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Comparison of Four Commercially Available Rapid Enzyme Immunoassays with Cytotoxin Assay for Detection of *Clostridium difficile* Toxin(s) from Stool Specimens

CINDY S. MERZ,* CHARLES KRAMER,† MICHAEL FORMAN, LINDA GLUCK, KELLY MILLS,‡ KELLY SENFT, IONEL STEIMAN, NATALIE WALLACE, AND PATRICIA CHARACHE

Microbiology Division, Department of Pathology, The Johns Hopkins Medical Institutions, Baltimore, Maryland 21287-7093

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Rapid (2.5- to 3.5-h) enzyme immunoassays (EIAs) for the detection of *Clostridium difficile* toxins have been developed. We report the results of simultaneous testing of 700 fresh stool specimens by the tissue culture cytotoxin assay and four EIAs (Bartels Prima System *C. difficile* Toxin A EIA, Cambridge Biotech Cytoclone A+B EIA, Meridian Diagnostics Premier *C. difficile* Toxin A EIA, and TechLab *C. difficile* Tox-A Test EIA). In cases of disagreement, culturing for toxigenic *C. difficile* was performed. A total of 61 (8.7%) specimens from 46 patients were positive for *C. difficile* toxin. The sensitivity of the cytotoxin assay was 87%, and that of culture was 93%. In comparison with the cytotoxin assay results, the sensitivity and specificity of the EIAs were as follows: Bartels, 87 and 96%; Cambridge, 89 and 99%; Meridian, 87 and 98%; and TechLab, 87 and 95%, respectively. In comparison with the cytotoxin assay plus toxigenic culture results, the sensitivity and specificity of the EIAs were as follows: Bartels, 84 and 97%; Cambridge, 85 and 99%; Meridian, 79 and 98%; and TechLab, 80 and 96%, respectively. The EIAs varied in positive predictive values (PPVs). A high PPV was seen with the Cambridge EIA (96%); lower PPVs were seen with the TechLab (64%), Bartels (72%), and Meridian (80%) EIAs because of high false-positive rates. The negative predictive values (98 to 99%) were excellent with all EIAs. Results were indeterminant with 0.3% of the samples by the Meridian EIA and 3% by all the other EIAs. Although the EIAs were less sensitive than the cytotoxin assay, they provide same-day results and may be useful in laboratories without tissue culture facilities.

The role of *Clostridium difficile* in antibiotic-associated diarrheal disease and pseudomembranous colitis has been well established. The organism's pathogenicity is associated with its ability to produce two extracellular toxins, toxin A (enterotoxin) and toxin B (cytotoxin). The designations A and B refer to the elution patterns of the toxins on anion-exchange resins; toxin A binds less tightly to the resin and elutes before toxin B. Although the exact mechanisms by which the toxins kill colonic mucosal cells and produce the diarrheal response are unknown, it is thought that the toxins act synergistically. Toxin A binds to receptors on the intestinal epithelium, causing colonic inflammation and a hemorrhagic exudate composed of serum components and cellular debris (11–13, 15, 17). Toxin B is a potent cytotoxin; subpicogram amounts cause rounding and death in most mammalian fibroblast cell lines in vitro. Although purified toxin B alone produces no effect when administered to ligated rabbit intestinal loops, it may play a role after toxin A has caused the initial binding and tissue damage (11, 13, 17).

The currently available methods that can be used to aid in the diagnosis of *C. difficile*-related disease include colonoscopy, tissue culture cytotoxin assay, stool cultures for toxin-producing *C. difficile* strains, molecular diagnostics including PCR, and immunodiagnostics including latex agglutination, counterimmunoelectrophoresis, and enzyme immunoassay (EIA). Of these approaches, colonoscopy is rapid and relatively specific, but it lacks sensitivity and is invasive, expensive, and often impractical. Stool cultures for *C. difficile* and the tissue culture cytotoxin assay are costly, time-consuming, and beyond the capabilities of many laboratories. PCR technology holds promise as a technique for the detection of *C. difficile*. Kuhl et al. (10) report the development of a PCR assay which can detect toxigenic *C. difficile* in stool samples by amplification of genomic sequences of the rRNA gene and the toxin A and B genes. However, until more user-friendly formats can be developed, use of PCR assays in the diagnostic laboratory, especially with large numbers of specimens, will be limited. The latex agglutination test, although rapid, targets its detection to glutamate dehydrogenase (8, 9), a bacterial enzyme present in both toxin- and non-toxin-producing *C. difficile* and other organisms. For this reason there is cross-reactivity and low specificity with this assay. Toxin B can be detected by counterimmunoelectrophoresis, but this assay lacks both sensitivity and specificity as a diagnostic test (9).

