BD GeneOhm™ Cdiff Assay

IVD

REF 441400 **48 Tests**
REF 441401 **200 Tests**

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Intended Use

The BD GeneOhm™ Cdiff Assay is a rapid *in vitro* diagnostic test for the direct, qualitative detection of *C. difficile* toxin B gene (*tcdB*) in human liquid or soft stool specimens from patients suspected of having *Clostridium difficile*-associated disease (CDAD). The test, based on real-time PCR, is intended for use as an aid in diagnosis of CDAD. The test is performed directly on the specimen, utilizing polymerase chain reaction (PCR) for the amplification of specific targets and fluorogenic target-specific hybridization probes for the detection of the amplified DNA.

Summary and Explanation of the Test

A liquid or soft stool specimen is collected and transported to the laboratory. A sterile dry swab is dipped into the liquid or soft stool material and processed. For testing, the swab is eluted in sample buffer and the specimen is lysed. An aliquot of the lysate is added to PCR reagents which contain the *tcdB* specific primers used to amplify the genetic target of *Clostridium difficile*, if present. The assay also includes an internal control (IC) to detect PCR inhibited specimens and to confirm the integrity of assay reagents. Amplified targets are detected with hybridization probes labelled with quenched fluorophores (molecular beacons). The amplification, detection and interpretation of the signals are done automatically by the Cepheid SmartCycler® software. The entire procedure takes about 75 to 90 minutes, depending on the number of specimens processed.

C. difficile is the etiologic agent of several diseases. Epidemiology studies demonstrated that an increased number of *C. difficile* associated outbreaks have been reported worldwide[1], some with increased mortality and morbidity[2]. Disease symptoms range from mild nuisance diarrhea to severe colitis and even to bowel perforation and death by dehydration. The pathogen is the major cause of antibiotic-associated diarrhea (AAD) and pseudomembranous colitis[3]. The predisposing risk factors are numerous and include age, length and number of hospital stays, invasive medical procedures, immunosuppressive treatments, and chronic pathologies (diabetes, cardiovascular syndromes or AIDS)[4]. These factors explain why some wards (intensive care, surgery, long-term care) in hospitals or institutions are more affected than others. The bacterium *C. difficile*, as the other intestinal flora bacteria, are killed by the treatment but not the *C. difficile* spores, which are insensitive to the majority of antimicrobial agents. When antimicrobial concentration is lower than the minimum inhibitory concentration, spores germinate into viable bacteria which produce toxins[5].

The diagnosis of toxigenic *C. difficile* is usually done by tissue culture cytotoxicity assay and/or by *C. difficile* culture identification and/or by enzyme immunoassay (EIA). The tissue culture cytotoxicity assay and *C. difficile* culture identification are labor and time consuming and results are obtained within 24 to 96 hours, whereas EIA assays display low specificity. Development of sensitive and specific molecular amplification techniques now allows the detection of only a few copies of bacterial DNA in clinical samples, and the discrimination of specific species[6]. In addition, rapid PCR technology can achieve this in about 1 hour. The combination of these characteristics may allow the prompt targeted treatment of CDAD patients and thus a potential improved patient outcome and reduced recovery time.

Principle of the Procedure

Following specimen lysis, amplification of the *tcdB* target, if present, occurs. Amplification of the internal control (IC), a DNA fragment of 333 base pairs (bp) including a 277 bp sequence not found in *C. difficile*, will also take place unless there are PCR inhibitory substances.

The amplified DNA target is detected with a molecular beacon, a hairpin-forming single-stranded oligonucleotide labelled at one end with a quencher and at the other end with a fluorescent reporter dye (fluorophore). In the absence of target, the fluorescence is quenched. In the presence of target, the hairpin structure opens upon beacon/target hybridization, resulting in emission of fluorescence. For the detection of *tcdB* amplicons, the molecular beacon contains the fluorophore FAM at the 5' end and the non-fluorescent quencher DABCYL at the opposite 3' end of the oligonucleotide. For the detection of the IC amplicons, the molecular beacon contains the fluorophore TET at the 5' end and the quencher moiety DABCYL at the 3' end. Each beacon-target hybrid fluoresces at a wavelength characteristic of the fluorophore used in the particular molecular beacon. The amount of fluorescence at any given cycle, or following cycling, depends on the amount of specific amplicons present at that time. The SmartCycler® software simultaneously monitors the fluorescence emitted by each molecular beacon, interprets all data, and provides a final result at the end of the cycling program (see Section "Interpretation of Results").

