Frequency of Sample Submission for Optimal Utilization of the Cell Culture Cytotoxicity Assay for Detection of Clostridium difficile Toxin

Anita P. Borek,* Deborah Z. Aird, and Karen C. Carroll

Division of Microbiology, Department of Pathology, The Johns Hopkins Medical Institutions, Meyer B1-193, 600 N. Wolfe St., Baltimore, Maryland 21287

Received 17 November 2004/Returned for modification 20 January 2005/Accepted 16 February 2005

Clostridium difficile is a leading cause of diarrhea in patients who have received antimicrobials and chemotherapeutic treatments. In the era of shrinking laboratory resources, the timely, accurate diagnosis of C. difficile continues to be a challenge. A variety of testing options are available. These include anaerobic culture on selective media, direct toxin testing for either toxin A alone or both toxins A and B, detection of C. difficile antigen with subsequent toxin testing of positive samples, and finally nucleic acid amplification techniques. Each of these methods has advantages and disadvantages (1–4, 7, 9).

Direct toxin testing is the most frequently used approach. Cell culture systems that are sensitive to the effects of toxin followed by neutralization of cytopathic effect have been the gold standard. Analytical sensitivity of the cell culture cytotoxicity assay (CCCA) is as low as 10 pg of toxin (4). Such testing requires the availability of cell culture lab facilities and skilled personnel. In addition, this system is labor-intensive and may require up to 72 h for a final result. For these reasons, many labs have abandoned CCCA in favor of enzyme immunonassays (EIAs). In general, EIAs are less sensitive than CCCA (2–4, 7, 9).

A previous publication from this tertiary-care, urban medical center reported the value in testing multiple samples from patients when an EIA is used (5). In the attempt to overcome suboptimal recovery, the laboratory received two, three, or more samples per week. After a series of problems related to consecutive use of two commercial toxin A/B EIAs in our laboratory, a decision was made to return to testing with an in-house CCCA previously reported to be more sensitive than an EIA method (5). The purpose of this study was to determine the utility of multiple sample submissions when a CCCA is used.

CCCA was performed using a modified version of an in-house procedure (5, 6). In brief, stool specimens were diluted 1:5 in sterile phosphate-buffered saline. Specimens were mixed by vortexing and were centrifuged at 4,000 rpm at 4°C for 30 min. Supernatants were filter sterilized through an 0.45-μm membrane filter (Millipore, Billerica, MA). Dilutions of the stool filtrates and positive control toxin (1:2 and 1:10) were prepared with sterile phosphate-buffered saline and antitoxin.

Twenty microliters of each dilution of control toxin, control toxin plus antitoxin, stool filtrate, and stool filtrate plus antitoxin was added to duplicate wells of microtiter plates containing confluent human foreskin fibroblast cells (Diagnostic Hybrids, Inc., Athens, OH). Thus, the final dilutions tested were 1:60 and 1:300. The plates were incubated at 37°C in 5% CO2 and were read for cytopathic effect at 24 and 48 h. Dilutions of toxin control reagent mixed with buffer were observed for the expected cell rounding in the 1:60 and 1:300 dilutions. Dilutions of toxin control reagent mixed with antitoxin were observed for neutralization in the 1:60 and 1:300 dilutions. In mixtures containing dilutions of fecal filtrate plus buffer, >50% cell rounding indicated the presence of cytotoxic activity. If this was neutralized in dilutions of fecal filtrate plus antitoxin, the presence of C. difficile toxin was confirmed. All consecutive stool samples with sufficient volume were accepted for testing 7 days per week.

CCCA results over a 3-month period were reviewed for multiple requests within 7 days. All data were entered into EXCEL and analyzed using STATA version 7.0.

There were 2,940 samples tested from 670 patients. Overall, there were 219 positives from 141 different patients for a sample positivity rate of 7.4%. In 1,101 instances a second sample from the same patient was submitted within 7 days. Of those 1,101 instances, two consecutive samples were negative for 1,063 specimens and in 38 instances both were positive. The first two samples were 100% concordant (Table 1). Two hundred forty-seven patients were tested using a third sample during the same week. For 245 of these 247 patients, results of the third sample matched the results of the initial two samples. Two hundred thirty-eight patients were negative, and nine were positive. Of the nine positive samples, two were first-time positives. Thus, 2 of the 247 patients (0.8%) had a third sample whose result was positive, compared to the initial two samples, whose results were negative in the same week. Chart review revealed that neither patient was treated. One patient had a
TABLE 1. Result concordance on repeated sample submissions

<table>
<thead>
<tr>
<th>Result</th>
<th>Second sample</th>
<th>Third sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>38</td>
<td>7</td>
</tr>
<tr>
<td>Negative</td>
<td>1,063</td>
<td>238</td>
</tr>
<tr>
<td>Total</td>
<td>1,101</td>
<td>245</td>
</tr>
</tbody>
</table>

* Two additional samples were first-time positives representing discordant results. See text for discussion and patient details.

We thank Richard Thompson for his assistance with the cost-effectiveness data analysis.

REFERENCES