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Evaluation of a Latex Agglutination Test for *Clostridium difficile* in Two Nursing Home Outbreaks

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The Culturette Brand *Clostridium difficile* test (CDT; Marion Laboratories, Inc., Kansas City, Mo.) is a latex agglutination test for *C. difficile*. The recent controversy involving the identity of antigens detected by CDT has made decisions on its use difficult. We compared the test results with those of selective culture and stool cytotoxin assays in investigations of two nursing home outbreaks of *C. difficile*-associated disease in order to formulate usage recommendations. Selective culture for *C. difficile* identified 27 (19%) of 142 subjects as carriers. CDT and the stool cytotoxin assay identified only 52 and 48% of these carriers, respectively. Compared with the stool cytotoxin assay, CDT had a high sensitivity (92%) and specificity (89%) for the detection of *C. difficile* disease, but the positive predictive value of the test was only 17% when the prevalence of disease was 2%. We conclude that the CDT should not be used to identify carriers but that it is a sufficiently sensitive and specific screening test for diagnosing *C. difficile* disease. However, since the positive predictive value of the CDT is low when the prevalence of disease is low, positive test results should be confirmed by the stool cytotoxin assay.

Clostridium difficile is the usual etiologic agent of pseudomembranous colitis and approximately 20% of cases of antibiotic-associated diarrhea (3, 4, 8, 9). *C. difficile* infection can be detected by culturing stool samples on a variety of selective media to isolate the putative agent (26, 27) or by analyzing stool samples for either of the two high-molecular-weight protein toxins (A and B) that the organism produces (22). Methods used to detect these toxins include tissue culture (17), enzyme-linked immunosorbent assays (12), counterimmunoelectrophoresis (24), and antibody-linked latex agglutination (20). The tissue culture assay remains the method of choice for the detection of cytotoxin, although this poses a major problem to most laboratories, since relatively few have tissue culture facilities. Until recently, none of the alternative methods was available except in reference laboratories.

In 1986, a rapid latex agglutination test for *C. difficile* (CDT), which was developed from the work of Banno and colleagues (2), was marketed by Marion Laboratories, Inc. (Kansas City, Mo.). Although more rapid and simpler than the other methods, this test was fraught with controversy regarding the antigen(s) that it detected. The original claim that the CDT detected toxin A was disputed by Lyerly and Wilkins (14). Our group and others have shown that the test detects antigens that are common to both cytotoxigenic and noncytotoxigenic strains of *C. difficile*, as well as to other species of clostridia and other genera of bacteria (13, 15; C. A. Gaydos, B. E. Laughon, L. M. Mundy, R. G. Bennett, and L. Bobo, Abstr. Annu. Meet. Am. Soc. Microbiol. 1987, B216, p. 61). As a result of the early controversy, the manufacturer withdrew the claim that the CDT detected toxin A (*CDT Position Paper*, Marion Laboratories, Inc., September 1986). Despite this problem, the clinical usefulness of the CDT can be determined by its performance in clinical tests. Recent reports have compared the use of the CDT with the use of the stool cytotoxin assay for the diagnosis of *C. difficile*-associated colitis and diarrhea (11, 19, 21). In this study we evaluated the test in investigations of two outbreaks of *C. difficile*-induced enteric disease. The CDT was compared with the tissue culture assay of stool samples for *C. difficile* cytotoxin, which we considered the standard diagnostic method, and selective culture of stool samples for *C. difficile*, which represented the standard method for detecting colonization.

MATERIALS AND METHODS

Study population and samples. Stool samples were obtained from patients in two Baltimore, Md., nursing homes. Demographic data were obtained from medical records, and a retrospective chart review was performed to determine whether diarrhea was reported in the 2-week period before samples were obtained to detect all carriers. Nursing home 1 (NH-1) was a 225-bed facility with four skilled- and intermediate-care wards and one chronic-care hospital ward. Nursing home 2 (NH-2) was a 240-bed facility with four skilled- and intermediate-care wards and three chronic-care hospital wards. Patients in the skilled- and intermediate-care wards were mostly elderly (age, >65 years) and had various degrees of debility. Patients in the chronic-care hospital wards ranged in age from 25 to 102 years and were generally severely debilitated. Anoxic encephalopathy, cerebral vascular accident, and severe decubitus ulcers were common diagnoses in patients in the chronic-care hospital wards.

