Comparison of Two Commercially Available Enzyme Immunoassays for Detection of Clostridium difficile in Stool Specimens

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Clostridium difficile is the cause of most cases of pseudomembranous colitis, the most severe form of antibiotic-associated diarrhea. Rapid diagnosis guides both the treatment and the control of nosocomial spread of infection. Two enzyme immunoassay (EIA) kits developed for the rapid detection of C. difficile toxin A in fecal specimens, Premier (Meridian Diagnostics, Cincinnati, Ohio) and Tox-A test (TechLab, Virginia Polytechnic Institute Research Park, Blacksburg), were evaluated by using 410 fecal specimens. Seventy-six specimens were positive for C. difficile toxin B by the cytotoxin assay (prevalence rate, 19%). The Meridian EIA was positive for 71 of the 76 samples, yielding a sensitivity of 93%. The TechLab EIA detected 75 of the 76 positive samples, yielding a sensitivity of 99%. The Meridian and TechLab EIAs had specificities of 100 and 93%, respectively. These data indicate that both EIAs are suitable alternatives to the cytotoxin assay in routine diagnostic laboratories. However, confirmation of TechLab EIA-positive test results by the cytotoxin assay remains necessary.

Clostridium difficile, the causative agent of antibiotic-associated colitis and pseudomembranous colitis (2, 6), is an important nosocomial pathogen (4). The toxigenic strains of the organism produce the disease by elaboration of two lethal toxins, toxins A and B (7, 8). Toxin A is a large (in its native form, its Mr is in the range of 400,000 to 600,000), potent enterotoxin with slight cytotoxic activity (8). Toxin A binds to cells that express the Galα1-3Galβ1-4GlcNAc trisaccharide receptor (5) in the brush border membrane, resulting in the erosion of the mucosa and then a fluid response in the intestine (8). Toxin B is also a large (in its native form, its Mr is in the range of 360,000 to 500,000), potent cytotoxin (8) which causes a number of nonspecific in vitro responses in mammalian cells, including the disorganization of the actin filaments, the loss of intracellular potassium, a decrease in the level of protein synthesis, and a decrease in the level of synthesis of RNA and DNA (3, 8, 10–12). Lyerly et al. (9) demonstrated that toxin B is not active in the intestine and suggested that toxin B exits through the damaged gut mucosa (as a result of the action of toxin A) and acts distally to the intestine. Demonstration of the presence of toxins in the stool and isolation of the organism and the subsequent demonstration of the isolate’s ability to produce toxins are the primary laboratory tests used for the presumptive diagnosis of C. difficile-related diarrheal disease in the appropriate clinical setting.

A number of rapid (enzyme immunoassay [EIA] and latex agglutination) and conventional (direct plate and tissue culture) tests have been developed as aids in the diagnosis of the disease. Tissue culture cytotoxicity assay is the best available laboratory test for determination of the role of C. difficile in the pathology of a given patient’s diarrhea, but its utility is limited because of its inherent technical complexity, time requirement (24 to 48 h), specimen-handling requirements, and expense. Thus, there is a need for alternative rapid, accurate, and easy-to-perform screening tests. We therefore evaluated the effectiveness of two rapid methods in detecting C. difficile toxin A from stool specimens of patients suspected of having C. difficile-associated illness. The new C. difficile Tox-A test (TechLab, Virginia Polytechnic Institute Research Park, Blacksburg) is a 6.5-h microtiter well EIA that detects C. difficile enterotoxin A; therefore, it is able to distinguish between toxigenic and nontoxigenic strains of C. difficile. The Premier C. difficile toxin A EIA (Meridian Diagnostics, Cincinnati, Ohio) is also a 2.5-h microtiter well EIA for detection of enterotoxin A in stool specimens. The tissue culture cytotoxin assay for C. difficile toxin B was used as the reference test method for comparison (13). All three tests were performed on each stool sample.

MATERIALS AND METHODS

Patient population. A total of 410 liquid stool specimens from patients suspected of having antibiotic-associated diarrhea were tested between August 1991 and December 1992 at the Children’s Hospital of Buffalo, Buffalo, N.Y. The patients’ ages ranged from 2 to 28 years.

