Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-015-2320-2

ARTICLE

Comparison of BD Max Cdiff and GenomEra *C. difficile* molecular assays for detection of toxigenic *Clostridium difficile* from stools in conventional sample containers and in FecalSwabs

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Received: 4 December 2014 / Accepted: 7 January 2015

Abstract In this study, the usability and performance of GenomEra™ *C. difficile* and BD Max™ Cdiff nucleic acid amplification tests (NAATs) for the detection of toxigenic *Clostridium difficile* were investigated in comparison with toxigenic culture and *C. difficile* toxin A- and toxin B-detecting immunochromatographic antigen (IA) test, the Tox A/B QuikChek®. In total, 302 faecal specimens were collected, 113 of which were in parallel to conventional sample containers and FecalSwab liquid-based microbiology (LBM) tubes. Seventy-nine specimens were considered true-positives for toxigenic *C. difficile*. The sensitivity and specificity were 97.5 % and 99.6 % and 93.7 % and 98.7 % for the GenomEra and BD Max assays respectively. Toxigenic culture and Tox A/B QuikChek had sensitivity and specificity of 91.1 % and 100 % and 34.2 % and 100 % respectively. Hands-on time for analysing 1 to 24 specimens using NAATs was 1 to 15 min. The rate of PCR inhibition was 0 % for both NAATs with faeces in LBM tubes, while with faeces in conventional sample containers the respective inhibition rates were 5.3 % and 4.4 % for the GenomEra and the BD Max assays. The NAATs demonstrated an excellent analytical performance, reducing significantly the overall workload of laboratory personnel compared with culture and IA test.

Introduction

Clostridium difficile, a Gram-positive anaerobic rod, is recognised as one of the commonest causes of hospital-acquired and antibiotic-associated diarrhoea throughout the world [1, 2]. *C. difficile* infection (CDI) results from the main virulence factors, toxin A (TcdA) and toxin B (TcdB), produced by the bacterium [3, 4]. In addition, a separate binary toxin, which is produced by a group of isolates with or without TcdA and/or TcdB [5, 6], has been suggested to play a part in the recurrence and in the severity of CDI [7]. As CDI is associated with an increase in the length of hospitalization and mortality, leading to augmented health-care costs [2, 6, 8], the rapid and reliable detection of toxigenic *C. difficile* is important.

Traditionally, culture-based methods, such as cytotoxigenic culture and cytotoxin assay, have been used as a gold standard for *C. difficile* screening. These are known to be cost-effective but time-consuming approaches [9–13]. An alternative approach is the detection of *C. difficile* toxins or glutamate dehydrogenase (GDH) in stool samples using immunochromatographic antigen (IA) tests or enzyme immunoassays (EIA) [14–16]. Although more rapid, these assays have been shown to be less sensitive and less specific than culture. Furthermore, relying only on GDH detection reveals nothing on the toxigenic nature of the possible *C. difficile* isolates.

In recent years, the direct detection of genes encoding *C. difficile* toxin A and/or toxin B with nucleic acid amplification tests (NAATs) has become a diagnostic target of interest [17–19]. NAATs have shown to be more sensitive than IA or EIA and in some studies even more sensitive than the cytotoxigenic culture or cytotoxin assay [14, 20–26]. The main advantage of NAATs, in addition to the high sensitivity and specificity, is the short turn-around time, compared with conventional culture. As the number of different NAATs for the detection of toxigenic *C. difficile* is rapidly increasing, comprehensive studies to determine the assay's quality and usefulness in clinical laboratories and for point-of-care (POC) settings are required.

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Published online: 24 January 2015

Here, we investigated the usability of two recently launched, *tcdB* gene-detecting, PCR assays, the GenomEra *C. difficile* (Abacus Diagnostica, Turku, Finland), and the BD Max Cdiff (Becton, Dickinson and Company, NJ, USA) for the detection of *C. difficile* in faecal specimens. Results were analysed in comparison with a single-use POC compatible IA test, the Tox A/B QuikChek® (Alere Limited, Stockport, UK) and toxigenic culture. Along with the assessment of performance, workload analysis and the ease of result interpretation were conducted for each test. Apart from the method comparison, the utility of a liquid-based microbiology (LBM) tube, the FecalSwab (Copan Italia, Brescia, Italy), for the screening of *C. difficile* was also investigated.

