Comparison of molecular and glutamate dehydrogenase tests in a multistep diagnostic algorithm in the laboratory diagnosis of Clostridium difficile

Erwin Ho*[1], Werner Van Nijnatten[1], Ingrid Mermans[1], Margareta Ieven[1]

[1]*Department of Clinical Biology – Microbiology, Antwerp University Hospital, Antwerp, Belgium *erwin.husin@gmail.com*

1. Introduction/aim

In recent years, the laboratory diagnosis of *Clostridium difficile* infection - the etiologic pathogen of antibiotic-associated diarrhea – has evolved to allow more rapid test results using novel principles.

Glutamate dehydrogenase is a metabolic enzyme produced by *Clostridium difficile* and encoded by the gluD gene. It is common in both toxigenic and non-toxigenic *Clostridum difficile*. By contrast, *Clostridium difficile* toxin A and B are encoded at the Pathogenicity Locus (PaLoc) by the tcdA and tcdB gene, and are only present in pathogenic *Clostridium difficile*.

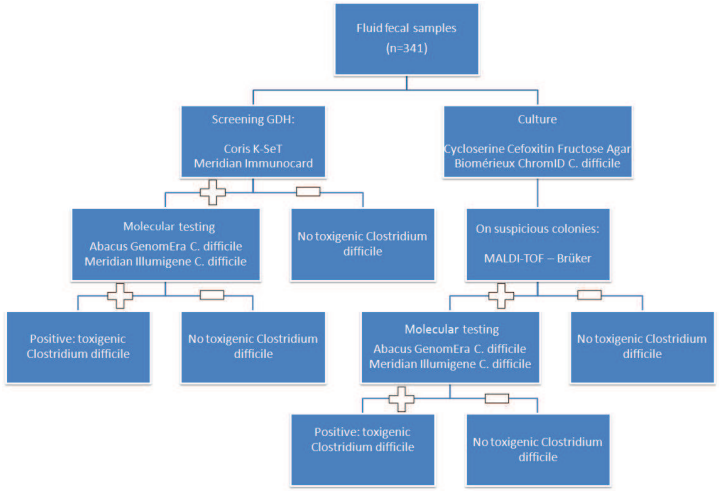
The aim of this study is to compare the performance characteristics of 2 new glutamate dehydrogenase tests (Meridian Immunocard GDH and Coris BioConcept Clostridium K-SeT) and 2 new molecular tests (Meridian Illumigene C. difficile and Abacus GenomEra C. difficile), in a multiple-step algorithm in the laboratory diagnosis of *Clostridium difficile*.

2. Methods and materials

341 fresh fecal samples were analyzed in one centre between 11/2012 and 5/2013. Only liquid fecal samples were included in the study.

Both GDH tests were performed on all samples. Both molecular tests were performed on GDH positive fecal samples. Molecular tests were also performed on suspicious colonies after culture on chromogenic Biomérieux ChromID C. difficile plates. Turnaround time for the Coris GDH test was 15 minutes, and for the Meridian GDH test 30 minutes. Both molecular tests yielded results within 60 minutes. The reference method was the cytotoxicity assay and toxigenic culture (on Cycloserine Cefoxitin Fructose Agar). Discordant results between tests and the reference method were evaluated by clinical background.

To compare the diagnostic performances, ROC curve Area-Under-the-Curve statistical analysis was performed.



3. Results

3.1. Diagnostic performance of GDH tests

Coris BioConcept K-Set GDH	CCFA (+)	CCFA (-)
(+)	18	25
(-)	2	297

Sensitivity: 90.0%
Specificity: 92.2%

Meridian ImmunoCard GDH	CCFA (+)	CCFA (-)
(+)	18	21
(-)	2	299

Sensitivity: 90.0%
Specificity: 93.4%

ROC curve AUC analysis: p= 0.78

3.2. Diagnostic performance of molecular tests ***on sample***

Abacus GenomEra C. difficile	Cytotoxicity assay/toxigenic culture (+)	Cytotoxicity assay/toxigenic culture (-)
(+)	7	2
(-)	0	20

Sensitivity: 100.0%
Specificity: 92.2%

Meridian Illumigene C. difficile	Cytotoxicity assay/toxigenic culture (+)	Cytotoxicity assay/toxigenic culture (-)
(+)	14	6
(-)	2	26

Sensitivity: 87.5%
Specificity: 81.3%

ROC curve AUC analysis: p= 1.00

3.3. Diagnostic performance of molecular tests ***after culture***

Abacus GenomEra C. difficile after culture	Cytotoxicity assay/toxigenic culture (+)	Cytotoxicity assay/toxigenic culture (-)
(+)	7	2
(-)	0	5

Sensitivity: 100.0%
Specificity: 71.4%

Meridian Illumigene C. dfficile after culture	Cytotoxicity assay/toxigenic culture (+)	Cytotoxicity assay/toxigenic culture (-)
(+)	15	6
(-)	0	9

Sensitivity: 100.0%
Specificity: 60.0%

ROC curve AUC analysis: p= 1.00

After culture, sensitivity improved to 100% for both the Abacus GenomEra test and the Meridian Illumigene test. Specificity data for molecular tests are underestimated due to lack of (true) negative results. False positive results were all found to be clinically suspicious.

4. Conclusion

- In a multiple step diagnostic algorithm combining GDH testing and PCR testing, both the Meridian and Coris GDH and the Meridian and Abacus molecular tests had similar performance characteristics
- In terms of user friendliness, the combination of the Coris BioConcept Clostridium K-Set with the Abacus GenomEra C. difficile PCR test could be recommended; however taking into account cost, a combination of the Meridian Immunocard GDH and Meridian Illumigene C. difficile molecular test was preferred in our patient population