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

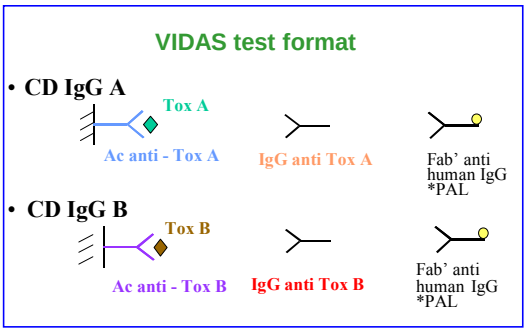
Clostridium difficile (CD) is a causative agent of antibiotic-associated pseudo membranous colitis (PMC). Since 2000, there has been a striking increase in the prevalence of *Clostridium difficile* infection and in associated mortality in the United States, Canada, and Europe. In this context, bioMérieux 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.



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.

Samples	CTA neutralisation 1/20	anti-toxin A IgG	anti-toxin B IgG
cutoff		signal positive > 300 RFV	
CDG29	Neg	1303	835
CDG32		250	201
CDG13		570	124
CDG8		503	197
CDG11		296	40
CDG19		1261	309
CDG22		261	100
CDG17		157	41
CDG15		377	1588
CDG16		424	981
CDG18		123	510
CDG23		161	40
CDG25		30	11
CDG26		198	231
CDG27		55	58
CDG36		17	11
CDG39		156	46
CDG33		208	58
CDG38		47	7
CDG20		63	77
CDG21		200	385
CDG40		194	113
CDG4		446	217
CDG12		108	24

Samples	CTA neutralisation 1/20	anti-toxin A IgG	anti-toxin B IgG
cutoff		signal positive > 300 RFV	
CDG14	- 4 logs	1186	4944
CDG6	- 3 logs	1290	5239
CDG43		2259	2616
CDG41		254	2251
CDG28		1545	1967
CDG44	- 2 logs	135	5472
CDG35		3484	4385
CDG30		195	3946
CDG45		619	3157
CDG34		561	2284
CDG5		119	2248
CDG42		159	1800
CDG31		1247	609
CDG10	-1 log	34	1895
CDG8		191	848
CDG7		155	548
CDG24		145	556
CDG37		552	386

	n	Correlation	CI 95%
anti-toxin B IgG	36/42	85.71%	[71.46-94.57]
anti-toxin A IgG	26/42	61.9%	[45.64-76.43]

Sensitivity	anti-toxin B IgG	anti-toxin A IgG
Positive samples	18	9
Negative samples	0	9
%	100% [81.47-100]	50%[26.02-73.98]

Specificiity	anti-toxin B IgG	anti-toxin A IgG
Negative samples	18	17
Positive samples	6	7
%	75% [53.29-90.23]	71% [48.91-87.38]

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.