Materials and methods

A total of 302 loose stool specimens, one specimen per patient, were prospectively collected from inpatients (the patients' mean age was 70 years, ages ranging from 7 to 95 years) at Vaasa Central Hospital, Finland, according to hospital routine practice in antibiotic-associated diarrhoea. Of these, 185 were collected into conventional sample containers and 113 were collected in parallel into one sample container and FecalSwab LBM tube. All specimens were analysed immediately after receipt into the laboratory using all four methods: the GenomEra™ *C. difficile*, the BD Max Cdiff™ assay, the Tox A/B QuikChek® and toxigenic culture.

Both NAATs and the IA test were performed as described in previous studies [27–29], according to the manufacturer's instructions. However, a minor variation with the BD Max Cdiff assay for sample collection was implemented when specimens were in FecalSwabs. Fifty microliters of homogenised stool was used for the assay run, rather than 10 μL, as it was found to be the optimal amount of sample in the BD Max buffer tube (data not shown). Toxigenic culture was performed by plating the specimen on cycloserine cefoxitin egg-yolk agar (CCEY) medium (Oxoid Limited, Basingstoke, UK) and incubating the plate for 48 h in an anaerobic atmosphere at +35 °C. Presumed growth of *C. difficile* and the toxigenic nature of the bacterium were confirmed by Gram staining, UV light, and the IA test (Wampole C. diff QuikChek Complete; Alere Limited) targeting *C. difficile*-specific glutamate dehydrogenase (GDH) and *C. difficile* toxins A and B. Hands-on time and total turnaround time for each test method were assessed by investigating the time elapsed for 1 to 24 specimens by three laboratory specialists.

Specimens were defined as true-positive for toxigenic *C. difficile* when the bacterium was isolated and its toxin production was confirmed by toxigenic culture, or when both NAATs reported a positive *tcdB* result regardless of a negative growth result in toxigenic culture, or when one of the NAATs in conjunction with the IA assay yielded a positive result regardless of a negative growth result in toxigenic culture. Fisher's exact test was used to determine the statistical significance of the differences among the various test methods.

Results

Of the 302 stool specimens, 79 (26.2 %) were considered true-positive for toxigenic *C. difficile*. Seventy-two (91.1 %) specimens yielded the growth of toxin-producing *C. difficile* and 7 (8.9 %) specimens were *tcdB* positive by both NAATs while being culture negative (Table 1). Furthermore, the BD Max Cdiff reported three additional positive results and the GenomEra *C. difficile* reported one positive result, which, however, remained negative according to all other methods and, thus, were determined as false-positives.

Table 1 Result cross-check of 302 stool specimens screened with toxigenic *C. difficile* culture, GenomEra *C. difficile*, BD Max Cdiff and ToxA/B QuikChek antigen test

Methods		GenomEra *C. difficile*		BD Max Cdiff		Tox A/B Quick Chek	
		Negative	Positive	Negative	Positive	Negative	Positive
Toxigenic culture	Negative	222	8[a]	220	10[b]	230	0
	Positive	2	70	5	67	45	27
GenomEra *C. difficile*	Negative			221	3[c]	224	0
	Positive			4[d]	74	51	27
BD Max Cdiff	Negative					225	0
	Positive					50	27

[a] Seven were also positive according to the BD Max and only one according to the GenomEra

[b] Seven were also positive according the GenomEra and three only by the BD Max

[c] Positive only according the BD Max

[d] Three were also positive according to toxigenic culture and only one according to the GenomEra

Of the 79 true-positive specimens, the BD Max Cdiff detected 74 and the GenomEra *C. difficile* detected 77 (Table 1). Using the Tox A/B QuikChek IA test, only 27 of the 79 specimens were detected as positive. The respective sensitivity and specificity were 91.1 % (95 % CI, 84.8–97.4 %) and 100 % for toxigenic culture, 93.7 % (95 % CI, 88.3–99.1 %) and 98.7 % (95 % CI, 97.2–100 %) for the BD Max, 97.5 % (95 % CI, 94.1–100 %) and 99.6 % (95 % CI, 98.8–100 %) for the GenomEra, and 34.2 % (95 % CI, 23.7–44.7 %) and 100 % for the Tox A/B QuikChek IA test. Positive and negative predictive values (PPV and NPV) were 100 % and 97.0 % for toxigenic culture, 96.3 % and 97.8 % for the BD Max, 98.8 % and 99.1 % for the GenomEra, and 100 % and 81.1 % for the Tox A/B QuikChek IA respectively.

