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Commercial Latex Test for *Clostridium difficile* Toxin A Does Not Detect Toxin A

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The rapid latex test recently marketed by Marion Scientific (Div. Marion Laboratories, Inc., Kansas City, Mo.) for the detection of *Clostridium difficile* toxin A does not react with the toxin, based on the following findings: (i) culture filtrates from nontoxigenic strains of *C. difficile* gave positive reactions in the test, (ii) culture filtrate in which toxin A had been removed gave positive reactions, (iii) purified toxin A did not react in the test, and (iv) the latex reagent bound an antigen which is distinct from toxin A and which is produced in various amounts by both toxigenic and nontoxigenic strains of *C. difficile*.

Clostridium difficile, the major cause of pseudomembranous colitis in humans, produces two toxins (A and B) which are involved in the development of the disease (1, 6). *C. difficile* isolates produce either both of the toxins or neither toxin (3, 5); no isolate has been characterized which produces only one of the toxins. Therefore, diagnostic tests which detect either toxin are suitable for the demonstration of toxigenic *C. difficile* in clinical specimens. Diagnostic tests should be specific for the toxins, because there are many nontoxigenic strains that do not cause disease. A rapid latex test was recently marketed for the detection of toxin A (enterotoxin) in fecal specimens. In the study described here, we examined the specificity of the test for toxin A and compared our findings with results obtained with a tissue culture assay and an indirect enzyme-linked immunosorbent assay (ELISA).

Ten *C. difficile* strains which vary in level of toxin production were obtained from the anaerobe collection of the Department of Anaerobic Microbiology of Virginia Polytechnic Institute and State University. The strains were grown in brain heart infusion broth for 36 h at 37°C. The cells were removed by centrifugation, and the supernatant fluids were passed through 0.2-μm (pore size) membranes. Culture filtrates prepared in this manner were analyzed for toxin by tissue culture assay (2), indirect ELISA using a monoclonal antibody against toxin A (4), and the rapid latex test manufactured by Marion Scientific, Div. Marion Laboratories, Inc., Kansas City, Mo. The cytotoxic titer determined by the tissue culture assay represented the level of toxin B, since we showed previously that the potent cytotoxic activity of toxin B masks the weaker cytotoxic activity of toxin A in culture filtrates (5). The specificity of the monoclonal antibody used in the ELISA for the detection of toxin A was demonstrated previously (4). The latex test was done as recommended by the manufacturer.

Our findings are presented in Table 1. There was a correlation between the cytotoxic titer and the ELISA titer: as the cytotoxic titer decreased, the ELISA titer also decreased. These results support our previous findings showing a close correlation of toxin A levels, as determined by ELISA, with toxin B levels, as determined by tissue culture assay (5). The results obtained with the commercial rapid latex test did not correlate with the ELISA and tissue culture assay. Weakly toxigenic as well as nontoxigenic strains of *C. difficile* gave positive reactions in the commercial assay.

To further examine the specificity of the rapid latex test, we removed toxin A from culture filtrates of *C. difficile* VPI 10463, a high toxin producer, by immunoadsorption with Affi-Gel 10 (Bio-Rad Laboratories, Richmond, Calif.) containing covalently bound PCG-4 monoclonal antibody. Analysis of the adsorbed culture filtrate by the ELISA showed that >99.9% of the toxin A was removed. In addition, the specific removal of the toxin by the gel was confirmed by crossed immunoelectrophoresis (data not shown). The adsorbed culture filtrate still gave strong positive reactions in the rapid latex test.

We analyzed several of our highly purified toxin A preparations in the latex test. Tenfold dilutions of toxin A, ranging from 500 ng to 500 μg/ml, were analyzed. None of the preparations of toxin A gave a positive reaction.

TABLE 1. Comparison of culture filtrates by tissue culture assay, indirect ELISA, and commercial rapid latex test

Organism and strain or medium	Assay results		
	Cytotoxic titer[a]	ELISA titer[b]	Latex test[c]
C. difficile			
10463	10^6	10^4	+ + +
26689	10^5	10^3	+ + +
7698	10^3	10^1	+ + +
24050-A	10^3	10^1	+
11011	10^2	−	+ + +
tox^R	10^2	−	+ + +
4474	10^1	−	+ +
2634	−	−	+ + +
2037	−	−	+ + +
11186	−	−	+ + +
Brain heart infusion broth	−	−	−

[a] The cytotoxic titer is expressed as the reciprocal of the highest dilution of culture filtrate which causes rounding of 100% of the tissue culture cells. −, Below the detectable limits of the assay.

[b] The ELISA titer is expressed as the reciprocal of the highest dilution of culture filtrate which gives an A_{405} of 0.2 to 0.4. −, Below the detectable limits of the assay.

[c] Test samples were diluted with an equal volume of diluent included in the Marion Scientific rapid latex test before the assay. Interpretation of results: + + +, strong rapid agglutination; + +, agglutination easily visible; +, weak agglutination; −, no detectable agglutination.

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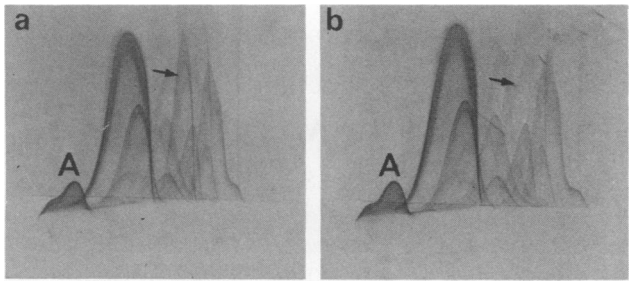

FIG. 1. Analysis, by crossed immunoelectrophoresis, of *C. difficile* VPI 10463 culture filtrate adsorbed with the negative (a) or positive (b) latex reagent from the Marion Scientific rapid latex test for *C. difficile* toxin A. Dimension 2 in each gel contains about 5 μl of goat antiserum against *C. difficile* VPI 10463 culture filtrate per cm^2. The arrows show the disappearance of a major immunoprecipitin arc in culture filtrate adsorbed with the positive latex reagent, indicating the adsorption of that antigen by the reagent. A indicates the toxin A immunoprecipitin arc. Note that the toxin A arc was not altered by the adsorption.

In an effort to identify the antigen(s) which reacted in the latex test, we adsorbed culture filtrate from *C. difficile* VPI 10463 with the positive latex reagent from the kit. Culture filtrate (1 ml) was mixed with 1.0 ml of the latex reagent at room temperature for 1 h. The latex beads were then removed by centrifugation. The adsorbed culture filtrate was concentrated and analyzed by crossed immunoelectrophoresis. Culture filtrate mixed with the negative latex reagent from the kit served as a control. Our findings are shown in Fig. 1. The positive latex reagent specifically adsorbed a major antigen from the culture filtrate, as shown by the disappearance of a major immunoprecipitin arc. Toxin A was not adsorbed by the latex reagent. Further analysis showed that culture filtrate adsorbed with the positive latex reagent did not react in the latex test even though toxin A was still present. Culture filtrate adsorbed with the negative latex reagent gave a strong positive reaction. Previous findings in our laboratory showed that the adsorbed antigen is a large molecule and that it is produced in various amounts by most *C. difficile* strains (unpublished data).

Our results show that the Marion Scientific rapid latex test for toxin A does not detect toxin A and that it reacts with nontoxigenic strains of *C. difficile*. The antibody used on the latex beads reacts with another antigen produced in various amounts by *C. difficile* strains. Thus, the test likely will give false-positive reactions, resulting in inappropriate therapy for the patient.

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