Utility of a Rapid Latex Test for the Detection of Clostridium difficile in Fecal Specimens

RAYMOND W. RYAN, PH.D., IRENE KWASNIK, M.S., DONNA CLOUT, B.S., and RICHARD C. TILTON, PH.D.

Department of Laboratory Medicine, University of Connecticut School of Medicine, Farmington, CT 06032

ABSTRACT

Currently, the method of choice for the laboratory diagnosis of Clostridium difficile disease is the detection of cytotoxin in stool filtrates by tissue culture. Since many hospital laboratories do not have tissue culture facilities, there is a need for a rapid test which is both sensitive and specific to diagnose C. difficile disease. A commercial latex agglutination was compared with the conventional cytotoxin tissue culture assay for the detection of C. difficile or its toxin(s) in fecal specimens. Of the 574 specimens evaluated, 111 were cytotoxin positive while 97 were positive by the latex agglutination test. There were 17 specimens positive by latex agglutination but negative by tissue culture assay. The overall sensitivity and specificity of the CDT latex test was 86.1 percent and 95.3 percent respectively. This rapid latex test can serve as an excellent screening procedure for the presence of C. difficile. Those specimens positive by the latex test should be further evaluated for the presence of cytotoxin by tissue culture.

Introduction

Clostridium difficile has been shown to be the major cause of antibiotic associated gastrointestinal disease in humans. The organism is known to produce at least two toxins, a cytotoxin, toxin B, and an enterotoxin, toxin A. Diagnosis of C. difficile disease has relied primarily upon the demonstration of toxin B in stool filtrates using tissue culture assay. Enzyme immunosorbent assays for both toxin A and toxin B have been developed. However, they remain largely a research tool and unavailable to most clinical laboratories.

Recently, a commercial latex agglutination test has become available for the detection of C. difficile antigen in stool filtrates. In this report, the cytotoxin tissue culture assay is compared with the latex test for the detection of C. difficile antigen.

Materials and Methods

Five hundred fifty-four fecal specimens from patients suspected of having

* Culturette Brand CDT Latex Test (Marion Laboratories, Kansas City, MO).
C. difficile disease were submitted to the Microbiology Division, Department of Laboratory Medicine, John Dempsey Hospital, University of Connecticut Health Center, Farmington, CT. Thirty-nine of the specimens had previously been shown to be positive for C. difficile cytotoxin and were frozen at —70°C. The remaining 515 specimens were examined for C. difficile toxin within 48 hr of arriving in the laboratory.

Fecal Filtrates

Liquid fecal samples were centrifuged for 30 min at 300 × g, and the supernatant was filtered through a 0.45 μm membrane filter. Formed stools were prediluted 1:1 in phosphate buffer (0.01M, pH 7.0) prior to centrifugation and filtration.

Cytotoxin B Assay

The cytotoxin assay was performed using an MRC-5 cell line. One tenth ml of sterile stool filtrate was diluted 1:1 with phosphate buffer (0.01M, pH 7.0). One tenth milliliter of the dilution was added to a tube of MRC-5 cells containing 0.9 ml tissue culture maintenance media. Filtrates causing cytopathic effects after 24 to 48 hr were further evaluated by preincubating the original filtrate with an equal volume of a 1/25 dilution of specific C. difficile antitoxin† and retesting. Those specimens not neutralized by the addition of antitoxin were considered negative for C. difficile toxin.

Latex Agglutination

The stool filtrates were tested undiluted using the Culturette Brand CDT Latex Test* for Clostridium difficile. The latex procedure was performed according to the manufacturers' instructions.

Results

In order to evaluate the CDT latex reagent, 75 fecal specimens known to be positive for C. difficile cytotoxin were tested (table I). Of these 75 specimens, 23 had been frozen at —70°C, and the remaining 52 were kept at 2° to 8°C. All positive specimens were tested with the latex reagent and retested for cytotoxin by the tissue culture assay. One hundred sixty-four specimens known to be negative for cytotoxin were also tested by latex agglutination and retested for cytotoxin.

All of the 75 known previously positive specimens remained positive when retested for cytotoxin. Of the 75 specimens, 66 (88 percent) were positive by the latex test. Three of the 164 cytotoxin negative specimens were positive when tested with the latex reagent. The cytotoxin titers of the nine specimens which were positive by tissue culture assay and negative by latex agglutination were determined. The titers ranged from $1 \times 10^{-2}$ to $1 \times 10^3$.