The PCR inhibition rate of the BD Max was 4.4 % (5 out of 113) with faeces in conventional containers and 0 % (0 out of 113) with faeces in FecalSwabs. The PCR inhibition rate of the GenomEra was 5.3 % (6 out of 113) with faeces in conventional containers and 0 % (0 out of 113) with faeces in FecalSwabs.

Hands-on time for analysing 1 to 4 specimens was 1 to 2.5 min for the GenomEra, 1.5 to 3 min for the BD Max, 2.5 to 5.5 min for the Tox A/B QuikChek IA test, and 5 to 10 min for culture (Table 2). Further, the test run time for the same amount of specimens was 55 min with the GenomEra, 85 min with the BD Max, and 25 min with the Tox A/B QuikChek. Using culture, approximately 48 h was needed for each specimen in the final results. To analyse 24 specimens, the hands-on time was 15 min for the GenomEra, 10 min for the BD Max, 38 min for the Tox A/B QuikChek, and 110 min for culture.

Result interpretation was considered to be easiest with the NAATs, and least agreeable with the Tox A/B QuikChek owing to the variable quality of the colour line indicating a positive result. With the GenomEra assay the test results were reported by the assay software in numerical form from −15 (negative) to +100 (strongly positive) for the *tcdB* together with a written conclusion: "*C. difficile tcdB* negative", "inconclusive", or "positive". The BD Max assay reported amplification curves and Ct values, together with a written conclusion: "*C. difficile* toxin B positive" or "negative". Oddly, in 2 cases the BD Max instrument reported a negative test result, while on the raw data sheet a low but definite amplification curve for the *tcdB* target was seen. These specimens were positive according to the GenomEra. In addition, in one case the BD Max reported a positive test result, although there was no amplification curve visible. This specimen was negative according to all other methods.

Table 2 Processing time of the different test methods in the detection of *C. difficile*

Number of samples	BD Max Cdiff		GenomEra *C. difficile*		Tox A/B QuikChek		Toxigenic culture	
	HOT (min)	TOT (min)	HOT (min)	TOT (min)	HOT (min)	TOT (min)	HOT (min)[d]	TOT (h)
1	1.5	87	1	56	2.5	28	5	48
4	3	88	2.5	58	5.5	30	10	48
24	10	145	15	308[b]	38	155[c]	110	48
48[a]	22	255	30	608	76	305	220	48

HOT hands-on time, *TOT* total turnaround time

[a] HOT and TOT measured with 1 to 24 samples and estimated for 48 samples

[b] Assay runs performed in a batch of four specimens owing to the limitation of the instrument's capacity

[c] Tests performed in a batch of four specimens

[d] Includes plating of a specimen and examination of the plated specimen

Discussion

The purpose of this study was to compare the performance of two new automated NAATs, the GenomEra *C. difficile* and the BD Max Cdiff, and to investigate their utility for the detection of toxigenic *C. difficile* in comparison with the POC-compatible toxin A/B IA test and toxigenic culture. Both NAATs demonstrated excellent sensitivity and specificity in our sample material (AAD patients). There were 7 (8.9 %) confirmed positive *C. difficile* specimens in which the infectious agent was only detectable by the NAATs and not by culture. This finding is consistent with previous studies investigating the performances of various test methods for the detection of toxigenic *C. difficile* [14, 20–27]. Differences in sensitivity between the NAATs and culture were not, however, significant (P value>0.5). An additional 4 specimens were reported to be positive by one of the NAATs (3 by the BD Max and 1 by the GenomEra), but these could not be confirmed by any other tests, and were thus considered false-positives.