A number of commercially produced EIA kits for the detection of *C. difficile* toxins are now available to the clinical laboratory. The advantage of the EIA format is predominantly the rapidity with which results can be reported. Many laboratories have experience with other EIA kits and can easily introduce another EIA into their current work flows. Unfortunately, EIAs typically lack sensitivity in comparison with reference assays. Investigators who have evaluated several commercially available EIA kits for the detection of *C. difficile* toxins have reported sensitivities, in comparison with the cytotoxin assay, ranging from 80 to 85% with the Cambridge

* Corresponding author. Mailing address: The Johns Hopkins Hospital, Meyer B1-193, 600 N. Wolfe Street, Baltimore, MD 21287-7093. Phone: (410) 955-5077. Fax: (410) 955-0767.

† Present address: 3275 Haviland Court, Apt. 101, Palm Harbor, FL 34684.

‡ Present address: 990 Irwin Street, Apt. B14, Morgantown, WV 26505.

Cytoclone A+B EIA, 69 to 88% with the Meridian Premier EIA, and 63 to 68% with the Vitek VIDAS EIA (1, 2, 4–6, 16).

It is difficult to compare published EIA results among different studies because of differences in patient populations, sample sizes, the methodologies used, positivity rates, etc. To eliminate such differences and permit direct comparison, we simultaneously tested specimens using four commercially available EIA kits for detection of *C. difficile* toxins and our reference tissue culture cytotoxin assay. The assays evaluated included the Bartels Prima System *C. difficile* Toxin A EIA (Baxter Diagnostics Inc., Deerfield, Ill.), Cambridge Biotech Cytoclone A+B EIA (Cambridge Biotech Corp., Worchester, Mass.), Meridian Diagnostics Premier *C. difficile* Toxin A EIA (Meridian Diagnostics, Inc., Cincinnati, Ohio), and TechLab *C. difficile* Tox-A Test EIA (TechLab, Blacksburg, Va.). The purpose of our study was to evaluate the usefulness of EIA for the detection of *C. difficile* toxins as a laboratory test.

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MATERIALS AND METHODS

Study protocol. A total of 700 stool specimens submitted from September 1991 through March 1992 for the *C. difficile* cytotoxin tissue culture assay were included in the study. This represented 650 specimens from 284 hospitalized patients and 50 specimens from 45 clinic patients. A total of 371 multiple specimens (two to seven specimens per patient) were sent from 101 patients. The stool specimens (<24 h from the time of collection) were refrigerated at 4°C until they were tested; an aliquot of each specimen was also frozen at −70°C. Testing was performed within 24 h of receipt of the specimens in the laboratory. The testing of specimens with the EIA kits from Bartels, Cambridge Biotech, Meridian Diagnostics, and TechLab was performed in parallel with the cytotoxin assay.

A specimen was considered positive for *C. difficile* toxin if either the cytotoxin tissue culture assay or the culture for toxigenic *C. difficile* was positive. When the cytotoxin assay was negative but the culture was positive, the stool supernatants were concentrated in a Minicon A2S concentrator (a filter system which decreases the fluid volume by half) and the cytotoxin assay was repeated. All specimens with indeterminant EIA results were repeat tested according to the manufacturers' instructions. Specimens which were indeterminant upon repeat testing were considered nonevaluable (i.e., neither positive nor negative). All specimens with discrepant results among the assays, all cytotoxin assay-positive specimens, and a subset of negative specimens were cultured for toxigenic *C. difficile*, and the clinical histories of the patients were obtained when possible. Clinical data included antibiotic usage, duration and frequency of diarrhea, presence of alternative explanations for diarrhea (other bacterial pathogens, gastrointestinal surgery, use of laxatives, underlying bowel disease, etc.), and sigmoidoscopy results, if a sigmoidoscopy was performed.