The latex test on 335 consecutive stool samples submitted for C. difficile toxin analysis was also evaluated. Results are shown in table II. Thirty-six specimens (10.7 percent) were positive by tissue culture assay. Of the 36 positive specimens, 31 were also positive by latex agglutination. Fourteen of the 299 cytotoxin negative specimens were positive by the latex assay. Two hundred eighty-five specimens (85 percent) were negative by both methods.

<table>
<thead>
<tr>
<th>Latex Agglutination</th>
<th>Cytotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>66</td>
</tr>
<tr>
<td>Negative</td>
<td>9</td>
</tr>
</tbody>
</table>

† VPI Anaerobe Laboratory, Blacksburg, VA.
### Table II

Comparison of Tissue Culture Assay and Latex Agglutination on 335 Consecutive Fresh Fecal Specimens Submitted for Clostridium difficile Cytotoxin Assay

<table>
<thead>
<tr>
<th>Latex Agglutination</th>
<th>Cytotoxin</th>
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</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Positve</td>
<td>31</td>
<td>14</td>
</tr>
<tr>
<td>Negative</td>
<td>Positve</td>
<td>5</td>
<td>285</td>
</tr>
</tbody>
</table>

The sensitivity, specificity, predictive value of a positive, and a negative (PVP, PVN) and prevalence of disease in the population are shown in Table III. These results, which are calculated on 335 consecutive samples, are as follows: sensitivity, 86.1 percent; specificity, 95.3 percent; PVP 69.0 percent; PVN 98.3 percent; and prevalence of disease, 10.7 percent.

### Discussion

Until recently, the only laboratory methods available for the diagnosis of C. difficile disease were tissue culture assay for the cytotoxin and direct culture of the organism from feces on selective media. The tissue culture assay is the most widely used; however, it lacks standardization. This procedure varies considerably among laboratories. Variables associated with the tissue culture procedure include: type of cell culture used, initial dilution of stool filtrate, time of incubation, and endpoint determination (percent CPE). Culture of C. difficile is laborious and time consuming and not available to most clinical laboratories.

Enzyme immunosorbent assays have been developed which are capable of detecting either toxin A and/or toxin B in stool filtrates; as yet, they have not become available for use in the clinical laboratory. Lyerly and Wilkins have recently shown that the CDT Latex Test does not detect toxin A as was previously thought but reacts with another cell associated antigen produced by both toxigenic and non-toxigenic strains of C. difficile. The question is: Is their justification valid for reporting only the presence of C. difficile antigen in stool filtrate or must a specific toxin, i.e., cytotoxin, be detected? The precise role of toxins A or B in the etiology of Clostridium difficile disease have not been defined.

Recent studies by Walker et al and Peterson et al have attempted to correlate clinical criteria of C. difficile disease with the presence of cytotoxin in the stool of patients. The tube cytotoxicity assay in those patients in each study, considered “very likely to have C. difficile disease”, was positive only 70 percent of the time. Peterson et al also reported that in the group of patients likely to have disease, the CDT latex test correlated at a rate of 90 percent with clinical criteria.

In this study, the results of cytotoxin assay were compared with the CDT Latex Test on 335 consecutive fecal specimens submitted for C. difficile toxin analysis. If it is assumed the standard for C. difficile disease is presence of cytotoxin in the stool, the prevalence of disease in our study was 10.7 percent. Thirty-six of the 335 specimens were positive by tissue culture assay, while only 31 of 36 tissue culture positive specimens were positive by the CDT Latex Test. Cytotoxin titers were determined on the five specimens positive by tissue
culture assay and negative by latex agglutination. All had titers of $1 \times 10^{-3}$. Fourteen samples positive by CDT but negative by cytotoxin may be explained by the presence of antigen from non-toxigenic strains of *C. difficile* in the stool specimens. These specimens were not available for *C. difficile* culture. The sensitivity and specificity of the CDT Latex Test when compared to the cytotoxin assay are 86.1 percent and 95.3 percent, respectively.

In summary, the culturette brand CDT Latex Test is rapid, sensitive, specific, and simple to perform. Even though this latex reagent does not detect specific *C. difficile* toxin, it allows the laboratory to screen suspected fecal specimens for *C. difficile* antigen within a relatively short time. The cytotoxin tissue culture assay may then be used to confirm latex positive specimens or for testing specimens from patients suspected of having *C. difficile* disease which were negative by the latex test.

References