Performance of two rapid, single-use immunoassays for the detection of
Clostridium difficile toxin A

Jean Baldus Patel*, Angela M. Donahue, Irving Nachamkin

Clinical Microbiology Laboratory, Department of Pathology & Laboratory Medicine, University of Pennsylvania Medical Center,
Philadelphia, Pennsylvania, USA

Received 28 May 2000; accepted 23 October 2000

Abstract

Two rapid, single-use immunoassays for C. difficile toxin A, the Clearview C. DIFF A (Wampole Laboratories, Cranbury, N.J.) and the ImmunoCard Toxin A assays (Meridian Diagnostics Inc., Cincinnati, Ohio) were compared to the cytotoxin assay for their ability to detect C. difficile toxin in fecal specimens. A total of 537 specimens were tested and 47 (8.8%) were positive by the cytotoxin assay. The sensitivity, specificity, positive predictive value, and negative predictive value of the toxin A assays were as follows: 70.2% (95% CI, 57.1 to 83.3), 98.8% (95% CI, 97.8 to 99.8), 84.6% (95% CI, 73.3 to 95.9), and 97.2% (95% CI, 95.7 to 98.6) respectively for the Clearview assay; and 74.5% (95% CI, 62.0 to 86.9), 99.0% (95% CI, 98.1 to 99.9), 87.5% (95% CI, 77.3 to 97.8), and 97.6% (95% CI, 96.2 to 98.9) respectively for the ImmunoCard assay. Both toxin A assays are less sensitive than the cytotoxin assay, however, these assays offer a rapid and easy-to-perform test that may be used in conjunction with the cytotoxin assay for laboratory confirmation of C. difficile-associated disease. © 2001 Elsevier Science Inc. All rights reserved.

1. Introduction

Toxigenic Clostridium difficile is an important cause of diarrhea and colitis in an adult patient population. Laboratory confirmation of C. difficile-associated disease (CDAD) is usually accomplished by detection of one or both of the bacterium’s toxins, toxin A and toxin B, in a fecal specimen (for review see Brazier, 1998). Assays for toxin A are based upon immuno-detection of the toxin either by a microplate enzyme immunoassay (EIA) or by a single-use, solid-phase immunoassay. Both methods are rapid (i.e., results in less than 24 hours), but microplate assays require a significant amount of hands-on time, so specimens must be tested in batches and specimen volume may not justify daily testing. In contrast, the single-use, solid-phase immunoassays require very little hands-on time, making a less than 24-hour turn-around time possible. Detection of C. difficile toxin B traditionally relies upon its cytotoxic activity for cells in tissue culture, although EIA’s that detects both toxin A and toxin B are available. The cytotoxin assay for toxin B is considered to be the “gold standard” for laboratory diagnosis because it is more sensitive than any of the immunoassays, but the cytotoxin assay requires significant hands-on time and at least 1–2 days for a final result. Moreover, like the microplate EIAs, many laboratories do not have the resources to set up the cytotoxin assay daily. There is no single assay for C. difficile toxins that has all of the desired characteristics of sensitivity, specificity, and rapid turn-around time. Therefore, each laboratory must choose a method or methods based upon their individual resources and patient population.

The single-use, solid-phase immunoassays for toxin A have largely been marketed to small community hospital laboratories and physician office laboratories. However, such assays are attractive to any laboratory that wishes to provide daily testing with a turn-around time of less than 24 hours. We evaluated the Clearview C. DIFF A and the ImmunoCard Toxin A assays for use in an adult tertiary care hospital laboratory by comparing the performance of these assays to the performance of the cytotoxin assay (Bartels Inc., Issaquah, WA) on specimens submitted to the laboratory for C. difficile toxin detection.

