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Evaluation of the BD Max Cdiff assay for the detection of toxigenic *Clostridium difficile* in human stool specimens

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Summary

The Becton Dickinson (BD) PCR-based GeneOhm Cdiff assay has demonstrated a high sensitivity and specificity for detecting *Clostridium difficile*. Recently, the BD Max platform, using the same principles as BD GeneOhm, has become available in Australia. This study aimed to investigate the sensitivity and specificity of BD Max Cdiff assay for the detection of toxigenic *C. difficile* in an Australian setting. Between December 2013 and January 2014, 406 stool specimens from 349 patients were analysed with the BD Max Cdiff assay. Direct and enrichment toxigenic culture were performed on bioMérieux ChromID *C. difficile* agar as a reference method. isolates from specimens with discrepant results were further analysed with an in-house PCR to detect the presence of toxin genes. The overall prevalence of toxigenic *C. difficile* was 7.2%. Concordance between the BD Max assay and enrichment culture was 98.5%. The sensitivity, specificity, positive predictive value and negative predictive value for the BD Max Cdiff assay were 95.5%, 99.0%, 87.5% and 99.7%, respectively, when compared to direct culture, and 91.7%, 99.0%, 88.0% and 99.4%, respectively, when compared to enrichment culture. The new BD Max Cdiff assay appeared to be an excellent platform for rapid and accurate detection of toxigenic *C. difficile*.

Key words: BD Max Cdiff assay, C. Diff Chek-60, *Clostridium difficile*, nucleic acid amplification test, toxigenic culture.

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INTRODUCTION

Clostridium difficile is the most important cause of hospital-associated infectious diarrhoea in the developed world, implicated as an aetiological agent in 20% of the antibiotic-associated diarrhoeal cases.[1] The two major virulence factors mediating the pathogenesis of *C. difficile* infection are toxins A and B.[1] Currently, toxigenic culture or faecal cytotoxicity assays are regarded as 'gold standards' for the diagnosis of *C. difficile* infection; however, these methods are time consuming (at least 24 h), and require expertise and specialised equipment. Therefore, their application is largely confined to the reference laboratory.[2] Toxin enzyme immunoassay (EIA) is a method for detecting *C. difficile* that is relatively quick but it has the disadvantage of lacking sensitivity.[3] Accurate and rapid detection of toxigenic *C. difficile* is necessary for infection control and patient management, however the task remains a challenge.

In 2008, the first United States Food and Drug Administration (US FDA) approved, nucleic acid based diagnostic test for *C. difficile* became commercially available.[4] The BD GeneOhm Cdiff assay is a real-time polymerase chain reaction (PCR) based technique, which consists of primers specific for a region of the toxin B (*tcdB*) gene and internal controls. The amplicons are detected with molecular beacon probe. The initial sample preparation is done manually, followed by an automated DNA amplification and detection, and data interpretation with Cepheid's SmartCycler platform.[5] The entire process takes approximately 2–3 h.[6,7] Studies have reported the BD GeneOhm assay to have a high sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 83.6–95.5%, 97.7–99.7%, 83.8–97.7% and 97.1–99.4%, respectively, when compared to toxigenic culture.[6–9]

In 2013, a new diagnostic tool produced by BD Diagnostics was approved by the US FDA. The BD Max Cdiff assay performs on the same principle and targets the same *tcdB* sequence as BD GeneOhm. However, the assay uses Taq Man as the hybridisation probe, BD Max as the data analysis platform and a different internal control ('specimen processing control'). The advantages of BD Max over BD GeneOhm include it being a fully automated procedure, which reduces the sample processing time from 40 to 10 min per 10 samples.[9] BD Max also has higher maximum capacity (24 as opposed to 14 specimens) and is able to be manipulated to perform an in-house PCR.[9,10] The few published studies evaluating the performance of the BD Max assay against toxigenic culture have shown a high sensitivity, specificity, PPV and NPV of 94.0–97.7%, 97.9–99.7%, 88.7–97.7% and 98.9–99.7%, respectively.[9,11,12]

As there have been limited studies of the BD Max Cdiff assay world-wide, and none in Australia, we aimed to investigate its sensitivity and specificity for the detection of toxigenic *C. difficile* in human stool samples in an Australian setting.

