

[C-306] EVALUATION OF *C. DIFF CHEK*TM, A SCREENING TEST FOR *CLOSTRIDIUM DIFFICILE*

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INTRODUCTION

Clostridium difficile is the most common pathogen identified as the cause of antibiotic-associated diarrhea, pseudomembranous colitis and hospital-acquired diarrhea. *C. difficile* is also considered an important cause of enteric infections in nursing home residents. Clinical symptoms range from mild diarrhea to toxic megacolon. The toxigenic disease caused by *C. difficile* results from the production of two toxins, A and B. Toxin A is a 308-kDa enterotoxin. Toxin B is a 270-kDa cytotoxin. Both toxins produce cytopathic effects in cell culture, but Toxin B is more active in in vitro cell culture assays. In combination with clinical symptoms and clinical history, in vitro laboratory tests aide in the diagnosis of *C. difficile* disease. Laboratory tests include detection of *C. difficile* common antigen (i.e., glutamate dehydrogenase), cell culture tests for Toxin A and/or Toxin B, EIA for Toxin A and /or B, bacterial culture and PCR (1). We evaluated a new screening test, the *C.DIFF CHEK*TM, in two formats, the *C.DIFF CHEK*TM-60 and the *C.DIFF CHEK*TM-30. The *C.DIFF CHEK*TM is an enzyme immunoassay for use as a screening test to detect *C. difficile* antigen, glutamate dehydrogenase, in fecal samples. The goals of this evaluation were to determine the sensitivity, specificity, positive predictive value, negative predictive value and correlation of the *C. difficile* common antigen test for two testing formats. The results from the two testing formats were compared first to the results of glutamase dehydrogenase (GDH) gene *gluD* PCR and then the results from cytotoxin detection in cell culture.

METHODS AND MATERIALS

Specimens

This study used 300 unpreserved fecal specimens. All the samples used in this study were tested for Toxin A and/or B using *C. DIFFICILE TOX A/B II* (Wampole Laboratories, Princeton, NJ). Staff in the Microbiology section of TriCore Reference Laboratories carried out the testing as per the manufacturers' package insert. This routine testing for *C. difficile* toxin occurred on fecal samples that were stored between 2°C and 8°C and tested within 48 hours Excess fecal sample was de-identified and given a study number in accordance with the approval of this study by the University of New Mexico Human Research Review Committee. Each sample was tested for *C. difficile* common antigen using the *C.DIFF CHEK*TM-60 and the *C.DIFF CHEK*TM-30. Each sample was plated on cycloserine-cefoxitin-fructose plates (CCFA), incubated anaerobically and examined for *C. difficile*. In addition, a vial of stool was labeled with the study number and frozen at -70°C for future testing by *ghd* gene PCR and cytotoxin testing by cell culture.

*C.DIFF CHEK*TM-30

The *C.DIFF CHEK*TM-30 is an enzyme immunoassay developed by TechLab Inc., as a screening test to detect *C. difficile* common antigen. Briefly, 3mm of formed stool or 50µl of liquid stool was suspended in 200µl sample diluent. Two drops (100µl) of diluted fecal specimen were added to 1 drop (50µl) conjugate, mixed by gently tapping the microwell plate and incubated in a shaking incubator for 20 minutes at 37°C. Microwell contents were disposed of and wells were washed with 1X wash solution to remove debris. After washing, wells were inverted and slapped on paper towels to remove excess solution. The washing step was repeated 4 times or until all particulate matter was removed. Two drops (100µl) of substrate were added, samples were mixed by

gently tapping the microwell plate and then incubated for 10 minutes at room temperature. One drop (50µl) of stop solution was added to each well after the substrate incubation. The optical density was measured at 450 nm (single wavelength) and at 450/620 nm (double wavelength) on a microwell plate reader.

C.DIFF CHEK™-60

The *C.DIFF CHEK™-60* is an enzyme immunoassay developed by TechLab Inc., as a screening test to detect *C. difficile* common antigen. Briefly, 3mm of formed stool or 50µl of liquid stool was suspended in 200µl sample diluent. Two drops (100µl) of diluted fecal specimen were added to 1 drop (50µl) conjugate, mixed by gently tapping the microwell plate and incubated in a shaking incubator for 50 minutes at 37°C. Microwell contents were disposed of and wells were washed with 1X wash solution to remove debris. After washing, wells were inverted and slapped on paper towels to remove excess solution. The washing step was repeated 4 times or until all particulate matter was removed. Two drops (100µl) of substrate were added, samples were mixed by gently tapping the microwell plate and incubated for 10 minutes at room temperature. One drop (50µl) of stop solution was added to each well after the substrate incubation. The optical density was measured at 450 nm (single wavelength) and at 450/620 nm (double wavelength) on a microwell plate reader.

