C. Diff Chek™ - 60: A Rapid and Cost Effective Method for Detection of *Clostridium difficile* in Fecal Specimens

Charles Vaughn, Marie Smith, David Bankert, and Arthur E. Crist, Jr.

Division of Clinical Microbiology, Department of Laboratory Services, York Hospital, York, PA 17405

BACKGROUND

Laboratory diagnosis of *Clostridium difficile* antibiotic-associated diarrhea or pseudomembranous colitis can be accomplished by a variety of methods. Most of the methods in routine use rely on detecting toxins (Toxin A or B) produced by the organism and excreted in the stool. Detection of *C. difficile* cytotoxin (Toxin B) in tissue culture is considered to be the gold standard but is labor intensive and requires 48 hours to report a negative result. Methods to detect Toxin A or A+B by enzyme immunoassay (EIA) are rapid and easy to perform but may not be as sensitive as the cytotoxin assay (1,3). Another approach is to isolate the organism from fecal samples on selective medium followed by toxin induction and detection. Although sensitive and specific, this method is not performed in most laboratories because it is labor intensive and requires several days to complete. The C.DIFF CHEK™ – 60 is an EIA that detects the presence of glutamate dehydrogenase (GDH), a common antigen produced in large amounts by all toxigenic and non-toxigenic strains of *C. difficile*. Positive results are then confirmed using a toxin specific assay (2).

OBJECTIVE

To compare the performance of the C.DIFF CHEK™ – 60 (GDH assay) to our routine cytotoxin assay and to determine if it would be cost effective to implement in our laboratory.

MATERIALS AND METHODS

Specimens. Three hundred and twenty seven fecal samples submitted for *Clostridium difficile*-associated disease were evaluated in the study. These were liquid or semi-solid stools collected primarily from adult inpatients.

Cytotoxin Assay. The Cytotoxicity Assay for *Clostridium difficile* Toxin (Bartels, Trinity Biotech Co., Carlsbad, CA) was performed according to the manufacturers instructions and has been described previously (1). Briefly, the specimen was emulsified in sample diluent, centrifuged, and the supernatant passed through a 0.45 –um-pore-size-filter. An aliquot of the filtrate was then mixed with either sample diluent or polyclonal *C. difficile* toxin antibody (antitoxin). Both aliquots were tested, using a microtiter tray format, in wells containing human foreskin fibroblast cells. This gave a 1:40 final dilution of the patient specimen. Cells were incubated at 35°C and examined under 100X magnification after 24 and 48 hours incubation for any characteristic cytopathic effect that was neutralized by specific antitoxin.

GDH Assay. The C. DIFF CHEK™- 60 test (TechLab, Blacksburg, VA) was performed according to the manufacturers instructions. The test uses antibodies specific for the GDH of C. *difficile.* The microassay plate format has wells that contain immobilized polyclonal antibodies that trap GDH if present in the patient sample. The bound antigen is detected with a horseradish peroxidase conjugated monoclonal antibody that is incubated for 50 minutes, without shaking, along with the diluted sample. After washing, addition of the substrate (tetramathylbenzidine and peroxide) will produce a colored reaction if GDH is present. Color development was detected visually after the addition of a stopping reagent.

RESULTS AND CONCLUSIONS

Three hundred and twenty seven fecal samples were evaluated. The *C. difficile* cytotoxin assay was positive for 56 (17%) of the samples, while the GDH assay was positive for 88 (27%) (see Table 1). There was one false negative result with the GDH assay. The sensitivity, specificity, positive predictive (PPV), and negative predictive values (NPV) for the GDH assay compared to the cytotoxin assay were 98%, 88%, 62%, and 99%, respectively. Although the PPV is poor, using the C. Diff Chek - 60 would decrease our turnaround time and allow us to report 88% of the negatives on the same day of testing. Performing a cost analysis using reagents, supplies, and labor we estimate a savings of $3.61 a test (see Table 2). This is based on performing the GDH assay on all specimens and the cytotoxin assay on 27% of the specimens. Implementation of the GDH assay in our laboratory would result in an annual savings of approximately fourteen thousand dollars.

REFERENCES

1. Doern, G.V., R.T. Coughlin, and L. Wu. 1992. Laboratory diagnosis of *Clostridium difficile*-associated gastrointestinal disease: comparison of a monoclonal antibody enzyme immunoassay for toxins A and B with a monoclonal antibody enzyme immunoassay for toxin A only and two cytotoxicity assays. J. Clin. Microbiol. 30 : 2042-2046.

2. Gleaves, C., J.J. Kohlhepp, M. Campbell, *et. al.* 2003. Evaluation of the TechLab C. DIFF CHEK –30 and C. DIFF CHEK- 60 for detection of *C. difficile* in fecal specimens. Abstracts from the Annual Meeting of the American Society for Microbiology, May 18-22, Washington, DC, L013, Pg. 388.

3. Wilkens, T.D., and D.M. Lyerly. 2003. *Clostridium difficile* testing: after 20years, still challenging. J. Clin. Microbiol. 41 : 531-534.

Table 1. Comparison of C. diff Chek - 60 to the Cytotoxin Assay

		CTA Pos	CTA Neg	Totals
C.Diff Chek - 60	Pos	55	33	88
	Neg	1	238	239
	Totals	56	271	327

Sensitivity	98.2%	PPV	62.5%
Specificity	87.8%	NPV	99.6%

Table 2. Cost Analysis for the C.Diff Chek - 60 and Cytotoxin Assay

	C.Diff Chek - 60	CTA Assay
Time*	18 Min.	38 Min.
Reagents & Supplies	$3.65	5.69
Labor costs**	$6.71	$13.42
Total Cost per Test	$10.36	$19.11

* Hands on Time

** Based on an average hourly rate of $21.90

Annual Expense

Method	Tests/Year	Subtotal	Total
CTA	3,898	$74,491	$74,491
C.Diff Chek - 60 and CTA***	3,898	$40,383	$60,410
	1,048	$20,027	
Annual Savings			$14,081

*** C. Diff Chek - 60 Positive Specimens