Clinical Comparison of the Molecular-Based BD GeneOhm Cdiff Assay to the Cytotoxicity Tissue Culture Assay for the Direct Detection of Toxin B Gene from Toxigenic Clostridium difficile Strains in Fecal Specimens

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INTRODUCTION (CONT)

Clostridium difficile (C. difficile) is the etiologic agent of several diseases, which may produce symptoms ranging from mild diarrhea to severe colitis and even bowel perforation and death by dehydration. It is the major cause of antibiotic-associated diarrhea (AAD) and pseudomembranous colitis. The predisposing risk factors are numerous and include age, length and number of hospital stays, invasive medical procedures, immunosuppressive treatments, chronic pathologies (diabetes, cardiovascular syndromes, AIDS). These factors explain why some wards (intensive care, surgery, long-term care) in hospitals or institutions are more affected than others. C. difficile is insensitive to the majority of antibiotics. As a result, the bacteria are killed by antimicrobial treatment, Cdiff spores survive. When antibiotic concentration is lower than the inhibitory concentration, spores germinate into growing bacteria which produce toxins.

The diagnosis of toxigenic C. difficile is usually done by cytotoxicity tissue culture assay and/or by Cdiff identification and/or by enzyme immunoassay (EIA). The cytotoxicity tissue culture assay and Cdiff identification are time consuming. Results are obtained usually within 48 to 96 hours. EIA assays are rapid; however, poor sensitivity often results in delayed diagnosis, treatment and containment measures. Because physicians may treat empirically while waiting for definitive positive test results, some patients may receive inappropriate empirical treatment. Development of sensitive molecular amplification techniques allows the detection of Cdiff by a few copies of bacterial DNA in clinical samples. In addition, rapid PCR technology can achieve this in about 1 hour. The combination of these two characteristics allows the prompt identification and isolation of patients with Clostridium difficile-associated disease (CDAD), allowing more rapid, appropriate treatment and containment of C. difficile outbreaks.

Previous studies have shown the benefit of using real-time PCR for detection of toxin B-producing C. difficile as a laboratory diagnostic test for confirmation of CDAD (Petersen, van den Berg, Belanger) and detection of the tcdB gene target is a good surrogate for detection of toxigenic Cdiff (Figure 1). The primary objective of this prospective evaluation was to demonstrate the use of the BD GeneOhm™ Cdiff assay (BD Diagnostics, San Diego, CA) real-time Polymerase Chain Reaction (PCR) assay as a diagnostic test for the detection of toxigenic Cdiff strains from fecal specimens. The BD GeneOhm™ Cdiff assay is a qualitative in vitro diagnostic test performed on the Cepheid SmartCycler (Cepheid, Sunnyvale, CA), a random-access real-time PCR instrument. The assay utilizes PCR for the amplification of the toxin B gene (tcdB) and fluorogenic target-specific hybridization probes for the detection of the amplified DNA. The amplification, detection and interpretation of the signals are done automatically by the SmartCycler® software.

METHODS

Surplus, de-identified, liquid to soft stool specimens from patients with Clostridium difficile-associated disease (CDAD) symptoms were included in this study (Figure 2). Briefly, stools were tested with the TechLab Cdiff chek™-60 enzyme immunoassay (EIA) for detection of the “common antigen”, glutamate dehydrogenase (GDH), and positive results were confirmed with the Tox A/B assay. If the Tox A/B was negative, a cytotoxicity tissue culture assay was also performed. Concurrently, the BD GeneOhm™ PCR assay was also performed on each stool specimen. Each stool was collected, processed, and tested according to the manufacturer’s standard of care and each assay was performed according to the manufacturer’s investigational test method (culture, cytotoxicity/neutralization testing) and were also performed on discordant specimens to aid in resolving discrepancies.

RESULTS

Of the 298 specimens included in this study, 247 (83%) tested negative with both PCR and cytotoxicity while 29 (10%) tested positive with both assays yielding 90.6% sensitivity and 92.9% specificity (Table 1)  

<table>
<thead>
<tr>
<th></th>
<th>Cytoxin Pos</th>
<th>Cytoxin Neg</th>
<th>Total</th>
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<tbody>
<tr>
<td>PCR Pos</td>
<td>32</td>
<td>266</td>
<td>298</td>
</tr>
<tr>
<td>PCR Neg</td>
<td>181</td>
<td>48</td>
<td>229</td>
</tr>
<tr>
<td>Total</td>
<td>213</td>
<td>314</td>
<td>527</td>
</tr>
</tbody>
</table>

90.6% Sensitivity (95/29)  
92.9% Specificity (247/266)

Table 1. PCR vs Cytoxin

After resolution of discordant results, the sensitivity and specificity was 93.6% and 98.4% respectively with a prevalence of nearly 15% (Table 2).

<table>
<thead>
<tr>
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<th>Cytoxin Pos</th>
<th>Cytoxin Neg</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR Pos</td>
<td>44</td>
<td>48</td>
<td>92</td>
</tr>
<tr>
<td>PCR Neg</td>
<td>3</td>
<td>247</td>
<td>250</td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>251</td>
<td>298</td>
</tr>
</tbody>
</table>

93.6% Sensitivity (44/47)  
98.4% Specificity (247/251)

Table 2. Resolved PCR vs Cytoxin

CONCLUSIONS

The diagnosis of toxigenic C. difficile is usually done by a combination of cytotoxicity assay, culture and EIA all of which are either labor intensive and time-consuming or lack sensitivity or specificity. The BD GeneOhm™ Cdiff assay (performed directly on stool specimens) offers sensitivity and specificity that is comparable to the cytotoxicity references.

RESOURCES


METHODS (CONT)

Analysis included all patients who participated in the study. If discrepant results were obtained between the BD GeneOhm™ Cdiff assay and cytotoxicity tissue culture assay, the culture isolation result was taken in consideration for the discrepant resolution and for sensitivity and specificity calculations.