Latex Agglutination Test for Detection of Clostridium difficile Toxin in Stool Samples

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A total of 163 stool specimens were tested for detection of Clostridium difficile and its toxin by cytotoxicity assay with tissue culture, latex agglutination test, and isolation of the organism. From 33 specimens which were positive for toxin by cytotoxicity, 30 were positive by the latex agglutination test; the organism was isolated from 21. The total number of samples which were positive with the latex agglutination test was 44. The predictive value of a positive latex agglutination result relative to the cytotoxicity test was 68%, and the predictive value of a negative result was 97.5%. The specificity and sensitivity of the latex agglutination test relative to the cytotoxicity assay and the low cost and simple facilities required indicate that the latex agglutination test is a useful procedure for screening for C. difficile toxins, provided that positive latex results are confirmed by cytotoxicity assay.

Clostridium difficile is the causative agent of antibiotic-associated pseudomembranous colitis in humans (1, 2, 7, 10). There are many reports regarding the laboratory diagnosis of C. difficile in stool samples. Some of these methods are based on the detection of C. difficile toxin in stool samples (4, 11, 15, 20), whereas others include mainly detection and isolation of the organism from the specimens (6, 8, 14, 22, 23). Detection of C. difficile toxin using tissue culture (3, 4, 7, 8, 11) is the diagnostic procedure that correlates best with pseudomembranous colitis. Some investigators have used counterimmunoelectrophoresis or enzyme-linked immunosorbent assay for demonstrating C. difficile toxin in fecal specimens (11, 12, 15, 20). However, development of a test for more simple and rapid laboratory detection of toxin is desirable. In this communication, we report the use of a rapid method for detection of C. difficile toxin in stool samples.

MATERIALS AND METHODS

Samples. A total of 163 stool samples were obtained from 152 patients in the Foothills Hospital, Calgary, Alberta. All of the patients were suspected of having pseudomembranous colitis. Samples were submitted to the Microbiology and Infectious Diseases Laboratory of the Foothills Hospital for isolation of C. difficile or for toxin testing. Samples were transferred to the laboratory at ambient temperature. Immediately after receipt (normally within 2 h after defecation), the samples were processed for culture and toxin testing.

Culture. Isolation of C. difficile was performed by the method of Koronsky et al. (9). Approximately 1 g of undiluted stool specimen was emulsified in an equal volume of 50% alcohol by vigorous mixing in a vortex mixer. The mixture was left at room temperature for 10 min. A few drops of the supernatant were cultured on cefoxitin-cycloserine agar plates, which were incubated anaerobically at 37°C. Resulting colonies of C. difficile were subcultured in chopped-meat broth (Oxoid Ltd., Hants, England), and toxin in the culture supernatant was assayed by the cytotoxicity test.

Toxin testing. A portion of each fresh specimen was diluted with an equal volume of phosphate-buffered saline and centrifuged at low speed for 10 min. The supernatant was passed through a filter (pore size, 0.45 μm), and the filtrate was assayed for toxicity. Samples from the same filtrate of each fecal specimen were used to test for toxicity by both tissue culture and latex agglutination. C. difficile antitoxin and control crude toxin were from T. D. Wilkins, Virginia Polytechnic Institute and State University, Blacksburg.

Cytotoxicity assay for C. difficile toxin. The tissue culture assays were by the method of Chang et al. (4), and either L cells or MRC-5 cells were used. In preliminary studies, we determined that L cells were as sensitive to the C. difficile cytotoxic effect as were either CHO or MRC-5 cells. Cells were grown in minimal essential medium supplemented with 5% fetal calf serum. The cell monolayers were prepared in 96-well, flat-bottomed microtiter plastic tissue culture plates. Serial 2- and 10-fold dilutions of the filtrates were prepared in minimal essential medium without calf serum and inoculated onto the monolayers of cells. Inoculated cell cultures were incubated at 37°C in 5% CO2. Plates were examined for cytopathic effect after 24 and 48 h of incubation, and 100% cytopathic effect was considered positive. In addition, 0.1 ml of each dilution of specimen was mixed with 0.1 ml of a 1:50 dilution of C. difficile antitoxin. The mixture was incubated at room temperature for 30 min and then assayed as described above. For positive control, 0.1 ml each of 104 to 106 dilutions of crude commercial control toxin was inoculated into tissue culture.