Reagents

BD GeneOhm™ Cdiff Assay	48 Tests	200 Tests
Sample buffer	60 X 1 mL	240 X 1 mL
Tris-EDTA buffer		
Lysis tube	50 tubes	200 tubes
Glass beads		
Master Mix	8 tubes	34 tubes
< 0.0005% DNA polymerase complex		
< 0.001% Internal Control (non-infectious DNA containing *tcdB* primer binding sequences with a unique sequence for probe hybridization)		
< 0.002% Primers		
< 0.002% Molecular probes		
< 0.05% Nucleotide mix (dATP, dCTP, dGTP, dTTP)		
Bovine serum albumin		
Carbohydrate		
$MgCl_2$		
< 0.001% Non-infectious genomic DNA of *Escherichia coli* (ATCC 25922)		
Control DNA	8 tubes	34 tubes
Tris-EDTA buffer		
Carbohydrate		
< 0.001% Non-infectious genomic DNA of C. difficile bearing *tcdB* gene (ATCC 43255)		
Diluent	8 X 700 µL	34 X 700 µL
Tris-HCl buffer		
$MgCl_2$		
$(NH_4)_2SO_4$		
KCl		

Precautions

- This test is for *in vitro* diagnostic use only.
- Do not use the kit if the outer carton safety seal is broken.
- Do not use reagents if their protective pouches are open or torn upon arrival.
- Close protective pouches of Master Mix and Control DNA quickly with the zip seal after each use.
- Do not remove desiccant from Master Mix and Control DNA pouches.
- Do not use reagents if desiccant is not present inside Master Mix and Control DNA pouches.
- Reagents are not interchangeable between lots.
- Never pool reagents from different tubes even if they are from the same lot.
- Do not use the reagents after their expiration date.
- Do not interchange caps among reagents as contamination may occur and compromise test results.
- Avoid microbial and deoxyribonuclease (DNAse) contamination of reagents when removing aliquots from tubes. The use of sterile DNAse-free disposable filter-blocked or positive displacement pipettor tips is recommended.
- To avoid contamination of the environment with *tcdB* gene amplicons, do not open the reaction tubes post-amplification.
- Use a new pipettor tip for each specimen or reagent.
- Performing the assay outside of the recommended time ranges can produce invalid results. Assays not completed within specified time ranges should be repeated.
- Additional controls may be tested according to guidelines or requirements of local, state, provincial and/or federal regulations or accrediting organizations.
- In cases where open-tube PCR tests are conducted in the same general area by the laboratory, separated and segregated working areas should be used for specimen preparation and amplification/detection activities. Supplies and equipment should be dedicated to each area and should not be moved from one area to another. Gloves must always be worn and must be changed before going from one area to another. Gloves must be changed before manipulating lyophilized reagents.
- Always handle specimens as if they are infectious and in accordance with safe laboratory procedures such as those described in *Biosafety in Microbiological and Biomedical Laboratories*[7] and in the CLSI Document M29[8].
- Wear protective clothing and disposable gloves while handling kit reagents. Wash hands thoroughly after performing the test.
- Do not pipet by mouth.
- Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
- Dispose of unused reagents and waste in accordance with country, federal, provincial, state and local regulations.

Materials Provided

- Sample buffer
- Lysis tube
- Master Mix
- Control DNA
- Diluent
- SmartCycler® reaction tubes, 25 µL
- Specimen identification labels

Storage, Handling and Stability

Collected Specimens

- Specimens should be kept between 2 °C and 25 °C during transport. Protect against freezing or exposure to excessive heat.
- Specimens can be stored up to 5 days at 2-8 °C before testing. Specimens can be kept at room temperature (15-25 °C) up to 48 hours before testing. Specimens can be tested after one freeze and thaw cycle.

Reagents

Note: Storage conditions must follow the specifications written on each pouch.