Cytotoxin assay. The stool specimen was diluted 1:5 in phosphate buffer (pH 6.9), vortexed, and centrifuged (4,100 rpm in a Jouan CT422 centrifuge) for 30 min at 4°C. The supernatant was filter sterilized (0.45-μm-pore-size unit; Millipore), and the filtrate was diluted 1:2 and 1:10. Wells of a 96-well microtiter plate containing human foreskin fibroblast monolayers (in-house propagation of ATCC Hs68) were inoculated with 20 μl of each dilution, making final dilutions of stool in the wells of 1/100 and 1/500, respectively. Plates were incubated in 5% CO_2 at 37°C and were examined at 24 and 48 h. A positive result was defined as at least 10% fibroblast rounding and cell death that was neutralized upon retesting with the addition of *C. difficile* antitoxin (AnaTox, Inc., Blacksburg, Va.) at the dilutions described above. When performing the neutralization, the original specimen was also repeat tested.

Culturing for *C. difficile*. Frozen aliquots of the stool specimens were thawed. Equal volumes of stool specimen and 95% ethanol were mixed and incubated for 1 h at 25°C; 0.1 ml was plated onto 5% sheep blood agar (SBA) and CCFA *C. difficile* agar (BBL, Cockeysville, Md.). The plates were incubated anaerobically and were examined for suspicious colonies on day 5. Two representative isolates were biochemically confirmed to be *C. difficile*. The isolates were nonmotile, negative for lipase and lecithinase production, positive for esculin hydrolysis, and fermented glucose but did not ferment mannitol, xylose, sucrose, lactose, or maltose. Salicin was fermented by one isolate. Other suspicious colonies with morphologies like those of the representative isolates and the characteristic "horsey" odor were tested for toxin production only.

Concurrently, a cotton swab was dipped into the stool specimen and was touched against the side of a tube of prereduced brain heart infusion (BHI) broth (5 ml) supplemented with 100 μl of Oxoid *C. difficile* supplement (final concentration; 500 μl of cycloserine and 16 μg of cefoxitin per ml of BHI broth). After 5 days of anaerobic incubation, 0.1 ml was subcultured onto 5% SBA for the recovery of *C. difficile*.

After 7 days of incubation, the BHI broths were centrifuged (3,000 rpm in a Jouan CT422 centrifuge) for 10 min, filtered, and tested for toxins by EIA and the cytotoxin assays. In cases in which the BHI broths tested negative for toxins but suspicious colonies were recovered on solid medium, the colonies were grown in chopped meat carbohydrate broth for 7 days (37°C). The chopped meat carbohydrate broths were then tested for toxin production in the cytotoxin assay described above.

Bartels Prima System *C. difficile* Toxin A EIA. The Bartels Prima System *C. difficile* Toxin A EIA (Bartels EIA) provides 12 1-by-8 non-breakaway-plastic microwell strips coated with mouse immunoglobulin G to *C. difficile* toxin A. The EIA was performed according to the manufacturer's instructions. The stools were diluted 1:2 (100 μl in specimen treatment buffer), vortexed, and centrifuged for 10 min at 2,000 × *g*. A positive control (two drops), three negative controls (two drops), and stool supernatant (100 μl) were added to the wells, and the plates were incubated at 37°C for 90 min. The plate was washed manually four times, after which one drop of primary antibody and then one drop of peroxidase-labeled anti-rabbit antibodies were added. The plate was incubated for 30 min at 37°C and was then washed manually four times. One drop each of substrate A and substrate B was then added, and the plate was incubated at 25°C in the dark for 15 min. Two drops of stop solution were added, and the plate was read spectrophotometrically. The absorbance values provided by the manufacturer were used for interpretation of controls and patient specimens.

