Evaluation of three commercially kits for toxigenic *Clostridium difficile* molecular detection in stools

Viala C[1]., Le Monnier A[2]., Maataoui N[2]., Rousseau C[1]., Cruaud P[1]., Collignon A[1]., Poilane I[1].
1 Laboratoire de Microbiologie, Hôpital Jean Verdier , Assistance Publique- Hôpitaux de Paris, Bondy, France, 2 Laboratoire de Microbiologie, Centre Hospitalier A. Mignot, Le Chesnay, France

Introduction and Purpose

The bacteriological diagnosis of *Clostridium difficile* associated diseases (CDAD) is based on toxins A/B detection in stools specimen with immunoenzymatic methods, associated to bacterial culture. However, if toxin enzyme immunoassays are rapid and easy to perform, they have a low sensitivity compared to cell cytotoxicity neutralization assay (CCNA) or toxigenic culture. Since few years, a highly virulent strain of *C.difficile* has emerged causing outbreaks and more severe cases. These new epidemiological aspects have led the laboratories to develop an accurate and rapid diagnosis. Molecular assays for various targets have been developed. In this study, we have evaluated three commercially available kits for toxigenic *C. difficile* molecular detection in stools.

Material and Methods

Stool specimens. Ninety four stool specimens were analysed. They were obtained from patients hospitalised in Jean Verdier Hospital (46 patients) and in Andre Mignot Hospital (40 patients).
Toxins A/B immunoassay testing. All samples were tested for both *C. difficile* toxins A and B using the ImmunoCard ToxAB assay (ICTAB-Meridian Bioscience) aacording to the manufacturer's recommendations.
Toxigenic culture. Samples were inoculated to a *C. difficile* selective media (CLO, bioMérieux). A presumptive identification of *C. difficile* colonies was performed. The ImmunoCard ToxAB assay was then performed on five to ten colonies, according to the manufacturer's recommendations.
Cytotoxicity assay. Suspension (1:10) of the stoll in MEM 2% were centrifugated, the supernatant was filtrated then inoculated on to MRC5 cells monolayer. The presence or absence of cytotoxicity effect was observed after 24 and 48 hours.

Toxins A/B molecular detection. The assays were performed restrospectively on frozen-thawed stool samples
XPert Cdifficile (Cepheid). This assay is a multiplex real-time PCR (Taqman) which amplifies *tcdB*, deletion 117 in *tcdC* and *cdtA* (binary toxin) for detection of *C. difficile* PCR-ribotype 027. The reaction, comprising the extraction step is performed in a cartridge placed in a Gene-Xpert Dx module. A result is obtained in less than one hour.
BD GeneOhm CDiff (BD Diagnostics). The BD GeneOhm CDiff assay is a real time PCR which amplifies a sequence within the *tcdB* gene. After a step of extraction, amplification was performed on a Cepheid SmartCycler using PCR parameters supplied by BD GeneOhm. A result is obtained in less than 3 hours.
Illumigene (Meridian). This assay is based on Loop Mediated Isothermal Amplification (LAMP) which targets a highly conserved sequence in *tcdA*. After DNA extraction, the amplification is performed in a specific instrument (Illumipro). A result is obtained in approximately one hour.

Results

Table 1. Comparison of results obtained with each evaluated test

N° of samples	XPert Cdifficile	BD GeneOhm Cdiff	Illumigene	Gold Standard	Result
47	Negative	Negative	Negative	Negative	Negative
39	Positive	Positive	Positive	Positive	Positive
4	Positive	Positive	Negative	Positive	Positive
1	Positive	Negative	Negative	Negative	Negative
1	Negative	Positive	Negative	Negative	Negative
1	Positive	Negative	Negative	Positive	Positive
1	Negative	Negative	Negative	Positive	Positive

Table 2. Performances of each test compared to gold standard assay

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Target(s)	Turnaround time (h)
XPertCdifficile	97.8	97.9	97.8	97.9	*tcdB, tcdC, cdtA*	< 1
BD GeneOhm Cdiff	95.5	97.9	97.7	96.0	*tcdB*	< 3
Illumigene	86.7	100	100	89.0	*tcdA*	1

PPV : Positive Predictive Value; NPV :Negative Predictive Value

- No discrepancy was observed between results with both « gold standard », toxigenic culture and cytotoxicity assay
- Discrepant results with molecular assays were observed for 8/94 samples
- False negative result was observed in one case with XPert, two cases with BD GeneOhm and six cases with Illumigene
- False positive result was observed in one case with XPert, one case with BD GeneOhm and none with Illumigene
- In one case, the toxigenic culture was positive whereas the three molecular tests were negative. The presence of *tcdA* and *tcdB* was confirmed by PCR (Lemée, JCM, 2004) (data not shown).
- With BD GeneOhm, in 6/94 cases, results were « unresolved » leading to repeat the test
- No PCR-ribotype 027 isolate has been identified in this study

Conclusions

- Performances of the molecular detection kits are significantly higher to those of the toxin enzyme immunoassays as already reported.
- With BD GeneOhm PCR, the extraction step is critical and requires an experienced operator. However, the assay is performed in a thermocycler not dedicated to a specific test or a specific manufacturer.
- With Illumigene, the assay is rapid, easy to perform and in this study the specificity is 100%. The relative low sensitivity compared to the other tests, could be explained by the use of frozen-thawed samples in this retrospective study.
- XPert PCR is an easy to perform, rapid and not time consuming assay. Moreover, it detects PCR-ribotype 027 isolates.
- These assays could be included in an algorithm to provide a rapid and accurate diagnosis of *C. difficile* associated diseases as cost-effective as possible