

Revised Abstract

Introduction

Laboratory diagnosis of *Clostridium difficile* infection (CDI) poses a significant challenge. No single test is adequate to diagnose all cases of CDI.<sup>1</sup> Rapid and accurate diagnosis is fundamental to the management of CDI cases and effective infection prevention and control. *Clostridium difficile* toxin assays though simple and easy to perform have been shown to have a wide range of sensitivities (30-70%).<sup>1,2</sup> In a setting of widespread testing (as in the UK) in all patients with diarrhoea >2 years of age the sensitivity and positive predictive value (PPV) of a positive test is highly variable. Therefore, there is a need for an efficient and effective diagnostic strategy. Following a recent evaluation,<sup>3</sup> in our laboratory a GDH-screening-based algorithm ('3 step CDI testing algorithm') is used for routine diagnosis. This has provided an opportunity to compare the performance characteristics of a commercial *Clostridium difficile* real-time PCR assay (C diff RT-PCR) in comparison with our '3 step CDI testing algorithm' and evaluate its potential use as a single test or as a component of a '2-step diagnostic algorithm'.

Methods

Three hundred and eighty one consecutive diarrhoeal stool specimens (stools taking the shape of the container) submitted for routine CDI testing (July-August 2009) were included. Each specimen was concurrently tested with BD GeneOhm™ C diff RT-PCR assay and our current routine diagnostic '3-step C diff testing algorithm'

The BD GeneOhm™ C diff RT-PCR assay is a rapid, qualitative PCR assay for the detection of the toxin B gene (*tcdB*), performed on the Cepheid SmartCycler® (Cepheid, Sunnyvale, CA). The amplification, detection and interpretation of the signals are done automatically by the SmartCycler® software.

'3 step CDI testing algorithm': (Fig 1)

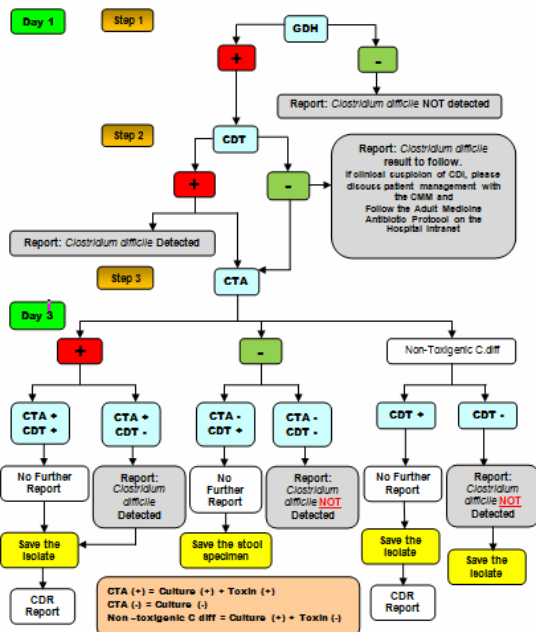
**Step 1:** Glutamate dehydrogenase antigen (GDH)- (C.DIFF CHEK™ 60;TECHLAB [Inverness Medical Innovations, Inc.]) screening

**Step 2:** A rapid confirmatory direct stool *Clostridium difficile* toxin (CDT) assay (Premier™ Toxins A & B EIA [Meridian Bioscience, Cincinnati, OH]) for GDH positive stools

**Step 3:** A 'culture-toxin assay' (CTA) i.e. anaerobic stool culture on Brazier's CCEY medium (Oxoid, Basingstoke UK) for 48h followed by confirmation of the isolate as *C. difficile* and confirmation of toxinogenicity of the isolate using Premier™ toxins A & B EIA for GDH screen (+) and CDT (-) specimens.<sup>4</sup>

All specimens, except those negative by both C diff RT-PCR and GDH were tested by CTA.

Fig 1: Laboratory *Clostridium difficile* infection (CDI) Testing Algorithm



Results

•Of the 381 specimens included in the study one (1/381, i.e. 0.3%) GDH (-) specimen after repeated testing yielded an unresolved PCR result and was excluded from the final result analysis. (Fig 2,3 & Table 2)

•The CTA was used as the 'gold standard'. The prevalence of 'CTA' (+) specimens was 5% (19/380).

•352/380 (93%) specimens yielded concordant results in both the C diff RT-PCR and '3 step CDI testing algorithm'.