Kit Component		Master Mix, Diluent and Control DNA (**white, black** strip and **red** strip labels, respectively)	Lysis Tubes (**yellow** cap)	Sample buffer (**blue** cap)
Sealed pouch	**Temperature**	2-8 °C	2-25 °C	2-25 °C
	Stability	Expiration date	Expiration date	Expiration date
Opened pouch[A]	**Temperature**	2-8 °C	2-25 °C	2-25 °C[B]
	Stability	1 month[C]	Expiration date	2 months[c]

[A] Once the original seal on the pouch is broken, carefully close the pouch with the zip seal after each use and store at appropriate temperature.
[B] Although these reagents can be stored at room temperature, they should be kept with their accompanying reagents of the same lot between 2-8 °C.
[C] Provided the bags are properly closed with the zip seal after each use.

Kit Components outside of their protective pouch			Master Mix and Control DNA (**white** and **red** strip labels)
Tubes containing unreconstituted reagents		**Temperature**	15-25 °C
		Stability	2 hours
Tubes containing reconstituted reagents [A]	**Original container**	**Temperature**	2-8 °C
		Stability	3 hours
	SmartCycler® tube	**Temperature**	2-8 °C
		Stability	1 hour

[A] Discard unused tubes after expiration of the indicated stability.

Materials Required but not Provided

- Dry sterile swab
- Dry sterile container for the collection of liquid or soft stool specimens
- Vortex Genie 2 (Scientific Industries Inc.) with 1.5 mL microtube holder or equivalent; for processing multiple samples, adapter with multiple holding sites can be used
- Micropipettors (accurate range between 1-10 µL, 10-100 µL and 100-1000 µL)
- Sterile DNAse-free filter-blocked or positive displacement micropipettor tips
- DNAse free microcentrifuge tubes
- Scissors (optional)
- Gauze
- Disposable gloves, powderless
- Microcentrifuge for low speed centrifugation
- Dry heating block for 1.5 mL tubes or water bath
- Ice or cooling block for 1.5 mL tubes
- Stopwatch or timer
- SmartCycler® starter system with Dx Software (processing block, user manual[9], accessory kit, and desktop computer) (Cepheid, Sunnyvale, CA, USA)

Instructions for Use

Specimen Collection

In order to obtain an adequate specimen, the procedure for specimen collection must be followed closely.

Liquid stool or soft stool specimen

Using a dry sterile container, liquid stool or soft stool specimens are collected according to the following procedure:

1. **Transfer liquid or soft stool (but not urine) into the container. Avoid mixing toilet paper, water, or soap with the sample.**
2. **Label the container.**
3. **Ship the container to the laboratory according to hospital standard operating procedures.**
4. **Refer to the section entitled Storage, Handling and Stability – Collected Specimens for storage and handling.**

Specimen Preparation

Note: One (1) sample buffer tube (**blue cap**) and one (1) lysis tube (**yellow cap**) are required for each specimen to be tested. An additional sample buffer tube (**blue cap**) is also required to dilute specimens. This sample buffer tube will also be used for the BD GeneOhm™ Cdiff Assay procedure. Remove the required number of tubes from their protective pouches, remove the excess air and close the pouches quickly with the zip seal.

1. **Prior to starting specimen preparation for the BD GeneOhm™ Cdiff Assay, vortex at high speed for 15 seconds and dip a sterile dry swab into the fecal material for testing. Remove excess of stool.**

2. **Place the swab in a sample buffer tube (blue cap).**
   Identify the sample buffer tube on the cap and/or the tube label.

3. **Break the swab stem and close the tube tightly.**
   Hold the swab by the stem near the rim of the tube (use gauze to minimize risks of contamination). Lift the swab a few millimeters from the bottom and bend the stem against the edge of the tube to break it. Alternative method: use clean scissors to cut the stem. Make sure the cap will close tightly.

4. **Vortex at high speed for one (1) minute.**
   For processing multiple samples, adapters with multiple holding sites can be used.

5. **Add 40 µL from the additional sample buffer tube (blue cap) to the lysis tube (yellow cap) (in order to dilute the specimen).**

6. **Transfer 10 µL of cell suspension (from Step 4) to the lysis tube (yellow cap) already containing 40 µL of sample buffer (blue cap) (from Step 5).**

7. **Vortex the lysis tube for five (5) minutes at high speed.**
   For processing multiple samples, adapters with multiple holding sites can be used.