An outbreak of *C. difficile* diarrhea occurred in the chronic-care hospital ward of NH-1 (NH-1A) in August 1985. One patient in NH-1A was diagnosed with pseudomembranous colitis and two others in that ward had *C. difficile* diarrhea over a 2-week period. During the following week, prevalence surveys were done in that ward (NH-1A) and four other wards (NH-1B). Stool samples were collected from 30 patients in NH-1A and from 67 patients in NH-1B. This survey was the first of seven monthly surveys done in ward NH-1A, and the epidemiology of this outbreak has been described previously (6).

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An outbreak of *C. difficile* diarrhea occurred in NH-2 in April 1986. Two patients in one chronic-care hospital ward and one patient in another ward developed diarrhea that was positive for *C. difficile* toxin. Stool samples were collected from 45 patients in these two wards over a 2-week period. Only the three index patients were reported as having diarrhea at the time of the investigation.

NH-1A and NH-2 were used as sites of established disease in which the organism was suspected of causing an outbreak or being endemic. NH-1B was presumed to be an area without *C. difficile* disease, and specimens were analyzed for comparison purposes. All stool samples were cultured on selective media and tested for cytotoxin on receipt, frozen at −70°C, and subsequently tested by CDT.

Selective culture. Stool samples were cultured anaerobically on taurocholate-cycloserine-cefoxitin-fructose agar (TCCFA) at 37°C for 48 h (27). Three to four colonies with characteristic morphology (flat, yellow, ground-glass texture) were subcultured anaerobically at 37°C for 24 h on anaerobic blood agar plates (CDC; BBL Microbiology Systems, Cockeysville, Md.). Several identical colonies were then inoculated into 5 ml of prereduced chopped meat-glucose broth (Scott Laboratories, Inc., Fiskeville, R.I.) and incubated at 37°C for 5 days. Isolates were tested for their cytotoxigenic potential in vitro by using cell-free filtrates of these broth cultures prepared with filters (pore size, 0.45 μm; Millex; Millipore Corp., Bedford, Mass.). Filtrates were diluted 1:10 with phosphate-buffered saline and tested by a tissue culture assay, as described below. Organisms with characteristic morphology on TCCFA which proved to be noncytotoxigenic were confirmed as *C. difficile* by using API 20A test strips (Analytab Products, Plainview, N.Y.), which were incubated anaerobically at 37°C for 48 h (10). Colonies whose morphologies bore some resemblance to *C. difficile* and which grew on TCCFA were also subcultured, identified by using API 20A test strips, and tested for cytotoxicity.

Tissue culture (cytotoxin) assay. Approximately 0.5 g of stool sample was mixed with an equal amount of phosphate-buffered saline and vortexed for 30 s. The resultant suspension was centrifuged to yield a supernatant from which a cell-free filtrate was prepared by using a filter (pore size, 0.45 μm; Millex; Millipore), and a 1:5 dilution of the filtrate was prepared with phosphate-buffered saline. A 25-μl portion was applied to a well of a 96-well microtiter plate (Becton Dickinson Labware, Oxnard, Calif.) containing confluent monolayers of WI-38 fibroblasts in 100 μl of modified Eagle essential medium with 10% fetal bovine serum. Another 25-μl portion was applied to another well containing 25 μl of *Clostridium sordellii* antitoxin (1:10 dilution; Bureau of Biologics, U.S. Food and Drug Administration, Bethesda, Md.). The inoculated tissue culture plate was incubated at 37°C in 5% CO_2 for 48 h. The criteria for the presence of *C. difficile* cytotoxin was an actinomorphic cytopathic effect in at least 50% of the fibroblasts that was neutralized by *C. sordellii* antitoxin.

