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Commercial Latex Agglutination Test for Detection of *Clostridium difficile*-Associated Diarrhea

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A commercially available latex agglutination test for *Clostridium difficile* was compared with a cell culture cytotoxin assay and bacteriological culture for the laboratory diagnosis of *C. difficile*-associated diarrhea and colitis (CAD). Stool specimens from 626 patients were tested by the three methods, and specimens from 118 patients (19%) were positive by at least one of the methods. The results of the three tests agreed in 88% of the specimens tested, overall, but they agreed in only 34% of the 118 positive specimens. Ninety-three patients were evaluated to assess the significance of positive and negative results for each assay. Of 40 patients found to have CAD, 70% were positive by the cytotoxin assay, 78% were positive by the latex agglutination test, and 90% were culture positive. Of 53 patients who did not have CAD, 2% were positive by the cytotoxin assay, 8% were positive by the latex test, and 4% were culture positive. The detection of CAD was improved by using the tests in combination, and 97% of specimens positive by two or three methods were from patients who had CAD. Testing of multiple specimens from individual patients also increased the sensitivity of detection of CAD. The results suggest that the latex agglutination test may be useful for rapid diagnosis of CAD, especially in laboratories that lack cell culture facilities. However, the accuracy of CAD detection is improved when the latex test is used in combination with culture or the cytotoxin assay.

Clostridium difficile is a major cause of diarrhea and pseudomembranous colitis (PMC) in patients who have received antimicrobial therapy (1). Bacterial cultures are often positive in *C. difficile*-associated diarrhea and colitis (CAD), but definitive laboratory diagnosis has required demonstration of a cytotoxin in stool specimens by a cell culture assay (3). Recently, a latex agglutination test for *C. difficile* has become commercially available (Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo.). Although this test apparently does not detect *C. difficile* enterotoxin as originally suggested (8), a recent preliminary study indicated that the test was more effective than the cytotoxin assay for detection of CAD (9). The present report describes an investigation of the performance of the latex agglutination test, compared with a cytotoxin assay and bacterial culture, for the laboratory diagnosis of CAD.

MATERIALS AND METHODS

Specimens. Stool specimens from adult patients at two university-affiliated hospitals were entered into the study sequentially as they were received in a central laboratory serving both institutions. Each specimen was cultured for *C. difficile* and tested in parallel by the latex agglutination and cytotoxin assays. Specimens that could not be tested immediately were held at 4°C until processing.

Cytotoxin assay. Each specimen was diluted 1:10 in phosphate-buffered saline (pH 7.2), vortexed, and centrifuged to clarify the resulting suspension. The supernatant fluid was diluted serially 1:10, and 0.1 ml of each dilution was added in triplicate to the wells of a microtiter plate containing human foreskin fibroblast cells in 0.1 ml of medium. The final dilutions tested ranged from 1:20 to 1:200,000 in 10-fold increments. The cell cultures were observed for cytopathic effect after 24 and 48 h of incubation. *C. difficile* cytotoxin activity was confirmed by neutralization of the cytopathic effect by using specific antiserum (Virginia Polytechnic Institute and State University, Blacksburg).

Latex assay. Stool specimens were tested by latex agglutination by using the Culturette brand Rapid Latex Test for detection of *C. difficile* (Marion Laboratories). Each specimen was diluted 1:2 in the buffer provided by the manufacturer, vortexed, and centrifuged. The supernatant fluid was tested by latex agglutination according to the instructions of the manufacturer as previously described (9).

***C. difficile* cultures.** Specimens tested by the latex and cytotoxin assays were also cultured for *C. difficile*. Stool specimens were inoculated to selective media for *C. difficile* (10), and isolates were identified by typical colony morphology and the RapID-ANA system (Innovative Diagnostic Systems, Atlanta, Ga.).

Clinical evaluations. Clinical information was obtained by chart review for 93 patients with positive or negative cytotoxin, latex agglutination, or culture results. Patients with positive tests for *C. difficile* were considered to have CAD when they had diarrhea with negative tests for other enteric pathogens, a history of recent antibiotic use, and/or colitis at endoscopy. Patients with positive or negative tests for *C. difficile* were considered not to have CAD when they had no diarrhea or diarrhea with no history of recent antibiotic use and with normal endoscopy.