Cambridge Biotech Cytoclone A+B EIA. The Cambridge Biotech Cytoclone A+B EIA (Cambridge EIA) provides 96 breakaway-plastic microwells coated with *C. difficile* toxin A- and B-specific monoclonal antibodies. The EIA was performed according to the manufacturer's instructions. The stools were diluted 1:5 (100 μl in 400 μl of kit diluent), vortexed, and centrifuged for 15 min at 2,500 × *g*. Positive and negative controls (100 μl each) and stool supernatant (100 μl) were added to the wells, and the plates were incubated at room temperature (20 to 25°C) for 2 h. The plate was washed manually five times, after which 100 μl of the Step 1 conjugate

TABLE 1. Comparison of EIAs and reference method results

EIA (no. of specimens)[a]	EIA versus cytotoxin assay (%)				EIA versus cytotoxin assay and toxigenic culture (%)			
	Sensitivity	Specificity	PPV[b]	NPV[c]	Sensitivity	Specificity	PPV	NPV
Bartels (n = 697)	87	96	65	99	84	97	72	98
Cambridge (n = 694)	89	99	90[d]	99	85	99	96[e]	99
Meridian (n = 699)	87	98	77	99	79	98	80	98
TechLab (n = 699)	87	95	60	99	80	96	64	98

[a] Although 700 specimens were included in the study, some test results were nonevaluable because of indeterminant results upon repeat testing.
[b] PPV, positive predictive value.
[c] NPV, negative predictive value.
[d] The PPV of the Cambridge EIA was significantly better than those of the Bartels, Meridian, and TechLab EIAs (P = 0.001, P = 0.053, and P = 0.001, respectively; chi-square test).
[e] The PPV of the Cambridge EIA was significantly better than those of the Bartels, Meridian and TechLab EIAs (P = 0.0005, P = 0.01, P < 0.0001, respectively; chi-square test).

from step 1 was added. The plate was incubated for 15 min (20 to 25°C) and was washed manually five times, and 100 μl of the diluted (1:100) Step 2 conjugate was added. After 15 min of incubation (20 to 25°C), the wells were again washed five times, and 100 μl of substrate solution (1:1 dilution of part A substrate and part B chromogen) was added. After 15 min of incubation at 20 to 25°C, one drop of stop solution was added and the plate was read spectrophotometrically. The absorbance values provided by the manufacturer were used for interpretation of control and patient specimens.

Meridian Diagnostics Premier *C. difficile* Toxin A EIA. The Meridian Diagnostics Premier *C. difficile* Toxin A EIA (Meridian EIA) provides 96 breakaway-plastic microwells coated with polyclonal antibodies to *C. difficile* toxin A. The EIA was performed according to the manufacturer's instructions. The stools were diluted 1:5 (50 μl in 200 μl of sample diluent) and vortexed. Enzyme conjugate (one drop) was added to each well. Positive and negative controls (one drop each) and diluted stool specimen (50 μl) were then added. The plate was incubated for 2 h at 37°C and was then washed manually five times. Substrate A (one drop) and substrate B (one drop) were added to the wells. After 10 min of incubation at 20 to 25°C, one drop of stop solution was added and the plate was read spectrophotometrically. The absorbance values provided by the manufacturer were used for interpretation of control and patient specimens.

TechLab *C. difficile* Tox-A Test EIA. The TechLab *C. difficile* Tox-A Test EIA (TechLab EIA) provides 96 breakaway-plastic microwells coated with polyclonal antibody against *C. difficile* toxin A. The EIA was performed according to the manufacturer's instructions. The stools were diluted 1:2 (0.4 ml in 0.4 ml of diluent) and vortexed. Conjugate (one drop), positive and negative controls (one drop each), and diluted stool (two drops) were added to the wells. After 2 h of incubation at 37°C, the wells were washed manually five times. Then, substrate A (one drop) and substrate B (one drop) were added and the plate was incubated for 15 min at 20 to 25°C. Intensifier (one drop) was added, and the plate was read spectrophotometrically. The absorbance values provided by the manufacturer were used for interpretation of control and patient specimens.

RESULTS

Reference method results. A total of 61 (8.7%) stool specimens from 46 patients were positive for *C. difficile* toxin by either or both of the reference methods. The positivity rates for hospitalized versus clinic patients were comparable: 8.6% (56 of 650) and 10% (5 of 50), respectively. Of the 700 stool specimens, 53 were positive by the tissue culture cytotoxin assay (42 were detected at the 24-h reading; 11 were detected at the 48-h reading). Of the 53 cytotoxin assay-positive specimens, 4 were culture negative for toxin-producing *C. difficile* and 2 specimens were not cultured because of an insufficient sample size. An additional eight specimens were cytotoxin assay negative even after sample concentration, but toxin-producing *C. difficile* was recovered from these eight specimens. Overall, the sensitivity of the cytotoxin assay was 87% (53 of 61 specimens); the sensitivity of culture was 93% (55 of 59 specimens).

