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 *C. difficile* toxins A and B in stool specimens was compared to the gold standard Cellular Cytotoxicity (CTA) and the Meridian Premier™ Toxins A&B EIA (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 colonic 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 compared to CTA were 88.3%, 99.8%, 98.1% and 98.4% respectively. The Sensitivity, Specificity, PPV and NPV of the Premier EIA compared to CTA were 86.4%, 97.4%, 83.2% and 97.9% respectively. The Positive agreement, Negative agreement and Total agreement of the CDAB compared to Premier were 81.3%, 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 9048. 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. 3 of the A-/B+ strains gave equivocal results. Conclusion: The Sensitivity, Specificity, PPV and NPV of the

toxins with good performance compared to the gold standard CTA.

INTRODUCTION

C. difficile has been found to be the major etiologic agent of antibiotic-associated pseudomembranous colitis (PMC). PMC is a clinically defined syndrome, associated with a recent history of antibiotic use, where pseudomembranous nodules or plaques form in the distal and sigmoid colon and rectum. If unrecognized or untreated, the disease can be fatal. Nosocomial acquisition of *C. difficile* is a serious consideration for some institutions, particularly those with high inpatient populations, chemotherapy wards, or long-term patient care. *C. difficile* can either be toxigenic or nontoxigenic. Toxigenic strains of *C. difficile* produce an enterotoxin (toxin A) as well as a cytotoxin (toxin B) in roughly equivalent amounts. However, some strains produce toxin B but not toxin A. It is possible that these strains are under-diagnosed due to the common use of diagnostic methods that detect only toxin A. The VIDAS® *C. difficile* Toxin A & B (CDAB) Assay is an automated test for use on the VIDAS instruments for the qualitative detection of *Clostridium difficile* toxin A and toxin B in stool specimens using the ELFA technique (Enzyme-Linked Fluorescent Assay).

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 EIA. 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 TRIS BSA 5% buffer were tested ten times with one VIDAS CDAB lot on two VIDAS instruments (n=20). Each dilution of toxins A and B was processed like a patient sample (1:6 dilution with the kit sample buffer). Stool Matrix condition: LoD was determined according to CLSI EP17-A using a negative human stool pool mixed with fetal calf serum (50%/50%) and spiked with various levels of toxin A or toxin B. In total, 60 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 one VIDAS CDAB reagent lot. 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 control using one VIDAS CDAB reagent lot. The bacteria were tested at a concentration of 1 x 10^7 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

RESULTS

The VIDAS CDAB assay was compared to cellular Cytotoxicity assay and to the Meridian Premier Toxins A&B assay. The results showed an overall Sensitivity, Specificity, PPV and NPV of 88.3%, 99.8%, 98.1% and 98.4% respectively for the VIDAS when compared to Cytotoxicity and a Positive and Negative agreement of 81.3% and 99.5% respectively when compared to Premier Toxin A&B assay.

Table 1: VIDAS CDAB Compared to Cellular Cytotoxicity Assay

		Cytotoxicity Assay		
		Positive	Negative	Total
VIDAS CDAB	Positive	106	2*	108
	Equivocal	12	30	42***
	Negative	14**	847	861
	Total	132	879	1011

Performance	Value (%)	95% CI
Sensitivity (%)	88.3	81.2 -93.5
Specificity (%)	99.8	99.2 - 99.9
PPV (%)	98.1	93.5 - 99.8
NPV (%)	98.4	97.3 - 99.1

*2 samples were VIDAS CDAB positive and Cytotoxicity test negative, of which both were Premier negative.
**14 samples were VIDAS CDAB negative and Cytotoxicity test positive, of which 10 were Premier negative and 4 Premier positive.
*** 42/1011(4.2%) samples were VIDAS CDAB equivocal and were not taken into account for the sensitivity, specificity, PPV & NPV calculations

Table 2: Premier Toxins A&B Compared to Cellular Cytotoxicity Assay

		Cytotoxicity Assay		
		Positive	Negative	Total
Premier Toxin A&B	Positive	114	23*	137
	Negative	18**	856	874
	Total	132	879	1011

Performance	Value (%)	95% CI
Sensitivity (%)	86.4	79.3 - 91.7
Specificity (%)	97.4	96.1 - 98.3
PPV (%)	83.2	75.9 - 89.0
NPV (%)	97.9	96.8 - 98.8

*23 samples were Cytotoxicity test negative and Premier positive, of which 20 were VIDAS CDAB negative and 3 were VIDAS CDAB equivocal.
**18 samples were Cytotoxicity test positive and Premier negative, of which 10 were negative, 6 were equivocal and 2 were positive with VIDAS CDAB.

Table 3: VIDAS CDAB Compared to Premier Toxins A&B Assay

		Premier Toxin A&B		
		Positive	Negative	Total
VIDAS CDAB	Positive	104	4*	108
	Equivocal	9	33	42***
	Negative	24**	837	861
	Total	137	874	1011

Performance	Value (%)	95% CI
Pos Agreement	81.3	73.4 - 87.6
Neg Agreement	99.5	98.8 - 99.9
Global Agreement	97.1	95.9 - 98.1

*4 samples were VIDAS CDAB positive and Premier negative, of which 2 were Cytotoxicity test positive.
**24 samples were VIDAS CDAB negative assay and Premier positive, of which 20 were Cytotoxicity test negative.
*** 4.2% (42/1011) samples were VIDAS CDAB equivocal, and were not taken into account for the positive, negative and global agreement calculation.

Table 4: Limit of Detection

Matrix	Toxin A	Toxin B
Buffer	3.00 ng/mL	1.00 ng/mL
Stool	7.73 ng/mL	4.55 ng/mL

Table 5: Cross Reactivity and Interference

List of the bacteria and viruses tested for cross reactivity and interference with the VIDAS CDAB assay.