- 338/380 (89%) specimens were tested (-) by both PCR and GDH and were considered 'true negative'.
- 14 GDH (+)/CTA (+) and PCR (+) specimens were considered 'true positive'.

Results cont'd.

•A total of 28 discrepant results were resolved using CTA.

- 16 GDH (+)/CTA (-) and PCR (-) specimens were considered 'true negatives'.
- An additional 5 GDH (+)/CTA (-) yielded a 'non-toxicogenic' *Clostridium difficile* isolate and were considered 'true negatives'
  - 4/5 were PCR (-), 1/5 was PCR (+)
- 4 GDH (+)/CTA (+) and PCR (-) specimens and one GDH (-)/CTA (+) and PCR (+) specimen, were considered 'true positives'
- 3 PCR (+) and CTA (-) specimens considered 'false (+) PCR' results

Fig 2: PCR vs. GDH Based 3 Step CDI Testing Algorithm

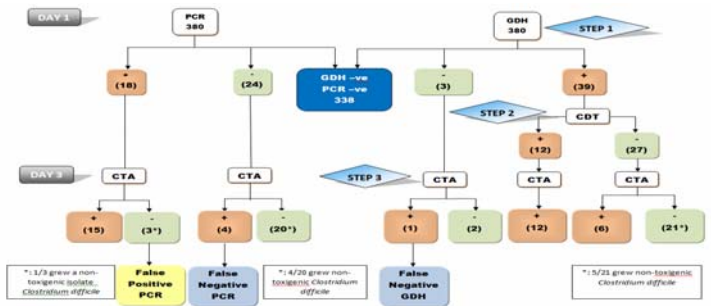


Table 1:

Test	Result	No. of Samples with CTA Result		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
		(+)	(-)				
GDH-screening	(+)	18	21	95	94	46	99.7
	(-)	1	340				
C diff RT-PCR	(+)	15	3	79	99	83	98.9
	(-)	4	358				

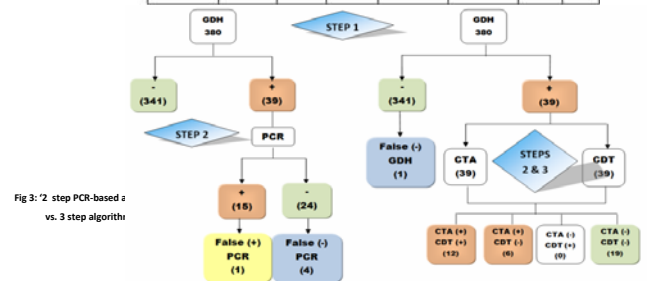


Fig 3: '2 step PCR-based vs. 3 step algorithm'

Discussion

- Due to previously reported high NPVs of both the C diff RT-PCR<sup>5-8</sup> and the GDH assays<sup>9-12</sup> the stool specimens that tested negative by both the C diff RT-PCR and GDH assays were considered true negative without further testing.
- Four C diff RT-PCR negative and CTA positive specimens represent true CDI cases.
  - 3/4 *Clostridium difficile* isolates were C diff RT-PCR (+) and 'cytotoxicigenic culture (+) using MRC-5 fibroblast culture (Hospital Saint Antoine, Rue Du Saint-Antoine, Paris France). 1 isolate was not tested
  - 5/5 specimens that grew a 'non-toxicogenic' *Clostridium difficile* isolate were all direct stool CDT negative and 4/5 were C diff RT-PCR negative (isolates were not tested by the C diff RT-PCR).
- The toxinogenicity testing of *Clostridium difficile* culture isolate using CDT assay however, is less sensitive than cell cytotoxicity neutralisation assay, but the latter requires 48 h to complete.
- Both false positive PCR and false negative PCR are a cause for a significant clinical concern.

Conclusions

- In a setting of CDI prevalence ranging from 2%-10% and widespread testing the GDH-screening-based '3 step CDI testing algorithm' is clinically and economically the most effective diagnostic strategy. i.e., £20 for C diff RT-PCR vs. £4 for (GDH [-] sample), ~ £14 for (GDH [+] sample),
- The GDH-screening-based '3 step CDI testing algorithm' combines the sensitivity of GDH, speed of CDT assay and high specificity of CTA. 93% of (GDH [-] and GDH [+] / CDT [+] specimens can be reported in <6 h and the remaining GDH (+) and CDT (-) within 48-72 h of receipt of specimens
- Additionally, this strategy facilitates further study of an isolate, such as antimicrobial susceptibility testing and investigating the molecular epidemiology of CDI

References

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