Sample handling and storage. Upon receipt, each stool specimen was mixed well and was split into two aliquots. Both aliquots were frozen at −70°C until they were tested. The first aliquot was thawed and the Premier EIA was performed. The second aliquot was thawed, and the tissue culture cytotoxin assay was performed. The TechLab EIA was sometimes performed by using the specimens used for the Premier EIA and was sometimes performed by using specimens from the cytotoxin assay, depending on the flow of the work in the laboratory on any given day. In total, the TechLab EIA was performed with the aliquot from the Premier EIA for 217 samples and with the aliquot from the

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cytotoxin assay for 193 samples. All samples were frozen only once.

**Cytotoxin assay.** The tissue culture procedure used in the present study was the Bartels *C. difficile* cytotoxin assay kit (Bartels Immunodiagnostics Supplies, Inc., Bellevue, Wash.) for *C. difficile* toxin B. The manufacturer's directions were followed, and a test well was considered positive if the cells showed 1+ or greater cytopathic effect.

**Premier C. difficile toxin A EIA.** The Premier *C. difficile* toxin A EIA was performed according to the manufacturer's instructions, and the results were interpreted visually. A test was considered positive, negative, or indeterminant if the test well had a definite yellow color, was colorless, or had a faint yellow color, respectively. All of the samples with indeterminant test results were repeat tested, and the results were reinterpreted as positive, negative, or indeterminant. The Premier EIA results were recorded without knowledge of the TechLab or toxin B assay results.

**TechLab Tox-A test.** The TechLab toxin A EIA was performed according to the manufacturer's instructions, and the results were interpreted visually. A result was considered positive, negative, or indeterminant if the test well had a definite yellow color, was colorless, or had a pale yellow color, respectively. All of the samples with indeterminant test results were repeat tested, and the results were reinterpreted as positive, negative, or indeterminant. The TechLab EIA results were recorded without knowledge of the Premier EIA or Toxin B assay results.

**Statistical analysis.** The McNemar test was used to detect differences between the results of the two EIAs on matched samples. For the statistical analysis, a true-positive sample was defined as one which was positive for *C. difficile* toxins by at least two of the three test methods.

**RESULTS**

Table 1 summarizes the initial results obtained for stool specimens from all patients in the present study. Both toxins A and B were found to be present in the stool specimens of 70 patients by all three methods. Neither toxin A nor toxin B was found to be present in the stool specimens of 311 patients by all three methods. Discrepant test results were found for stool specimens from 29 patients. Thus, 29% (29 of 99) of positive results were discrepant.

Comparisons of the TechLab toxin A and Premier toxin A EIA results with cytotoxin assay results are given in Table 2. Of the 76 toxin B-positive specimens, the TechLab toxin A EIA identified 75 and the Premier toxin A EIA identified 71. In comparison with the cytotoxin assay, the sensitivity of the TechLab toxin A EIA was 99% and the specificity was 93%. Predictive values depend on the prevalence of a condition (in the present study, the presence of *C. difficile*-associated toxins) as well as the specificity and sensitivity of the test. On the basis of an overall prevalence of 19% (76 of 410 specimens) found by the cytotoxin assay, the predictive value of a positive TechLab toxin A EIA result was 77% and the predictive value of a negative TechLab toxin A EIA result was 100%. When the Premier toxin A EIA was compared with the cytotoxin assay, the sensitivity was 93% and the specificity was 100%. The predictive value of a positive Premier toxin A EIA result was 100%, and the predictive value of a negative Premier toxin A EIA result was 99%.

Thirty specimens (7% of all specimens) were indeterminant by the TechLab EIA, whereas 7 specimens (2% of all specimens) were indeterminant by the Premier EIA. Upon repeat testing of the specimens with indeterminant results, 18 of the 30 specimens tested by the TechLab EIA and all the specimens tested by the Premier EIA converted to negative results.

