ABSTRACT

Background: Clostridium difficile infection (CDI) is a leading cause of infectious diarrhea in the U.S. healthcare environment. Although the organism is not invasive, disease is caused by toxigenic strains that produce toxin A and B, and less frequently, a binary toxin, 027/B1/NAP1). Several commercial tests encompassing enzyme immunoassays (EIA) that detect Glutamate Dehydrogenase (GDH) and toxin, and PCR targeting the gene that codes for tcdA, tcdB or the combination of tcdB/NAP1027/B1 are available for the laboratory diagnosis of CDI. The objective of this study was to compare the Simplexa™ C. difficile Direct PCR assay to our in-house two-step/three-step algorithm (GDH, EIA, PCR) for the detection of the gene that codes for tcdB and NAP. METHODS: A total of 196 liquid stool specimens were tested by our in-house GDH, toxin A and B enzyme immunoassay method (C. Diff Quik Check Complete, Alere, TechLab, Blacksburg, VA) and three molecular assays: Great Basin (Salt Lake City, UT); Simplexa™ C. difficile Direct (DiaSorin Molecular LLC, Cypress, CA), and the Xpert™ C. difficile Direct/Epi performed on the GeneXpert Infinity (Cepheid, Sunnyvale, CA). All samples were stored at 4°C and prospectively tested within 24 hrs. Results were analyzed by comparing both methods (Simplexa™ and Infinity) to the two-step/three-step algorithm in which the Great Basin PCR assay was used as a confirmatory test if the EIA toxin result was negative. RESULTS: There was complete agreement (100%) between the Simplexa™ and Infinity with our in-house two-step/three-step testing method when the GDH and toxin assay (EIA) were positive. Twenty five samples were GDH positive and EIA negative; of these, the Great Basin and Simplexa™ demonstrated 100% agreement; the Infinity detected one additional positive and negative, respectively. The two-step/three-step method 159 negatives (GDH and EIA negative). Of these, the Simplexa™, 157 and the Infinity, 156 which resulted in two false-positives by the Simplexa™ versus 3 false-positives by the Infinity. The overall agreement between the Simplexa™ and Infinity was 98.5%. Conclusion: The Simplexa™ compared favorably to the Infinity and our in-house two-step/three-step testing algorithm supporting its utility as a reliable method for confirmatory testing in laboratories using algorithmic testing as routine diagnostic platform.

INTRODUCTION

Clostridium difficile accounts for the majority of infectious diarrhea in the healthcare environment in the developed world. In the United States alone, healthcare costs associated with CDI are estimated to range from $500 million to $1.5 billion annually (1). In an effort to reduce healthcare costs including reduced length of hospital stays related to CDI, clinical microbiology laboratories are challenged and pressured to provide rapid, accurate, and cost-effective diagnostic test results. Current diagnostic tests detect toxigenic C. difficile or its toxins. Many institutions have adopted molecular testing technologies for C. difficile that are more sensitive compared to conventional methods, namely immunoassays that detect the glutamate dehydrogenase antigen (GDH) and toxin (2). Both molecular and conventional methods are replacing the traditional culture-based methods, toxigenic culture and cell cytotoxicity neutralization.

In this study, we compared the performance of two PCR assays with our current in-house testing algorithm using PCR as a confirmatory test.

RESULTS

1. Both PCR methods demonstrated complete agreement with our in-house testing algorithm in which specimens were GDH positive and Toxin positive.
2. Of the 25 specimens that were GDH positive, 15 were toxin positive and 10 were toxin negative. There was 100% agreement between the Great Basin and Simplexa™. Of these the Xpert Infinity detected 16 positives and 9 negatives resulting in one false-positive.
3. Of the 159 GDH negative, Toxin negative specimens, 3 false positives were recorded, 2 for Simplexa™ and 1 for the Xpert Infinity.
4. In the single incident involving a negative GDH and positive Toxin, all three PCR methods agreed with the EIA toxin results.
5. The overall agreement between the Simplexa™ and Xpert Infinity was 98.5% (Table 2).

METHODS

A. Study Design (Figure 1)

1. Samples: Total of 196 liquid stools (conform to shape of container) from hospitalized patients
   a) Tested prospectively within 24 hours
   b) Stored at 4°C

2. In-House Testing Algorithm
   a) Glutamate Dehydrogenase Antigen plus Toxin EIA (C. Diff Quik Check Complete; Alere, TechLab, Blacksburg, VA)
   b) Confirmatory Molecular Assays:
      1) Great Basin (Salt Lake City, UT)
      2) Xpert™ C. difficile/Epi (GeneXpert Infinity; Cepheid, Sunnyvale, CA)™
      3) Simplexa™ C. difficile Direct (DiaSorin Molecular; LLC, Cypress, CA)™ performed on the Integrated Cycler (Figure 2)

   Table 1: Results of Two-Step/Three-Step Testing Algorithm and PCRs for Detection of C. difficile

<table>
<thead>
<tr>
<th>Method</th>
<th>GDH Pos</th>
<th>GDH Neg</th>
<th>Toxin Pos</th>
<th>Toxin Neg</th>
<th>Combined Positive</th>
<th>Combined Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDH</td>
<td>15</td>
<td>125</td>
<td>10</td>
<td>25</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>EIA</td>
<td>125</td>
<td>70</td>
<td>25</td>
<td>25</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>PCR</td>
<td>10</td>
<td>25</td>
<td>10</td>
<td>10</td>
<td>8</td>
<td>12</td>
</tr>
</tbody>
</table>

   Table 2: Agreement of Simplexa™ versus Infinity

<table>
<thead>
<tr>
<th>Simplexa™</th>
<th>Infinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GDH/Toxin</td>
<td>GDH/Toxin</td>
</tr>
<tr>
<td>POS/POS</td>
<td>POS/POS</td>
</tr>
<tr>
<td>POS/NEG</td>
<td>NEG/POS</td>
</tr>
<tr>
<td>NEG/POS</td>
<td>POS/POS</td>
</tr>
<tr>
<td>NEG/NEG</td>
<td>NEG/NEG</td>
</tr>
</tbody>
</table>

   Upper 95% CI

   Positive Percent Agreement (PPA)
   Negative Percent Agreement (NPA)

   *All specimens tested (Direct PCR) independent of two-step algorithm

REFERENCES


SUMMARY

1. There was complete agreement between the Simplexa™ C. difficile Direct and Xpert Infinity with our in-house two-step/three-step algorithm.
2. Overall agreement of Simplexa™ C. difficile Direct with Xpert Infinity was 98.5%.
3. A limitation to our study was the small sample size (196 samples).
4. We demonstrated that the Simplexa™ C. difficile Direct assay is a rapid and reliable method for confirmatory testing in laboratories that follow a two-step testing algorithm.

ACKNOWLEDGMENTS:
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