EIA results. Of the 61 positive specimens in the present study, 45 were positive with all four EIA kits. As seen in Table 1, the Bartels EIA detected 71 positive specimens, of which 51 were positive by the reference methods, and 626 negative specimens, of which 616 were negative by the reference methods. With the Cambridge EIA, of the 52 positive specimens, 50 were positive by the reference methods, and of the 642 EIA-negative specimens, 633 were negative by the reference methods. Of the 60 positive specimens detected with the Meridian EIA, 48 were positive by the reference methods, and of the 639 EIA-negative specimens, 626 were negative by the reference methods. The TechLab EIA detected 77 positive specimens, of which 49 were positive by the reference methods, and 622 negative specimens, of which 610 were negative by the reference methods.

Sensitivities of EIAs. In comparison with the cytotoxin assay alone, the Cambridge EIA had a sensitivity of 89%; all other EIAs had sensitivities of 87% (Table 1). When compared with cytotoxin and culture, the Cambridge EIA again had the best sensitivity (85%); this was followed by the Bartels EIA (84%), the TechLab EIA (80%), and the Meridian EIA (79%). Of the true-positive specimens, multiple specimens from nine patients were sent (range, two to four per patient). All 22 of the multiple specimens from these nine patients were positive by all EIAs.

All false-negative EIA results occurred with 14 specimens from 13 patients (Table 2). For this group of specimens, 63% of the cytotoxin assays were positive only at 48 h, suggesting

TABLE 2. Discrepant EIA results

EIA	No. of specimens	
	False-positive EIA (n = 59)	False-negative EIA (n = 14)
Bartels	20	10
Cambridge	2	11
Meridian	12	13
TechLab	28	10

TABLE 3. Clinical characteristics of patients who had specimens with false-positive EIA results

Patient group	Mean age (yr)	Mean duration of diarrhea (days)	Mean frequency of diarrhea (per day)	Mean no. of antibiotics administered	Mean duration (days) of antibiotic use
Negative controls (n = 26/639)	58	8.3	3.2	3	8.3
All assays positive (n = 9/45)	59	6.5	3.2	1.3	14
False-positive results by:					
Bartels EIA (n = 9/20)	65	5.4	3.7	2.5	5.5
Cambridge EIA (n = 1/2)	52	3	2	4	9.5
Meridian EIA (n = 6/12)	59	9	3.2	1.2	7.2
TechLab EIA (n = 12/28)	65	4.4	3.8	2.3	4.8

the presence of low titers of toxin, which may explain the false-negative EIA results.

Specificities of assays. The number of cytotoxin assay-negative specimens was 639. Of these, 15 randomly selected cytotoxin-negative specimens were cultured, and none grew toxigenic *C. difficile*. The specificity was high with all EIAs compared with the cytotoxin assay alone or in conjunction with culture (Table 1); the specificity of the Cambridge EIA was 99%, that of the Meridian EIA was 98%, that of the Bartels EIA was 96 to 97%, and that of the TechLab EIA was 95 to 96%. Of the true-negative specimens, multiple specimens from 68 patients were sent (range, two to seven per patient). All 170 of these specimens were negative by all EIAs.

Fifty-nine specimens from 51 patients were positive by one or more EIAs but were negative by both reference methods (Table 2). Fifty-six specimens tested positive with only a single EIA kit. The TechLab EIA had the most false-positive results (28 specimens); the Cambridge EIA had the fewest (2 specimens).

False-positive results were obtained with the Bartels EIA for specimens from 18 patients. Five patients had multiple specimens for testing, for a total of 13 specimens. Except for one patient, only one positive EIA result for specimens from each patient was obtained; no other specimen tested by the Bartels EIA or the reference method was positive. The one patient whose two specimens were both positive by the Bartels EIA had a history of multiple antibiotic usage and a 4-day duration of diarrhea with a frequency of 2 to 10 stools per day. However, no other assay was positive with this patient's specimens.