Organism	Strain #	Organism	Stain #
Adenovirus 40	bMx 98007	*Clostridium tetani*	bMx 8808010C
Adenovirus 41	bMx 98008	*Enterobacter aerogenes*	ATCC 2121421
Rotavirus	bMx 60500455	*Enterobacter cloacae*	ATCC 2121422
Aeromonas hydrophila ssp hydrophila	ATCC 35654	*Enterococcus faecalis*	ATCC 29212
Bacillus cereus	ATCC 2231042	*Escherichia coli*	ATCC 25922
Bacillus subtilis	ATCC 2231010	*Escherichia coli 0157:H7*	ATCC 43889
Bacteroides fragilis	ATCC 25285	*Helicobacter pylori*	ATCC 2413042
Campylobacter coli	ATCC 33559	*Klebsiella pneumoniae*	ATCC 2121411
Campylobacter jejuni spp jejuni	ATCC 33560	*Peptostreptococcus anaerobius*	ATCC 27337
Candida albicans	ATCC14053	*Porphyromonas asaccharolytica*	bMX 8907158
Clostridium bifermentans	ATCC 638	*Proteus vulgaris*	ATCC 2121517
Clostridium butyricum	ATCC19398	*Pseudomonas aeruginosa*	ATCC 27853
Clostridium difficile (nontoxigenic)	VPI 0210114	*Salmonella enteritidis*	ATCC 25928
Clostridium haemolyticum	ATCC 2425083	*Salmonella Groupe B (paratyphi B)*	ATCC 8759
Clostridium histolyticum	ATCC 19401	*Salmonella typhimurium*	ATCC 2121212
Clostridium innocuum	bMx 8206019	*Serratia liquefaciens*	ATCC 2121441
Clostridium novyi	ATCC 7659	*Shigella dysenteriae*	bMx 8802065
Clostridium perfringens	ATCC 13124	*Shigella flexneri*	ATCC 12661
Clostridium septicum	ATCC 12464	*Shigella sonnei*	bMx 8303066
Clostridium sordelli	ATCC 9714	*Staphylococcus aureus ssp aureus*	ATCC 12599
*Clostridium sordelli**	VPI 9048	*Staphylococcus epidermidis*	ATCC 2222101
Clostridium sporogenes	ATCC 19404	*Vibria parahaemolyticus*	ATCC 2122103
Clostridium subterminale	bMx 8508074	*Vibrio cholerae*	bMx 8208005
Clostridium tertium	ATCC 14573	*Yersinia enterocolitica*	bMx 0001031

* Cross-reactivity with *C. sordelli* VPI 9048 is observed depending on the culture conditions used.

None of the bacteria or viruses tested showed Cross reactivity or interference when tested with the VIDAS CDAB assay.

Table 6: *C. difficile* Toxigenic Strains

Strains A+/B+	Test value	Interpretation	Strains A-/B+ Ribotype 017	Test value	Interpretation
R8366	5.74	POSITIVE	R2140	1.86	POSITIVE
R14935	5.66	POSITIVE	R13167	1.23	POSITIVE
R14988	3.22	POSITIVE	R11092	1.08	POSITIVE
R14990	4.87	POSITIVE	R12878	0.86	POSITIVE
R15262	5.38	POSITIVE	R10430	0.93	POSITIVE
R15657	0.66	POSITIVE	R10205	0.78	POSITIVE
R15826	5.3	POSITIVE	R16792	1.11	POSITIVE
R15832	0.92	POSITIVE	R16509	0.31	EQUIVOCAL
R15947	4.92	POSITIVE	R16486	1.33	POSITIVE
R16326	4.79	POSITIVE	R16475	1.09	POSITIVE
R16810	3.02	POSITIVE	R16480	4.25	POSITIVE
R4622	5.45	POSITIVE	R16090	2.13	POSITIVE
R15673	0.77	POSITIVE	R15452	0.96	POSITIVE
R16631	5.04	POSITIVE	R13695	1.44	POSITIVE
R16808	1.27	POSITIVE	R15652	1.04	POSITIVE
R15516	3.19	POSITIVE	**Strains A-/B+ Ribotype 047**	**Test value**	**Interpretation**
R16690	5.51	POSITIVE	R10542	0.7	POSITIVE
R15508	1.24	POSITIVE	**Strains A-/B+ Ribotype 110**	**Test value**	**Interpretation**
R15808	5.21	POSITIVE	R7771	0.29	EQUIVOCAL
R16448	1.83	POSITIVE	R7981	0.16	EQUIVOCAL
R16705	5.88	POSITIVE	***C. difficile* types**	**% VIDAS CDAB Postive**	
R16809	1.5	POSITIVE	A+/B+	100% (23/23)	
R16811	2.97	POSITIVE	A-/B+	83% (15/18), 3 strains were VIDAS Equivocal	

CONCLUSION

The sensitivity, specificity, PPV and NPV of the VIDAS CDAB test in comparison to Cytotoxicity were 88.3%, 99.8%, 98.1% and 98.4% respectively*. The VIDAS CDAB provided higher specificity and PPV than the Premier Toxins A&B test and equivalent sensitivity and NPV when both assays were compared to the gold standard Cytotoxicity test. The VIDAS CDAB showed cross-reactivity only with *C. sordelli*, no interference and the ability to detect different strains of toxigenic *C. difficile*, including A+/B+ and A-/B+ strains. The VIDAS CDAB assay detected toxin A at a level of 7.73 ng/mL and toxin B at a level of 4.55 ng/ml in human stool. The VIDAS CDAB test enables automated, rapid and reliable detection of *C. difficile* toxins with good performance compared to the gold standard Cytotoxicity test.

*Extended 510k claims pending with US FDA.