GDH GENE *gluD* PCR

DNA extraction and electrophoretic identification was performed by TechLab, Inc. (Blacksburg, VA). For PCR, DNA was extracted from fecal material using the QIAamp DNA Stool Mini Kit (Qiagen, Valencia, CA) according to manufacturer's instruction. The extracted DNA was stored at -70°C until use. PCR was performed using Ready-To-Go PCR beads (Amersham Biosciences, Piscataway, NJ) with 15µL of primers, each at a concentration of 1.67µM and 10µL of extracted fecal DNA. The PCR reaction for the GDH gene *gluD* was done using 45 cycles of 94°C 30sec, 56°C 1min and 72°C 1min. Primers were designed based on *C. difficile gluD* sequence (2) and included: a forward primer, 5'GGAAAAGATGTAAATGTC TTCGAGATG3' and a reverse primer, 5'CTGATTTACACCATTTCAGCCATAGC3'. The presence of a 750 bp *gluD* gene amplicon was determined electrophoretically on 1% agarose gel. In each assay, aliquots (1ng) of DNA from *C. difficile* VPI 11186 or water were used as positive and negative controls, respectively. The minimal amount of DNA detected was 1 copy of *gluD* gene per reaction, which is equivalent to about 100 copies per mL of feces. Discrepant samples with negative PCR results, along with more than 50 negative samples were checked for PCR inhibition. No PCR inhibition was observed with these samples.

Bacterial Culture

Approximately 100µl of sample was plated onto CCFA agar plates (Anaerobe Systems, Morgan Hill, CA). Inoculated plates were incubated at 37°C in anaerobic chambers for 48 to 72 hours. Presumptive *C. difficile* colonies were identified by their large size (4mm), yellow color, ground-glass appearance, circular shape with slight filamentous edge, and low umbonate to flat profile and a horsy smell. All specimens in which we detected *C. difficile* in culture were tested for cytotoxic activity using the *TOX B TEST*, TechLab, Inc. (Blacksburg, VA).

RESULTS

TABLE 1.

Comparison of the *C. DIFF CHEK™* - 60, *C. DIFF CHEK™* - 30, GDH gene *gluD* PCR, and bacterial culture for detecting *C. difficile* in fecal specimens.

TEST	RESULTS													
<i>C. DIFF CHEK™</i> - 60 ^a	+	-	+	-	-	+	+	-	-	+	-	-	+	+
<i>C. DIFF CHEK™</i> - 30 ^a	+	-	+	-	-	+	-	+	+	-	-	+	+	-
GDH gene <i>gluD</i> PCR	+	-	+	-	+	-	-	-	+	-	+	+	-	+
Bacterial Culture	+	-	-	+	-	-	-	-	-	+	+	-	+	-
Number	70	169	17	19	2	6	6	3	0	3	2	1	1	1
Percentage	23.3	56.3	5.7	6.3	0.7	2.0	2.0	1.0	0.0	1.0	0.7	0.3	0.3	0.3

TriCore Reference Laboratories, N=300

^aAlthough the results of the *C. DIFF CHEK™* test can be measured visually, by single wavelength at 450 nm, and by dual wavelength at 450/620 nm, only the dual wavelength measurements were evaluated for this study.

A total of 300 fecal samples were analyzed by the 30 and 60 minute *C.DIFF CHEK™*, bacterial culture and GDH gene *gluD* PCR (Table 1). There were 70 samples (23%) positive and 169 samples (56%) negative in all four tests. The remaining 61 (20%) gave discrepant results among the four tests. We compared the 30 and 60 minute *C.DIFF CHEK™*- and bacterial culture to GDH gene *gluD* PCR (Table 2). The 60 minute *C.DIFF CHEK™* test had a sensitivity (SEN), specificity (SPEC), positive predictive value (PPV), negative predictive value (NPV), and correlation CORR of 94.6%, 92.3%, 84.6%, 97.4%, and 93% respectively when compared to GDH gene *gluD* PCR. The 30 minute *C.DIFF CHEK™* test had a SEN, SPEC, PPV, NPV and CORR of 94.6%, 95.2%, 89.8%, 97.5% and 95% respectively when compared to GDH gene *gluD* PCR. Bacterial culture had a SEN, SPEC, PPV, NPV and CORR of 77.4%, 88.9%, 75.8%, 89.8%, and 85.3% respectively when compared to GDH gene *gluD* PCR.