8. **Centrifuge the lysis tube briefly (quick spin).**
   At low speed for two (2) to five (5) seconds to bring the solid contents at the bottom of the tube.

9. **Heat the lysis tube at 95 ± 2 ºC between five (5) and seven (7) minutes.**
   Use a dry heating block for 1.5 mL tubes or a water bath.

10. **Place the lysis tube on ice or on a cooling block.**
    Lysates are stable up to four (4) hours at 2-8 ºC.

BD GeneOhm™ Cdiff Assay Procedure

NOTE: One (1) reconstituted Master Mix tube (**white label**) will yield enough reagents **to run eight** (8) reactions. Allow one SmartCycler® tube per specimen to be tested and two (2) additional SmartCycler® tubes for the **positive and the negative controls**. One (1) **positive** and one (1) **negative** control must be included in each BD GeneOhm™ Cdiff Assay run. One (1) Control DNA (**red strip label**) is required per assay run. One (1) diluent tube (**black strip label**) is required for the reconstitution of up to three (3) Master Mix tubes. Remove the required number of tubes from their protective pouches, **remove the excess air, and close the pouches quickly with the zip seal.**

Prepare only enough SmartCycler® tubes to fill available I-CORE® modules on the SmartCycler® instrument.

1. **Place the required number of Master Mix tubes on ice or on a cooling block for 1.5 mL tubes.**
2. **Add 225 µL of diluent (black strip label) to each Master Mix tube.**
   Insert the micropipettor tip through the septum of the cap of the Master Mix tube. Do not insert the tip too deeply into the cap. Deliver the diluent. Discard the unused diluent afterward.
3. **Vortex the tube(s) for 5-10 seconds.**
4. **Place the tube(s) on ice or on a cooling block for 1.5 mL tubes until ready to use.**
   Place a Control DNA tube (red strip label) on ice or on a cooling block for 1.5 mL tubes.
5. **Add 225 µL of sample buffer (blue cap) to the Control DNA tube.**
   Use the additional sample buffer tube (blue cap) from the specimen preparation (Step 4). Insert the micropipettor tip through the septum of the cap of the Control DNA tube. Do not insert the tip too deeply into the cap. Deliver the sample buffer.
6. **Vortex the tube for 5-10 seconds.**
   Place the tube on ice or on a cooling block for 1.5 mL tubes until ready to use.
7. **Place the required number of SmartCycler® tubes on the SmartCycler® cooling block.**
   Allow one (1) SmartCycler® tube per specimen and two (2) more SmartCycler® tubes for the controls. Avoid touching the optical detection windows at the bottom edges of the tube and the lower diamond-shaped area.

THE FOLLOWING STEPS MUST BE PERFORMED WITHIN A ONE (1) HOUR TIME FRAME:

8. **Add 25 µL of reconstituted Master Mix to the SmartCycler® tubes (appropriate pipetting technique required to ensure proper transfer of the solution).**
   Remove the cap before pipetting the reagent. Deliver the liquid into the reservoir (upper part) of the SmartCycler® tubes. Identify the SmartCycler® tubes on the cap. Specimen identification labels can be used (provided with the kit). Discard the unused Master Mix.
9. **Add 3.0 µL of each lysed specimen to a different SmartCycler® tube previously filled; close the tubes.**
   Be careful not to aspirate beads when pipetting into the lysis tube. After addition of the specimen, pipet up and down 2-3 times in the reservoir to ensure transfer of the complete volume. Use a new micropipettor tip for each specimen.
10. **Add 3.0 µL of the reconstituted Control DNA to the next to last SmartCycler® tube (Positive Control); close the tube.**
    After addition of the DNA, pipet up and down 2-3 times in the reservoir to ensure transfer of the complete volume. Identify as the positive control. Discard the unused control DNA.
11. **Add 3.0 µL of sample buffer (blue cap) to the last SmartCycler® tube (Negative Control); close the tube.**
    Use the sample buffer tube from Step 5. This will monitor PCR contamination that might occur during the manipulation of the specimens. Identify as the negative control. Discard the unused sample buffer afterward.
12. **Centrifuge all reaction tubes for 5-10 seconds.**
    Use the specially adapted microcentrifuge provided with the SmartCycler® instrument.
13. **Keep the tubes at 2-8 °C on the SmartCycler® cooling block before loading on the instrument.**
    The remaining lysates should be frozen at -20 ± 5 °C for later use, if necessary.
14. **Create a run with the BD GeneOhm™ Cdiff Assay protocol.**
    Refer to the SmartCycler® Dx Software Operator Manual[9] if needed. It is recommended to enter the identification parameters for the specimens before starting the run.
15. **Insert each reaction tube into an I-CORE® module of the SmartCycler® and close the I-CORE® lid.**
    Place the positive and negative controls at their appropriate positions (see the section entitled “Quality control”). Press all the tubes firmly down into place.
16. **Start the run.**

