DIAGNOSIS OF CLOSTRIDIUM DIFFICILE INFECTION IN CLINICAL LABORATORIES: A DIFFICULT COMPROMISE BETWEEN PERFORMANCE, PRACTICABILITY AND COST

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ABSTRACT

The current guidelines for the diagnosis of *Clostridium difficile* infection (CDI) combine rapid detection of toxins A and B and toxigenic culture. However, there is no definitive consensus on the best tests and their combination. Thus, the diagnosis of CDI still remains tricky for many clinical microbiology laboratories. The results of an audit conducted during 2008 in our institution highlighted the limits for the rapid detection of both toxins and a low rate of isolation of the bacteria and suggested several propositions for improving our algorithm. The aim of our study was to evaluate prospectively technical characteristics, feasability, and performance of several commercially available tests performed systematically for rapid detection of toxin A and B (ImmunoCard® toxins A & B (ICTAB, Meridian), Vidas® *C. difficile* A & B, (CDAB, bioMérieux)), toxigenic culture, and real-time PCR GeneOhm CDiff (BD). All consecutive diarrheic stools from adult patients, sent to the laboratory for suspicion of CDI during a five-month period, were included in the study. The results of each tests were compared to the cytotoxcity neutralization assay (CCNA), as reference test, and the clinical data for each episode of CDI (≥ 3 months between two episodes for the same patient). A total of 28 cases of CDI (11.8%) were diagnosed among the 237 fresh stools tested (215 patients). Systematic and rapid culture of sample, use of UV (312 nm) enrichment step in broth to isolate and identify suspect colonies have significantly improved the rate of isolation of *C. difficile* (Se: 93%, Spe: 100%). Toxigenic culture, using the ICTAB test, showed a total concordance with the CCNA. The performance of both rapid tests Vidas CDAB and ICTAB were similar (Se: 77.5%, Spe: 98% ; Se: 78.5%, Spe: 96.5% respectively). We observed an important number of equivocal results (2.5%) with VIDAS CDAB distributed either within positive (n=1) or negative (n=5) cases. Real-time PCR Cdiff GeneOhm provides rapid and relevant results (Se: 100%, NPV: 100%). One false positive result has been reported and may be explained by a succession of freeze-thawing of the sample. The choice of tests for CDI diagnosis must not be only based on their performance but should also integrate cost, feasability, and practicability for a routine use in clinical laboratory. The analysis of the results of this study led to the optimization of our organization and to define the respective position for each test in a new algorithm. It associates rapid detection of toxins and toxigenic culture, whereas CCNA is still used as a confirmatory technique for difficult or discordant cases. Despite excellent sensitivity, PCR was ruled out because of excessive costs for yet.

INTRODUCTION

Since few years, a highly virulent strain of *Clostridum difficile* has emerged causing outbreaks and more severe cases. These new epidemiological aspects have led the laboratories to develop an accurate and rapid diagnosis of *Clostridium difficile* infection (CDI) for the early instauration of adapted treatment and appropriate isolation precautions to prevent complications and spread into the health-care structures.

The current guidelines (ESCMID) for the diagnosis of CDI combine rapid detection of toxins A and B in stools specimen with immunoenzymatic methods, associated to toxigenic culture. However, the bacteriological diagnosis of CDI is so far non-consensual, hard working and time consuming. Indeed, the results of an audit conducted during 2008 in our institution highlighted the limits for the rapid detection of both toxins and a low rate of isolation of the bacteria suggesting several propositions for improving our algorithm.

The aims of our study was : 1) to evaluate prospectively technical characteristics, feasibility, and performance of several commercially available tests performed systematically for rapid detection of toxin A and B [ImmunoCard® toxins A & B (ICTAB, Meridian), Vidas® C. difficile A & B, (CDAB, bioMérieux)], toxigenic culture, and real-time PCR GeneOhm CDiff (BD) ; 2) to determine the respective position of each tests evaluated for constructing a new algorithm of CDI diagnosis according to the local specificities of our institution.

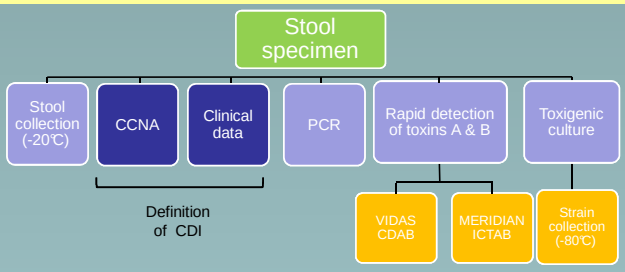
PATIENTS, MATERIAL AND METHODS

Study design

- Inclusion prospectively in the study of all consecutive diarrheic stools from adult patients, sent to the laboratory for suspicion of new episode of CDI during a five-month period.

- Exclusion: children, non diarrheic specimen, relapse or treatment failure, and « control » for the same patient.

-The results of each tests were compared to the cytotoxcity neutralization assay (CCNA), as reference test, and the analysis of clinical data for each episode of CDI (≥ 3 months between two episodes for the same patient).



A total of 237 fresh stools corresponding to 215 different patients were included in the study.
28 cases of CDI (11.8%) were diagnosed during this study and 10 cases of colonization with a non toxigenic strain of *C. difficile*.