RESULTS

Stool specimens from 626 patients were tested by cytotoxin assay, latex agglutination test, and culture for *C. difficile*, and specimens from 118 patients (19%) were positive by at least one of the methods. Cytotoxin assays, latex agglutination tests, and cultures were positive for 67, 78, and 85 patients, respectively. Specimens from 45 patients were positive by both the cytotoxin and latex assays, and 40 of

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TABLE 1. Isolation of *C. difficile* from stool specimens positive or negative by *C. difficile* cytotoxin and latex agglutination assays

Cytotoxin/ latex results	No. of specimens	No. of specimens culture positive (%)
+/+	45	40 (89)
+/−	22	15 (68)
−/+	33	12 (36)
−/−	526	18 (3)

these patients were also culture positive (Table 1). Specimens from 22 patients were positive by the cytotoxin assay but negative by the latex agglutination assay, and cultures were positive in 15 of these specimens. Specimens from 33 patients were latex positive but cytotoxin negative, and 12 of these specimens yielded *C. difficile*. Specimens from 526 patients had negative latex and cytotoxin assays, and 18 of these specimens were culture positive. Overall agreement among the three methods was 88% (548 of 626 patient specimens), but of the 118 positive patients, only 40 (34%) were positive by all three of the methods.

To compare the value of the cytotoxin assay, latex agglutination test, and culture for the diagnosis of CAD, clinical findings were reviewed for 93 patients, of whom 40 were found to have CAD and 53 were found to have non-CAD. The cytotoxin assays, latex agglutination tests, and cultures were positive in 70, 78, and 90% of the CAD patients and in 2, 8, and 4% of the non-CAD patients, respectively (Table 2). Of the 93 patients, 19 had positive results for all three of the tests, and all of these patients had CAD (Table 3). Nine patients were positive only by the latex agglutination test and culture, and all of these patients also had CAD. Eight patients were positive only by the cytotoxin assay and culture, and seven (88%) of these patients had CAD. One patient with positive cytotoxin and latex assays but a negative culture had CAD. Overall, 36 (97%) of 37 patients positive by two or three test methods had CAD, but only 4 (44%) of 9 patients positive by a single test method had CAD.

Multiple specimens were received from 13 patients, and 39 specimens from these patients were tested by the three methods during the course of the study. The results of the repeat testing for six representative patients are presented in Table 4. Patient 9 had PMC, and the first specimen received from this patient was positive by all three test methods. The patient initially responded to treatment, and the latex agglutination test became negative, whereas the cytotoxin assay and *C. difficile* culture remained positive. The patient subsequently had a relapse of PMC, and stool specimens again became positive for all three tests. Patient 58 also had PMC, but the initial stool specimen received from this patient was positive only by the latex test and culture. In a repeat specimen collected on day 35, only the culture remained positive. However, another repeat specimen nine days later

TABLE 3. Value of combinations of positive and negative cytotoxin, latex agglutination, and culture results in the diagnosis of CAD

Results of cytotoxin/latex agglutination/culture	No. of patients	No. of patients with CAD (% of total)
+/+/+	19	19 (100)
−/+/+	9	9 (100)
+/−/+	8	7 (88)
−/+/−	6	2 (33)
−/−/+	2	1 (50)
+/−/−	1	1 (100)
+/+/−	1	1 (100)
−/−/−	47	0 (0)

was positive by the cytotoxin assay, latex test, and culture. Specimens from patient 87, who had PMC, were initially positive by all three assays. Treatment was associated with a decreasing titer of cytotoxin, and the latex test became negative after 30 days. The culture remained positive in all the specimens received from this patient. The first stool specimen received from patient 66 was cytotoxin positive, latex negative, and culture positive, but subsequent specimens were positive by all three methods. Specimens received from patient 68 were initially negative by all three assays, but those received after 8 and 35 days were positive by all three assays. Specimens from patient 71 were also initially negative, but those received on days 3 and 39 were positive by the latex test and culture. These findings demonstrate that the results of the cytotoxin assay, latex agglutination test, and culture for *C. difficile* may vary with time for individual patients and that treatment affects the outcome of the tests.

DISCUSSION

The diagnosis of *C. difficile* diarrhea has been based on cultures for the organism, detection of the cytotoxin in stool filtrates, or demonstration of PMC by endoscopy. The latter has been recommended as the definitive method for diagnosis, but pseudomembranes may be present without evidence of *C. difficile* involvement (2, 4), and *C. difficile* has been implicated in diarrhea and colitis without the presence of pseudomembranes (7; R. Fekety, Clin. Microbiol. Newsl. 3:63–65, 1981). Cultures for *C. difficile* are often positive in patients with antibiotic-associated diarrhea, but the organism can also be recovered from normal individuals (6). Demonstration of the cytotoxin in stool filtrates has been the most widely used method for diagnosis, but both false-positive and false-negative results occur with this test. In addition, the cytotoxin assay is technically demanding and costly, and it is not available in many laboratories (5). Accordingly, there is a need for a reliable, convenient, and inexpensive alternative test. The purpose of the present evaluation was to determine whether the latex agglutination test can fill this need.

