THE SIMOA ASSAY FOR DETECTION OF *CLOSTRIDIUM DIFFICILE* TOXINS HAS A BETTER SENSITIVITY THAN THE CYTOTOXICITY ASSAY.

Gateau C. [1], Gouot C. [1], Lapidus N.[2], Couturier J.[1,3,5], Lalande V.[3], Foussadier A. [4], Banz A.[4], Barbut F. [1,3,5].

[1]National Reference Laboratory for C. difficile, Saint-Antoine Hospital, AP-HP, Paris France; [2]Department of Public Health, Saint-Antoine Hospital, AP-HP, Paris France; [3]Department of Microbiology, Saint-Antoine hospital, AP-HP, Paris, France; [4]bioMérieux, Marcy l'Etoile, France; [5]INSERM S-1139, University Paris Descartes.

Background- Objective

Clostridium difficile is a major agent responsible for healthcare associated diarrhoea. The European guidelines recommend the use of a two-step algorithm for the diagnosis of *Clostridium difficile* infection (CDI) based on a sensitive screening method (GDH detection or NAAT) followed by a more specific test detecting toxins. Commercial EIA tests for detection of toxins display a suboptimal sensitivity and currently cannot be used as standalone test. An ultrasensitive assay detecting free toxins A and B has been recently developed by bioMérieux using the single molecule array technology (SIMOA). The objective of this study was to evaluate the analytical performances of SIMOA toxins A and B assay.

Materials and Methods

We compared the sensitivity and specificity of the assay using 100 frozen stools of patients previously diagnosed with CDI by toxigenic culture (see Barbut *et al.,* EJCMID 2017). Among these patients, 67 had a positive cytotoxicity assay (CTA) on MRC-5 cell culture and 33 a negative CTA.

We also tested stools of 38 patients negative for *C. difficile* by culture and 32 patients harboring a non-toxigenic strain of *C. difficile*.

SIMOA : SIMOA consists of toxin capture by specific anti-toxin A and B antibodies coated on paramagnetic beads (Quanterix Corporation), followed by detection with specific antibody conjugated to an enzyme β-galactosidase. Beads are then incubated with enzyme substrate and individually isolated in microwells of an array for digital imaging.

The threshold of positive result with SIMOA was set up **at 22 and 18.8 pg/ml**, for toxins A and B detection, respectively.

Faecal calprotectin concentrations were determined using a quantitative immunoassay according to the manufacturers' instructions (Quantum Blue, Bülhmann).

Fecal lactoferrin concentrations were determined with a quantitative ELISA according to the manufacturers' instructions (IBD Scan, TechLab, Blacksburg, VA).

Statistical analysis: Toxins titers were arbitrarily classified into 3 groups and compared to different biological and clinical parameters. Continuous variable were compared by the Kruskall Wallis test whereas categorical variable were compared by the Fisher exact test.

Results

Among the 67 patients with a positive CTA assay, only 3 (4.5%) were negative for both toxins A and B by SIMOA (**fig.1**). The remaining stool samples were positive for both toxins (n=59) or for toxin B only (n=5). Among the 33 patients with CDI but negative for CTA, 9 (27.3%) were positive for both toxins by SIMOA, 5 (15.1%) were only positive for toxin A and 2 (6.1%) were only positive for toxin B. Among the 38 patients negative for *C. difficile* by culture, 2 (5.2%) were positive for toxin A only with a very low titer (43 and 40 pg/ml). The 32 patients harboring a non-toxigenic strain of CDI were all negative for both toxins. Toxins A and B concentrations determined by SIMOA were significantly correlated (Pearson correlation=0.89, p<0.001) (**fig.2**). There was a statistical association between levels of toxins A or B and digestive inflammation markers as determined by levels of fecal lactoferrin or calprotectin. Severity was not correlated to levels of toxin production whereas there was a trend for a higher mortality rate in patients with a level of toxins >1000 pg/ml (**Table 1**).