MATERIALS AND METHODS

Between December 2013 and January 2014, a total of 406 stool specimens from 349 patients were obtained from PathWest Laboratory Medicine microbiology

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laboratories at Royal Perth Hospital, Fremantle Hospital, and Sir Charles Gairdner Hospital. Samples were stored at 4°C and processed within 24 h of their arrival. Only the first specimen from the patient and any subsequent specimens collected more than 14 days later were included in the analysis.

All samples were analysed with BD Max Cdiff assay (BD Diagnostic, USA) and C. Diff Chek-60 (Alere, France), an EIA designed to detect glutamate dehydrogenase (GDH), an enzyme that is produced by all *C. difficile*.[13] Both tests were performed according to the manufacturer's instructions. As a reference method, toxigenic culture was performed by direct plating onto ChromID *C. difficile* agar (bioMérieux, France).[14] In addition, enrichment in cooked meat broth supplemented with gentamicin, cefoxitin and cycloserine was undertaken.[15] Taurocholic acid was added to the enrichment broth as a germinant and, after incubation, broths were alcohol shocked and plated onto ChromID *C. difficile* agar. All agar plates were incubated in an A35 anaerobe chamber (Don Whitley Scientific, Australia) for up to 48 h. Presumptive *C. difficile* colonies on ChromID agar were subcultured onto pre-reduced blood agar plates and colonies identified by morphology, horse-dung odour and fluorescence chartreuse (yellow-green) under 360 nm UV light. The identity of ambiguous colonies was further confirmed with Gram staining and L-proline amino peptidase test.

Isolates from specimens with discrepant results were further screened with an in-house PCR for the presence of toxin A (*tcdA*)[16] and toxin B (*tcdB*) genes,[17] binary toxin (*cdtA* and *cdtB*) genes[18] and for changes in repeating region of *tcdA*.[16] PCR products were run on QIAxcel capillary electrophoresis platform and visualised on QIAxcel ScreenGel software (Qiagen, Germany). BD Max negative/culture positive isolates that gave positive results with an in-house PCR were deemed false negatives on BD Max, while isolates that gave negative results with an in-house PCR were removed from the analysis (non-toxigenic isolates).

RESULTS

After removing the duplicate specimens ($n = 55$), specimens with inconclusive BD Max results ($n = 7$), and specimens with non-toxigenic *C. difficile* as determined by an in-house PCR ($n = 10$), 334 of 406 samples remained for analysis. When tested with the BD Max Cdiff assay, 7.5% (25/334) and 92.5% (309/334) of the samples were positive and negative, respectively (Table 1). When tested with C. Diff Chek-60, 6.3% (21/334) and 93.7% (313/334) of the specimens were positive and negative for GDH, respectively. By culture, 6.6% (22/334) of the specimens were positive via direct plating, while 0.6% (2/334) of the specimens grew *C. difficile* only after the enrichment procedure (Table 1). The overall prevalence of toxigenic *C. difficile* was 7.2% (24/334). Concordance between direct or enrichment culture and the two assays occurred for 97.9% (327/334) of the specimens. Concordance between the BD Max Cdiff assay and enrichment culture occurred in 98.5% (329/334) of the specimens.

The sensitivity, specificity, PPV and NPV of C. Diff Chek-60 were 95.5%, 100.0%, 100.0% and 99.7%, respectively, when compared to direct culture, and 87.5%, 100.0%, 100.0% and 99.0%, respectively, when compared to enrichment culture. With direct culture as a gold standard, the sensitivity, specificity, PPV, NPV and likelihood ratio (LR) of BD Max Cdiff assay were 95.5%, 99.0%, 87.5%, 99.7% and 98.6%, respectively (Table 2). When two additional specimens that were positive only after enrichment culture were included in the analysis, the sensitivity, specificity, PPV, NPV and LR of BD Max Cdiff assay were, 91.7%, 99.0%, 88.0%, 99.4% and 94.7%, respectively (Table 2).