Specimens from two patients gave false-positive results with the Cambridge EIA. Two specimens from one patient were tested; one was EIA positive and the other was EIA negative.

The Meridian EIA yielded false-positive results for specimens from 12 patients. Five patients had multiple specimens for testing, for a total of 16 specimens. In each case, only one specimen from each patient was positive by the Meridian EIA; all other assays of these specimens were negative.

The TechLab EIA yielded false-positive results for specimens from 19 patients. Six patients had multiple specimens for testing, for a total of 22 specimens. For four patients who had multiple specimens, only a single specimen was positive by the TechLab EIA; all other assays of these specimens were negative. Of interest was one patient with Whipple's disease who had undergone a segmental resection of the small bowel. Six samples from this patient had positive assay results with the TechLab EIA only over a 1-month period; 11 additional specimens from this patient sent to the laboratory during that interval (which were not tested in the present study) were also negative by the cytotoxin assay.

Predictive values. All EIAs had excellent negative predictive values, ranging from 98 to 99%. The positive predictive values (PPVs) of the Cambridge EIA were statistically significantly better than those of the other EIAs (chi-square test): 90% compared with the cytotoxin assay alone and 96% compared with cytotoxin assay and toxigenic culture. The PPVs were lower with the other EIAs; compared with the cytotoxin assay alone or in conjunction with culture, the PPVs of the Meridian EIA were 77 and 80%, those of the Bartels EIA were 65 and 72%, and those of the TechLab EIA were 60 and 64%, respectively.

Indeterminant results. With the Meridian EIA, only two specimens (0.3%) had initial indeterminant results and required repeat testing. With the other EIAs, approximately 3% of all specimens had indeterminant results. Resolution of indeterminant results was good in all cases; both positive and negative results were obtained when the assays were repeated. With the Bartels EIA, 19 of the 22 specimens with indeterminant results were negative upon retesting; with the Cambridge EIA, 13 of 21 specimens retested negative and 2 specimens retested positive; with the Meridian EIA, 1 of 2 specimens retested positive; and with the TechLab EIA, 19 of 23 specimens retested negative and 3 retested positive. Three specimens were nonevaluable with the Bartels EIA, six specimens were nonevaluable with the Cambridge EIA, and one sample each was nonevaluable by the Meridian and TechLab EIAs.

Clinical data. The information obtained for patients whose specimens tested true positive by the toxin assays, false-positive, by the EIAs and randomly selected patients whose specimens were negative by the toxin assays (negative control group) is summarized in Table 3. We found no major differences in age, antibiotic usage, or duration and frequency of diarrhea among the groups. Length of antibiotic use, which is widely held as a risk factor for antibiotic-associated *C. difficile* disease, was longer in the true-positive group; however, the number of patients in each category evaluated clinically was too small for the results to be analyzed statistically.

DISCUSSION

The first assay to be developed for the diagnosis of *C. difficile*-induced intestinal disease was the tissue culture cytotoxin assay. Although considered the "gold standard" against which the results of other methodologies are compared, the cytotoxin assay has never been fully standardized. Laboratory protocols vary in the dilutions of stool tested, the cell lines inoculated (some cell lines are more susceptible to toxin B than others), and the interpretation of the assay endpoint (any amount of rounding versus 50 to 75% monolayer involvement). When appropriate neutralization with antitoxin is performed to rule out nonspecific cytopathic effects, the cytotoxin assay is considered to be the most specific assay for the detection of *C. difficile* toxin B. The sensitivity of the assay, however, may be compromised because of toxin B degradation by proteases in

the stool, especially if the specimen is held at room temperature.

Culturing for *C. difficile* is considered to be the most sensitive assay, although toxin assays must subsequently be performed to differentiate toxigenic from nontoxigenic strains. Culturing is time-consuming and labor-intensive; however, it does permit epidemiological analysis of the organisms isolated. Strain typing may prove to be helpful in assessing outbreaks of *C. difficile*. Lastly, culturing may result in the isolation of other enteric pathogens. Heavy growth of *Clostridium perfringens*, for example, may indicate the cause of the diarrhea in the absence of recovery of *C. difficile*.

