Clinical and Analytical Performance of a New Molecular C. difficile Direct Assay

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Introduction: C. difficile, a gram-positive, anaerobic, spore-forming bacillus, is associated with significant morbidity and mortality. C. difficile has been linked to 50% - 70% of antibiotic-associated colitis cases, up to one-third of all antibiotic-associated diarrhea cases and over 90% of all antibiotic-associated pseudomembranous colitis.

The DiaSorin Molecular Simplexa® C. difficile Direct Assay is FDA- cleared for sample-to-answer detection of C. difficile toxin B. It gains (gains) in liquid or unformed stool samples from individuals suspected of infection (CID). This test utilizes real- time PCR amplification without any nucleic acid extraction.

The analytical performance characteristics, including analytical sensitivity/limit of detection (LoD), reproducibility, analytical specificity, cross-reactivity, microbial inhibition and interfering substances, were determined. Clinical agreement studies compared Simplexa performance to direct and combination direct-enriched culture and toxin assay. Positive and negative agreement were also determined for Simplexa versus three FDA cleared NAAT assays.

Methods: The analytical sensitivity/limit of detection was tested for two toxigenic C. difficile strains, ATCC 43255 and NAP1A. A reproducibility panel with both strains was tested at 3 different laboratories. For the method comparison study, the performance of Simplexa C. difficile Direct was compared to direct culture and direct-enriched culture with toxin assay. Samples from the method comparison study were also tested with one of three FDA-cleared molecular NAAT assays. Analytical reactivity was determined for an additional 32 toxigenic C. difficile strains. In order to evaluate analytical specificity, a panel of 127 bacteria, viruses, fungi, and fungi were tested for cross-reactivity. Microbial inhibition was evaluated by testing low levels of ATCC 43255 or NAP1A in a background of high levels of each of the same 127 microorganisms. The potential inhibitory effect of 33 endogenous and exogenous substances on detection of ATCC 43255 and NAP1A was evaluated.

Results: The LoD for the Simplexa C. difficile Direct Assay was 0.95 CFU/mL for the ATCC 43255 strain and 0.43 CFU/mL for NAP1A. Across all 3 sites in the reproducibility study, 100% detection was observed for ATCC 43255 CFU. ATCC 43255 NP and NAP1A NP. For NAP1A LP detection was 99.5%. In comparison to direct culture and toxin assay, Simplexa C. difficile Direct had a positive agreement of 99.7% and a negative agreement of 99.2%. When Simplexa C. difficile Direct was compared to direct-enriched culture and toxin assay, the sensitivity was 95.8% and the specificity was 99.1%. The performance of Simplexa C. difficile Direct compared to 3 FDA-cleared NAAT assays, demonstrated 93.4% and 93.9% and 84 positive and negative agreements and 96.8%, 94.0% and 96.1% negative agreements. Simplexa C. difficile Direct was able to detect 32 toxigenic strains tested for analytical reactivity. The assay did not have any cross-reactivity in the 127 microorganisms tested at the same time; none of the organisms tested inhibited the detection of the ATCC 43255 or NAP1A strains when tested at 2.4X LoD. Simplexa C. difficile Direct inhibition was seen in the presence of each potentially interfering substance tested.

Revised Abstract

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Real-time PCR Amplification and Detection: Simplexa C. difficile Direct Assay (MOL2950) contains all reagents for on- board extraction and real-time PCR. Swabs were dipped into stool specimens and excess liquid was removed. The swab was placed into Sample Prep Buffer and swirled to release the stool. For each reaction on the Direct Amplification Disc (DAD), 50 μL of sample was loaded into the sample port and 50 μL of C. difficile Direct Reaction Mix was added to release the RNA. All testing was performed using the LIAISON MDx instrument.

Limit of Detection: The LoD for C. difficile ATCC 43255 and NAP1A (Zooidontph) strains was determined as the lowest concentration with 99% detection in negative stool-TE matrix.