**DISCUSSION**

Rapid diagnosis of *C. difficile* in patients with pseudomembranous colitis and antibiotic-associated diarrhea is very important and guides both the treatment and the control of nosocomial spread of infection. No single laboratory test yields a definitive diagnosis at present. Visualization of the characteristic pseudomembranous plaques by colonoscopy remains the method of choice for the documentation of pseudomembranous colitis, although microscopic lesions may not be grossly visible in patients with less severe or early cases of infection (1). Pseudomembranous lesions are also produced in the colon in patients with ischemic enterocolitis, shigellosis, amoebiasis, and other conditions that could be mistaken morphologically for *C. difficile*-associated pseudomembranous colitis, and colonoscopy subjects patients to unnecessary trauma and cost.

The diagnosis of *C. difficile*-associated pseudomembranous colitis generally depends upon the demonstration of the toxins and isolation of the toxigenic organism from stool specimens, coupled with clinical findings. Because no toxin

<table>
<thead>
<tr>
<th>No. of specimens</th>
<th>Tissue culture (toxin B)</th>
<th>TechLab EIA (toxin A)</th>
<th>Premier EIA (toxin A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>311</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>23</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

**TABLE 2. Comparison of TechLab toxin A and Premier toxin A EIAs with the cytotoxin assay**

<table>
<thead>
<tr>
<th>EIA</th>
<th>% (no. positive/total no. tested)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TechLab toxin A</td>
<td>99 (75/76)</td>
<td>93 (311/334)</td>
<td>77 (75/98)</td>
<td>100 (311/312)</td>
<td>99 (334/339)</td>
</tr>
<tr>
<td>Premier toxin A</td>
<td>93 (71/76)</td>
<td>100 (334/334)</td>
<td>100 (71/71)</td>
<td>99 (334/339)</td>
<td></td>
</tr>
</tbody>
</table>

a The TechLab toxin A EIA was significantly more sensitive than the Premier toxin A for detection of *C. difficile* toxin A in stool specimens (*P* < 0.0001; McNemar's test).

b The TechLab toxin A EIA was significantly less specific than the Premier toxin A EIA for detection of *C. difficile* toxin A in stool specimens (McNemar's, *P* < 0.0001; McNemar's test).

c The TechLab toxin A EIA had a significantly lower positive predictive value than the Premier toxin A EIA for detection of *C. difficile* toxin A in stool specimens (McNemar's test).
B-positive, toxin A-negative *C. difficile* isolate has been reported to date, the tissue culture cytotoxicity assay is the most sensitive and accepted laboratory test for the detection of toxigenic *C. difficile* in stool specimens. Its use, however, is limited because of its inherent technical complexity and expense. Therefore, the need for rapid, accurate, and easy-to-perform diagnostic screening tests led us to evaluate the TechLab toxin A EIA and the Meridian Premier *C. difficile* toxin A EIA kits for their ability to detect *C. difficile* toxin A in stool specimens. Both test kits were comparable in hands-on time and the ease of assay performance. However, the TechLab toxin A EIA tended to have more specimens with indeterminant test results than the Premier toxin A EIA (30 versus 7). Of the 30 specimens that were indeterminant by the TechLab toxin A EIA, 18 converted to negative upon repeat testing and the remaining specimens were interpreted as positive upon repeat testing. All seven specimens with indeterminant test results by the Premier toxin A EIA converted to negative upon repeat testing. None of the specimens with initial indeterminant test results by either EIA were found to be toxin B positive in the cytotoxin assay. The overall correlations of the Premier toxin A and TechLab toxin A EIAs with the cytotoxin assays were 99 and 94%, respectively.

The sensitivity, specificity, and overall correlation of both EIAs in comparison with the cytotoxin assay in tissue culture indicate that they are suitable alternatives for routine diagnostic laboratories that lack access to tissue culture facilities, especially in the case of the Premier toxin A EIA, because it gave no false-positive results. During the present study, only 18% (5 of 28) of the specimens that were positive by the TechLab toxin A EIA were confirmed to be toxin B positive by the cytotoxin assay; thus, confirmation of TechLab toxin A EIA positive results by the cytotoxin assay remains necessary.