In determining *C. difficile* disease, the relationship between the laboratory results and the clinical diagnosis is not always clear-cut. Culture for toxigenic *C. difficile* and cytotoxin assays may be positive for patients without enteric disease. In the first months of life, up to 50% of infants may be colonized with highly toxigenic strains yet do not express any clinical symptoms (3, 7, 9). Likewise, 35% of patients with cystic fibrosis can be asymptomatic carriers of toxigenic *C. difficile* (14). In our retrospective review of the patients' charts, we could not identify specific symptoms which were consistent indicators of *C. difficile* disease. Antibiotic usage and the duration and frequency of diarrhea were not dramatically different between patients in whom *C. difficile* toxin was detected and a negative control group. Although we were not comfortable making determinations of *C. difficile*-associated diarrhea solely on the basis of clinical symptoms to resolve assay discrepancies, certainly clinicians must use laboratory results in conjunction with the patient's clinical history to make a final diagnosis.

The need for rapid, sensitive, and accurate diagnostic tests has encouraged the development of EIAs for many analytes. As also reported by other investigators (1, 2, 4–6), we found that the sensitivities of the EIAs that we evaluated approached or equaled that of the cytotoxin assay (87 to 98%). Most of the specimens that were false negative by the EIAs had low toxin titers, as evidenced by delayed (48-h) or negative cytotoxin detection and poor recovery of the organism by culture. In cases in which multiple specimens were sent, consistent results were usually obtained by each assay. With a subset of specimens for which false-positive results were obtained by EIA, when multiple specimens were sent, only a single specimen was positive by one EIA. Given negative culture, cytotoxin assay, and the multiple other negative EIA results obtained with this specimen and the other specimens from patients who had multiple specimens, it is likely that the one specimen that had a positive EIA result represented a false-positive result. Thus, analysis of results for multiple specimens may help in test interpretation. Although similar in sensitivities, the EIAs varied greatly in their PPVs because of a wide range of false-positive results. The Cambridge EIA had the highest PPV (96%), whereas lower PPVs were seen with the Meridian EIA (80%), the Bartels EIA (72%), and the TechLab EIA (64%). All of the EIAs had excellent specificities (96 to 99%) and negative predictive values (98 to 99%) in a population with a low positivity rate of 8.7%.

The rapid availability of results (2.5 to 3.5 h) is the main advantage of EIA technology. With the Meridian EIA, interpretable results were obtained upon initial testing for 99.7% of the specimens. With the other EIAs, interpretable results were possible for 97% of the specimens. When indeterminant results were obtained, resolution by retesting was good; overall, 75% of the specimens with indeterminant results were negative upon repeat testing. The Cambridge EIA had the greatest number of unresolved results (6 of 21), but even this number represented less than 1% of all specimens tested.

All of the EIAs were easy to perform. The Cambridge, Meridian, and TechLab EIA kits were formatted with 96 breakaway-plastic wells; any size run can be accommodated without waste. In contrast, the Bartels EIA kit has 12, 1-by-8 microwell strips which were not of the breakaway type. The waste of unused wells in a run can be a problem in a cost-conscious environment. Reagents provided in the Bartels, Meridian, and TechLab EIA kits were prediluted to the proper concentrations. Dilution of two reagents was required to perform the Cambridge EIA. All EIAs had objective endpoints determined with an EIA reader. The Meridian and TechLab EIA results can also be interpreted visually, although this capability was not tested in our study. Other investigators have compared visual and spectrophotometric readings and have reported good correlations (4).

The simultaneous testing of the different EIA kits with the same stool specimens allowed for direct comparison of results. The incidence of disease was also controlled for in the study. This is important, because the incidence of disease can influence the data obtained, and the incidence of disease varies among patient populations. In the present study, the Cambridge EIA consistently had the best sensitivity, specificity, PPV, and negative predictive value when compared with the results of the cytotoxin assay alone or in conjunction with the toxigenic culture. Overall, the EIAs were less sensitive than the cytotoxin assay; however, they provided same-day results, could be used as a screening test, and may be useful in laboratories without tissue culture facilities.

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