Quality Control

Positive and Negative Controls

Quality control procedures are designed to monitor assay performance. The positive control is intended to monitor substantial reagent failure. The negative control is used to detect reagent or environmental contamination (or carry-over) by either DNA containing *tcdB* genes or *tcdB* amplicons. Positive and negative controls are assay controls (run controls). An invalid control invalidates the run. Finally, an internal control incorporated into each reaction mixture is intended to monitor the reagent integrity and PCR inhibition in each specimen.

One positive control and one negative control must be included in each assay run on the SmartCycler®. The software automatically assigns the position of the controls on the instrument (refer to the SmartCycler® Dx Software Operator Manual[9]).

Specimen Processing Controls

Control strains may be tested according to guidelines or requirements of local, provincial and/or federal regulations or accreditation organizations. A reference toxigenic *C. difficile* strain bearing the *tcdB* gene (e.g. American Type Culture Collection, ATCC 43255 or a well characterized *C. difficile* clinical isolate identified to be carrying the *tcdB* gene) may be used as a positive specimen processing control while a culture of a non toxigenic *C. difficile* strain (e.g. ATCC 700057) may be used as a negative specimen processing control.

Incubated colonies are isolated after 18 to 24 hours on anaerobe 5% sheep blood agar. Resuspend colonies in saline to a turbidity of 0.5 McFarland (~1.5 X 10^7 CFU/mL). Dilute with saline to obtain a suspension of ~10^6 CFU/mL. Dip a dry swab into the bacterial suspension, press out the excess fluid. Process and test as a clinical specimen (refer to the sections entitled "Specimen Preparation" and "BD GeneOhm™ Cdiff Assay Procedure"), including controls. All specimens and controls should yield valid results (no invalid positive or negative control; no failed internal control; and no incorrect specimen processing control results – when specimen processing controls are performed). In the event of an incorrect specimen processing control result, it is recommended that a new aliquot be obtained from the original stool specimen, and that this specimen be retested along with new controls before reporting results.

For general QC guidance, the user may wish to refer to CLSI MM3[10] and C24[11].

Culturing of Clinical Specimens

To perform species identification directly from stools, clinical specimens can be cultured using the hospital standard procedures.

Interpretation of Results

The decision algorithm for the BD GeneOhm™ Cdiff Assay is embedded in the SmartCycler® DX software. The interpretation of assay results is done according to the following criteria:

Sample Type	Instrument-Reported Assay Result[9]	Instrument-Reported IC Result[9]	User Interpretation of Results
Clinical Specimen	NEG	PASS	No *tcdB* gene DNA detected
	POS	NA	*tcdB* gene DNA detected
	Unresolved	FAIL	Unresolved – inhibitory specimen or reagent failure
	ND	ND	Not determined due to I-CORE® Module failure (with Warning or Error Codes[9])
Positive Control	Valid	NA	Valid Positive Control; valid run when Negative Control is also valid.
	Invalid	NA	Invalid Positive Control; invalid run.[A] Assay results are invalid and must not be reported.
Negative Control	Valid	PASS	Valid Negative Control; valid run when Positive Control is also valid.
	Invalid	FAIL	Invalid Negative Control; invalid run.[A] Assay results are invalid and must not be reported.

IC - Internal Control; NA – not applicable; ND – not determined

[A] Invalid assay run or instrument error codes or warnings are flagged on-screen and on reports. Before reporting *C. difficile* results, always verify that the assay run is valid.

