

# Multicenter clinical evaluation of the Portrait Toxigenic *C. difficile* Assay for detection of toxigenic *C. difficile* from clinical specimens

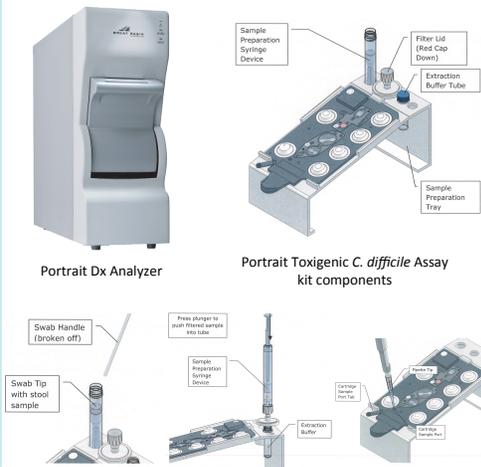
Tami-Lea A. Mackey<sup>1</sup>, Kim T. Insyxiengmay<sup>1</sup>, Judy A. Daly<sup>3</sup>, Garrison Alger<sup>3</sup>, Gerald A. Denys<sup>4</sup>, Lance R. Peterson<sup>5</sup>, Sue C. Kehl<sup>6</sup>, Blake W. Buchan<sup>1,2</sup>, and Nathan A. Ledebor<sup>1,2</sup>

<sup>1</sup>Dynacare Laboratories and <sup>2</sup>Medical College of Wisconsin, Milwaukee, WI, <sup>3</sup>University of Utah, Salt Lake City, UT, <sup>4</sup>Indiana University Health Pathology Laboratory, Indianapolis, IN, <sup>5</sup>NorthShore University Health System, Evanston, IL, <sup>6</sup>Childrens Hospital of Wisconsin, Milwaukee, WI,

### Introduction

*Clostridium difficile* associated disease affects >500,000 people in the United States alone and results in up to 20,000 deaths. Toxigenic strains of *C. difficile* produce cytotoxin B (TcdB), which is primarily responsible for clinical symptoms. Early identification of *C. difficile* strains carrying *tcdB* in patients suffering diarrheal episodes is important for infection control and can aid in decisions relating to antibiotic therapy.

### Methods and Instrumentation



- Step 1: Collect stool on swab, break into sample preparation syringe containing extraction buffer. Vortex to homogenize.
- Step 2: Attach filter to syringe and transfer homogenized sample into extraction buffer tube.
- Step 3: Transfer 180 µl of filtrate into test cartridge sample port. Cartridge contains all reagents to complete test.
- Step 4: Insert cartridge into analyzer and start assay. Run time approx. 2 hrs.

The Portrait Toxigenic *C. difficile* Assay (Great Basin, West Valley City, UT) is a nucleic acid amplification assay that uses chip-based detection for the *tcdB* gene present in toxigenic strains of *C. difficile*. A total of 540 stool specimens were collected from patients with suspected *C. difficile* disease at four geographically distinct study sites. Each specimen was tested within 5 days of collection using the Portrait test and results were compared to toxigenic bacterial culture/cell cytotoxicity neutralization assay (TBC/CCNA) as the gold standard. Additionally, each specimen was also tested using each sites standard of care method.

**Table 1. Clinical Trial Data. Portrait Toxigenic *C. difficile* Assay compared to toxigenic culture (gold standard)**

Site	Total Tested	True Positive	False Positive	True Negative	False Negative	Sensitivity (95% C.I.)	Specificity (95% C.I.)
A	169	37	4	127	1	97.4% (86-99)	96.9% (92-99)
B	219	44	16	159	0	100% (91-100)	90.9% (85-94)
C	96	15	9	72	0	100% (78-100)	88.9% (79-94)
D	56	13	2	40	1	92.9% (66-99)	95.2% (83-99)
<b>Total</b>	<b>540</b>	<b>109</b>	<b>31</b>	<b>398</b>	<b>2</b>	<b>98.2% (93-99)</b>	<b>92.8% (89-95)</b>

**Table 2. Comparison of the Portrait Toxigenic *C. difficile* Assay to other molecular methods**

Method	Total Tested	Portrait (+) Comparator (+)	Portrait (+) Comparator (-)	Portrait (-) Comparator (+)	Portrait (-) Comparator (-)	Agreement
Gene Xpert	275	72	3 <sup>a</sup>	4 <sup>b</sup>	196	97.5%
GeneOHM	169	37	4 <sup>c</sup>	2 <sup>d</sup>	126	96.4%
Illumigene	96	18	6 <sup>e</sup>	0	72	93.8%
<b>Total</b>	<b>540</b>	<b>127</b>	<b>13</b>	<b>6</b>	<b>394</b>	<b>96.5%</b>

<sup>a</sup>All 3 were culture negative  
<sup>b</sup>3 were culture negative, 1 was culture positive  
<sup>c</sup>3 were culture negative, 1 was culture positive  
<sup>d</sup>1 was culture negative, 1 was culture positive  
<sup>e</sup>5 were culture negative, 1 was culture positive

**Table 3. Reproducibility, *C. difficile* toxinotype O (A+, B+) ATCC 43255**

Site	Moderate Positive <sup>a</sup>	Low Positive <sup>b</sup>	High Negative <sup>c</sup>
A	100% (30/30)	100% (30/30)	90.0% (27/30)
B	100% (30/30)	100% (30/30)	96.7% (29/30)
C	93.3% (28/30) <sup>d</sup>	90.0% (27/30) <sup>d</sup>	100% (30/30)
<b>Total</b>	<b>97.8% (88/90)</b>	<b>96.7% (87/90)</b>	<b>95.6% (86/90)</b>

<sup>a</sup> Approximately 3x limit of detection (LoD)  
<sup>b</sup> C<sub>95</sub> Concentration  
<sup>c</sup> C<sub>5</sub> Concentration (Should not be detected 5.0% of the time.)  
<sup>d</sup> Repeat testing of the reproducibility panel demonstrated 100% concordance at this site

### Conclusion

The portrait Toxigenic *C. difficile* Assay provides a robust method with high sensitivity and specificity for detection of toxigenic *C. difficile* in clinical specimens.

Compared to gold standard toxigenic culture the Portrait Toxigenic *C. difficile* Assay was > 98% sensitive and > 93% specific for the detection of toxigenic *C. difficile*

The Portrait Toxigenic *C. difficile* Assay demonstrated 93.8% - 97.5% agreement with alternative FDA-cleared molecular assays.

The Portrait Toxigenic *C. difficile* Assay can be completed in approximately 2 hours with 15 min. of hands on time.