Compared with the POC-compatible toxin A/B IA test, NAATs improved significantly the detection of toxigenic *C. difficile* (P value<0.0001), a finding that is also familiar from earlier reports [14, 20–26]. Thus, when toxin A/B IA tests are used as stand-alone tests in clinical microbiological laboratories, many clinical presentations compatible with CDI may remain without confirmation or may be erroneously considered *C. difficile*-negative. Moreover, IA tests are prone to subjective result interpretation, unlike the automated NAATs. The detection of CDI with IA or EIA tests may be improved using 2- to 3-step diagnostic algorithms [30]. These approaches combine a preliminary GDH screening test (more sensitive test) with toxin A- and/or toxin B-detecting EIA, NAATs or culture. Recent studies have, however, demonstrated that the sensitivity of 2- to 3-step algorithms may still be as low as 41–68 % [31]. In addition, these approaches

undoubtedly increase the workload of laboratory personnel and extend the total turnaround time.

It should be noted, though, that the level of sensitivity needed for CDI diagnostic testing is not yet clear, as stated recently by Stellrecht et al. [28]. It has been assumed that sensitive methods, such as culture or NAATs, are not able to discriminate between CDI and asymptomatic colonisation, as the asymptomatic carriage of toxigenic *C. difficile* among children and elderly inpatients, and among patients in extended care facilities (i.e. nursing homes), can be common [32–35]. However, the detection of asymptomatic carriage may have relevance, if the diagnostic purpose is to investigate the transmission of *C. difficile* in different health care settings [32, 35]. Thus, good practice requires careful consideration of testing indication, and in the case of CDI, attention should be paid to performing NAATs on symptomatic patients only [31].

In some recently published studies, NAATs have been stated to be an uneconomical choice for the screening of toxigenic *C. difficile* because of the higher cost of reagents and consumables compared with culture or IA tests [15, 16]. However, Brecher et al. highlighted in their recent review that although the cost of a test is low, it is of little value if the result is inaccurate and has to be repeated many times over several days to get an accurate result [31]. We observed that hands-on time in addition to the total processing time was considerably shorter with the BD Max Cdiff and with the GenomEra *C. difficile* than with IA or culture. Hence, NAATs reduces labour costs. Furthermore, using the NAATs, the results were reported more reliably and more rapidly, which we believe to be essential in decreasing the need for retesting and in reducing unnecessary treatment and isolation of patients.

As regards PCR inhibition, it is known to have a notable effect on the diagnostic performance of the NAATs [36]. Thus, by eliminating the problems due to inhibitors, the NAATs are clearly superior to the conventional test methods. In our study, no PCR inhibition was observed with faeces in FecalSwabs, while with faeces in conventional sample containers the inhibition rates were 4.4 % and 5.3 %, depending on the test used. Although the number of specimens tested for this particular study phase was quite low (n=113), this finding may still provide useful information for further experiments on LBM tubes, improving the utility and performance of the automated NAATs. Because of a more homogenised and diluted form, specimens in FecalSwab tubes are more suitable for use with the NAATs than specimens in conventional containers. Furthermore, LBM tubes have proved to be suitable for extended storage and transportation of enteric pathogens, including toxigenic *C. difficile* [37], enabling successful and reliable microbiological analysis when specimens are sent to either a local laboratory or a more distant reference laboratory.

In conclusion, the BD Max Cdiff and the GenomEra *C. difficile* assays are both accurate and well-performing diagnostic tests for the rapid and reliable detection of *C. difficile*. The GenomEra *C. difficile* have proved to be optimal for smaller laboratories performing less than 24 analyses per day, and the BD Max Cdiff proved to be suitable for medium-sized laboratories performing 24 or more analyses per day. When the overall process is optimised, including appropriate sample selection, collection and transportation, reduction of PCR inhibition, and reporting the results promptly to physicians, these NAATs can be of maximal use for improving CDI diagnostics and patient outcome.

Acknowledgements Nancy Lahtinen, Mikaela Eur, and Marianne Nynäs are gratefully acknowledged for their help in sample preparations and workload analysis. We have no conflicts of interests to declare.

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