Table 2. Comparison of the *C. DIFF CHEK™* - 60, *C. DIFF CHEK™* - 30, and bacterial culture to GDH gene *gluD* PCR

Test	Results	<i>gluD</i> PCR		Sensitivity	Specificity	PPV	NPV	Correlation
		Positive	Negative					
<i>C. DIFF CHEK™</i> - 60	positive	88	16	94.6%	92.3%	84.6%	97.4%	93.0%
	negative	5	191					
<i>C. DIFF CHEK™</i> - 30	positive	88	10	94.6%	95.2%	89.8%	97.5%	95.0%
	negative	5	197					
Bacterial Culture	positive	72	23	77.4%	88.9%	75.8%	89.8%	85.3%
	negative	21	184					

We compared the 30 and 60 minute *C.DIFF CHEK™*, bacterial culture, and GDH gene *gluD* PCR to cytotoxin detection by cell culture (Table 3). The 60 minute *C.DIFF CHEK™* test had a SEN, SPEC, PPV, NPV, and CORR of 97%, 83.3%, 62.5%, 99%, and 86.3% respectively when compared to cytotoxin detection by cell

culture. The 30 minute *C. DIFF CHEK*TM test had a SEN, SPEC, PPV, NPV, and CORR of 95.5%, 85.4%, 65.3%, 98.5%, and 87.7% respectively when compared to cytotoxin detection by cell culture. GDH gene *gluD* PCR had a SEN, SPEC, PPV, NPV, and CORR of 91%, 86.3%, 65.6%, 97.1%, and 87.3% respectively when compared to cytotoxin detection by cell culture. Bacterial culture had a SEN, SPEC, PPV, NPV, and CORR of 79.1%, 82%, 55.8%, 93.2%, and 81.3% respectively when compared to cytotoxin detection by cell culture.

Table 3. Comparison of the *C. DIFF CHEK*TM - 60, *C. DIFF CHEK*TM - 30, GDH gene *gluD* PCR and bacterial culture to cytotoxin cell culture assay

Test	Results	Tissue Culture Assay Results		Sensitivity	Specificity	PPV	NPV	Correlation
		Positive	Negative					
<i>C. DIFF CHEK</i> - 60	positive	65	39	97.0%	83.3%	62.5%	99.0%	86.3%
	negative	2	194					
<i>C. DIFF CHEK</i> - 30	positive	64	34	95.5%	85.4%	65.3%	98.5%	87.7%
	negative	3	199					
PCR for <i>gluD</i>	positive	61	32	91.0%	86.3%	65.6%	97.1%	87.3%
	negative	6	201					
Bacterial Culture	positive	53	42	79.1%	82.0%	55.8%	93.2%	81.6%
	negative	14	191					

CONCLUSIONS

- Both formats of the *C. DIFF CHEK*TM have good sensitivity, specificity, PPV, and NPV when compared to GDH gene *gluD* PCR.
- Bacterial culture has lower sensitivity, specificity, PPV, and NPV than the *C. DIFF CHEK*TM when compared to GDH gene *gluD* PCR.
- Both formats of the *C. DIFF CHEK*TM have good sensitivity, reduced specificity, a low PPV and a reasonable NPV when compared to cell culture cytotoxin assay.
- The GDH gene *gluD* PCR has good sensitivity, reduced specificity, a low PPV and a reasonable NPV when compared to the cell culture cytotoxin assay.
- In this evaluation bacterial culture has the lowest sensitivity, specificity and PPV with a reasonable NPV when compared to cell culture cytotoxin assay.
- *C. DIFF CHEK*TM is comparable to GDH gene *gluD* PCR when the cell culture cytotoxin assay is used as the “Gold Standard”.
- The observation that an antigen assay has performance equivalent to a PCR assay is interesting. Although we attempted to exclude inhibition as the source of this observation, it is still possible that inhibition did cause low level *gluD* target to be missed.
- The *C. DIFF CHEK*TM is a simple, rapid method with good performance characteristics that would facilitate the use of this assay in the routine clinical laboratory.

REFERENCES

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