Invalid Assay Run

Using frozen lysate(s), prepare new reaction tubes for all clinical specimens within that assay run along with new control tubes.

Unresolved Specimen

Repeat testing with the corresponding frozen specimen lysate. The effect of a freeze-thaw cycle has been shown to reduce the effect of PCR inhibitory substances.

Specimen Not Determined Due to I-CORE® Module Failure

Repeat testing with the corresponding frozen specimen lysate. For the interpretation of warning or error code messages, refer to the SmartCycler® Dx Software Operator Manual[9].

Limitations of the procedure

- The performance characteristics of this assay have not been established with automated real-time PCR instruments other than the SmartCycler® instrument.
- This test is for use only with liquid or soft stools; performance characteristics of other clinical specimen types have not been established.
- Negative test results may also occur from improper specimen collection, handling or storage, presence of inhibitors, technical error, sample mix-up or because the number of organisms in the specimen is below the analytical sensitivity of the test. Careful compliance to the instructions given in this insert and in the SmartCycler® Dx Software Operator Manual[9] is necessary to avoid erroneous results. Use of this test should be limited to personnel trained on the procedure and on the use of the SmartCycler®.
- The BD GeneOhm™ Cdiff Assay may generate unresolved or invalid results due to an invalid control; therefore, retesting of the lysate kept between -20 ± 5 °C is required and will lead to a delay in obtaining results.
- A positive test result does not necessarily indicate the presence of viable organisms. It does however indicate the presence of the *tcdB* gene and allows for a presumptive detection of the *C. difficile* toxigenic organism. The BD GeneOhm™ Cdiff Assay cannot be used for species identification as it does not contain primers and probes specific to *C. difficile*.
- Mutations or polymorphisms in primer or probe binding regions may affect detection of variant *tcdB* gene of *C. difficile* resulting in a false negative result with the BD GeneOhm™ Cdiff Assay.
- Although there is no need for reagent preparation and the main technical operation is pipetting, good laboratory techniques are essential for the proper performance of this assay. Due to the high analytical sensitivity of this test, extreme care should be taken to preserve the purity of all reagents, especially in cases where multiple aliquots are taken from a tube.

Interfering Substances

Potentially interfering substances include, but are not limited to blood and mucus. The presence of excessive blood may inhibit PCR and may give unresolved results.

Performance Characteristics

Performance characteristics of the BD GeneOhm™ Cdiff Assay were determined in a multi-site prospective investigational study. Four (4) medical centers, two (2) in Canada and two (2) in the United States, participated in the study. For any specimen to be enrolled in the study, proper *Clostridium difficile* testing had to be ordered for the patient and leftover liquid or soft stool specimen had to be eligible by the study criteria.

The Reference Cytotoxicity Assay was performed using a tissue culture Cytotoxicity assay on liquid or soft specimens within 48 hours of collection. The procedure was performed according to the Manufacturer's Instructions for Use.

In total, 1143 specimens were found compliant and were tested with both the Reference Assay described above and the BD GeneOhm™ Cdiff Assay (Tables 1 and 4). The first dataset includes 876 fresh specimens tested at three (3) clinical sites. The second dataset includes 267 frozen specimens tested at a fourth clinical site. In comparison to the Reference Assay, the BD GeneOhm™ Cdiff Assay identified 93.9% and 100% of the *C. difficile* positive specimens in the fresh and frozen datasets, respectively (Tables 2 and 5). For the population tested this resulted in a Negative Predictive Value of 99.1% and a Positive Predictive Value of 64.8%. For the frozen dataset tested this resulted in a NPV of 99.2% and PPV of 80.3%.

Out of 876 fresh specimens tested with the BD GeneOhm™ Cdiff Assay, 39 were initially reported as unresolved (4.5%). Upon repeat testing from the frozen lysates, 22 were resolved and 17 remained unresolved (1.9%) (Table 6). Out of 267 frozen specimens tested with the BD GeneOhm™ Cdiff Assay, only one specimen (0.4%) was initially reported unresolved. The specimen remained unresolved upon repeat testing from the frozen lysate (0.4%) (Table 7). One (1) run was reported invalid due to Run Control failure (0.6%). The run was reported valid upon repeat testing of the specimen lysates (Table 8).

