Clostridium difficile

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Diagnosis of Clostridium difficile: Real-time PCR Detection of Toxin Genes in Faecal Samples is More Sensitive Compared to Toxigenic Culture

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Introduction

Clostridium difficile (CD) is the most frequent cause of nosocomially acquired diarrhoea and the rates of incidence and severity are increasing. A hyper-virulent strain of CD, PCR ribotype 027 (CD027), has emerged, shown to hyper-produce the two major virulence factors of CD, toxins A and B. Characteristic of CD027 is a deletion at position 117 (Δ117) in the putative negative regulator gene *tcdC* of toxins A and B, and the production of a third toxin the binary CD toxin (CDT). (Figure 1).

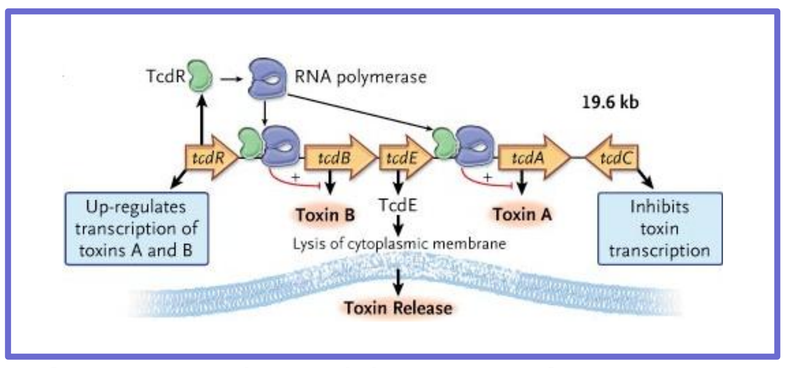

Figure 1. Pathogenicity Locus of CD
(*Kelly & LaMont, 2008. NEJM)*

Objectives

The aim of this study was to evaluate four DNA-amplification methods (Table 1) detecting toxigenic CD, including CD027, directly from faecal samples. Thereby to establish a rapid routine diagnostic test of CD based on the evaluation.

Toxin Gene Target	*a) In-house*	*b) Illumigene® C.difficile*	*c) PCRFast® C.difficile*	*d) Xpert® C.difficile*
tcdA	+	+	+	
tcdB	+	(+)	+	+
tcdC/Δ117 tcdC	+/+			-/+
cdt	+			+
CD027	+			+

Table 1. Toxin gene targets.

Methods

A total of 300 faecal samples from 284 Danish hospitalized patients with diarrhoea were included consecutively from mid February to the beginning of April 2011. CD was detected routinely by anaerobic culture on cycloserine cefoxitin fructose agar for 48 hours. Genotypic toxin profiling by PCR and PCR ribotyping were performed on CD culture positive samples. In parallel, the samples were analysed for toxigenic CD by a) an "in-house" multiplex Real-time (RT) PCR two-step algorithm and three commercial DNA-amplification methods: b) Illumigene® *C. difficile* [Meridian Bioscience]; c) PCRFast® *C. difficile A/B* [Simoco Diagnostics] and d) Xpert® *C. difficile* [Cepheid]. Assay a) and d) detects presumptive CD027; b) and c) detects *tcdA/B* only (Table 1).

Results

Assay a) and d) were significantly more sensitive compared to toxigenic culture. The concordance between a) and d) was 97.3% (292/300). Re-culture and/or prolonged incubation time (3-7 days) of the culture negative, but a) and d) CD positive samples, increased the culture positive rate by 29% (from 38 to 49/300). Compared to initial culture as the reference standard (Figure 2A), the re-culture data (Figure 2B) resulted in an increased positive predictive value (PPV) (> 80%) and specificity (> 95%) for all assays, although a reduced negative predictive value (NPV) (> 90%) and sensitivity (63-95%) of all but d) which had a NPV and sensitivity of 100%. RT PCR inhibition was <1% (0 and 2/300) in a) and d) respectively i.e. in Bovine Serum Albumin (BSA) optimized assays. In contrast, PCR inhibition was 13% (38/300) in c) not containing BSA. Preliminary results from PCR ribotyping showed 94% and 97% concordance of a) and d) respectively for the detection of CD027.

	a) In-house		*b) Illumigene® C.difficile*		*c) PCRFast® C.difficile*		*d) Xpert® C.difficile*		
Toxigenic Culture	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Total
Positive	37	1	36	2	29	9	38	0	38
Negative	21	241	11	251	5	257	20	242	262
Total	58	242	47	253	34	266	58	242	300
PPV	63.8%		76.6%		85.3%		65.5%		
NPV	99.6%		99.2%		96.6%		100.0%		
Sensitivity	97.4%		94.7%		76.3%		100.0%		
Specificity	92.0%		95.8%		98.1%		92.4%		

Figure 2A. Routine culture as the reference standard.

	a) In-house		*b) Illumigene® C.difficile*		*c) PCRFast® C.difficile*		*d) Xpert® C.difficile*		
Toxigenic Culture	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Total
Positive	47	2	41	8	31	18	49	0	49
Negative	11	240	6	245	3	248	9	242	251
Total	58	242	47	253	34	266	58	242	300
PPV	81.0%		87.2%		91.2%		84.5%		
NPV	99.2%		96.8%		93.2%		100.0%		
Sensitivity	95.9%		83.7%		63.3%		100.0%		
Specificity	95.6%		97.6%		98.8%		96.4%		

Figure 2B. Re-culture as the reference standard.

Conclusion

Assay a) and d) are sensitive diagnostic methods and their high concordance suggests that a) and d) CD positives, but culture negative faecal samples are true positives. Both methods are usable as rapid primary diagnostic tests for toxigenic CD and for presumptive identification of CD027. The current toxigenic culture method is sub-optimal for primary diagnostics of CD. BSA efficiently reduces RT PCR inhibition and could be considered included in RT PCR protocols for detection of CD genes in faecal samples.

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