PERFORMANCE OF THE PORTRAIT TOXIGENIC *C. DIFFICILE* ASSAY COMPARED TO THE BD GENEOHM CDIFF PCR ASSAY AND TOXIGENIC CULTURE FOR DIAGNOSING *CLOSTRIDIUM DIFFICILE* INFECTION

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ABSTRACT

Background: Early detection of toxin-producing strains of *Clostridium difficile* infections is essential for patient management and prevention of nosocomial transmission. The Portrait Toxigenic *C. difficile* assay from Great Basin Diagnostics (Salt Lake City, UT) is a rapid, automated, qualitative assay performed on the Portrait Dx Analyzer. The assay utilizes thermophilic helicase-dependent amplification (tHDA) technology targeting the *C. difficile tcdB* gene. Toxigenic *tcdB* specific C. difficile DNA probes are immobilized on a silicon chip surface to enable detection of the amplified DNA. The objective of this study was to investigate the performance of the Portrait Toxigenic *C. difficile* assay compared to commercially available BD GenOhm Cdiff PCR assay and the gold standard toxigenic culture.

Methods: In total, 214 liquid and soft stool specimens were prospectively collected and included in the study. The Portrait Toxigenic *C. difficile* and BD GenOhm Cdiff PCR assays were performed according to the manufacturers' protocols. Toxigenic bacterial cultures were performed by heat shocking an aliquot of stool, followed by inoculation onto cycloserine cefoxitin fructose agar with horse blood (Anaerobe Systems, Morgan Hill, CA) and prereduced chopped-meat glucose broth (CMG). The *C. difficile* TOX-B test (TechLab, Blacksburg VA) was used for toxin testing of recovered isolates grown in CMG and inoculated into tissue culture plates containing human foreskin fibroblasts (Diagnostic Hybrids, Athens, OH). Using toxigenic culture as the reference method, the sensitivity, specificity, positive/negative predictive values (PPV/NPV) of each molecular method was calculated.

Results: The overall prevalence of specimens positive by toxigenic culture and toxin production was 20% (43/214). The sensitivity, specificity, PPV, and NPV were for BD GenOhm Cdiff 97.7%, 98.8%, 95.5%, and 99.4%, for Portrait Toxigenic *C. difficile* 97.6%, 96.4%, 87.2%, and 99.4%. Three samples initially tested on the Portrait as invalid were unresolved upon retesting.

Conclusion: The performance characteristics of the Portrait Toxigenic C. difficile assay in our laboratory compared favorably with the BD GenOhm Cdiff PCR assay and toxigenic culture for the detection of toxigenic *C. difficile* directly from stool specimens.

INTRODUCTION

Toxigenic C. difficile is a major cause of nosocomial cases of infectious antibiotic-associates diarrhea and pseudomembranous colitis. The prevalence of *C. difficile* has increased in both hospital and community-acquired infections. Accurate and rapid diagnosis of *Clostridium difficile* –associated disease (CDAD) is important for patient management and prevention of nosocomial transmission. A new FDA cleared molecular assay, Portrait Toxigenic *C. difficile* assay (Great Basin Diagnostics, Salt Lake City, UT), was developed for the rapid, qualitative detection of the *tcdB* gene of *C. difficile*. The Portrait Dx Analyzer utilizes a novel blocked-primer-mediated helicase-dependent amplification (bpHDA) technology which utilizes the isothermal amplification method helicase-dependent amplification to exponentially amplify target DNA sequences coupled with blocked primer/ RNase H2 mediated target-specific "hot start" (Figure 1).

The Portrait Toxigenic *C. difficile* Automated System includes the Portrait Dx Analyzer, controller laptop PC with Portrait program data analysis software, and a single use Portrait toxogenic *C. difficile* test cartridge (Figure 2). Performance of the Portrait Toxigenic *C. difficile* assay and the BD GenOhm Cdiff PCR assay were compared to the gold standard toxigenic culture.

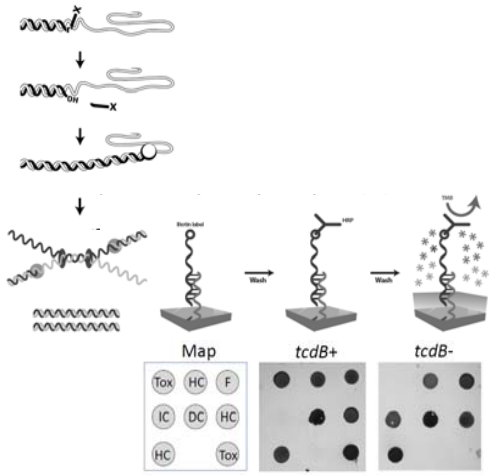

Figure 1: Portrait Toxigenic C. difficile assay scheme. (A) Amplification. Cell lysis releases genomic DNA, which is unwound by a helicase. In bpHDA, modified blocked-primers are utilized, which are constructed with a single ribonucleotide linkage 4 bases inserted upstream of a 3'-end block added to prevent primer extension (X). Once blocked primers hybridize to complementary target sequences, thermostable RNase H2 derived from *Pyrococcus abyssi* is activated, cleaving the ribonucleotide linkage in the primer present in duplex DNA. The short segment of the primer 3'of the ribonucleotide dissociates, liberating the block and creating a free 3'-hydroxyl (OH) which is now capable of primer extension. Exponential amplification occurs with the HDA method once primers are unblocked. In HDA, a DNA helicase unwinds double stranded DNA, instead of heat as is done in PCR. Once double-stranded DNA is unwound and target annealed primers are de-blocked, DNA polymerase extends the primers. RNase H2 used is highly-active at 65°C, the temperature required for HDA to amplify target sequences optimally.
(B) Detection. After bpHDA amplification, resultant amplicons are detected by hybridization to a probe set. After washing, an anti-biotin antibody conjugated to Horseradish Peroxidase (HRP) produces a visual signal via precipitation of a tetramethylbenzidine (TMB) cleavage product that detects *tcdB* gene amplicon and controls onto a modified silicon chip surface.
(C) Visual readout. Imaging of chip patterns for positive and negative *tcdB* results. Features: IC, internal control; HC and DC, hybridization and detection controls; F, chip orientation feature; Tox, *tcdB*.

