RESEARCH NOTE

Effect on diagnostic yield of repeated stool testing during outbreaks of *Clostridium difficile*-associated disease

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ABSTRACT

The effect on diagnostic yield of testing sequential stools was assessed during two hospital epidemics of *Clostridium difficile*. Using a rapid immunoassay, *C. difficile*-associated disease was diagnosed in 237 diarrhoeal patients, of whom 204 (86%) were diagnosed from the first faeces sample and 12 (5%) were diagnosed from follow-up samples obtained within 1 week. The remaining 21 (9%) patients yielded a positive test from stools obtained >1 week after the initial negative sample. It was concluded that repeated testing of stools for *C. difficile* toxin is of value in controlling outbreaks of *C. difficile* infection.

Keywords *Clostridium difficile*, diagnostic yield, diarrhoea, immunoassay, repeated tests, toxin detection

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Clostridium difficile-associated disease (CDAD) is one of the most common hospital-acquired infections [1]. Early recognition of CDAD patients is of prime importance to prevent spread and to enable rapid implementation of adequate isolation and hygiene procedures and the initiation of CDAD-specific therapy. For rapid diagnosis, a fast, one-step immunoassay (ICTAB; Meridian Bioscience Europe, Boxtel, The Netherlands) is available for the detection of *C. difficile* toxins A and B in faeces samples. Using the cell cytotoxicity test as a reference standard, the relative sensitivity and specificity, and positive and negative predictive values of the ICTAB assay were 91%, 97%, 70% and 99%, respectively [2]; similarly, Diederen *et al.* [3] reported a relative sensitivity of 88.6% compared with the cytotoxicity test.

Current guidelines for the diagnosis of CDAD recommend analysis of additional samples for *C. difficile* toxin when the first sample is negative and clinical suspicion is high [4,5]. This recommendation has been disputed in two published studies [6,7]; however, both of these studies were performed in an endemic situation. The purpose of the present study was to assess the effect of sequential analysis of stools on diagnostic yield when using the ICTAB immunoassay as an alternative to the cytotoxicity test in CDAD outbreaks caused by *C. difficile* strains belonging to PCR ribotypes 027 and 017.

A CDAD epidemic caused by *C. difficile* PCR ribotype 027/toxinotype III occurred in hospital A between April and September 2005, with the incidence of CDAD increasing rapidly from 3.8 to 58.4/10 000 admissions. At a distance of 35 km, a second epidemic occurred in hospital B between May 2005 and October 2006, caused by *C. difficile* PCR ribotype 027/toxinotype III and PCR ribotype 017/toxinotype VIII. Physicians were instructed to collect stools from all diarrhoeal patients who were hospitalised for >3 days and/or who were clinically suspected of CDAD. Samples were tested within 24 h of arrival at the laboratory because of possible toxin degradation. The ICTAB immunoassay was performed at least twice-daily for as long as the epidemics continued. Following a negative result, the responsible clinicians were requested to resample diarrhoeal patients, preferably within 48 h. When both tests were negative, CDAD was considered unlikely, and a new test was requested and the corresponding sample was

Table 1. Value of repeated testing with immunocard toxins A and B (ICTAB) for patients with *Clostridium difficile*-associated disease

	Hospital A	Hospital B			
			Subdivided by PCR-ribotype[b]		
Patient characteristics	All ribotypes[a]	All ribotypes	027	017	Other ribotypes
Number of CDAD patients	50	187	40	47	60
ICTAB-positive with first sample (% of all positive patients)	43 (86%)	161 (86%)	36 (90%)	40 (85%)	51 (85%)
ICTAB-positive with repeated sample ≤1 week (cumulative % of all positive patients)	4 (94%)	8 (90%)	1 (93%)	3 (91%)	1 (87%)
ICTAB-positive with repeated sample >1 week (cumulative % of all positive patients)	3 (100%)	18 (100%)	3 (100%)	4 (100%)	8 (100%)

[a]PCR-ribotyping was only performed for ribotype 027 in hospital A (25 isolates, 76%).
[b]Not all isolates were available for typing.

cultured only if clinical suspicion remained. Toxin-positive faeces were cultured for the presence of *C. difficile* and isolates were identified as described previously [8]. PCR-ribotyping was also performed as described previously [9].

During the epidemic in hospital A, 50 patients eventually yielded an ICTAB-positive sample, with 43 (86%) patients being ICTAB-positive on initial testing (Table 1). Within 7 days, a second sample was collected from 131 patients who were initially ICTAB-negative, of whom three (2%) were positive with the second sample; thus, 46 (92%) patients were diagnosed correctly with CDAD following two sequential samples. One additional patient was ICTAB-positive with a third sample, also obtained within 7 days, and three (2%) patients were positive with samples taken within, on average, 24 days of the first sample. Considering the interval between samples, this suggested a new infection. The final four samples mentioned above were confirmed by specific culture of *C. difficile*. Of the ICTAB-positive samples, 37 were available for culture, with 33 (90%) yielding *C. difficile*. Twenty-five (76%) isolates were identified as *C. difficile* PCR ribotype 027. The remaining eight isolates belonged to various other PCR ribotypes. A comparison of patients with CDAD caused by PCR ribotype 027 and other PCR ribotypes revealed no differences in the test results.

In hospital B, 187 patients were diagnosed with CDAD, of whom 161 (86%) were found to be ICTAB-positive on initial testing (Table 1). Following a negative first test, 15 patients were resampled within 1 week, of whom eight were positive. Thus, CDAD was diagnosed in <1 week in 169 (90%) of 187 patients. In addition, two patients were found to be ICTAB-positive with a second sample obtained 10 days after the first negative sample. The remaining 16 patients were diagnosed as positive with samples taken >14 days after the initial sample. Of the total of 187 ICTAB-positive samples, 165 were cultured for the presence of *C. difficile*, with 149 being culture-positive. Isolates from 147 samples were available for further typing (Table 1). The epidemic strains isolated from patients in hospital B were identified as PCR ribotypes 017 (toxinotype VIII; $n = 47$) and 027 (toxinotype III; $n = 40$). The remaining 60 isolates belonged to a range of PCR ribotypes.

Thus, overall, 12 (5%) of 237 diarrhoeal patients from hospitals A and B were diagnosed following the analysis of one or more additional samples within a week of the initial negative result. An additional 21 (9%) samples became positive within, on average, 45 days of the initial sample, which probably reflects the development of CDAD in diarrhoeal patients after the observation period of 1 week. Of 202 positive samples from hospitals A and B, 20 (10%) were negative by culture for *C. difficile*. Importantly, in both hospitals, all retested and subsequently cultured ($n = 9$) ICTAB-positive samples that were taken within 1 week of the first negative sample yielded a positive culture for *C. difficile*.

In conclusion, testing of multiple stool samples, collected at an interval of a few days, for *C. difficile* toxin appears to be of value for combating outbreaks of toxin-producing *C. difficile*. In particular, when highly epidemic strains are involved, the additional costs of repeated testing may be rapidly offset by the benefits associated with prevention of spread of the disease, including preventing closure of wards and expensive treatment of patients.

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