[C-309] Comparison of Screening Tests for Detection of *Clostridium difficile* in Fecal Specimens ASM 2004 New Orleans, LA

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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 will reduce the labor and turn-around time for reporting the negative results. In this study we evaluated several tests as screens for subsequent tissue culture assay for detection of C. difficile and its toxins. These screening tests are:

- *C. DIFF CHEK™ – 60* and *C. DIFF CHEK™ – 30*, both are microwell enzyme immunoassay formats for the *C. difficile* common antigen glutamate dehydrogenase (GDH). GDH is called "the common antigen" because this enzyme is expressed at a high level in all *C. difficile* strains (2).
- An in-house polymerase chain reaction assay for the GDH gene gluD (PCR for gluD).
- Bacterial culture using selective cycloserine–cefoxitin-fructose agar (CCFA) plates.

METHODS

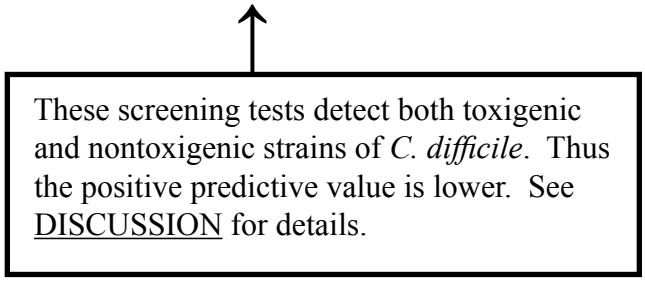
- Two hundred and twelve AAD fecal specimens, submitted for routine *C. difficile* toxin testing from AAD patients, were collected from hospitals and clinical laboratories. The specimens included solid, semi-solid and liquid samples. Stool samples from babies (8-months to 2 years) were not excluded from this study because only the presence of *C. difficile* and its toxins 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™ – 60* and *C. DIFF CHEK™ – 30* from TechLab, Inc. based on the manufacturer's instruction. *C. DIFF CHEK™ – 60* has a 50-minute incubation plus 10-minute color development. *C. DIFF CHEK™ – 30* has a 20-minute incubation in a shaking incubator plus 10-minute color development.
  - PCR for *gluD*, an in-house polymerase chain reaction assay for the *gluD* gene. Fecal DNA was extracted using the Qiagen QIAamp DNA Stool Mini Kit and analyzed for *C. difficile* GDH gene by polymerase chain reaction amplification, followed by electrophoretic identification of the amplicons. Primers were based on previously published sequences (2).
  - Bacterial culture. Fecal samples were plated on CCFA plates and presumptive colonies were recognized as described by Summanen et al (3).
- The results of the screening tests were compared to that of the TC assay, the gold standard. The tissue culture assay was performed using the TechLab *C. DIFFICILE TOX-B TEST* and cultured human foreskin cells or CHO cells.

RESULTS

- Compared to the tissue culture assay, the gold standard, the sensitivities of the *C. DIFF CHEK™ – 60*, *C. DIFF CHEK™ – 30*, PCR for *gluD*, and bacterial culture assay were 100%, 100%, 100%, and 73.3%, respectively. The negative predictive values for the screening tests were 100%, 100%, 100%, and 97.6%, respectively. The correlations of the tests to tissue culture assay were 90.1%, 91.0%, 89.6% and 82.1%,

Table 1. Comparison of the Screening Tests to Tissue Culture Assay

Test	Results	Tissue Culture Assay		Sensitivity	Specificity	PPV	NPV	Correlation
		Positive	Negative					
C. DIFF CHEK™-60	Positive	15	21	100.0%	89.3%	41.7%	100.0%	90.1%
	Negative	0	176					
C. DIFF CHEK™-30	Positive	15	19	100.0%	90.4%	44.1%	100.0%	91.0%
	Negative	0	178					
PCR for *gluD*	Positive	15	22	100.0%	88.8%	40.5%	100.0%	89.6%
	Negative	0	175					
Bacterial Culture	Positive	14	34	73.3%	82.7%	24.4%	97.6%	82.1%
	Negative	1	163					



- Twenty-one samples that were positive by the *C. DIFF CHEK™-60* test were negative by the TC assay. Nineteen of these samples were positive by the *C. DIFF CHEK™-30* test. Eighteen of these samples were confirmed positive for *gluD* gene by PCR, demonstrating a high correlation (96.7% to 97.6%) of the *C. DIFF CHEK™* test to the PCR test (Table 2). The PCR method used here is highly sensitive, detecting 1 copy of the *gluD* gene in each reaction.

Table 2. Comparison of the Screening Tests to PCR for *gluD*

Test	Results	PCR for *gluD*		Sensitivity	Specificity	PPV	NPV	Correlation
		Positive	Negative					
C. DIFF CHEK™-60	Positive	33	3	89.2%	98.3%	91.7%	97.7%	96.7%
	Negative	4	172					
C. DIFF CHEK™-30	Positive	33	1	89.2%	99.4%	97.1%	97.8%	97.6%
	Negative	4	174					
Bacterial Culture	Positive	18	27	52.9%	84.8%	40.0%	90.4%	79.7%
	Negative	16	151					

DISCUSSION

- The *C. DIFF CHEK™* test detected all 15 TC positive samples in this study, comparable to that by the PCR assay and outperformed the bacterial culture. **The high sensitivity and high negative predictive value, along with a rapid turnaround time demonstrated that the *C. DIFF CHEK™* test is a suitable cost-effective screening test for laboratories using the tissue culture assay or PCR for toxin genes.** Using the *C. DIFF CHEK™* as a screen could eliminate approximately 80% (>83% in our study) of the negative samples in an hour or less from further toxin testing, which translates into cost savings on

unnecessary patient isolation and extra precaution used for *C. difficile* disease patients.

- Fifteen of 37 fecal specimens positive for *C. difficile* common antigen by the *C. DIFF CHEK*™ test and/or by PCR 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 predictive positive value of the *C. DIFF CHEK*™ 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 this test as a screen for patients with AAD.

CONCLUSIONS

The *C. DIFF CHEK*™ test is an excellent screening test for laboratories using the tissue culture assay or toxin-PCR for detecting *C. difficile* in stool samples from patients with AAD. The test should be followed with toxin testing, because the *C. DIFF CHEK*™ test does not distinguish toxigenic or non-toxigenic strains of *C. difficile*.

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