Reproducibility: The panel included a positive control (PC), a negative control and 4 controlled samples: a low positive (LP) and a medium positive (MP) for the C. difficile ATCC 43255 and NAP1A strains. The C. difficile reproducibility panel was confirmed in negative stool-TE matrix.

Method Comparison: A panel of clinical specimens from 5 geographically diverse sites was evaluated using the Simplexa Direct and Direct-Enriched Culture methods as well as 3 commercially available FDA-cleared molecular NAAT Assays. Analytical reactivity was determined for an additional 32 toxigenic C. difficile strains. In order to evaluate analytical specificity, a panel of 127 bacteria, viruses, fungi, and fungi were tested for cross-reactivity. Microbial inhibition was evaluated by testing low levels of ATCC 43255 or NAP1A in a background of high levels of each of the same 127 microorganisms. The potential inhibitory effect of 33 endogenous and exogenous substances on detection of ATCC 43255 and NAP1A was evaluated.

Analytical Reactivity: Thirty-two toxigenic C. difficile strains were individually spiked into negative stool-TE matrix at 2.4X LoD and tested.

Cross-reactivity: The test panel consisted of 10 CFU/mL for bacteria and fungi, 105 cells/mL for parasites or 105 TCID50/mL for viruses and was formulated in negative stool-TE matrix.

Microbial Inhibition: The organisms in the cross-reactivity panel were spiked into a separate baseline sample of C. difficile ATCC 43255 or NAP1A at 2.4X LoD in negative stool-TE matrix.

Results (continued)

Analytical Reactivity: All toxigenic C. difficile strains tested were detected at 12f CFU/mL or lower. No cross-reactivity or microbial inhibition was observed with the organisms tested in Table 9

Table 7. C. difficile Strains Tested

<table>
<thead>
<tr>
<th>Strain Toxinotype</th>
<th>Toxin Ribotype</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC 43255</td>
<td>A+B+</td>
<td>Detected</td>
</tr>
<tr>
<td>NAP1A</td>
<td>A+B+</td>
<td>Detected</td>
</tr>
<tr>
<td>R10870</td>
<td>XIV A+B+</td>
<td>Detected</td>
</tr>
<tr>
<td>BAA-1871</td>
<td>XXII A+B+</td>
<td>Detected</td>
</tr>
<tr>
<td>BAA-1872</td>
<td>VIII A-B+</td>
<td>Detected</td>
</tr>
<tr>
<td>BAA-1873</td>
<td>V A+B+</td>
<td>Detected</td>
</tr>
<tr>
<td>BAA-1874</td>
<td>I A+B+</td>
<td>Detected</td>
</tr>
<tr>
<td>R9385</td>
<td>XV A+B+</td>
<td>Detected</td>
</tr>
<tr>
<td>R8366</td>
<td>IV A+B+</td>
<td>Detected</td>
</tr>
<tr>
<td>R2016</td>
<td>V A+B+</td>
<td>Detected</td>
</tr>
</tbody>
</table>

Table 8. Potentially Interfering Substances Tested

<table>
<thead>
<tr>
<th>Substance</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>No interference detected</td>
</tr>
<tr>
<td>KY Jelly</td>
<td>No interference detected</td>
</tr>
<tr>
<td>Naproxen</td>
<td>No interference detected</td>
</tr>
<tr>
<td>SPF 50 Sunscreen</td>
<td>No interference detected</td>
</tr>
</tbody>
</table>

Conclusions

• The Simplexa C. difficile Direct kit and LIAISON® MDx from DiaSorin Molecular provide sample-to-answer detection of C. difficile from retracted liquid or unformed stool in approximately one hour, with a simple workflow.

• Simplexa C. difficile Direct does not show cross-reactivity, nor inhibition by, other organisms found in human stool. The assay could detect all toxigenic C. difficile strains tested.

• This assay is FDA-cleared with a CLIA moderate complexity rating and is CE-marked.

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