Latex agglutination test. The Culturette Brand *C. difficile* test kit (CDT; Marion Laboratories, Inc.) contained vials of detection, negative control and positive control latex reagents, and buffer. Equal mass/volume suspensions of stool samples and buffer were homogenized by Vortex mixing and were centrifuged at 12,000 × *g* for 3 min. Single drops of each supernatant were mixed with the latex reagent on a slide and gently rotated for 3 min. Tests were read as strong, weak, or negative agglutination. Tests with weak agglutination were repeated. The negative control latex reagent was tested with the supernatant of any filtrate producing agglutination. A positive control test was performed with each batch of filtrates that was processed. Criteria for a positive CDT were (i) strong agglutination or (ii) weak agglutination on duplicate assays with the detection reagent and no agglutination with the negative control reagent. Criteria for a negative CDT were (i) no agglutination with the detection reagent or (ii) agglutination in both the detection and the negative control assays. All samples could be categorized as positive or negative.

TABLE 1. Results of selective culture, cytotoxin assay of stool samples, and CDT for outbreak and comparison wards

Ward and selective culture	No. of samples	No. of stool samples positive by:	
		Cytotoxin assay	CDT
Outbreak ward			
Cytotoxigenic *C. difficile*	22	12	12
Noncytotoxigenic *C. difficile*	5	0	1
Other clostridia	6	0	2[a]
No growth[b]	42	0	3
Comparison ward			
Cytotoxigenic *C. difficile*	5	1	2
Noncytotoxigenic *C. difficile*	1	0	0
Other clostridia	5	0	0
No growth[b]	56	0	4

[a] Both specimens that were positive by CDT contained *Clostridium innocuum*.

[b] No characteristic colonies on TCCFA.

RESULTS

The results for stool samples from the outbreak areas NH-1A and NH-2 were combined and are shown in Table 1. Selective culture of stool samples from these wards showed that 22 (29.3%) of 75 samples yielded cytotoxigenic strains of *C. difficile*; 5 (6.7%) yielded noncytotoxigenic strains; 6 (8.0%) yielded other clostridial species, including *Clostridium innocuum* and *Clostridium sporogenes*; and 42 (56.0%) had no growth of characteristic colonies. The tissue culture assay and CDT of stool specimens were positive for 12 (54.5%) of 22 samples that yielded a cytotoxigenic strain by selective culture. The cytotoxin assay was positive and CDT was negative for one sample that yielded cytotoxigenic *C. difficile*, and CDT was positive and the cytotoxin assay was negative for another similar sample. Otherwise, all samples that were cytotoxin positive were also CDT positive. None of the 57 samples that yielded noncytotoxigenic strains or other clostridial species or that showed no growth of characteristic colonies were positive by the cytotoxin assay of stool specimens. However, the CDT was positive for 6 (10.5%) of these 57 specimens.

A similar analysis for the comparison area NH-IB (Table 1) revealed 5 (7.5%) of 67 samples with a cytotoxigenic strain of *C. difficile*, 1 (1.5%) with a noncytotoxigenic strain, 5 (7.5%) with another clostridial species, and 56 (83.6%) with no growth of characteristic colonies. The cytotoxin assay of stool samples was positive for only one (20%) of five samples from which cytotoxigenic strains were isolated. The CDT was also positive for this specimen and one other specimen containing a cytotoxigenic strain as well as for 4 (7.1%) of 56 samples showing no growth of characteristic colonies.