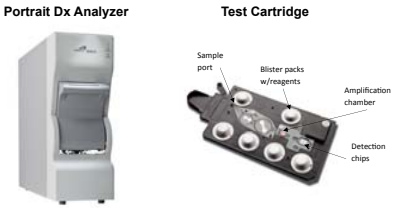

Figure 2. Portrait Toxigenic *C. difficile* Automated System. The Portrait toxigenic *C. difficile* assay is built into an injection-molded single use cartridge that contains blister packs, fluidic channels, processing chambers, and the assay chip coated with sequence-specific detection probes. A filtered stool sample is placed into a sample port of the test cartridge for processing. The operator inserts the cartridge into the Portrait Dx Analyzer and initiates the test. Multiple fluidic channels move reagents from integrated blister packs to chambers where boiling, reagent mixing and sample processing occur. Software automatically returns a result within 90 min.

METHODS

Specimens. A total of 214 liquid and soft stool specimens were prospectively collected from patients age >2 years and suspected of having CDAD. Stool specimens were tested daily or stored a 4°C and tested within 24 hours.

Toxogenic Bacteria Culture (TBC). All specimens were processed for anaerobic culture and culture isolates were characterized further by cytotoxin testing using the *C. difficile* TOX-B test (TechLab, Blacksburg VA). A spore enrichment step was carried out by heating the specimen for 10 min on a dry heat block at 80°C and then cooling to room temperature. Anaerobic cultures were performed by plating specimens onto prereduced cycloserine cefoxitin fructose agar with horse blood (CCFA-HB, Anaerobe Systems, Morgan Hill, CA). Additional sample was added to pre-reduced chopped meat glucose (CMG) broth. Plates and tubes were incubated anaerobically at 35°C for up to 48h. *C. difficile* was identified by colony morphology, "horse barn" odor, susceptibility to vancomycin, no growth at 35°C under 5% CO_2, and positive Pro-disk test (L-proline). CMG broth cultures were subcultured onto CCFA-HB only if the primary plate was negative and molecular assays and/or TOX B assay were positive. For isolates testing positive by the TOX-B test, 0.2ml of CMG culture was diluted (1:10), vortexed, and inoculated into tissue culture plates containing human foreskin fibroblast. The results were read and interpreted according to manufacturer's protocol.

Molecular assays. The Portrait Toxigenic *C. difficile* assay and BD GenOhm Cdiff PCR assay were preformed according to manufacturer's instructions. Invalid results were repeated.

Data Analysis. Sensitivity, specificity, positive predictive value, and negative predictive value of the Portrait Toxigenic *C. difficile* assay and BD GenOhm Cdiff PCR assay were compared to TBC

RESULTS

The overall prevalence of specimens positive by TBC and toxin production was 20% (43/214). Table 1 shows the performance characteristics of both molecular assays compared to TBC for the presence of tcdB gene. The sensitivity, specificity, PPV, and NPV of the Portrait Toxigenic *C. difficile* assay were 97.6%, 96.4%, 87.2%, and 99.4%, respectively and those of the BD GenOhm Cdiff assay were 97.7%, 98.8%, 95.5%, and 99.4%, respectively. Three samples initially tested by the Portrait Toxigenic C. difficile assay as invalid were unresolved upon retesting.

Table 1. Comparison of the Portrait Toxigenic *C. difficile* assay to the BD GenOhm Cdiff PCR assay and Toxigenic Bacteria Culture (TBC).

Assay/result	TBC		Performance[a] of assay compared to TBC			
	POS	NEG	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Portrait Tox *C. difficile*						
POS	41	6	97.6	96.4	87.2	99.4
NEG	1	163				
Total (n=211)[b]	42	169				
BD GenOhm Cdiff PCR						
POS	42	2	97.7	98.8	95.5	99.4
NEG	1	169				
Total (n=214)	43	171				

[a]PPV, positive predictive value; NPV, negative predictive value.
[b]Three samples were excluded from analysis due to unresolved invalid result.

CONCLUSIONS

- The performance of the Portrait Toxigenic *C. difficile* assay compared favorably to both the BD GenOhm Cdiff PCR assay and TBC.
- The Portrait Dx Analyzer is a small, automated bench-top analyzer with low cost disposable cartridges for performing on-demand testing during any shift.
- The BD GenOhm Cdiff PCR assay requires more hands-on time (20-35 min vs less than 2 min) compared to Portrait Toxigenic *C. difficile* assay and is more adapted to batch testing.
- The approximate costs per test are $25 for the Portrait Toxigenic *C. difficile* assay and $25 for the BD GenOhm Cdiff PCR assay.
- The turn-around–times for Portrait Toxigenic C. difficile assay (90 min) and BD GenOhm Cdiff PCR assay (75-90 min) were similar.
- In summary, the Portrait Toxigenic *C. difficile* assay is a rapid and reliable test for the detection of toxogenic *C. difficile* directly from clinical specimens.

ACKNOWLEDGMENTS

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