Evaluation of VIDAS® *C. difficile* Toxin A IgG and VIDAS® *C. difficile* Toxin B IgG prototypes on blood bank sera characterized with cell cytotoxicity neutralisation assay

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**INTRODUCTION**

*Clostridium difficile* (CD) is a causative agent of antibiotic-associated pseudo membranous colitis (PMC). Since 2000, there has been a striking increase in the prevalence of *Clostridium difficile* infection and in associated mortality in the United States, Canada, and Europe. In this context, bioMérieux commercialize currently a VIDAS® *C. difficile* Toxin A&B test for toxins A and B detection. It seems necessary to investigate the impact of antibody responses to *Clostridium difficile* toxins on the risks of colonization, diarrhea and asymptomatic carriage.

**OBJECTIVES**

The aim of this study was to evaluate the results obtained on blood bank samples characterized with cell cytotoxicity neutralisation assay (CCNA) on the VIDAS® *C. difficile* Toxin B IgG (CDB IgG) prototype and on the VIDAS® *C. difficile* Toxin A IgG (CDA IgG) prototype for the determination of *C. difficile* serological status.

**MATERIALS AND METHODS**

**VIDAS test:**

The prototypes VIDAS CDB IgG and VIDAS CDA IgG use specific native toxin B and toxin A antigens respectively coated on the solid phase with the help of specific monoclonal antibodies against these toxins respectively. During the first step of the test, the specific IgG are captured by this solid phase. The complexes are revealed during the second step using an anti-human IgG conjugated to Alkaline Phosphatase (PAL).

The result is presented in signal (RFV = relative fluorescent value).

The serum volume is 100 µl for each test. The results are obtained in 35 minutes. Results are given as a relative fluorescence value for each sample and compared to results obtained with cell cytotoxicity neutralisation results.

For this study, the cut off is established at 300 RFV.

**VIDAS test format**

- **CD IgG A**
  - Ac anti - Tox A
  - IgG anti Tox A
  - Fab' anti human IgG

- **CD IgG B**
  - Ac anti - Tox B
  - IgG anti Tox B
  - Fab' anti human IgG

+PAL

Cell Cytotoxicity Neutralisation Assay (CCNA):

100 µl of human sera, diluted to 1/20 are dispensed in duplicate on Vero cell microtiter plates and then, ten fold logarithmic dilution of *C. difficile* toxin B (110 µg/ml) are dispensed, 100 µl per well. The first well of each row received the dilution medium as negative control. Dilution of toxin B are dispensed in two wells as positive control. Microtiter plates are incubated at 37°C under 5% CO2 during 48 h. The cell monolayer are checked by microscopy after 24 and 48 h in order to note the typical cell rounding effect. The results are expressed as the lower dilution of toxin B neutralized by the specific antibodies present in the sera.

**Samples:**

Forty two sera from blood bank origin are tested on VIDAS with each prototype. These sera were characterized with the cell cytotoxicity neutralisation assay currently used in bioMérieux.

**RESULTS**

Of the 18 samples characterized as positive in the cell cytotoxicity neutralisation assay, 18 samples showed positive results with the VIDAS CDB IgG prototype and 9 with the VIDAS CDA IgG prototype. Of 24 samples characterized as negative in the cell cytotoxicity neutralisation assay, 18 samples showed negative results with the VIDAS CDB IgG prototype and 17 with the VIDAS CDA IgG prototype. The agreement with the cell cytotoxicity neutralisation assay is 86% for VIDAS CDB IgG prototype and 62% for VIDAS CDA IgG prototype on the 42 samples. The results are detailed in the tables below.

**CONCLUSION**

With the VIDAS *C. difficile* Toxin A IgG and the VIDAS *C. difficile* Toxin B IgG prototypes, it is possible to detect the *C. difficile* toxins A and B IgG in blood samples. The results obtained with the VIDAS *C. difficile* Toxin B IgG prototype are better correlated with the cytotoxicity neutralisation assay than the results with the VIDAS *C. difficile* Toxin A IgG prototype (p<0.05). The VIDAS *C. difficile* Toxin B IgG seems to be more relevant for studying the immunological response for *C. difficile* infection. Larger studies with different population in the context of *C. difficile* infection in hospital settings (patients at risk, colonized patients, infected patients and patients with asymptomatic carriage....) will need to be conducted to establish the optimal neutralisation cut off of the VIDAS *C. difficile* Toxin B IgG.