Of the 27 carriers that were identified (22 from the outbreak wards and 5 from the comparison wards), 10 (37%) had

TABLE 2. CDT versus cytotoxin assay of stool samples for the diagnosis of disease and versus selective culture for the diagnosis of carriage

Result	Outbreak ward (n = 75)		Comparison ward (n = 67)	
	No. positive by CDT	No. negative by CDT	No. positive by CDT	No. negative by CDT
Cytotoxin[a]				
+	11	1	1	0
−	7	56	5	61
Culture[b]				
+	12	10	2	3
−	6	47	4	58

[a] Compared with the cytotoxin assay of stool samples from the outbreak ward, the CDT sensitivity was 92%, the specificity was 89%, and the positive predictive value (PPV; calculated as PPV = number of true positive tests/number of positive tests) was 61%. Similarly, for the samples from the comparison ward, the CDT sensitivity was 100%, the specificity was 92%, and the PPV was 17%.

[b] Compared with the selective culture of stool samples from the outbreak ward, the CDT sensitivity was 55%, the specificity was 89%, and the PPV was 67%. Similarly, for the samples from the comparison ward, the CDT sensitivity was 40%, the specificity was 94%, and the PPV was 33%.

diarrhea and none had a severe illness, which is consistent with the diagnosis of pseudomembranous colitis. The cytotoxin assay and CDT were each positive for 7 (70%) samples from the 10 symptomatic carriers.

The CDT was compared with the cytotoxin assay of stool samples to determine the sensitivity and the specificity for detecting *C. difficile*-induced enteric disease (Table 2). The sensitivity of the CDT, when used for the samples from the outbreak and the comparison areas, was high. For the outbreak areas, the CDT was positive for 11 of 12 cytotoxin-positive stool samples, for a sensitivity of 92%. It correctly identified the only cytotoxin-positive stool sample from the comparison wards. The specificity of the CDT was also high. It was negative for 56 of 63 cytotoxin-negative stool samples from the outbreak wards, resulting in a specificity of 89%, and for 61 of 66 cytotoxin-negative stool samples from the comparison wards, resulting in a specificity of 92%.

Comparison was also made between CDT and selective culture for detecting *C. difficile* carriage (Table 2). CDT was positive for 12 of 22 samples that yielded cytotoxigenic *C. difficile* from the outbreak wards, for a sensitivity of 55%, and for 2 of 5 similar samples from the comparison wards, for a sensitivity of 40%. In contrast to these low sensitivities, the specificities were high. CDT was negative for 47 of 53 samples without cytotoxigenic *C. difficile* from the outbreak wards, for a specificity of 89%, and negative for 58 of 62 similar samples from the comparison wards, for a specificity of 94%.

DISCUSSION

Diarrhea caused by *C. difficile* may be the most common nosocomial infection. Both sporadic cases and outbreaks in hospitals are common, and an outbreak has been reported recently in a long-term-care facility (6). The results of tests for *C. difficile* infection must be interpreted correctly by clinicians caring for patients in hospitals and nursing homes so that appropriate treatment decisions for individuals can be made and so that proper infection control procedures can be implemented to contain outbreaks. For example, a patient with postantibiotic diarrhea, fever, abdominal pain, and a positive test for *C. difficile* cytotoxin in the stool has a presumptive diagnosis of pseudomembranous colitis and needs emergent evaluation and treatment. On the other hand, an asymptomatic patient with a positive cytotoxin test identified in a prevalence survey may not need treatment, but may need to be isolated for infection control purposes if diarrhea develops. Clinical laboratories use a variety of methods to diagnose *C. difficile* infection. We compared two standard methods, the tissue culture assay and selective culture on TCCFA, with the newly marketed CDT for *C. difficile* from Marion Laboratories. The rationale for this approach deserves comment.

Prior studies from our laboratory showed that 136 (97%) of 141 patients with antibiotic-associated pseudomembranous colitis had positive cytotoxin assays of stool samples, whereas cytotoxin was not detected in any of 60 healthy adults and in only 2 (1.8%) of 110 asymptomatic antibiotic recipients (5). Other investigators have reported a similar experience. By contrast, the carrier rate for *C. difficile* has been reported to be 2 to 3% for ambulatory, healthy adults (1, 5) and 10 to 20% for hospitalized patients and people recently exposed to antibiotics but without enteric disease (23). Because the available data suggest that the best correlation of symptoms with disease is the cytotoxin assay rather than culture, we have always considered the cytotoxin assay to be the best method for diagnosing *C. difficile* disease.