Table 1 A comparison of assay results for the BD Max Cdiff assay, C. Diff Chek-60 and toxigenic culture*

BD Max Cdiff assay	C. Diff Chek-60 (GDH)	Toxigenic culture (% direct)	No. specimens
Neg	Neg	Neg	307
Neg	Neg	Pos (0%)	1
Neg	Pos	Pos (100%)	1
Pos	Neg	Neg	3
Pos	Neg	Pos (50%)	2
Pos	Pos	Pos (100%)	20
Total			334

* Duplicate specimens ($n = 55$), specimens with indeterminate BD Max results ($n = 7$) and specimens with non-toxigenic *C. difficile* ($n = 10$) were not included in the table.
GDH, glutamate dehydrogenase; Neg, negative; Pos, positive.

DISCUSSION

Clostridium difficile infection accounts for approximately 20% of antibiotic-associated infectious diarrhoea in the developed world.[1] For healthcare services to be able to provide treatment to the right patient in an appropriate and timely manner, a rapid and accurate diagnostic tool is essential. In the current study, the performance of BD Max Cdiff assay in detecting toxigenic *C. difficile* was evaluated against toxigenic culture. After removing 55 duplicates from 406 specimens obtained, the discordance between two assays occurred in 6.3% (22/351). Among these, 12 (3.4%) were BD Max negative but culture positive. Further examination of these isolates with an in-house PCR indicated that 10 were non-toxigenic *C. difficile* and this explained the lack of detection by the BD Max Cdiff assay, which targets *tcdB*. Of the two false negatives observed, one was culture positive via enrichment. Possible explanations for this include the presence of dormant spores, which only germinated following enrichment incubation with taurocholic acid, a spore germinant.[19] Additionally, the presence of aberrant or mutated *tcdB*, which

Table 2 BD Max performance characteristics using toxigenic culture as the gold standard

	n				% (95% CI)				
Culture method	TP	FP	FN	TN*	Sensitivity	Specificity	PPV	NPV	LR
Direct†	21	3	1	307	95.5 (77.2–99.9)	99.0 (97.2–99.8)	87.5 (67.6–97.3)	99.7 (98.2–100.0)	98.6
Enrichment‡	22	3	2	307	91.7 (73.0–99.0)	99.0 (97.2–99.8)	88.0 (68.8–97.5)	99.4 (97.7–99.9)	94.7

* TP and TN are specimens with positive and negative results, respectively, for both toxigenic culture and BD Max assay. FP are specimens with negative toxigenic culture but positive BD Max assay results. FN are specimens with positive toxigenic culture but negative BD Max assay results.
† Only direct culture positive specimens were included in the analysis ($n = 332$).
‡ Both direct and enrichment culture positive specimens were included in the analysis ($n = 334$).
CI, confidence interval; LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

render it unrecognisable by BD Max primers, may have contributed to this discordance. The possibility of variant *tcdB* isolates was not investigated in this study. Additionally, three specimens (0.9%) were BD Max positive but GDH and culture negative. The positive results may due to BD Max assay detecting the DNA of dead bacteria present in stools following antibiotic treatment. However, as the history of antibiotic usage was not available, this could not be evaluated. Other explanations for culture negativity include the presence of viable but non-culturable *C. difficile* and a low bacterial load in stool, which may be resolved by repeat sampling.

According to BD Diagnostics, results obtained from BD Max Cdiff assay may include positive, negative, unresolved, indeterminate and incomplete. The positive, negative and unresolved results are based on the amplification status of the target sequence or the internal control, and the indeterminate or incomplete results are due to BD Max system failure.[20] In this study, four and three specimens were unresolved and indeterminate, respectively. All seven specimens (2.0%) were GDH and culture negative. Similar observations were reported by Stamper *et al*.[8] and Barbut *et al*.,[7] who found 0.7% (3/401) and 7.3% (22/300) of unresolved cases, respectively, all of which were negative for cytotoxicity assay and toxigenic culture. The explanation for unresolved results is unclear but may include the presence of PCR inhibitory substances in stool samples.

