibodies, washing, and substrate development. The enclosed cassette minimizes exposure to the fecal specimen. The reaction is determined within 20-25 minutes, and specimens can be processed individually or batched. In on-site analyses, the TOX A/B QUIK CHEKTM was compared with the TECHLAB TOX A/B IITM and with another commercial A+B ELISA. **Results:** When compared to the TOX A/B IITM (N=466), the TOX A/B QUIK CHEKTM exhibited a sensitivity and specificity of 95.9% and 99.7%, respectively. The predictive positive and negative values were 98.6% and 99.2%, with a correlation of 99.1%. Compared to the other commercial A+B ELISA (N=256), the sensitivity and specificity were 98.0% and 99.0%. The predictive positive and negative values were 96.2% and 99.5%, with a correlation of 98.8%.

Conclusion: These results demonstrate that the TOX A/B QUIK CHEKTM performs comparably to commercial *in vitro* diagnostic A+B ELISAs, and demonstrate its value as a suitable rapid diagnostic aid for *C. difficile* disease.

C-028. Evaluation of C. DIFF Quick CHEK and Toxin A/B Quick CHEK for Diagnosis of *Clostridium difficile*-Associated Diarrhea

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Recently, TechLab (Blacksburg, VA) has marketed two test kits. One for the detection of Glutamate dehydrogenase (GDH), a common *Clostridium difficile* antigen, C. DIFF Quik CHEK (QC-Ag) and the other Tox A/B Quik CHEK (QC-AB) for the detection of toxins A/B. We evaluated these kits by comparing them with the Biosite Triage *C. difficile* panels (San Diego, CA), which detects GDH (T-Ag) and toxin A (T-A). In total 403 unformed fresh stool specimens from adult patients clinically suspected of having *Clostridium difficile*-associated diarrhea (CDAD) were studied. All samples were tested in parallel with TechLab and Triage kits and were cultured for *C. difficile* using CCFA plates (Quelabs, Quebec). Specimens positive for GDH but negative for toxin by any method were also tested with an in-house cytotoxin B tissue culture assay (CTA). Of 403 stool samples, 320 (79.4%) were negative for all *C. difficile* markers. Forty-four tests (10.9%) were considered to be diagnostic of CDAD. Of these, 34/44 (8.4%) were positive for T-Ag, QC-Ag, T-A and T-A/B. In another 10 GDH and CTA-positive specimens, the Triage kit failed to detect toxin A, eight of these were also negative by QC-A/B. Twenty-nine patients were carriers of *C. difficile*; their stool samples were positive for *C. difficile* by culture plus QC-Ag and/or T-Ag but tested negative for *C. difficile* toxins by T-A, QC-A/B and CTA. QC-Ag produced false positive results in 8 samples. Two culture positive samples were not detected by T-Ag and QC-Ag. These two were also negative for toxins by all three methods. The Triage panels could not be evaluated in 10 cases due to blackening of the test area. In conclusion, QC-Ag and QC-A/B were as sensitive as the Triage panel and easier to perform. The added advantage of the TechLab kits is that unlike Triage panels the panels for the detection of GDH and toxin are separate, thus stools samples can first be screened by QC-Ag and only positive samples require further testing. Without CTA, Triage panels and TechLab kits would have not confirmed 10/44 (22.7%), and 8/44 (18.2%) of toxin-positive stools respectively.



COMPARISON OF TRIAGE® TO TECHLAB® TESTS FOR DETECTION OF CLOSTRIDIUM DIFFICILE ASSOCIATED DIARRHEA (CDAD) IN STOOL SAMPLES

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Introduction:

Toxicigen *Clostridium difficile* causes antibiotic associated diarrhea (CDAD) which is the most common bacterial cause of nosocomial diarrhea. Indeed the incidence of CDAD is higher than all other traditional enteric pathogens (e.g. *Salmonella*, *Shigella*, *E.coli* O157:H7 etc) combined. Disease severity ranges from mild diarrhea to pseudo membranous colitis, toxic mega colon and death. This organism has recently been associated with increased incidence and disease severity in Quebec and in the USA. CDAD poses many challenges as a major nosocomial infection. There are multiple approaches to the diagnosis of CDAD but antigen detection for Toxin A and B are most widely used. The recent increased incidence and disease severity has focused attention on rapid and accurate diagnosis of CDAD in health care facilities.

Objective:

The aim of this study was to evaluate two antigen detection tests, the Triage® test and the TechLab® test.

Materials & Methods:

Ninety consecutive stool samples submitted for assessment of CDAD were included in the evaluation. The CPE assay using human foreskin fibroblast (HFF) cells were used to resolve discordants for the Triage test and when the results of the two methods being compared were different. For TechLab testing, the GD card was tested first and testing with the Toxin A/B card was only performed for those stools that were positive for the GD test card. For Triage testing, there is only one test card that detects both GD and Toxin A, however, additional testing with the CPE assay was done for discordant test results (i.e. GD(+), ToxA(-) or GD(-), ToxA(+)).