A recent study by Peterson et al. (16) questioned whether the cytotoxin assay of stool samples should be the reference method for detecting disease, because the percentage of results of cytotoxin assays of stool samples from patients with antibiotic-associated diarrhea was less than the percentage with a positive CDT (70 versus 90%, respectively). Although this observation suggests that the CDT might be a superior test for detecting disease, in a subsequent letter from the same group of investigators, the rates of positivity for the two tests were found to be similar (70 versus 67%, respectively) (L. R. Peterson, J. J. Holter, C. J. Shanholtzer, C. R. Garrett, and D. N. Gerding, Letter, Am. J. Clin. Pathol. **87**:298–299, 1987).

Kelly et al. (11) have reported that the sensitivity of the cytotoxin assay for detecting *C. difficile* disease was 70% and that the sensitivity of the CDT was 78%. An explanation of the lower sensitivity of the cytotoxin assay compared with that of CDT may be in the definition that is used for *C. difficile* diarrhea. Since these investigators included some patients with a positive CDT and a negative cytotoxin assay and culture result in the group with *C. difficile* diarrhea, it is possible that the sensitivity of the cytotoxin assay reported by them was understated because the CDT is known to cross-react with other bacteria (13, 15). We would not make the diagnosis of *C. difficile* diarrhea in the absence of a positive tissue culture assay result or positive culture for toxin-producing *C. difficile* and maintain that the tissue culture assay for cytotoxin in stool samples should continue to be the standard test for diagnosing *C. difficile* disease.

In contrast to diagnosing *C. difficile* disease in individual patients, the approach to investigating and managing an outbreak may depend on the identification of patients with an asymptomatic infection. Cycloserine-cefoxitin-fructose agar (CCFA) was one of the initial selective media used for the isolation of *C. difficile* (25). Wilson and colleagues (26, 27) have shown that the addition of taurocholate to CCFA increases the recovery of *C. difficile* from clinical specimens. Since carriers may be at risk for developing disease or spreading infection, we wished to identify all carriers by using a sensitive epidemiologic tool in our investigations.

Therefore, we used the most sensitive method, TCCFA culture, to define carriage.

In an epidemiologic investigation, the ability to quickly define the penetration of disease into a population is important. The introduction of the CDT in 1986 potentially allowed the rapid identification of toxigenic *C. difficile* in stool specimens during epidemiologic investigations. We tested specimens that were obtained from two investigations of outbreaks in nursing homes. These specimens were frozen at −70°C after they were initially tested for cytotoxin and cultured on TCCFA. Comparison of the CDT results obtained by using frozen specimens may have biased our results. However, freezing at −70°C has been shown to preserve cytotoxic activity for 5 days (7) and has preserved activity for up to 10 years in clinical specimens sent to our laboratory (unpublished data). If freezing affected the test results of the CDT in the present study, the change would have been in the direction of decreasing the yield of positive CDT results. Therefore, our results would be biased toward a decreased sensitivity for the CDT with respect to the cytotoxin assay or the TCCFA culture. However, our results showed that the CDT had a high sensitivity (92%) compared with the cytotoxin assay for diagnosing disease and that the CDT had a higher sensitivity than the cytotoxin assay compared with selective culture for the detection of carriage (52 versus 48%, respectively). Therefore, we do not believe that the testing of frozen stool samples interjected important biases into this study.

Compared with TCCFA culture, the CDT was not a sensitive test for identifying carriers. For the 27 carriers, CDT and the cytotoxin assay of stool samples identified only 14 (52%) and 13 (48%) specimens, respectively. At present there are no data to quantify the risks of *C. difficile* carriage, so that the relatively low sensitivity of CDT compared with that of culture may be irrelevant from the standpoint of treating a single patient. In an epidemiologic investigation, however, the low sensitivity of CDT and the cytotoxin assay for identifying carriers means that the true prevalence of infection may be significantly higher than would be apparent if either of these two tests was used alone.

