**Ultra-Sensitive *C. difficile* Toxin A/B Assay in Development for the Sgx Clarity System from Singulex**

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**BACKGROUND**

*Clostridium difficile* is the most common cause of healthcare-associated infection in the US, at an annual cost of $3 billion in hospital settings and $39 billion in community settings. Diagnosis of *C. difficile* infection (CDI) now involves 2 or 3-step algorithms,11 such as initial screening using sensitive glutamate-dehydrogenase (GID) antigen immunoassays (ELISA) or nucleic acid amplification tests (NAAT), followed by detection of C. difficile toxins A and/or B. Another common approach is to rely exclusively on rapid lateral flow tests and toxin A, which can rule out CDI in up to 50% of patients. Highly sensitive NAAT assays are used as a stand-alone test, although a growing body of evidence indicates that up to 45% of positive results may be attributed to colonization and not to true toxigenic *C. difficile* infection (CDI).4 Detection by toxigenic cultures and cytotoxicity assays are labor intensive with long turnaround time (TAT).

There is a strong desire to move away from algorithms that rely on multiple distinct tests and toward a single assay that can rapidly and accurately rule out CDI. For decades, traditional toxins assays were flawed methods to rule out CDI, but recent studies have shown that there is a need for a toxin IA with improved sensitivity that maintains the high specificity associated with toxin IA.2

Singulex has developed Single Molecule Counting technology that can detect analytes at picogram-per-milliliter levels and has 100-1000 times higher analytical sensitivity than conventional IAs. The Sgx Clarity platform from Singulex is a CE-marked and fully-automated IA platform that incorporates Single Molecule Counting for rapid, ultra-sensitive, and specific detection of analytes.

**Objective**

To evaluate the feasibility of a ultra-sensitive C. difficile toxin A and B detection using the Sgx Clarity system from Singulex.

**METHODS**

The Sgx Clarity C. difficile toxin A/B assay (in development, not available for commercial sale) is an ultra-sensitive IA that uses single photon fluorescence detection for the simultaneous detection of toxins A and B in stool samples.

Before analysis 100 µL of stool is extracted and diluted 1:10 with 3 mL of standard buffer (Tris buffer with 0.8% BSA). The samples are centrifuged at 13,200 g, for five minutes, and any 0.5 µL of the supernatant is transferred into a Sgx Clarity tube. Samples are automatically transferred by the Sgx Clarity system from Singulex.

**RESULTS**

**Figure 1.** The fully-automated Sgx Clarity system from Singulex. Single Molecule Counting technology allows for the detection of low-abundance biomarkers that were previously undetectable using conventional immunoassays.

**Figure 2.** The capture and detection antibodies are incubated with samples in a one-step reaction, followed by a washing step. The immunoassay sandwich is subsequently cleaved and the fluorescent signal is measured by the Sgx Clarity system.

<table>
<thead>
<tr>
<th>Toxin A</th>
<th>Toxin B</th>
<th>Toxin A &amp; B</th>
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<tbody>
<tr>
<td>Buffer</td>
<td>0.021</td>
<td>0.023</td>
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<tr>
<td>Stool</td>
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<td>0.007</td>
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<table>
<thead>
<tr>
<th>Type</th>
<th>Sample</th>
<th>Limit of Detection (ng/mL)</th>
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<tbody>
<tr>
<td>Buffer</td>
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<td>0.006</td>
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<tr>
<td>Stool</td>
<td>0.014</td>
<td>0.016</td>
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</table>

**Table 1.** Limit of detection of the Sgx Clarity C. difficile toxin A/B assay (preliminary data) and commercially available assays for detection of toxins A and B. The Sgx Clarity C. difficile toxin A/B assay is currently under development.

**Conclusions**

- The Sgx Clarity system from Singulex, a CE-marked and fully-automated IA platform, can detect analytes at picogram levels.
- The Sgx Clarity C. difficile toxin A/B assay (in development) is designed to be 100 times more sensitive than conventional, commercially available assays.
- The TAT for the Sgx Clarity C. difficile toxin A/B assay is <1 h, allowing for rapid detection and rule-out of suspected CDI.
- The clinical goal for the Sgx Clarity C. difficile toxin A/B assay is to provide the sensitivity of NAAT while providing clinically actionable toxin specificity.

**References**


