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Comparison of *illumigene® C. difficile Assay* and Portrait Toxigenic *C. difficile* Assay for the Detection of Toxigenic *Clostridium difficile* in Pediatric Patients

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ABSTRACT (Revised)

Background: Toxigenic *Clostridium difficile* (CDT) is the causative agent of a spectrum of clinical manifestations ranging from mild diarrhea to pseudomembranous colitis and death. Accurate detection is imperative for disease management and control with molecular detection being well accepted as the diagnostic standard. We sought to compare the performance of two molecular assays, *illumigene® C. difficile* (Meridian Bioscience) and Portrait Toxigenic *C. difficile* (Great Basin Corporation) for detection of CDT from stool samples. In addition, we investigate the workflow capabilities of the two assays in the microbiology laboratory.

Methods: Both Molecular assays were performed on 103 liquid or semi-formed clinical stool specimens collected from pediatric patients within 24 hour of receipt. The assay was batched once daily and performed by Clinical Laboratory Scientist (CLS). In contrast, up to two stools were tested at a time using Portrait Toxigenic *C. difficile* (two bench top analyzers were available); Clinical Laboratory Technicians (CLT) primarily performed all testing for this assay. *illumigene® C. difficile* was considered the reference method and all discrepant and invalid results were repeated.

Results: When comparing Portrait Toxigenic *C. difficile* with *illumigene® C. difficile*, 32 and 29 positives were identified respectively. Both assays correctly identified 28 positives cases; Portrait Toxigenic *C. difficile* detected 2 more positives and missed 1 positive. Overall sensitivity, specificity, and concordance of Portrait Toxigenic *C. difficile* were 96.6%, 97.0% and 96.8%, respectively. The positive predictive value was 98.5% and the negative predicative value was 93.3%. *illumigene® C. difficile* had 6 (8/103, 7.8%) more invalid results compared to Portrait Toxigenic *C. difficile* (2/103, 1.9%). Two specimens that was invalid by *illumigene® C. difficile* was found to be positive by Portrait Toxigenic *C. difficile*. With regards to workflow *illumigene® C. difficile* is performed once daily by the CLS, yielding an average turn-around-time (TAT) of 15 hours. In contrast, Portrait Toxigenic *C. difficile* is semi-automated and tested on arrival by the CLT, significantly decreasing the TAT to about 2-3 hours.

Conclusion: The performance characteristics of Portrait Toxigenic C. difficile are comparable to *illumigene® C. difficile* and is an appropriate option for the diagnosis of *C. difficile* infection. In addition, a lower number of invalids were detected using Portrait Toxigenic *C. difficile*, decreasing the need for repeat testing. The semi-automated and sample-to-answer capability of Portrait Toxigenic *C. difficile* allows the true 'stat' testing of CDT in the microbiology laboratory, allowing for prompt therapeutic response and infection control.

INTRODUCTION

- *Clostridium difficile* has emerged as a major nosocomial pathogen and is a leading cause of antibiotic-associated diarrhea and pseudomembranous colitis. Treatment with certain antibiotics or anti-neoplastic agents can disrupt the normal flora and allow *C. difficile* to become predominant bacteria in the colon. When the toxigenic strains overgrow, *C. difficile* infection (CDI) results, and can lead to mild diarrhea, pseudomembranous colitis and death.
- With the number of *Clostridium difficile* Infections (CDI) on the rise, accurate and rapid diagnosis is imperative to aid in therapy selection, improve patient outcome, prevent disease spread and lessen negative impacts on healthcare systems.
- *Clostridium difficile* can carry a genetically variable pathogenicity locus (PaLoc), which encodes clostridial toxins A and B.
- The *illumigene® C. difficile* targets a conservative region in the toxin A gene (tcdA) while the Portrait Toxigenic *C. difficile* targets the bacterium's toxin B gene (tcdB).

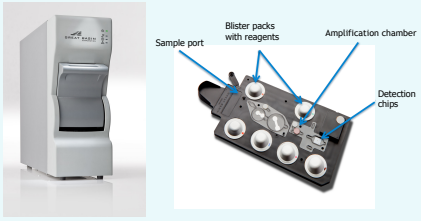
Goals of this study

- To compare the performance of the Portrait Toxigenic *C. difficile* Assay (sensitivity, specificity, positive and negative predicative values) for the laboratory diagnosis of CDI in pediatric patients.
- To evaluate the changes in workflow and TAT between the two molecular assays in a clinical microbiology laboratory.

METHODS

- A total of 103 liquid or semi-formed clinical stool specimens collected from pediatric patients suspected of having CDI were tested by both methods.
- Stools specimens were tested daily by *illumigene® C. difficile* as per the manufacturer's protocol and then stored at 4˚C for up to 72 hours or stored immediately at > -20˚C until additional testing using Portrait Toxigenic *C. difficile* (Figure 1).
- All discrepant results are repeated.
- Sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), concordance are calculated for the Portrait Toxigenic *C. difficile* using the *illumigene® C. difficile* as the reference method.

