Evaluation of the VIDAS *Clostridium difficile* Toxin A&B Assay Clinical Performance Compared to the Cellular Cytotoxicity and Meridian Premier Toxins A&B Assays and Assessment of the Assay’s Limit of Detection, Cross-Reactivity and Interference Characteristics

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**ABSTRACT**

Background: The performance of the VIDAS *C. difficile* Toxin A&B (CDAB) qualitative test for the detection of free-toxigenic *C. difficile* (tA+B) in stool specimens was compared to the gold standard Cellular Cytotoxicity (CTA) and the Meridian Premier™ Toxins A&B (Premier) assay. The limit of detection (LoD) of the CDAB assay for toxin A and toxin B was evaluated. The cross-reactivity and interference of colonial flora bacteria and viruses was assessed and the reactivity of toxigenic *C. difficile* strains was evaluated. Methods: 1011 fresh stool specimens were prospectively collected and tested at two clinical sites (US and UK) using the CDAB and Premier tests. CTA testing was centralized and performed at bioMérieux, SA. Dilutions of toxin A and toxin B were performed in buffer and stool matrix to determine the LoD. 44 bacteria and 2 viruses were diluted in the CDAB negative and positive controls and then processed like a patient sample to assess cross-reactivity and interference. 23 *C. difficile* A+B+ and 18 *C. difficile* A+B- strains were tested for reactivity with the CDAB assay. Results: The Sensitivity, Specificity, PPV and NPV of the CDAB and Premier were compared to CTA and 98.3%, 98.8%, 98.1% and 98.4% respectively. The Sensitivity, Specificity, PPV and NPV of the Premier EIAs compared to CTA were 94.4%, 97.4%, 97.2% and 97.5% respectively. The Positive agreement, Negative agreement and Total agreement of the CDAB compared to Premier were 99.5% and 97.1% respectively. The LoD of the CDAB for toxin A and toxin B was determined to be 3 ng/ml and 1 ng/ml respectively in buffer and 7.73 ng/ml and 4.55 ng/ml respectively in stool matrix. Cross-reactivity was observed only with *C. sordelli* strain VPI 0948. No interference was detected. The CDAB detected 23/23 (100%) of the *C. difficile* A+B+ strains and 15/18 (83%) *C. difficile* A+B- strains. No false-positive results were detected.

**INTRODUCTION**

*C. difficile* has been found to be the major alpha-toxin secreted by *C. difficile* strains and is a necrotizing enteritis that causes ileus, pseudomembranous colitis and generally results in a systemic exacerbation of the illness. For the past decade, *C. difficile* has been increasing in prevalence as hospitals and long-term care facilities become more crowded and antibiotic use continues to increase. *C. difficile* strains can be either toxigenic or non-toxigenic. Toxigenic strains of *C. difficile* produce an enterotoxin (tA) as well as a cytotoxin (tB) in roughly equal amounts. However, some strains produce a tB but not a tA. It is possible that these strains are under-diagnosed due to the common practice of using diagnostic methods that detect only toxin A. The VIDAS *C. difficile* Toxin A & B (CDAB) Assay is an automated test for use in the VIDAS instruments for the quantification of *C. difficile* Enterotoxins A and B in stool samples. The test is a latex agglutination detection of *Clostridium difficile* toxin A and B in stool samples using the ELISA technology. The results of the test are visualized using a fluorescent microplate reader.

**METHODS**

**Clinical Study**

A total of 1011 fresh stool specimens were collected and tested at site 1 (TriCore Reference Laboratories) and site 2 (Addenbrooke’s Hospital). Each sample was tested using the VIDAS *C. difficile* Toxin A & B assay on the VIDAS instrument and the Meridian Premier Toxins A&B. Cellular cytotoxicity assay (gold standard) testing of each sample was centralized and performed at bioMérieux, SA using vero cells. Limit of Detection (LoD): Buffer condition: Serial dilutions of recombinant toxin A and B in TTBS 5% buffer were tested ten times with one VIDAS CDAB list on two VIDAS instruments (n=20). Each dilution of toxins A and B was processed as a patient sample (1:1 dilution with the kit sample buffer). Stool matrix condition: LoD was determined according to CSLI EP17-A using a negative human stool pool mixed with fetal calf serum (50%) and spiked with various levels of toxin A or toxin B. In total, 40 replicates of each dilution were tested for each toxin. The smallest amount corresponding to the limit where truly positive samples produce a positive result 95% of the time was defined as the LoD.

Cross-reactivity and Interference: To test for cross-reactivity, each bacteria or virus was diluted in the VIDAS CDAB negative control, processed like a patient sample, and tested in singlicate using two VIDAS CDAB reagent lists. To test for interference, each bacteria or virus was diluted in the VIDAS CDAB toxin A and toxin B controls, processed like a patient sample and tested in singlicate for each conjugate in one VIDAS CDAB reagent list. The bacteria were tested at a concentration of 1 x 10^8 CFU/ml (1 McFarland). Test values obtained with the spiked C1, C2 and C3 controls were compared to the kit specific expected values of the controls. If the results showed conformity to the expected range, no cross-reactivity or interference was present.

Toxigenic *C. difficile* Strain Study: *C. difficile* strains were grown in Yeast Peptone broth and tested for reactivity with the VIDAS CDAB assay. Supernatant culture material from each strain was processed like a patient sample. A single replicate of each strain was tested using one