* Corresponding author. Tel.: 1-(215)-662-6651; fax: 1-(215)-662-6655.
E-mail address: jbpatel@mail.med.upenn.edu. (J.B. Patel).
2. Materials and methods

2.1. Specimen handling

Upon receipt in the laboratory, specimens were first processed for the cytotoxin assay. Processing included specimen dilution and centrifugation; the remaining processing steps (e.g., filtration, etc.) occurred when the cytotoxin assay was set up. Specimen supernatants were stored at 2–8°C if the cytotoxin assay was to be set up that same day or stored at 20°C for one to three days depending upon when the assay would be set up next. A portion of the remaining unprocessed specimen was saved for possible retesting (20°C storage) and the rest of the unprocessed specimen was saved for the toxin A assays (2–8°C storage). Although the manufacturer’s instructions for both toxin A assays state that the tests can be performed on specimens stored at 2–8°C for up to 72 hrs, we chose to eliminate specimens from the study if the toxin A assays could not be performed within 24 hrs of collection since this would better reflect test conditions if either toxin A assay were implemented in our laboratory. In addition, specimens were eliminated from the study if they did not meet our laboratory’s criteria for the toxin B assay. Reasons for specimen rejection were: 1) specimens received in preservatives, 2) receipt of a rectal swab, 3) formed stool specimens, 4) specimens from patients who have had a previous positive in the last seven days, 5) specimens from patients for which two specimens were submitted for testing in the previous seven days and were both negative.

2.2. C. difficile toxin assays

All assays for C. difficile toxin were performed according to the manufacturer’s instructions. A specimen filtrate for the cytotoxin assay (Bartels Inc., Issaquah, WA) was made by adding an equal volume of phosphate buffered saline to a portion of the specimen, centrifuging, and passing the supernatant through a 0.45 μm filter. This filtrate was diluted 1:4 with toxin-titer diluent prior to inoculation of the fibroblast cell line. If nonspecific changes were noted in the cell culture, the specimen filtrate could be further diluted to 1:12 and 1:120 and then retested. For the Clearview assay, the specimen was processed using the centrifugation procedure rather than with the optional filtration unit.

Two rotating technologists were responsible for performing the toxin A assays. The cytotoxin assays were performed as part of the routine laboratory workload by technologists who were unaware of the toxin A results.

2.3. Calculation of performance characteristics

The performance characteristics of the toxin A assays were determined by comparing the results of these assays to the results of the reference method, the cytotoxin assay. If either or both toxin results disagreed with the cytotoxin assay result, all three tests were repeated on a frozen portion of the same specimen. However, only the initial results were used for calculation of the performance characteristics.

3. Results

Between April 20, 1998 and January 24, 1999 a total of 586 consecutively submitted stool specimens were included in the study. Twenty-seven specimens were eliminated from the study because the assays for toxin A could not be performed within 24 hrs. Another 20 specimens were eliminated because the specimens did not meet the requirements for the cytotoxin assay (see Materials and Methods). Finally, two specimens were eliminated because they were suspected of being mislabeled and definitive patient identification could not be resolved. This left a total of 537 specimens from 397 patients for analysis. Of these, 47 (8.8%) were positive for toxin B by the cytotoxin assay. The Clearview assay detected toxin A in 33 of these specimens for a sensitivity of 70.2% (95% CI, 57.1 to 83.3) and the ImmunoCard assay detected toxin A in 35 of these specimens for a sensitivity of 74.5% (95% CI, 62.0 to 86.9) (Table 1).

Table 1
Comparison of results from the Clearview and ImmunoCard assays with the results from the cytotoxicity assay

<table>
<thead>
<tr>
<th>Test</th>
<th>Result</th>
<th>Cytotoxicity Assay Results (No. of specimens)</th>
<th>Sensitivity (%), (95% CI)</th>
<th>Specificity (%), (95% CI)</th>
<th>Positive Predictive Value (%), (95% CI)</th>
<th>Negative Predictive Value (%), (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearview</td>
<td>Positive</td>
<td>33</td>
<td>6</td>
<td>70.2, (57.1 to 83.3)</td>
<td>98.8, (97.8 to 99.8)</td>
<td>84.6, (73.3 to 95.9)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>14</td>
<td>484</td>
<td>74.5, (62.0 to 86.9)</td>
<td>99.0, (98.1 to 99.9)</td>
<td>87.5, (77.3 to 97.8)</td>
</tr>
<tr>
<td>ImmunoCard</td>
<td>Positive</td>
<td>35</td>
<td>5</td>
<td>74.5, (62.0 to 86.9)</td>
<td>99.0, (98.1 to 99.9)</td>
<td>87.5, (77.3 to 97.8)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>12</td>
<td>485</td>
<td>74.5, (62.0 to 86.9)</td>
<td>99.0, (98.1 to 99.9)</td>
<td>87.5, (77.3 to 97.8)</td>
</tr>
</tbody>
</table>