Table 1: Fresh Stool Results Obtained with the BD GeneOhm™ Cdiff Assay in Comparison with the Reference Assay

		Reference Cytotoxicity Assay +	Reference Cytotoxicity Assay -	
BD GeneOhm™ Cdiff Assay	+	77	39	116
	-	5	738	743
		82	777	859

Table 2: Performance Obtained with Fresh Stools using the BD GeneOhm™ Cdiff Assay in Comparison with the Reference Method

Clinical Sites	Prevalence	Sensitivity with 95% CI*	Specificity with 95% CI*
Site 1	11.0% (43/390)	90.7% (39/43) (77.9%, 97.4%)	95.1% (328/345) (92.2%, 97.1%)
Site 2	6.6% (18/274)	94.4% (17/18) (72.7%, 99.9%)	95.6% (239/250) (92.3%, 97.8%)
Site 3	10.4% (22/212)	100% (21/21) (83.9%, 100%)	94% (171/182) (89.4%, 96.9%)
Overall	9.5% (83/876)	93.9% (77/82) (86.3% - 98.0%)	95.0% (738/777) (93.2% - 96.4%)

* CI: Confidence Intervals

Discrepant samples of the first dataset were resolved by isolating *C. difficile* from fecal specimens on selective media and retesting the isolates with the Reference Assay (Table 3). From 23 of the 39 apparent false PCR positive samples *C. difficile* was isolated and produced toxin B by the Reference Cytotoxicity Assay and thus were considered as true positives. Of the remaining 16 false positive samples, *C. difficile* was identified in six (6) of them but no detectable levels of toxin were measured by the Reference Assay and no *C. difficile* isolates were recovered by culture on ten (10) of them. From two (2) of the 5 false negative specimens *C. difficile* was recovered by culture, and only one of these two was reported as toxigenic. From three (3) false PCR negative specimens no *C. difficile* was recovered by culture.

Table 3: Summary of Clinical Performance of the BD GeneOhm™ Cdiff Assay in Comparison with Culture isolation and Cytotoxicity Testing.

		Resolved Culture and Toxigenicity Testing +	Resolved Culture and Toxigenicity Testing -	
BD GeneOhm™ Cdiff Assay	+	100	16	116
	-	5	738	743
		105	754	859

Table 4: Frozen Stool Results Obtained with the BD GeneOhm™ Cdiff Assay in Comparison with the Reference Assay

		Reference Cytotoxicity Assay +	Reference Cytotoxicity Assay -	
BD GeneOhm™ Cdiff Assay	+	34	6	40
	-	0	226	226
		34	232	266

Table 5: Performance Obtained with Frozen Stools using the BD GeneOhm™ Cdiff Assay in Comparison with the Reference Method

Clinical Site	Prevalence	Sensitivity with 95% CI*	Specificity with 95% CI*
Site 4	12.7% (34/267)	100.0% (34/34) (89.7%, 100%)	97.4% (226/232) (94.5%, 99%)

* CI: Confidence Intervals

Table 6: Fresh Stool Unresolved Rates

Clinical Sites	Initial unresolved rate with 95% CI*		Unresolved rate after repeat with 95% CI*	
Site 1	0.8% (3/390)	(0.2% - 2.2%)	0.5% (2/390)	(0.1% - 1.8%)
Site 2	6.6% (18/274)	(3.9% - 10.2%)	2.2% (6/274)	(0.8% - 4.7%)
Site 3	8.5% (18/212)	(5.1% - 13.1%)	4.2% (9/212)	(2.0% - 7.9%)
Overall	4.5% (39/876)	(3.2% - 6.0%)	1.9% (17/876)	(1.1% - 3.1%)

* CI: Confidence Intervals

Table 7: Frozen Stool Unresolved Rates

Clinical Site	Initial unresolved rate with 95% CI*		Unresolved rate after repeat with 95% CI*	
Site 4	0.4% (1/267)	(0.0% - 2.1%)	0.4% (1/267)	(0.0% - 2.1%)

* CI: Confidence Intervals

Table 8: Overall Invalid Run Rates

Site	Invalid Run Rates with 95% CI*	
Site 1	2.5% (1/40)	(0.1% - 13.2%)
Site 2	0.0% (0/45)	(0.0% - 7.9%)
Site 3	0.0% (0/58)	(0.0% - 6.2%)
Site 4	0.0% (0/24)	(0.0% - 14.2%)
Overall	0.6% (1/167)	(0.0% - 3.3%)