References

1- Crobach MJ, Planche T, Eckert C, **Barbut F,** Terveer EM, Dekkers OM, Wilcox MH, Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for *Clostridium difficile* infection. Clin Microbiol Infect. 2016 Aug;22 Suppl 4:S63-81.

2-**Barbut F.** et al., Faecal lactoferrin and calprotectin in patients with *C. difficile* infection : a case control study. Eur J Clin Microbiol Infect Dis. 2017 Dec;36(12):2423-2430

Fig. 1 : Comparison of SIMOA results in the different patients' groups

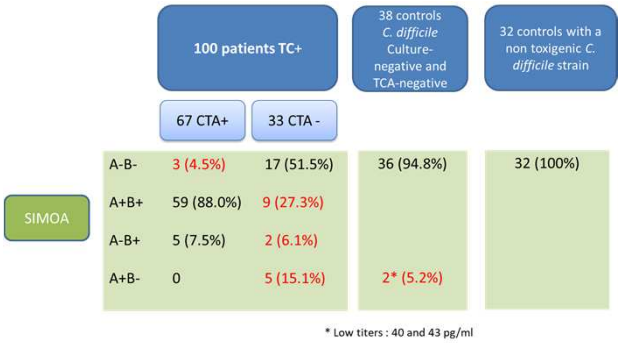

Fig. 2 : . Correlation between toxinA and B levels as determined by SIMOA technique (Person correlation = 0,89, p<0,001)

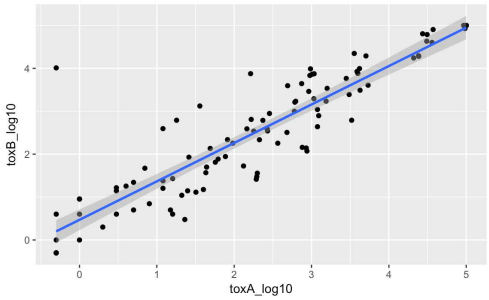

Table 1A and B. Correlation between toxin production determined by SIMOA and calprotectin and lactoferrin levels, CDI severity and mortality at D30 et D90.

A

Toxin A	N	Fecal lactoferrin (µg/g), median	Fecal calprotectin (µg/g), median	Severity IDSA score N (%)	Severity Zar Score N (%)	Mortality D30 N (%)	Mortality D90 N (%)
<22 pg/ml	28	10.2	122	9 (32.1%)	5 (17.9%)	1 (4.2%)	3 (15.0%)
22-1000 pg/ml	40	32.2	166.5	10 (25.0%)	11 (27.5%)	7 (18.4%)	10 (28.6%)
> 1000 pg/ml	32	63.1	453.75	8 (25.8 %)	7 (21.9%)	8 (25.0%)	11 (34.4%)
p		0.0011	0.0207	0.84	0.63	0.09	0.32

B

Toxin B	N	Fecal lactoferrin (µg/g), median	Fecal calprotectin (µg/g), median	Severity IDSA score N (%)	Severity Zar Score N (%)	Mortality D30 N (%)	Mortality D90 N (%)
<18.8 pg/ml	25	9.0	119.5	7 (28.0%)	4 (16.0%)	1 (4.5%)	3 (15.0%)
18,8-1000 pg/ml	36	18.6	162.7	10 (27.8%)	10 (27.8%)	7 (21.2%)	8 (29.7%)
> 1000 pg/ml	39	69.3	567.0.75	10 (26.3%)	9 (23.0%)	8 (20.5%)	13 (35.1%)
p		<0.001	0.002	0.95	0.57	0.20	0.27

Conclusions

This study confirmed that the sensitivity of the SIMOA assay is higher than the cytotoxicity assay and can detect fecal toxins in 48.5 % of samples found to be negative using the CTA which is currently the reference technique for detecting free toxins from stools. *C. difficile* toxin detection using SIMOA technology has the potential to improve and simplify the CDI diagnosis.