a CI, confidence interval
of toxin B that are close to the detection limit of the assay. A review of medical records for the four patients who produced these specimens revealed that three of the four patients received *C. difficile*-appropriate therapy (500 mg metronidazole po tid) for 4 to 10 days immediately before the specimen was submitted for toxin detection. Two of the patients had laboratory-confirmed CDAD, both were clinically improved, and the specimen in question was sent at the end of therapy as a test of cure. The third patient was suspected of having CDAD but a specimen was not submitted for laboratory testing until she had received 4 days of therapy, the diarrhea resolved on day 5 of therapy. These data suggest that all three patients had partial or complete treatment of their disease, which may account low concentration of *C. difficile* toxins and thus for the false-negative toxin A results.

Both toxin A assays produced positive results for specimens that were negative by the cytotoxin assay. The Clearview assay had 6 apparent false positive results for a specificity of 98.8% (95% CI, 97.8 to 99.8) and the ImmunoCard assay had 5 apparent false positive results for a specificity of 99.0% (95% CI, 98.1 to 99.9) (Table 1). Of the apparent false-positive specimens, two specimens were positive by both toxin A assays and negative by the cytotoxin assay. For one of the two specimens we found strong evidence that the positive toxin A assay results were correct and the cytotoxin assay was falsely negative. Repeat testing of the frozen specimen by the cytotoxin assay was positive and a second specimen from the same patient was positive by all three assays. Although the performance characteristics were not recalculated, these results suggest that the initial specimen was a true toxin A positive.

For our patient population, the positive predictive value of the Clearview assay was 84.6% (95% CI, 73.3 to 95.9) and 87.5% (95% CI, 77.3 to 97.8) for the ImmunoCard assay. The negative predictive values were 97.2% (95% CI, 95.7 to 98.6) for Clearview and 97.6 (95.5% CI, 96.2 to 98.9) for ImmunoCard (Table 1).

4. Discussion

The two toxin A assays evaluated in this study had very similar performance characteristics. As expected, both assays were less sensitive than the cytotoxin assay. These results are consistent with the results of other studies. The Clearview assay was found to have a sensitivity of 83.1% compared to the cytotoxin assay in a study by Bentley et al., 1998, and sensitivities of 84% (Staneck et al., 1996) and 58.2% (Fedorko et al., 1999) were reported for the ImmunoCard when compared to the cytotoxin assay. Since we compared assays that detect toxin A to the cytotoxin assay which detects toxin B, it is possible that the difference in sensitivity actually reflects the emergence of *C. difficile* isolates that produce toxin B but not toxin A. Such isolates have been reported in the literature and some have been associated with clinical disease (Al-Barrak et al., 1999; Lyerly et al., 1992; Borriello et al., 1992; Depitre et al., 1993; Kato et al., 1998; Brazier et al., 1999). However, these isolates are extremely rare and are unlikely to account for many, if any, of the specimens that were cytotoxin positive and toxin A negative. It is more likely that these toxin A assays simply have a lower analytical sensitivity than the cytotoxin assay. This is supported by our finding that specimens from patients with predictable low concentrations of toxin (i.e., patients with treated or partially treated disease) can be positive by the cytotoxin assay but negative by the toxin A assays. It also should be noted that the cytotoxin assay is not a perfect assay for the diagnosis of CDAD. Unfortunately, clinical criteria and other laboratory tests are equally or more unreliable, so we offer the cytotoxin test as a reasonable estimate of disease.

Any laboratory that considers replacing the cytotoxin assay with either toxin A assay must weigh the benefits of a shorter turn-around time with the benefits of a more sensitive assay. Although the cytotoxin assay is sensitive, the turn-around time of the assay limits its clinical utility. This problem is compounded if laboratories do not have personnel resources to set up the assay seven days a week. Alternatively a single-use toxin A card assay is easily performed on a daily basis and can be performed as specimens are received, but because they lack sensitivity, clinically significant infections may be missed or more specimens may have to be submitted for testing before a positive is detected. The optimal solution may be the use of both a toxin A test and the cytotoxin test. Recently, several authors have cautioned against relying on a toxin A assay alone for detection of *C. difficile* toxins (Kader et al., 1998; Brazier et al., 1999). Two reasons for concern are the decreased sensitivity of toxin A assays and the appearance of toxin A-negative, toxin B-positive *C. difficile* isolates.