TABLE 2. Cytotoxin, latex agglutination, and culture results for patients with or without CAD

Test	No. (%) of positive patients			Sensitivity (%)	Specificity (%)	PV (%)[a]		Accuracy (%)
	With CAD (n = 40)	Without CAD (n = 53)	Total			+	−	
Cytotoxin	28 (70)	1 (2)	29 (31)	70	98	97	81	86
Latex agglutination	31 (78)	4 (8)	35 (38)	78	92	89	84	86
Culture	36 (90)	2 (4)	38 (41)	90	96	95	93	94

[a] PV, Predictive value.

TABLE 4. *C. difficile* cytotoxin, latex agglutination, and culture results for patients tested more than once

Patient no.	Diagnosis	Day of testing	Results[a] for:		
			Cytotoxin	Latex agglutination	Culture
9	PMC	1	10^{-2}[b]	2+[c]	+
		72	10^{-5}	−	+
		80	10^{-3}	−	+
		103	10^{-4}	3+	+
58	PMC	1	−	2+	+
		35	−	−	+
		44	10^{-4}	3+	+
87	PMC	1	10^{-4}	3+	+
		16	10^{-3}	3+	+
		30	10^{-2}	−	+
66	UNK[d]	1	10^{-5}	−	+
		16	10^{-5}	3+	+
		33	10^{-5}	3+	+
68	UNK	1	−	−	−
		2	−	−	−
		8	10^{-2}	2+	+
		35	10^{-4}	4+	+
71	UNK	1	−	−	−
		3	−	4+	+
		39	−	4+	+

[a] +, Positive test result; −, negative test result.
[b] Titer of cytotoxin activity in stool specimens.
[c] Intensity of reaction.
[d] UNK, Clinical information not available.

The results of the evaluation indicate that the latex test had 78% sensitivity and 92% specificity for the detection of CAD. The comparable values for the cytotoxin assay and *C. difficile* culture were 70 and 98% and 90 and 96%, respectively. This difference in sensitivity does not appear to be caused by limitations in our cytotoxin assay, because our level of case detection by the cytotoxin assay exceeds that reported by other investigators (5). Although the latex test was more sensitive than the cytotoxin assay for detection of CAD, the test also failed to detect 22% of patients who were positive only by the cytotoxin assay or culture or both. Culture for *C. difficile* was the most sensitive of the three methods evaluated for the detection of CAD, but 10% of cases would have been missed if cultures had been used alone. Therefore, it appears that the tests should be used in combination for optimal detection of CAD. Use of the three tests together provided detection of 100% of the CAD cases, but this approach would be costly and would not provide results rapidly. Use of two of the methods in combination may provide the best approach to laboratory diagnosis of CAD (Table 5). A positive result by one or both of any two methods used in combination provided ≥93% accuracy for detection of CAD. The choice of methods for use in individual laboratories will depend on the relative importance of cost, speed, and accuracy and the availability of cell culture facilities. The latex test used in conjunction with culture is one approach that may have merit, especially for laboratories that lack cell culture facilities. The latex test provides rapid, presumptive results, and a positive latex test with a positive culture confirms the diagnosis of CAD. All specimens in the present study that were positive by both the latex test and culture came from patients who had CAD. Specimens positive by the latex test or culture alone could be referred for cytotoxin testing. Our results suggest that whatever combination of methods is used, testing more than one specimen, collected on different days, may increase the diagnostic yield, as is the case with many other enteric pathogens.

TABLE 5. Value of paired tests for the diagnosis of CAD

Test combination[a]	No. of patients positive by one or both tests		Sensitivity (%)	Specificity (%)	PV[b]		Accuracy (%)
	With CAD (n = 40)	Without CAD (n = 53)			+	−	
CT-CU	38	2	95	96	95	96	96
LA-CU	39	6	98	89	87	98	93
LA-CT	39	5	98	91	87	98	94

[a] CT, Cytotoxin assay; CU, *C. difficile* culture; LA, latex agglutination test.
[b] PV, Predictive value.

Our findings are similar to those recently reported by Peterson et al. (9). In their study of 161 stool specimens, the latex test was found to be more sensitive than the cytotoxin assay for detection of CAD. Another recent report suggested that the latex assay does not detect the enterotoxin of *C. difficile* (8). Our findings do not relate directly to the specificity of the latex assay for detection of the enterotoxin, but they do suggest that the results of the latex assay are closely correlated with CAD. Since the latex test does not appear to be specific for the enterotoxin, it is possible that the test is an indicator, comparable to culture, of the presence of the organism. However, the results of the latex test were not closely correlated with the culture results in the present study. Of 111 specimens positive by latex, culture, or both, only 47% were positive by both methods; 26 specimens were latex positive and culture negative, and 33 specimens were latex negative and culture positive. Whatever is being detected by the latex agglutination test, our results suggest that it is a useful, easily performed, and readily available test for the rapid diagnosis of CAD. However, our results also indicate that the test should be used in conjunction with culture or the cytotoxin assay for optimum laboratory diagnosis of CAD.

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