Toxins A/B immunoassay testing
ImmunoCard® toxins AB (ICTAB-Meridian Bioscience) and Vidas® *C. difficile* A&B (bioMérieux) were performed on each stool specimen according to the manufacturer's recommendations.

Toxigenic culture
Fresh stool samples were cultured on selective media (CLO® ,bioMérieux) under anaerobic atmosphere and subcultured after an enrichment step in Schaedler broth (BD). Suspect colonies were located by using UV, 312 nm, identified (API 32A, bioMérieux) and the susceptibility to antimicrobial was tested. The ImmunoCard ToxAB assay was then performed on five to ten colonies, according to the manufacturer's recommendations.

BD GeneOhm CDiff (BD):
real time PCR 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.

Cytotoxcity neutralisation assay (CCNA)
Suspension (1:10) of the specimen 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.

RESULTS

Comparison of performance and characteristics of each tests

Toxigenic culture

Toxigenic culture		CCNA + clinical data	
		Positive	Négative
Toxigenic culture CLO medium	Positive	26	0
	Négative	2	209
	Total	28	209

Se = 93%
Spe = 100%
PPV =100%
NPV = 99%

Modifications for improving the sensitivity of toxigenic culture for the detection of *C. difficile*:
- Information to medical and nurse staffs to reduce the delay for transport of specimen to the laboratory,
- Reduction of the delay of inoculation of fresh specimens,
- Use of UV (312 nm) to detect *C. difficile* colonies,
- Use of enrichment step in broth and subculture.

The modifications made have significantly increased the rate for *C. difficile* recovery and provide more reading comfort.

- In our study, the sensitivity of toxigenic culture is similar to that of the reference technique (CCNA) as it was already described in other laboratories.
- The two false negative results may be explained by the delay for bacterial culture (n=1) and previous administration of metronidazole (n=1).
- Toxigenic culture, using the ICTAB test, showed a total concordance with the CCNA including cases of colonization by nontoxigenic *C. difficile* (data not shown).

Although very sensitive and optimizable, culture remains a demanding and time-consuming test giving definitive results to clinicians in 48 hours in the best case. However, culture remains essential as it constitutes the only technique that allows antimicrobial susceptibility testing and epidemiological surveillance of emerging strains.

Rapid detection of toxin A and B

ICTAB MERIDIAN		CCNA + clinical data	
		Positive	Négative
Immunocard toxins A et B*	Positive	22	7
	Négative	6	200
	Total	28	207*

Se = 78,5%
Spe = 96,5%
PPV = 75,9%
NPV = 97,1%

* 2 non interpretable results

VIDAS CDAB		CCNA + clinical data	
		Positive	Négative
Vidas® *C. difficile* A & B	Positive	21	4
	Négative	6	200
	Equivocal	1	5
	Total	28	209

Se = 77,5%
Spe = 98%
PPV = 84%
NPV = 97,1%

The performance of both toxin enzyme immunoassays evaluated in this study are somewhat similar.
However, although these tests are rapid and easy to perform, they have a low sensitivity compared to cell cytotoxicity neutralization assay (CCNA) or toxigenic culture missing the diagnosis in one fifth of cases.
The two tests differ in their technical characteristics.

MERIDIAN ICTAB
- Adapted for single test but not suitable for series
- Technical time (15 ')
- Reading may be difficult (problem for interpretation in some cases of weak positive (n=2) or non interpretable (n=2) signal)

VIDAS CDAB
- Adapted either for single test or series
- Requires the VIDAS system
- Standardized , reproducible and information-rich values
- Longer delay for result (1h)
- Comfort of reading at the expense of a equivocal zone (2.5% of cases distributed either within positive (n=1) or negative (n=5) cases), for which uncertainty remains about the meaning of equivocal results.

PCR

PCR		CCNA + clinical data	
		Positive	Négative
PCR GeneOhm® Cdiff	Positive	27	1
	Négative	1	208
	Total	28	209

Se = 96,4%
Spe = 99,5%
PPV =96,4%
NPV = 99,5%

(Se= sentivity ; Spe= specificity ;
PPV= positive predictive value ; NPV= negative predictive value)

Molecular assays for various targets have been developed. In this study, we have evaluated the Real-time PCR Cdiff GeneOhm for toxigenic *C. difficile* molecular detection in stools.
- Rapid test giving results within 2 hours (adapted in first line diagnosis for rapid isolation and treatment of patients)
- Highly performant like the gold standard (one false positive result has been reported and may be explained by a succession of freeze-thawing of the sample).
- Fully adapted for series of tests ; single tests may be performed but with a significant additional cost.

- Detect only the toxin B
- Requires a laboratory equipped for molecular biology and experience staff
- Very expensive test up to now …

CONCLUSIONS
How to choose an algorithm that fit our specificities ?

The choice of tests for CDI diagnosis must not be only based on their performance but should also integrate: the cost, the feasibility, and practicability for a routine use in clinical laboratory.
The analysis of the results of this study led to the optimization of our organization and to define the respective position for each test in a new algorithm.
It associates the rapid, automatized, and easy to perform VIDAS test for detection of toxins A&B and systematic and optimized toxigenic culture, whereas CCNA is still used as a confirmatory technique for difficult or discordant cases.
Despite excellent sensitivity, PCR was ruled out because of excessive costs for yet.