After removing duplicates, specimens with inconclusive BD Max results and those with non-toxigenic *C. difficile*, the performance of BD Max Cdiff assay was assessed. The assay had a high sensitivity, specificity, PPV and NPV (95.5%, 99.0%, 87.5% and 99.7%, respectively) comparable to that previously reported for BD GeneOhm (83.6–95.5%, 97.7–99.7%, 83.8–97.7% and 97.1–99.4%, respectively).[6–9] The results were similar to the previously reported sensitivity, specificity, PPV and NPV figures for BD Max (94.0–97.7%, 97.9–99.7%, 88.7–97.7% and 98.9–99.7%, respectively).[9,11,12]

Using direct culture as a gold standard, we found the sensitivity of C. Diff Chek-60 to be 95.5%. This is comparable to that reported previously (93.0–94.0%).[21–23] The sensitivity decreased to 87.5% when compared with enrichment culture, a result more closely resembling that reported by Zheng et al. (71.0%);[23,24] however, they assessed C. Diff Chek-60 against direct culture method. The reasons for this difference are unclear.

Not surprisingly, the sensitivity of the BD Max assay dropped slightly (95.5% to 91.7%) when enrichment culture positive specimens were included in the analysis. This is because half the enrichment culture positive specimens in this study were BD Max (and GDH) negative (1/2). The presence of dormant *C. difficile* spores in stool samples may explain the observed discrepancies as previously suggested. The high NPV of BD Max assay (99.7%) indicates its suitability as a first-line screening tool in diagnostic settings, which allows physicians to quickly exclude *C. difficile* as a possible aetiological agent.

A large prospective observational study conducted to evaluate the association between results of *C. difficile* testing and clinical outcomes suggested that poorer clinical outcomes correlated with the presence of pre-formed toxin (positive cytotoxin assay) as opposed to *C. difficile* with toxigenic potential (positive toxigenic culture but negative cytotoxin assay).[25] To improve diagnostic test accuracy, the study recommended a multistep algorithm. This involves the use of a highly sensitive, first-stage test to detect *C. difficile*, followed by a second more specific test, which detects the presence of free toxin, and identifies the true cases of *C. difficile* infection.[25] By using this algorithm, the second test needs to be performed only on a small number of samples. The initial screening step also increases the prevalence of true positive cases, resulting in an increase in the PPV of the second test. The BD Max assay appears to be suitable for use as part of such an algorithm.

The cost of the BD Max Cdiff assay is approximately AU$20 per sample; however, the exact cost depends on the ordering volume. This assay is currently being used routinely at PathWest Laboratory Medicine, WA, as the sole method of *C. difficile* detection. Prior to the introduction of the BD Max assay, an algorithm involving initial screening of samples with a GDH EIA (approximately AU$6.50 per sample) was used. Only GDH positive samples (approximately 20%) were further tested with the BD GeneOhm assay for the presence of *tcdB* at a cost of approximately AU$30 per sample, again with the exact cost dependent on volume. Thus on initial scrutiny there appears to be no economic advantages to the BD Max Cdiff assay. However, the hands-on time required for the BD Max Cdiff assay was approximately 30 min per 24 samples compared to approximately 3 h for the same number of samples using BD GeneOhm. The shift to the BD Max Cdiff assay has greatly reduced the waiting time associated with initial screening of samples with the GDH EIA (50 min). Therefore, using the BD Max Cdiff assay significantly improved the work flow in the diagnostic laboratory. Although the clinical impact of detecting additional cases of *C. difficile* with toxigenic potential (toxin gene positive isolates) is unclear, the improved workflow and the shorter time to results notification will assist physicians in decision making processes.

The high accuracy, higher sample capacity (as compared to BD GeneOhm), and the ability to perform in-house PCR are some features that make BD Max Cdiff assay desirable as a rapid molecular diagnostic test. Furthermore, as the procedure to perform the BD Max Cdiff assay is relatively simple, the laboratory may reduce costs associated with staff training. The short turnaround time will also increase efficiency and workflow within the laboratory. In conclusion, BD Max Cdiff assay appeared to be a suitable first-line diagnostic tool for rapid and accurate detection of toxigenic *C. difficile*.

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