Figure 1. The Portrait Toxigenic *C. difficile Semi*-Automated System



Portrait Dx Analyzer Test Cartridge

Portrait Toxigenic *C. difficile* procedure is as follows:

The Portrait Toxigenic *C. difficile* kit (pouch) is brought to room temperature.

An aliquot of stool is mixed in sample diluents and passed through a filter. 180µl of filtered stool specimen is then pipetted into the Portrait Toxigenic *C. difficile* test cartridge and the Sample Port Tab is locked to prevent leakage.

The Test Cartridge is the placed in the Analyzer and the door is closed.

Pertinent cartridge and patient information is entered into the Portrait Dx Analyzer Interface and start the Assay. Run time is approximately 2 hours.

RESULTS

- Based on *illumigene® C. difficile* results, a total of 29 positive, 66 negative and 8 indeterminants were included in the study.
- The Portrait *Toxigenic C. difficile* correctly identified 28/29 positive specimens, 64/66 negative specimens and 1/8 indeterminants when compared to *illumigene® C. difficile* (Table 1).

Table 1. Method Comparison: Reference Vs. Portrait Toxigenic *C. difficile*

Great Basin Corporation	Meridian *illumigene®* C. difficile		Specificity: 97.0% Sensitivity: 96.3% Concordance: 96.8%	PPV: 98.5% NPV: 93.3%
	Positive	Negative		
Positive	28	2		
Negative	1	64		

Figure 2. Comparison of *C. difficile* Detection Methods

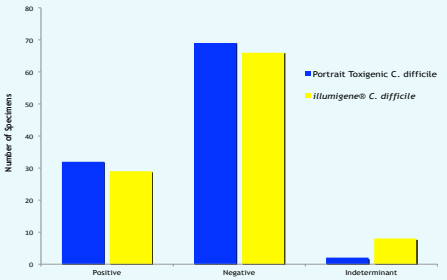
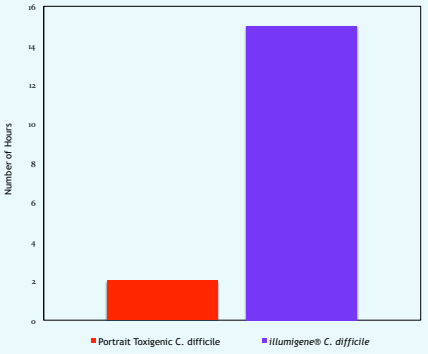


Figure 3. Comparison of Turn-around time based on workflow



RESULTS

- Looking at just the 95 specimens that had a negative or positive result by *illumigene® C. difficile*, the sensitivity and specificity of the Portrait Toxigenic *C. difficile* assay are 96.3% and 97.0 % , respectively.
- The NPV was 93.3% and the PPV was 98.5%. The concordance rate is 96.8%.
- Importantly, the indeterminant rate decreased from 7.8% (8) to 1.9% (2) negating the need to repeat testing or request resubmission of new specimen.
- Overall, the Portrait Toxigenic *C. difficile* assay detected a total of 32 positive and 69 negative (Figure 2).
  - 2 additional positive were detected by Portrait Toxigenic *C. difficile* that was reported as negative by *illumigene® C. difficile*.
  - 1 true positive specimen was missed by Portrait Toxigenic *C. difficile*.
  - 2 indeterminant specimens were reported with Portrait Toxigenic *C. difficile*; 1 concurred with *illumigene® C. difficile* and second specimen was called negative by *illumigene® C. difficile*.
  - Of the 7 additional specimens that were called indeterminant by *illumigene® C. difficile* but resolve by Portrait Toxigenic *C. difficile*, 5 were negative and 2 were positive (total of 4 new positive cases).
- With regards to work flow, *illumigene® C. difficile* is batched in our laboratory and performed once daily by Clinical Laboratory Scientist (CLS), yielding an average turn-around time of 15 hours.
- The Portrait Toxigenic *C. difficile* is more 'sample-to-answer' with minimal steps which allows it to be performed upon arrival in the laboratory. The assay is performed by the Clinical Laboratory Technician (CLT) and the results are analyzed by a CLS.
- The expected TAT for this assay is approximately 2 - 3 hours, allowing for prompt therapeutic response and control of infection (Figure 3).

CONCLUSIONS

- The performance characteristics of Portrait Toxigenic *C. difficile* are comparable to illumigene® *C. difficile* and is an appropriate option for the diagnosis of *C. difficile*.
- The number of repeat testing due to indeterminant results is expected to decrease significantly with Portrait Toxigenic *C. difficile* as a 5.9% decline was noted in this study.
- The semi-automated and sample-to-answer capability of Portrait Toxigenic *C. difficile* allows the true 'stat' testing of CDT in the microbiology laboratory, allowing for prompt therapeutic response and infection control.
- The Portrait Dx Analyzer is a small, automated bench-top analyzer with low cost disposable cartridges for performing on-demand testing during any shift.

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