* CI: Confidence Intervals

Analytical Specificity

Genomic DNA from one non toxigenic *C. difficile* strain, two strains of Toxinotype XI lacking *tcdB* gene[13] and 29 other-*Clostridium* strains (including *C. sordellii*), along with 99 closely related organisms and other pathogenic and commensal flora found in the intestine and stools (representing 96 species) were tested. All strains were tested at a concentration of approximately $1X10^8$ CFU/mL or $1X10^8$ target copies/mL. None of these species tested positive with the BD GeneOhm™ Cdiff Assay, hence the analytical specificity is 100 %.

Analytical Sensitivity

Quantitated culture and purified genomic DNA diluted in the BD GeneOhm™ Cdiff Assay sample buffer were tested in five (5) replicates. The LOD was defined as the lowest concentration, in DNA copy number per reaction and CFU per reaction, at which five replicates out of five were found positive.

The analytical sensitivity (limit of detection or LOD) of the BD GeneOhm™ Cdiff Assay was determined with one strain of Toxinotype 0 *Clostridium difficile* carrying the *tcdB* gene (ATCC 43255).

The BD GeneOhm™ Cdiff Assay LOD is 10 DNA copies per reaction. The LOD in Colony Forming Units (CFU) is established at 4 CFU per reaction.

The analytical sensitivity in CFU per reaction was confirmed with a second Toxinotype 0 (ATCC 9689) and with Toxinotypes IIIa (SE844[12]), V (SE881[12]), VII (57267[12]) and VIII (1470[12]) *Clostridium difficile* toxigenic strains.

In addition to strains used for LOD determination, one hundred (100) other toxigenic *C. difficile* strains (including 17 other Toxinotypes), representing 21 countries, from well-characterized clinical isolates or public collections were evaluated using the BD GeneOhm™ Cdiff Assay. *C. difficile* strains were tested at a concentration of approximately 6.7 DNA copies/µL or 1 CFU/µL. The assay correctly identified all 100 *C. difficile* strains carrying the *tcdB* gene.

Reproducibility

The reproducibility panel consisted of three (3) simulated specimen categories where each tube contained 100 µL of simulated bowel flora inoculated with *C. difficile* (ATCC 43255). Additionally, two (2) Specimen Processing Controls (ATCC 9689 and ATCC 25922) and, two (2) Run Controls (Positive and Negative) were included. The specimens were tested, in triplicate per panel run, on five (5) distinct days (consecutive or not), wherein each day two (2) panels were tested, one for each of two (2) technologists, at three (3) clinical sites with one (1) lot of reagents. One (1) of these clinical sites participated in the extended study where two (2) additional lots of reagents were tested.

The overall correct percentage rate for the low *C. difficile* specimen category is 96.7%; the moderate *C. difficile* specimen category is 100% and the negative specimen category gave 100% for the Site-to-Site Reproducibility (Table 9).

The overall correct percentage rate for the low *C. difficile* specimen category is 100%; the moderate *C. difficile* specimen category is 97.8% and the negative specimen category gave 100% for the Lot-to-Lot Reproducibility (Table 10).

Table 9: Site-To-Site Reproducibility Study Results using One Lot

Category	SITE						Overall Agreement (%)	
	Site 1		Site 2		Site 3			
	Percent Agreement		Percent Agreement		Percent Agreement			
NEG	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90	100.0%
LOW POS	28/30	93.3%	29/30	96.7%	30/30	100.0%	87/90	96.7%
MOD POS	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90	100.0%

Table 10: Lot-To-Lot Reproducibility Study Results using Three Lots

Category	SITE						Overall Agreement (%)	
	Lot 1		Lot 2		Lot 3			
	Percent Agreement		Percent Agreement		Percent Agreement			
NEG	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90	100.0%
LOW POS	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90	100.0%
MOD POS	29/30	96.7%	29/30	96.7%	30/30	100.0%	88/90	97.8%

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Index of Symbols

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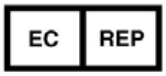	Authorized European Representative
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