The decision of how to use two different assays for *C. difficile* toxin depends in part upon the prevalence of CDAD among an institution’s patient population. In this study, less than 9% of the specimens sent for *C. difficile* toxin detection were positive. As a result, the negative predictive value of either toxin A test was quite high (approximately 97%) despite the sensitivity problems associated with the toxin A assays. In light of the data, we decided to implement a toxin A test as our primary test for *C. difficile* toxin. All reports include a description of the assay’s performance characteristics and a comment informing the physician that the more sensitive cytotoxin assay is available upon request. Testing is limited to two toxin A assays per patient per week. Resquests for the cytotoxin assay are honored at any point including requests on a third specimen from a patient who had two negative toxin A assays in the same week. Since implementing this policy, the cytotoxin testing has been reduced to a total of 1–3 cytotoxin assays per week. This represents approximately 3% of specimens submitted for toxin detection. The positivity rate for specimens tested by
Table 2
Assay Characteristics

<table>
<thead>
<tr>
<th>Assay</th>
<th>Manual Steps</th>
<th>Incubations</th>
<th>Total Time</th>
<th>Equipment &amp; Supplies Not Provided</th>
</tr>
</thead>
<tbody>
<tr>
<td>ImmunoCard</td>
<td>1. Specimen dilution</td>
<td>5 min</td>
<td>15 min</td>
<td>test tubes, application sticks</td>
</tr>
<tr>
<td></td>
<td>2. Specimen application</td>
<td></td>
<td></td>
<td>timer</td>
</tr>
<tr>
<td></td>
<td>3. Add wash reagent</td>
<td>5 min</td>
<td></td>
<td>microfuge tubes, application sticks or transfer pipette</td>
</tr>
<tr>
<td></td>
<td>4. Add substrate reagent</td>
<td></td>
<td></td>
<td>centrifuge (or filtration unit)</td>
</tr>
<tr>
<td></td>
<td>5. Read results</td>
<td></td>
<td></td>
<td>micropipette</td>
</tr>
<tr>
<td>Clearview</td>
<td>1. Specimen dilution</td>
<td>10 min*</td>
<td>44 min</td>
<td>* The centrifugation step can be eliminated if the optional filtration unit is used.</td>
</tr>
<tr>
<td></td>
<td>2. Centrifugation*</td>
<td>10 min*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Specimen application</td>
<td>30 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Read results</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

the cytotoxin assay is less than 7%, suggesting that most cases are detected by the toxin A assay. Laboratories with a higher prevalence rate may decide to perform the cytotoxin assay on any specimen that is negative for toxin A (Kader et al., 1998).

Since the primary benefit of toxin A assays are their rapid turn-around time, the decision of whether or not to use a toxin A assay also depends upon how quickly a laboratory can perform the test. For most laboratories, this decision is based upon the amount of technologist’s time required to perform the assay. Both toxin A assays evaluated in this study have very short turn-around times, approximately 44 min for the Clearview assay and 15 min for the ImmunoCard assay (Table 2). Although the Clearview assay has a longer turn-around time, the majority of this time consists of a centrifugation step (10 min) and an incubation step (30 min) in which the technologist can be occupied with other tasks. Using the optional Clearview filter unit rather than centrifuging the specimen can also shorten the turn-around time. In contrast, the ImmunoCard assay does not have an incubation period long enough (i.e., greater than 5 min) for a technologist to perform other functions. Both assays are more efficiently performed when specimens are batched but batches can potentially be run one or more times per day since the assays are easy to perform. At our institution, specimens are tested in batches with 2 to 3 batches tested per day.

We conclude that single-use immunoassays for C. difficile toxin A can play a role in a large tertiary care hospital laboratory. However, physicians should be informed of the assay’s performance characteristics and have the opportunity to order a more sensitive assay such as the cytotoxin assay when the toxin A assay is negative but clinical suspicion of CDAD is high.

Acknowledgment

We thank the staff of the Clinical Microbiology Laboratory at the Hospital of the University of Pennsylvania for their help with this study. This study was supported in part by Wampole Laboratories and Meridian Diagnostics, Inc.

References