Compared with the cytotoxin assay of stool samples, CDT showed sensitivity and specificity rates greater than 90% in this study, making it a potentially valuable diagnostic test. This observation is in agreement with those of other investigators (11, 19, 21), who have reported high sensitivities and specificities for CDT. Despite these high rates, the positive predictive value of the CDT in this study and others has proved to be quite variable. This is shown in Table 3, which summarizes the results from our study and others in which the cytotoxin assay has been used to define *C. difficile* disease. As anticipated, the positive predictive value fell with a decreasing prevalence of disease, although this fall was more dramatic than one might have predicted a priori. Our study of the comparison wards, in which the disease prevalence was low, shows the potential limitation of CDT. The prevalence of *C. difficile* disease was 2%, and the positive predictive value of the CDT was only 17%. Kaplan et al. (R. L. Kaplan, K. W. Mayer, W. Laudau, and L. J. Goodman, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, C313, p. 380) have reported a prevalence of 4% and a calculated positive predictive value of 40%. The outbreak wards in our investigation had a disease prevalence of 16% and a positive predictive value of 61%. Sherman et al. (21) have reported a disease prevalence of 17%, with a calculated positive predictive value of 73%. Ryan et al. (R. W. Ryan, I. Kwansik, and R. C. Tilton, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. abstr. no. 163, 1985) have reported the highest prevalence of disease, and the CDT had a correspondingly high positive predictive value.

TABLE 3. Summary of data from available studies of CDT which used the cytotoxin assay of stool samples as the standard for diagnosing *C. difficile* disease

Study	%			
	CDT sensitivity	CDT specificity	Prevalence of disease[a]	CDT positive predictive value
Comparison wards	100	92	2	17
Kaplan et al.[b]	85	95	4	40
Outbreak wards	92	89	16	61
Sherman et al. (21)	83	93	17	73
Ryan et al. (19)	88	98	31	95

[a] Prevalence of disease expressed as the percentage of patients with a positive cytotoxin assay result.
[b] Abstr. Annu. Meet. Am. Soc. Microbiol. 1986; see text.

To place these observations in perspective, it should be noted that most clinical studies of antibiotic-associated diarrhea show a *C. difficile* disease prevalence of 10 to 20% (18). This reflects the frequency of positive tests in patients with diarrhea when the tissue culture assay is used for cytotoxin detection in clinical laboratories. It is presumed that most of these specimens are obtained in symptomatic patients and that the majority of them have antibiotic-associated diarrhea or colitis. A review of our experience at the Johns Hopkins Hospital is perhaps typical and shows that about 11% of the specimens submitted to the clinical laboratory for *C. difficile* cytotoxin assay were positive. This experience, combined with the reported experience with analogous populations, suggests that in the usual clinical setting CDT has a positive predictive value of 60 to 80%.

The economical consequences of reliance on CDT could be profound if vancomycin, an expensive antibiotic, is used for the treatment of patients with positive test results. In our studies, 37% of patients on the outbreak wards and 83% on the comparison wards had false-positive CDT results. If treatment decisions were made by using these test results alone, unnecessary treatment might have been prescribed. Although CDT is sufficiently sensitive to use as a screening test because of its high sensitivity, we recommend the confirmation of a positive CDT result with the standard tissue culture assay for cytotoxin in the stool because of the problem with false-positive test results. Of course, in clinical laboratories the final decision regarding the need for confirmation depends on interrelated factors, including prevalence rates, available resources, cost, and management strategies.

Finally, although CDT enables a rapid and presumptive diagnosis of *C. difficile* disease to be made in an individual symptomatic patient, neither the CDT nor the tissue culture assay can be used for identifying carriers in an outbreak. Asymptomatic carriage is common in certain endemic environments, and selective culture should be used to identify carriers, as neither the CDT nor the cytotoxin assay of stool samples is sufficiently sensitive for this purpose.

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