Single Molecule Counting Technology for Ultrasensitive Detection of *Clostridioides difficile* Toxins: Improved Sensitivity Compared to a Standard-of-Care Algorithm

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**ABSTRACT**

Background: The diagnosis of *Clostridioides difficile* (formerly *Clostridium difficile*) infection (CDI) is challenging due to lack of tests with high sensitivity and specificity. Currently available tests, including nucleic acid amplification tests (NAAT), are limited by high rates of false negative results. The Singulex Clarity assay is a novel ultrasensitive diagnostic test for detection of the *Clostridium difficile* toxins A and B (TcdA and TcdB).

Methods: The Clarity assay employs a novel technology based on Singulex’s Single Molecule Counting Technology, for the detection of TcdA and TcdB toxins in stool. The assay is based on the following steps: stool sample is mixed (1:20) with diluent buffer and centrifuged at 14,000 x g for 10 minutes and 200 µL of clear supernatant is loaded onto the Singulex Clarity system. There are three capture microparticles labeled with a proprietary anti-Toxins A or B antibody and a second antibody labeled with iron oxide nanoparticles. The resulting mixture is exposed to a magnetic field and 300 µL of the resulting supernatant is loaded onto the Singulex Clarity system again. The sample is subjected to a negative control and positive control reaction. The control reactions are detected using a proprietary algorithm that counts detected events and compares these with the negative control reaction. The software then interpolates the molecule concentrations found in each of these reactions into a combined TcdA/TcdB concentration. The limit of detection for TcdA and TcdB are 0.8 and 0.3 pg/mL in buffer, and 2.0 and 0.7 pg/mL in stool, respectively. The cutoff for a positive test is 5 pg/mL.

Results: There were 87 (8.7%) samples positive by the SOC algorithm, and Clarity had 90.8% sensitivity. Among these samples, Clarity detected toxins in six (42.8%) of the GDH+/toxin-/NAAT+ samples, which were negative by NAAT. Clarity detected toxins in 16 (34.8%) of the GDH+/toxin-/NAAT- samples, of which 14 (87.5%) were NAAT negative.

Conclusion: The Singulex Clarity assay is more sensitive than the NAAT and can detect toxins in the stool on the Singulex Clarity system and has been approved for use in clinical laboratories.

**RESULTS**

I. Clarity Compared to Standard-of-Care Algorithm

Table 1. Clarity detected toxins in 16/46 (34.8%) of the GDH+/toxin-/NAAT- samples, of which 14 (87.5%) were NAAT negative (90.8% sensitivity).

<table>
<thead>
<tr>
<th>Clarity</th>
<th>NAAT</th>
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<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>16/46</td>
<td>14/14</td>
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<tr>
<td>34.8%</td>
<td>87.5%</td>
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</table>

**METHODS**

The Singulex Clarity C. diff toxins A/B assay is more sensitive than the NAAT and can detect toxins in the stool on the Singulex Clarity system and has been approved for use in clinical laboratories.

**CONCLUSIONS**

- The Singulex Clarity C. diff toxins A/B assay was more sensitive than a toxin EIA and may detect a proportion of samples missed by NAAT.
- Clarity provided improved specificity compared to a SOC laboratory algorithm that utilize NAAT.
- The Clarity assay may offer a standalone solution for the rapid detection of *C. difficile*.

**REFERENCES**