Latex agglutination test. Antitoxin obtained from T. D. Wilkins was passed through a column of DEAE-Sephadex A50 (Pharmacia Fine Chemicals, Piscataway, N.J.) by the method of Dedmon et al. (5). Samples (3 ml) of antitoxin were applied, and the column was eluted with 0.02 M phosphate buffer (pH 7.6). The gamma globulin fraction was collected, concentrated to 3 ml in a speed vacuum concentrator (model RH20-12; Savant Instruments, Inc., Hickle, N.Y.), and diazylized against phosphate-buffered saline. Coating of latex with gamma globulin was performed by the method of Newman et al. (13) and Severin (18). Briefly, latex particles (0.8 μm; Difco Laboratories, Detroit, Mich.) was diluted 1/3 in glycine buffer (0.1 M glycine, 0.15 M NaCl [pH 8.2]). The concentrated antitoxin was diluted 1/20 (3 mg of

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protein per ml) in the glycine buffer. One part of gamma globulin was mixed well with one part of the latex particle suspension in glycine buffer and incubated at 37°C for 2 h. After the incubation period, two parts of glycine buffer containing 0.1% bovine serum albumin were added to the tube and stored at 4°C for 3 to 5 days. When the latex particles sedimented to the bottom of the tube, the clear supernatant was carefully removed and replaced by the same volume of fresh glycine buffer containing bovine serum albumin. For negative control, unimmunized rabbit gamma globulin was similarly purified and used to coat latex particles. The test was carried out on a glass slide or on a dark agglutination plate (Wellcome Research Laboratories, Inc., Beckenham, England) by mixing 50 μl of coated latex with 50 μl of stool filtrate. The mixture was gently shaken continuously, and the results were recorded after 5 min. For examination of the specificity of the test, 0.1 ml of antitoxin was added to 0.1 ml of stool filtrate (five samples), incubated at room temperature for 15 min, and then used in the latex agglutination test.

**Purification and absorption of reagents.** Toxin A was purified from crude commercial toxin by column chromatography and acetic acid precipitation by the method of Sullivan et al. (17).

To remove the antibody to toxin A from the commercial antitoxin, 700 μg of purified toxin A in 0.5 ml was mixed with 0.5 ml of antitoxin containing 3 mg of protein. For control, 0.5 ml of the antitoxin was mixed with 0.5 ml of saline. The mixtures were left at 25°C for 1 h and then centrifuged for 2 min in a microcentrifuge (model 235A; Fisher Scientific Co., Pittsburgh, Pa.) to remove antigen-antibody complexes. Serial twofold dilutions were prepared in glycine buffer and used for coating latex particles.

The commercial antitoxin also was adsorbed with whole cells of a toxigenic strain of *C. difficile* (ATCC 9689) by the method of Ryan et al. (16).

**RESULTS**

The results are presented in Table 1. All toxigenic specimens were neutralized by *C. difficile* antitoxin.

The sensitivity and specificity of the latex agglutination test relative to those of the cytotoxicity test were 91 and 91.5%, respectively (Table 2). *C. difficile* was isolated from 64% of the specimens that were cytotoxic. No agglutination occurred with culture supernatants of *Clostridium sordellii* (ATCC 9714) or *Clostridium bifermantans* (ATCC 17838).

Purified toxin A at a concentration of 0.2 μg in 50 μl reacted strongly with antitoxin-coated latex particles.

Undiluted antitoxin absorbed with toxin A was negative in the latex agglutination test against purified toxin A. Of eight stool samples that were positive by the cytotoxicity assay and that reacted in the latex agglutination assay with 1/16 unabsorbed antitoxin, seven were still positive with latex particles coated with serum that had been adsorbed with toxin A. Four of these samples were positive at dilutions of 1.4, and three were positive at a dilution of 1.8. These results show that toxin A was one of the major components detected by latex particles coated with commercial antitoxin, but that other reactive components were present in all but one of these sample filtrates.

The antitoxin that was adsorbed with cells of a toxigenic strain of *C. difficile* was nearly as reactive with stool filtrates as it was before adsorption.

**DISCUSSION**

For detection of *C. difficile* toxin in stools, the latex agglutination test is more rapid than assay by cytotoxicity, counterimmunoelectrophoresis, or enzyme-linked immunoabsorbent assay. The sensitivity of the test is high. As little as 0.2 μg of toxin A gave positive agglutination.

There were 11 specimens that were positive by the latex agglutination test, but negative by cytotoxic assay. The discrepant results could either be false-positives or they could reflect enhanced sensitivity of the latex agglutination test. When we adsorbed the antitoxin with purified toxin A, the titer of the antitoxin was reduced by two- to fourfold, but significant activity remained in seven of the eight samples tested. The antibodies that could not be removed by toxin A could be antibodies to toxin B, to other *C. difficile* antigens, or to both. It is possible that the higher number of apparent false-positive reactions was due to nonspecific reaction between the nontoxin antibodies and some other *C. difficile* antigens. West and Wilkins (21) have reported that the commercial antitoxin contains antibodies against other *C. difficile* antigens that react with some strains of nontoxigenic *C. difficile*. However, absorption of the antitoxin with cells of toxigenic strain of *C. difficile* did not reduce the titer of cytotoxic strains appreciably. Also, the results were negative when the antitoxin-coated latex particles were tested against the culture supernatants of *C. sordellii* and *C. bifermantans*.