References


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Letters to the Editor
Tox-A Test for Clostridium difficile

In recent articles (1, 4), the results of studies comparing several of the Clostridium difficile toxin enzyme-linked immunoasorbent assays (ELISAs) with tissue culture assay were presented and points were made concerning the low specificity and high rate of indeterminate results with the Tox-A Test. We point out that the Tox-A Tests used in those studies were labeled “For Investigational Use Only” and that they are not the same test that has been approved for in vitro diagnostic use.

The approved Tox-A Test exhibits higher specificity and fewer indeterminate results than those in these two articles. This statement is based on the results from a number of studies performed at various locations around the country. In our clinical trials, which were performed at six different locations and which involved the analysis of 1,130 specimens, the Tox-A Test exhibited a sensitivity and specificity of 91.9 and 97.4%, respectively, and the predictive positive and negative values were 84.5 and 98.7%, respectively, when the test was compared with tissue culture and/or toxigenic culture. The overall correlation of the Tox-A Test with tissue culture assay and/or toxigenic culture was 96.7%, and the indeterminate rate was 1.7%.

In studies presented at the 93rd ASM General Meeting last year (2, 3), the Tox-A Test was compared with other C. difficile toxin ELISAs, including the Baxter EIA, the Analytab (Cytoclone) EIA, and the Premier test. The results showed that the Tox-A Test exhibited performance characteristics similar to those of the other ELISAs. Specificities of 98.3 and 100% were reported for the Tox-A Test in those studies. In one of the studies, indeterminate rates of 5.1, 0.5, and 2.6% were reported for Cytoclone, Premier, and the Tox-A Test, respectively. The results from a reference laboratory that routinely uses the Tox-A Test indicated a sensitivity of 80.3% and a specificity of 98.8% compared directly with tissue culture assay when used with more than 400 specimens. In addition, the predictive positive and negative values were 87.5 and 96.7%, respectively; the overall correlation was 95.4%; and the indeterminate rate was 3.0%.

REFERENCES

Tracy D. Wilkins, President
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Author’s Reply
Because most laboratories cannot evaluate each new product that reaches the marketplace, they are dependent upon laboratories with sufficient staff and interest to perform and report their findings of new product evaluations. These evaluations are dependent upon manufacturers supplying their products to the investigators. Most manufacturers attempt to supply investigators with products that are as close as possible to those which they will market. However, products may not perform in these evaluations as well as the manufacturer would hope. When this occurs, three choices are left to the manufacturer: (i) do nothing to the product and hope for the best, especially if it has received Food and Drug Administration approval, (ii) modify the product to improve its performance characteristics, or (iii) abandon the product. Without published, critical evaluations of the product the first strategy would be much more common than many of us would like to think. Because of critical evaluations, the second strategy is frequently followed.

Recently published abstracts indicate that improvements have been made to the TechLab Tox-A Test for detection of C. difficile toxin A. It is clear that the manufacturer deemed the second strategy to be more appropriate than the first strategy. For this, it should be commended. My coauthors and I await with interest published data in refereed journals corroborating these findings.

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Additional Data on Clinical Isolates of Campylobacter mucosais

Figura et al. (3) reported the first isolation of Campylobacter mucosais from children with enteritis. We disputed this identification (5), indicating that phenotypic tests were unreliable and that molecular studies must be done for positive identification of C. mucosais. Dr. Figura (3) indicated that these presumed C. mucosais strains had been deposited at the National Collection of Type Cultures (NCTC), London, as NCTC 12407 and NCTC 12408. We obtained strain NCTC 12408, but strain NCTC 12407 was not available from NCTC. Dr. Figura was unable to supply us with these strains (2a).

The Red Cross Hospital microbiology laboratory uses filtration and incubation in both a microaerophilic and an H2-enhanced (Oxoid BR 38; no catalyst) microaerophilic atmosphere for the isolation of bacterial pathogens (4). In a