Evaluation of a novel molecular assay to detect toxigenic strains of *Clostridium difficile*

C. ECKERT[1], T. DEVALLIÈRE[1], R. SYED-ZAIDI[1], J. COUTURIER[1], V. LALANDE[2], F. BARBUT[1]

(1) CNR *Clostridium difficile* Hôpital Saint-Antoine, Paris, FRANCE; (2) Bactériologie hôpital Saint-Antoine, Paris, FRANCE

Introduction

Guidelines for the diagnosis of *C. difficile infection* were recently updated (1). Briefly, an optimal strategy is based on a 2-steps algorithm detecting both free toxins in stools and a toxigenic strain. The combination of rapidity, specificity and sensitivity characteristics allows the prompt isolation of patients with CDI and thus prevents the spread of *C. difficile* in clinical settings.

The Amplidiag *C. difficile+027*® (Mobidiag Ltd., Finland) assay is a multiplex real-time PCR amplification assay with fluorophore-labeled probes for the identification of toxigenic *C. difficile* directly from unformed (liquid or soft) stool specimens collected from patients suspected of having CDI. The assay targets the toxin B gene of *C. difficile* PaLoc. It also detects a putative conjugative transposon *pct* present in 027 strains (positive marker for 027 strains). A TetR family transcriptional regulator protein hydR present in non-027 strains (negative marker for 027 strains) and an internal control are also targeted.

Objective

The objective of this study was to assess the performances (sensitivity, specificity, predictive positive and negative values, including their 95% confidence intervals) of Amplidiag *C. difficile+027*® assay in comparison to the TC for the detection of a toxigenic strain.

Methods

309 consecutive diarrheal stool samples were collected from January to March 2016 from patients suspected of having CDI hospitalized in four hospitals in Paris. Culture was performed on the selective medium ChromId (Biomérieux). After DNA extraction, specific primers for *tpi*, *tcdA*, *tcdB*, *cdtA*, *cdtB*, *tcdC* and for non toxigenic strains (lok) were used for the *in vitro* determination of the cytotoxicity of *C. difficile* isolates by an in-house multiplex PCR (TC). The cytotoxicity assay (CTA) was performed using MRC-5 cells monolayers. PCR-ribotypes (PR) of the strains were determined after capillary electrophoresis, using the probes described by Bidet *et al.* (2) and analyzed with GenMapper software. The Chemagic Prepito Cyto Pure kit (Perkin Elmer) was used to extract DNA directly from stools. Amplidiag assay was performed after DNA extraction according to the manufacturer's instructions. In case of discordant results, the samples which were stored at -80°C were tested again by molecular assay or enriched toxigenic culture (ETC).

Results

The prevalences of positive CTA, positive culture and positive TC were 8.1% 19.4% and 14.6%, respectively. Five results (1.6%) were invalid with Amplidiag. After repeating the test, all the results could be interpreted either as positive (n=2) or negative (n=3). Compared to the TC, the sensitivity, specificity, positive and negative predictive values of the Amplidiag *C. difficile+027*® assay were 84.4% (CI95% 69.9-93), 98.5% (CI 95% 95.9-99.5), 90.5% (CI 95% 76.5-96.9) and 97.4 (CI 95% 94.4-98.8), respectively. After resolving discrepant results, the sensitivity, specificity, positive and negative predictive values of the Amplidiag assay were 91.1% (CI95% 77.9-97.1), 99.6% (CI 95% 97.6-100), 97.6% (CI 95% 85.9-99.9) and 98.5 (CI 95% 96-99.5), respectively.

None PR027 strain was isolated during this study, but both negative and positive markers for 027 were positive for 3 stool samples and the conclusion was "hypervirulent 027 strain cannot be excluded". The strains isolated were characterized as PR056 (binary toxin negative, no deletion in the negative regulator *tcdC*), PR014/020/077 (binary toxin negative, no deletion in the negative regulator *tcdC*) and PR106 (binary toxin negative, 18 pb-deletion in the negative regulator *tcdC*). Additionally, the result for 2 samples was "mixed population cannot be excluded".

The total hands-on-time was 45 min for extraction and 20 min for the preparation of the samples for the PCR respectively.Turn-around-time for extraction was about 1.30 h per 12 samples and 2h for PCR.

Table 1: results of the different tests

	First Test			
No with results	CTA	TC	Amplidiag	Ct *tcdB* Amplidiag
260	-	-	-	
25	+	+	+	
13	-	+	+	
4	-	-	+	37.69-34.37-33.67-28.06
7	-	+	-	
Total 309				

Table 2: analysis of the 11 discrepant results

No of samples	**TC**	**ETC**	**Amplidiag 1st test**	**Amplidiag 2nd test**	**Amplidiag on colonies**	**Conclusion**
3	+	ND	-	+	ND	True positive
4	+	+	-	-	+	False negative Amplidiag
3	-	-	+	-	ND	True negative
1	-	-	+ (Ct *tcdB* 34.37)	+ (Ct *tcdB* 37.57)	ND	False positive Amplidiag

Conclusion

Amplidiag C. difficile+027® is a sensitive method to detect toxigenic strains of *C. difficile*. It would be of interest to evaluate the performances of this assay on PR027 strains.

Bibliography:

1. Crobach MJT, Planche T, Eckert C, Barbut F, Terveer EM, Dekkers OM, et al. European Society of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document for *Clostridium difficile* infection. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2016 Aug;22 Suppl 4:S63–81
2. Bidet P, Barbut F, Lalande V, Burghoffer B, Petit JC. Development of a new PCR-ribotyping method for Clostridium difficile based on ribosomal RNA gene sequencing. FEMS Microbiol Lett. 1999;175(2):261–6.