Background: Clostridium difficile infection (C. difficile) remains a formidable pathogen. While controversial, many laboratories use a molecular test for the diagnosis of C. difficile infection (CDI). We report a single-center’s experience from a multi-center study for FDA 510K approval of a new qualitative real-time PCR assay, i.e. the GenePOCTM Cdiff assay (GenePOC Inc., Quebec, Canada).

Methods: The Johns Hopkins Hospital (JHH) Microbiology Laboratory tested 200 unformed stool specimens. To run the GenePOC Cdiff test, approximately 5 µL of the stool sample was tested according to the manufacturer's package insert. 500 µL of the stool sample was transferred to an Anaerobe Transport Medium and shipped to an off-site reference lab for direct culture on CCFA agar and enriched culture using CCMB-TAL broth in case direct culture was positive. Recovered isolates were determined to be cytotoxin positive by a cell culture cytotoxin neutralization assay. In addition, since the standard-of-care in our clinical laboratory is the BD MAX™ Cdiff test (BD Diagnostics, Inc., USA), the GenePOC Cdiff assay was also compared with the BD MAX results.

Results: Two hundred samples were tested at JHH. Eleven samples were eliminated due to an exceeded transit time to the reference lab for culture. Thirty-six samples were excluded because SBT and PIES were mixed with different lot numbers. Therefore, a total of 176 stool specimens had valid test results. Twelve samples were positive for toxigenic C. difficile by direct culture. All direct culture positive samples were positive by GenePOC Cdiff (sensitivity, 100%). An additional nine specimens were direct culture negative, but positive by the GenePOC Cdiff assay (specificity, 94.5%). Twenty-three samples were positive by combined culture, sixteen of which were positive by the GenePOC assay (69.6% sensitivity). There were five GenePOC positive, culture negative samples for a specificity of 96.7%. Compared to the BD MAX (n = 189), the specificity and sensitivity of the GenePOC test were 95.5% and 100%, respectively.

Conclusions: The GenePOC Cdiff test performs favorably to direct culture on CCFA and to another FDA-cleared NAAT that detects the toxin B gene in unformed fecal samples.

INTRODUCTION

Clostridium difficile infection (CDI) is a major cause of nosocomial infections, particularly in the elderly and immunocompromised patients. Accurate and rapid diagnosis is essential, and recent guidelines recommend early initiation of appropriate antibiotic therapy for C. difficile infection to reduce disease morbidity and mortality. The gold standard for diagnosis is stool culture, which provides definitive identification of C. difficile and toxin production. However, culture methods are time-consuming and not always feasible, particularly in settings with limited laboratory resources. Nucleic acid amplification tests (NAATs) have been developed as alternative diagnostic tools for CDI, offering rapid results and high sensitivity and specificity.

The goal of this study was to investigate the performance of a novel qualitative NAAT for the detection of C. difficile toxin B gene, compared to the BD MAX™ Cdiff test, a FDA-cleared NAAT with proven clinical performance.

MATERIALS AND METHODS

Test Method: GenePOC™ Cdiff Assay

Controls:
- Each PIE contains reagents for the detection of tcdB and process controls to monitor processing, amplification, and the absence of reaction inhibitors.
- Positive external controls, provided by GenePOC, were tested each day.
- 150 µL of SBT was used as a negative external control.

Testing Algorithm:
- The stool samples were placed in the SBTs for testing. Afterwards, the SBTs were placed at 2-8°C in the event repeat testing is required. If the sample did not require repeat testing, the SBT was frozen at 70°C or colder.
- 500 µL of stool sample was transferred into an Anaerobe Transport Medium kept at room temperature for shipment to a reference lab (Microbiology Specialists, Inc.) for toxigenic culture (reference method).
- The remaining (minimum of 250 µL) of the homogenized stool sample was frozen at -70°C or colder for further testing (if needed).

GenePOC Specimen Processing:
- 5µL of uniformed stool was transferred into the appropriately labeled Sample Buffer Tube (SBT) using a disposable inoculating loop.
- The SBT was closed and vortexed as described in the GenePOC Cdiff test package insert.
- Mixed SBT was transferred into the PIE using the disposable transfer pipette.
- The PIE was loaded onto the GenePOC instrument and run.

Comparative Methods

Toxicogenic Culture (Reference Method):
- Specimens were inoculated onto a CCFA plate for direct culture and a CCMB-TAL broth for enriched culture.
- After incubation, the broth was sub-cultured to another CCFA plate.
- The plates were examined for colonies characteristic of C. difficile.
- Suspected isolates were confirmed as C. difficile by gas liquid chromatography after incubation in chopped meat carbohydrate broth for 48 h.
- Toxicogenicity of isolates was determined by cell culture cytotoxicity neutralization assay.

BD MAX (Standard of care):
- Specimens were inoculated into SBT tube, vortexed, and run on BD MAX instrument.
- Real-time PCR for the amplification of C. difficile toxin B gene.
- Detection of the amplified DNA uses fluorogenic target specific hybridization probes.
- Results are available in 100 minutes.

RESULTS

Table 1: GenePOC™ Cdiff results compared to direct culture results.

<table>
<thead>
<tr>
<th>Test Method</th>
<th>GenePOC™ Cdiff</th>
<th>GenePOC™ Cdiff vs Direct Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>POS</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>NEG</td>
<td>0</td>
<td>155</td>
</tr>
</tbody>
</table>

Table 2: GenePOC™ results compared to BD MAX™ results

<table>
<thead>
<tr>
<th>Test Method</th>
<th>GenePOC™ Cdiff</th>
<th>GenePOC™ Cdiff vs BD MAX™ Cdiff</th>
</tr>
</thead>
<tbody>
<tr>
<td>POS</td>
<td>5</td>
<td>94.5%</td>
</tr>
<tr>
<td>NEG</td>
<td>7</td>
<td>95.5%</td>
</tr>
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</table>

Table 4: Analysis of GenePOC™ results versus comparative methods.

<table>
<thead>
<tr>
<th>GenePOC™ Cdiff vs Direct Culture</th>
<th>GenePOC™ Cdiff vs Combined Culture</th>
<th>GenePOC™ Cdiff vs BD MAX™ Cdiff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>69.6%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>69.6%</td>
<td>95.5%</td>
</tr>
<tr>
<td>Specificity</td>
<td>94.5%</td>
<td>96.7%</td>
</tr>
<tr>
<td>Specificity</td>
<td>96.7%</td>
<td>100%</td>
</tr>
</tbody>
</table>

CONCLUSIONS

The GenePOC™ Cdiff assay is comparable to toxigenic culture for C. difficile detection, but less sensitive than enriched culture (CCMB-TAL).

The GenePOC™ Cdiff assay is comparable to the FDA-cleared BD MAX™ Cdiff test, a NAAT that detects the toxin B gene found in C. difficile.

The GenePOC™ Cdiff assay requires little sample preparation for testing and will provide accurate results within 70 minutes, allowing for rapid diagnosis of C. difficile infection.

References