

Evaluation of the TechLab C. DIFF CHEK™-30 and C. DIFF CHEK™-60 for Detection of *C. difficile* in Fecal Specimens

C. Gleaves, S. J. Kohlhepp, M. Campbell, C. Kviz, E. Wohlsein, K. Lublink, S. Mortensen, R. Casciato, and J. Welle

Providence Portland Medical Center, 4805 NE Glidsn St., Portland OR 97213 USA

Abstract

Clostridium difficile is the leading cause of antibiotic-associated diarrhea and is responsible for nearly all cases of pseudomembranous colitis. The new TechLab C. difficile Antigen-Chek™ assay is an enzyme immunoassay specific for the C. difficile glutamate dehydrogenase common antigen in fecal samples. This test can detect both toxigenic and non-toxigenic strains of C. difficile. The test has a 20 and a 50-min format and both formats were compared to our in-house Cytotoxin assay for the detection of C. difficile Toxin B. In this preliminary study, a total of 254 specimens were collected and tested. Between the two formats, there were 7 discrepant samples, 1 positive in the 50 min format and negative in the 20 min format and 6 samples were positive in the 20 min format but negative in the 50 min format. When compared to the cytotoxin assay the 20 min format had a sensitivity of 92%, a specificity of 91.6%, a positive predictive value of 54.8%, and a negative predictive value of 99%. The 50 min format had a sensitivity of 88%, a specificity of 93.4%, a positive predictive value of 59.5%, and a negative predictive value of 98.6%. Further testing is ongoing, but the preliminary data suggest that fecal samples that test negative with the TechLab C. difficile Antigen-Chek assay can be considered true negatives and further testing of C. difficile can be eliminated. Additionally, it is recommended that samples that test positive with the assay be further tested to identify specific C. difficile toxins.

Introduction

At the present time laboratory diagnosis of *Clostridium difficile* infection relies on either detection of toxins (A or B) produced by the bacterium in the gut and excreted in the stool. Isolation of C. difficile in culture is difficult and can require several days. Toxin B is a cytotoxin detected in cultured cells and usually takes two days to perform. Toxin A is an endotoxin and there are EIA tests that detect A or A and B in a time span measured in hours. TechLab's new EIA detects C. difficile glutamate dehydrogenase, a marker for all Clostridia, both toxigenic and non-toxigenic. The new assay has two incubation formats. One called C. DIFF CHEK™-30 has a 20 minute, shaken incubation period and C. DIFF CHEK™-60 has a 50 minute non-shaken incubation period. We have compared these assays to an in-house cytotoxin assay (CTA) for toxin B, and anaerobic culture.

Methods

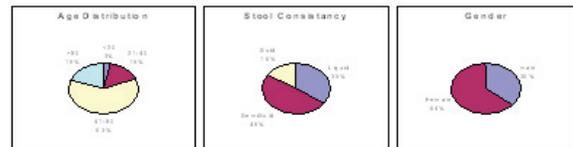
TechLab of Blacksburg VA supplied the reagents necessary to perform the assay for C. difficile specific glutamate dehydrogenase. Our in-house Cytotoxin assay for the detection of C. difficile Toxin B (CTA) was assayed with human foreskin cells purchased from Diagnostic Hybrids, Athens, OH and antitoxin purchased from TechLab, Blacksburg, VA.

The TechLab glutamate dehydrogenase assay has two microassay plate formats; 20-minute incubation with shaking and 50-minute incubation without shaking. Both plates have polyclonal antibody against C. difficile glutamate dehydrogenase immobilized in the wells and detect the presence of antigen with horseradish peroxidase induced color detected at single wavelength, 450 nm or dual wavelength, 450/630 nm.

Results

There were 619 stools included in the study. 3.4% were from individuals

younger than 20 years, 15.4% from individuals 21 to 40 years, 61.9% from individuals 41 to 80 years and 19.4% from individuals older than 80 years. 35% of the stools were classified as liquid, 48.6% as semisolid and 16.2% as solid. 36% were from men and 64% were from women.



		C. DIFF CHEK™-30		
		Pos	Neg	
C. DIFF	Pos	82	4	Discrepant assays 18/635 = 2.8%
CHEK™-60	Neg	14	535	

		CTA		
		Pos	Neg	
C. DIFF	Pos	56	39	Sensitivity 90.30%
CHEK™-30	Neg	6	534	Specificity 93.20%
				PPV 58.90%
				NPV 98.90%

		CTA		
		Pos	Neg	
C. DIFF	Pos	55	33	Sensitivity 87.30%
CHEK™-60	Neg	8	539	Specificity 94.20%
				PPV 62.50%
				NPV 98.50%

		Culture		
		Pos	Neg	
C. DIFF	Pos	35	61	Sensitivity 92.10%
CHEK™-30	Neg	3	536	Specificity 89.80%
				PPV 36.50%
				NPV 99.40%

		Culture		
		Pos	Neg	
C. DIFF	Pos	35	52	Sensitivity 92.10%
CHEK™-60	Neg	3	545	Specificity 91.30%
				PPV 40.20%
				NPV 99.50%

Conclusions

The C. DIFF CHEK™ -30 and C. DIFF CHEK™-60 are slightly different formats of the same assay for the presence of glutamate dehydrogenase, the common antigen of *Clostridium difficile*. Sensitivity and specificity of both formats of the common antigen assay are greater than 87% compared to cytotoxin assay and culture.

A stool testing negative for the common antigen is greater than 98% likely to contain no *Clostridium difficile* (NPV => 98% compared to both CTA and culture). This data suggest that fecal samples that test negative with the TechLab C. difficile Antigen-Chek assay can be considered true negatives and further testing of C. difficile can be eliminated.

This will facilitate the patient's release from isolation procedures in several hours instead of several days, thereby realizing a saving of material and time on the nursing unit.

Additionally, it is recommended that samples that test positive with the assay be further tested to identify specific C. difficile toxins A and/or B.