Triage® Cdifficile Test

Samples were diluted 1:10 and processed as per the manufacturer's recommendations. Briefly, this involved placing the diluted stool sample into a filter device and centrifuging it. The filtrate was then inoculated onto the surface of the test cartridge and conjugate added. The test was held at room temperature for 3 minutes. The device was washed twice. Then a substrate was added and the device incubated at room temperature for 5 minutes. Immediately after the incubation, the results were read. The appropriate reactions for both the positive and negative controls were required. Stool samples that showed a colored band (any amount of color development detected visually was considered positive) for both the Toxin A and GD antigens were considered positive. If only one of the Toxin A or GD antigens showed a color reaction, this was considered discordant, and no color reaction for either Toxin A or GD antigens was considered a negative test result.

Tissue Culture Cytotoxin Assay

Human foreskin fibroblasts (HFF) were used for this assay. The Human Foreskin Fibroblast (HFF) cell line was maintained in-house and monolayers of this cell line in 96 well trays were used for cytotoxin testing. The stool sample was diluted 1:5, centrifuged to pellet debris and organisms and the supernatant was filtered through a 0.45 um syringe filter unit. The filtered sample with and without polyclonal anti-*C. difficile* toxin B anti-toxin (Bareis) was inoculated into separate wells of the 96-well tissue culture tray where each well contained a confluent HFF monolayer. Cytotoxin effect (CPE) that was characterized by rounding of at least 50% of the HFF cells within 48h that was neutralized by anti-toxin was considered positive for the presence of *C. difficile* cytotoxin. *C. difficile* toxin B (Bareis) with and without anti-*C. difficile* anti-toxin (Bareis) were used as the positive and negative controls, respectively.

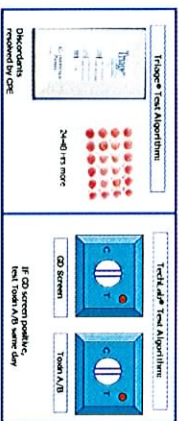
TechLab® Cdiff Quick

The fecal sample was added to diluent and conjugate. The mixture was transferred to the sample well and the device was incubated for 15 minutes. During this time, glutamate dehydrogenase could bind to the conjugate and the complexes were captured by the antibodies in the test line. The Reaction Window was washed and substrate was added. After a 5-10 minute incubation, the result was read.

Proper test performance is indicated by the formation of a blue line at the control side of the Reaction Window.

A positive result is indicated by the formation of a blue line at the test side of the Reaction Window.

When a positive result was obtained, the TechLab® Tox A/B Quick Chek was performed. The same procedure was followed. In this test, toxin A and toxin B were the antigens that formed complexes with the conjugate and were captured by the antibodies in the test line.



Results:

The results of the assay for TechLab are shown in Table 1. The results of the assay for Triage are shown in Table 2.

Table 1

TechLab® Test result:		Triage Test result:	
	Pos	Neg	
Positive	20	0	
CD Pos & ToxA/B Pos			
Negative	0	53	
GD Neg			
Negative	5	12	
CD Pos & ToxA/B Neg			
Total:	25	65	

Sensitivity: 20/25 = 80%
 Specificity: 53/65 = 81%
 PPV: 20/29 = 69%
 NPV: 53/70 = 92.9%

Table 2

Triage® Test result:		TechLab Test result:	
	Pos	Neg	
Positive (N = 19)	9	12	
(GD Pos & Tox A Pos)			
(GD Neg & Tox A Pos)	14	0	
Negative (N = 50)	1	53	
(GD Neg & Tox A Neg)			
Total:	25	65	

Sensitivity: 24/25 = 96%
 Specificity: 65/66 = 100%
 PPV: 24/24 = 100%
 NPV: 65/66 = 98.2%

Triage® Algorithm:

Discussion:

The TechLab test advantages include:
 - no discordants testing needed so that TAT for positives was faster,
 - no centrifugation step required,
 - results easy to read as most were strong reactions and the sample volume needed was small.

The Triage test advantages include:
 - one card to complete assay,
 - filtered sample used so less biohazard risk and sensitivity was excellent (i.e. doesn't miss true positives).

If discordants were not resolved for the Triage test, the TechLab test provided superior PPV (100% vs 66.7%) and specificity (100% vs 82%) but lower sensitivity (80% vs 96%) compared to the Triage test.

If the CPE assay was used in conjunction with the Triage test provided optimal sensitivity, specificity, PPV and NPV compared to TechLab testing.

Conclusions:

TechLab® test:
 -Excellent TAT
 -Less sensitivity

Triage® test:
 -Discordants an issue regarding TAT
 -Excellent sensitivity when used with CPE to resolve discordants

References

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