**INTRODUCTION**

*Clostridium difficile* infection (CDI) is an inflammation of the large intestine due to an infection with a spore-forming bacteria, *C. difficile*, causing diarrhea. It is a healthcare-associated disease linked to the use of antibiotics. The mortality rate varies from 0.6-3% for the "simple" form of the disease to 35-50% in cases of pseudomembranous colitis. Infection with *C. difficile* species is common, serious, and costly. The presence of *C. difficile* toxin in fecal samples is the most reliable indicator of true CDI, but immunassays for toxin testing are not as stand-alone tests due to a lack of sensitivity. All guidelines strongly recommend a two-step assay algorithm based on the detection of the bacteria by a GDH assay followed by toxin detection. Since 2010, many laboratories are performing molecular assays for toxin gene detection and an increase in CDI incidence has been observed. Nevertheless, the presence of genes does not always correlate with the presence of functional toxins, leading to an inability to distinguish a disease state from colonization. An automated 1-step assay with high sensitivity for *C. difficile* toxins (similar to the gold standard, the cytotoxicity test) could strongly improve the accuracy of CDI diagnosis and reduce costs.

**OBJECTIVE**

The objective of this study was to evaluate the Simoa digital technology for the detection of very low quantities of A & B toxins in human fecal samples: 10 pg/ml or lower quantity of toxin detected.

**METHODS**

A Simoa assay consists of a standard ELISA conducted with paramagnetic microbeads, followed by the isolation of individual beads in microwells of an array for digital imaging.

The analyte is first captured by an antibody coated on paramagnetic microbeads, and then detected by a biotinylated antibody, followed by incubation with a streptavidin-conjugated β-galactosidase. After the final wash, the capture beads are re-suspended in a buffer containing the substrate: resorufin beta-D-galactopyranoside and transferred into an array containing 216 000 microwells fitted to hold no analyte. The whole signal range is determined using imaging analysis software to obtain an Average Enzyme per Bead (AEB).

**RESULTS**

The Simoa *Clostridium difficile* toxin A and B assay consists of two ELISAs, one for toxin A and one for toxin B. Anti-toxin A & B antibodies (Abs) were selected according to their affinity to detect purified native toxins from *C. difficile* strains (Native Antigen Company) and recombinant toxins (bioMérieux).

The ability of the assays to detect several *C. difficile* strains from different ribotypes was evaluated using 24 *C. difficile* A+, B+, A- B+ strains, 6 *C. difficile* A- B- strains, 6 non-toxigenic *C. difficile* A-B- strains. Ten bacteria known to potentially induce cross-reactivity were also tested. Bacterial suspensions were tested at 1.10^8 cfu/ml. Figure 1 shows that both assays clearly differentiate the detection of toxigenic from non-toxigenic strains.

![Detection of toxins A and B from *C. difficile* strains by Bead-based ELISA](image)

The detection curves for native toxins on the Simoa Instrument are shown in Figure 3. The whole signal range is determined using imaging analysis software to obtain an Average Enzyme per Bead (AEB).

![Detection of positive and negative samples by Bead-based ELISA on Simoa instrument](image)

The two assays were also evaluated with clinical samples characterized for toxin detection by VIDAS® C diff Toxins A& B (BDCA) and for bacterial detection by VIDAS GDH and/or Xpert Cdiff PCR assay (Cepheid) (Figure 2). The samples were tested with Simoa reagents on either a fluorescent reader (Bead-based ELISA) or a Simoa instrument. All positive toxins A & B samples were detected with both assays (A&B), except two samples which gave a negative result with the toxin A assay. One of these two samples was confirmed as A-negative B-positive sample (* in Table 1). Two positive samples for toxin B gene and negative for A & C difficile toxins by immunassay gave a negative result for toxins A & B on Simoa technology.

**CONCLUSION**

PRELIMINARY RESULTS are encouraging allowing the detection of very low quantities of A and B toxins in fecal samples. The opportunity to improve the sensitivity of current immunnoassays for CDI diagnosis, as well as the specificity by toxin detection instead of gene detection, is now possible with this new Simoa technology. Further studies are required to determine specific cut-offs on this assay for detection of both toxins A & B.

**REFERENCES**

2. **ADH/HCAL/ infectious disease. Updated Guidance on the diagnosis and reporting of Clostridium difficile. NHS.** 2012; 1-25
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