Culture results showed greater agreement with the latex agglutination than with cytotoxicity (Table 3). Of 38 specimens positive by culture, 21 were cytotoxic and 28 reacted in the latex agglutination test. However, although we did not analyze all clinical cases, significant diarrhea generally correlated better with the cytotoxicity assay than either of the other two tests.

The calculated predictive value (16) of a positive latex agglutination result relative to the cytotoxicity test was 68%, and the predictive value of a negative result was 97.5%. The high predictive value of a negative makes the latex agglutination very useful as a screening test for detection of toxin-positive specimens. However, specimens positive by the

**TABLE 1. Comparison of different tests for detection of *C. difficile* and its toxin in 163 stool specimens**

<table>
<thead>
<tr>
<th>Test</th>
<th>No. positive</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>LA</td>
<td>44</td>
<td>26.9</td>
</tr>
<tr>
<td>C</td>
<td>38</td>
<td>23.3</td>
</tr>
<tr>
<td>Cyto</td>
<td>33</td>
<td>20.2</td>
</tr>
<tr>
<td>C-LA*</td>
<td>28</td>
<td>17.1</td>
</tr>
<tr>
<td>Cyto-LA*</td>
<td>30</td>
<td>18.4</td>
</tr>
<tr>
<td>Cyto-C*</td>
<td>21</td>
<td>12.8</td>
</tr>
<tr>
<td>Cyto-LA-C*</td>
<td>19</td>
<td>11.6</td>
</tr>
</tbody>
</table>

* LA, Latex agglutination; C, culture; Cyto, cytotoxicity assay.

* Positive by both procedures.

* Positive by three procedures.

**TABLE 2. Comparison of bacterial isolation, cytotoxicity assay, and latex agglutination test for detection of *C. difficile* organisms or toxin in 163 stool specimens**

<table>
<thead>
<tr>
<th>Cytotoxicity assay (n)</th>
<th>Latex agglutination*</th>
<th>Bacterial isolation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (33)</td>
<td>30</td>
<td>3</td>
</tr>
<tr>
<td>Negative (130)</td>
<td>11</td>
<td>119</td>
</tr>
</tbody>
</table>

* All tests were performed in duplicate, and no discrepant results were found on repeated tests.
latex agglutination assay must be confirmed by the cytotoxicity assay. In our cases, the three cytotoxicity-positive specimens that were negative by latex agglutination were not associated with clinical diarrhea or PMC.

The latex agglutination test is 100% reproducible, simple to do as compared with the cytotoxicity assay, and can be performed in 10 to 15 min. In our laboratory, the cost of testing a specimen for C. difficile toxin by the latex agglutination test is about one-eighth of that of the cytotoxicity assay. In addition, because the latex agglutination test does not require special facilities and equipment, it would be useful in small hospitals.

In conclusion, the latex agglutination test for detection of C. difficile toxin seems clearly useful as a screening test. In small health centers where no facilities for tissue culture are available, a rapid answer can be obtained by the latex agglutination test. We recommend that positive specimens be sent to a larger health center for confirmation by cytotoxicity testing. In larger centers, latex agglutination also could be used to reduce the number of cytotoxicity assays. In our study, for example, 119 of 163 cytotoxin assays could have been eliminated.

The major disagreement between cytotoxicity and latex agglutination was cytotoxicity-negative and latex agglutination-positive results. It is possible that by using a more specific antisera prepared against purified C. difficile toxins, the number of false-positives may be reduced or eliminated, and closer to 100% correlation with the cytotoxicity assay could be obtained. Ryan et al. (16) have adsorbed the commercial antisera with the whole cells of C. difficile, thus removing the cross-reacting antibodies. Using this adsorbed antisera, they succeeded in reducing the nonspecific reactions in the counterimmunoelectrophoresis test. In our study, use of preadsorbed antisera to toxigenic strains of C. difficile did not appreciably affect the reactivity of cytotoxicity-positive specimens in latex agglutination test. We are accumulating false-positive specimens which are cytotoxicity-negative to test them with the preadsorbed antisera. We are also in the process of preparing specific antisera to purified toxin A. It is possible that using these antisera may reduce the number of false-positives in the latex agglutination test.

ACKNOWLEDGMENT

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LITERATURE CITED


TABLE 3. Comparison of bacterial isolation and latex agglutination test for detection of C. difficile organisms and toxin in 163 stool specimens

<table>
<thead>
<tr>
<th>Bacterial culture (n)</th>
<th>Latex agglutination</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive (38)</td>
<td>28</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Negative (125)</td>
<td>16</td>
<td>109</td>
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