References


Comparison of Four Methods in the Diagnosis of Clostridium difficile Disease

A.R. Mattia1,2, G.V. Doern3, J. Clark3, J. Holden1, L. Wu3, M.J. Ferraro1,2*

Nine hundred forty-five stool specimens from patients suspected of having Clostridium difficile disease were examined using a cell culture cytotoxicity assay (CTA), two enzyme immunoassay (EIA) kits (Cytocline for toxins A and B; VIDAS for toxin A) and a latex agglutination assay (CTD). One hundred nineteen specimens had positive titers (≥ 90) in the CTA; clinical review of 16 discordant samples and 49 controls supported the significance of 90 as the positive cut-off titer. The performance of the two EIAs and the latex assay was assessed relative to CTA titers of the samples. Sensitivity was ≤ 50 % for all three assays for the 24 specimens with CTA titers of 90, but it reached 97-100 % for the two EIAs and 84 % for the latex assay at titers of ≥ 2,250. The Cytocline EIA exhibited higher sensitivity at the lower positive titers. Overall, specificity of the methods ranged from 96.7 % (CTD latex assay) to 99.1 % (Cytocline EIA).

1. Clinical Microbiology Laboratories—Gray S, Massachusetts General Hospital, Boston, Massachusetts 02114, USA.
2. Harvard Medical School, Boston, Massachusetts, USA.
3. University of Massachusetts Medical Center, Worcester, Massachusetts, USA.
Clostridium difficile-associated disease (CAD) includes a spectrum of syndromes ranging from antibiotic-associated diarrhea to pseudomembranous colitis (1). Clostridium difficile elaborates two major toxins, toxins A and B (2, 3). Definitive diagnosis of CAD is problematic and requires both clinical and laboratory evidence. Detection of Clostridium difficile toxin B in stool using the cell culture cytotoxicity assay (CTA) is considered the most reliable and accurate diagnostic method (1). However, CTA is labor intensive, poorly standardized and unavailable in many hospital laboratories. Culture methods for Clostridium difficile are also difficult to perform, non-specific and not widely used (1, 4). Alternative methods include latex agglutination assays that detect a Clostridium difficile glutamate dehydrogenase (5–8) and enzyme immunoassays (EIA) that detect toxin A or toxins A and B (9–13). As yet, no clear consensus exists to define their choice as primary diagnostic options.

The purpose of this study was to evaluate two selected EIAs (Cytoclone A+B EIA [CAB], Cambridge Biotech, USA; Vidas Clostridium difficile toxin A assay [VA], bioMerieux Vitek, USA) and a latex agglutination method (Clostridium difficile test [CDT], Becton Dickinson, USA) to determine their performance relative to CTA, and, most importantly, to correlate results of these tests with CTA titers. In addition, using relevant clinical data obtained in selected cases, we evaluated the significance of low-CTA titer levels in order to establish a clinically useful positive cut-off value.

Materials and Methods. Nine hundred forty-five unselected fecal specimens submitted to two laboratories (Massachusetts General Hospital and University of Massachusetts Medical Center) from patients suspected of having CAD were examined over a 15-week period in 1992. Specimens were stored at 4 °C and processed within 24 h of collection. All tests were performed simultaneously; samples yielding indeterminate results were immediately retested and the second result considered definitive.

The CTA assay used an MRC-5 cell line in a microtiter format. Equal volumes of specimen and phosphate-buffered saline (PBS) were mixed and centrifuged at 3,000 x g for 15 min at 2–8 °C, followed by filtration of the supernatant through a 0.45 µm pore-size filter. Twenty-five µl of the filtrate (original 1:2 dilution) was added to 200 µl of MRC-5 cells in the wells, followed by serial dilutions for final specimen dilutions of 1:18, 1:90, 1:450, 2:2,250 and 1:11,250. Clostridium difficile antitoxin (polyclonal goat anti-A and anti-B, Tech Labs, USA) was used as a control for observed cytotoxic activity. Plates were incubated and examined after 24 and 48 h for characteristic cytopathic effect. The titer was defined as the reciprocal of the highest dilution exhibiting 50 % cytopathic effect.

The CAB EIA for the detection of toxins A and B was performed according to the manufacturer’s instructions, with a final specimen dilution of 1:5. Following toxin capture and detection, the OD readings were determined spectrophotometrically. Results were recorded as positive (≥ 0.2), indeterminate (0.150–0.199) or negative (< 0.150). Total performance time was approximately 3 h.

The VA EIA for toxin A detection was also performed according to the manufacturer’s instruc-

Table 1: Results of assays of 924 fecal specimens for detection of Clostridium difficile disease relative to cytotoxin titer.

<table>
<thead>
<tr>
<th>Cytotoxicity assay result*</th>
<th>Titer</th>
<th>No. of specimens</th>
<th>Percent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CAB</td>
</tr>
<tr>
<td>Negative</td>
<td>&lt; 18</td>
<td>788</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>Positive</td>
<td>90</td>
<td>24</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>450</td>
<td>33</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>2,250</td>
<td>31</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>&gt; 11,250</td>
<td>31</td>
<td>100</td>
</tr>
</tbody>
</table>

*Equivocal results (total number of specimens per assay, after immediate repeat on same sample) excluded from tabulations: CAB, n = 5; VA, n = 12; CDT, n = 4.

CAB = Cytoclone assay for toxins A and B; VA = VIDAS assay for toxin A; CDT = latex agglutination assay.