510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT COMBINATION TEMPLATE

A. 510(k) Number:

K081920

B. Purpose for Submission:

To determine substantial equivalence of the device for the detection of *C.diffcile* toxin B gene (*tcdB)* in human liquid or soft stool specimens.

C. Measurand:

C. difficile toxin B gene

D. Type of Test:

Real-time polymerase chain reaction (PCR)

E. Applicant:

BD Diagnostics (GeneOhm Sciences Canada Inc.)

F. Proprietary and Established Names:

BD GeneOhm C. diff Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.2660, reagents, *Clostridium difficile* toxin

2. Classification:

   Class I

3. Product code:

   LLH

4. Panel:

83

H. Intended Use:

1. Intended use:

   The BD GeneOhm™ C diff 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.

2. Indications for use:

   The BD GeneOhm™ C.diff 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.

3. Special conditions for use statement:

   For prescription use

4. Special instrument requirements:

   Cepheid Smart Cycler

I. Device Description:

The BD GeneOhm C.diff Assay kit consists of sample buffer, lysis tube, master mix control DNA, diluent, 25 µL Smart Cycler reaction tubes, and specimen identification labels.

J. Substantial Equivalence Information:

1. Predicate device names:
   Techlab *C.difficile* Tox-B Test
   Techlab *C.difficile* Toxin/Antitoxin Kit

2. Predicate 510(k) numbers:
   K 935296
   K923463

3. Comparison with predicate:

Similarities		
Item	Device	Predicates
Specimen	Stool	Same
Analyte	Toxin B gene	Toxin B

Differences		
Item	Device	Predicates
Assay technique	PCR	Cytotoxicity cell culture
Assay Time	90 mins.	48 hrs

K. Standard/Guidance Document Referenced:

1. "Review criteria for devices assisting in the diagnosis of C. difficile associated diseases" ODE guidance 5/31/1990

L. Test Principle:

An aliquot of stool is lysed by eluting in sample buffer. The lysate is then 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. 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 amplification, detection and interpretation of the signals and provision of a final result are done automatically by the Cepheid SmartCycler® software. The entire procedure takes about 75 to 90 minutes, depending on the number of specimens processed.

M. Performance Characteristics:

1. Analytical performance:

a. Reproducibility:

The reproducibility panel consisted of three simulated specimen categories where each tube contained 100 µL of simulated bowel flora; the two positive panel members were also inoculated with *C. difficile* (ATCC 43255). Additionally, two Specimen Processing Controls (ATCC 9689 and ATCC 25922) and two Run Controls (Positive and Negative) were included. The specimens were tested in triplicate per panel run, on five distinct days. Each day two panels were tested, one for each of two technologists, at three clinical sites with one lot of reagents. Only one of these clinical sites participated in the extended study where two additional lots of reagents were tested.

The overall percent agreement for the low positive *C. difficile* specimen category is 96.7%; the moderate positive *C. difficile* specimen category is 100% and the negative specimen category is 100% for the Site-to-Site Reproducibility (Table 8).

The overall percent agreement for the low positive *C. difficile* specimen category is 100%; the moderate positive *C. difficile* specimen category is 97.8% and the negative specimen category is 100% for the Lot-to-Lot Reproducibility (Table 9).
Cycle threshold (Ct), an internal criteria used to determine a final assay result, was selected as an additional means of assessing assay reproducibility. Overall mean Ct values with variance components (SD and %CV) are shown in Tables 8 and 9.

An additional reproducibility study was performed, in accordance with the original reproducibility study protocol, to assess high negative specimens below the BD GeneOhm™ Cdiff Assay limit of detection (LOD). A sample containing simulated bowel flora was inoculated with *C. difficile* (ATCC 43255) at a concentration equivalent to the assay LOD. 100-fold and 10-fold dilutions of this sample were prepared, respectively, to obtain the two (2) high negative panel members. Overall percent agreement for negative test results and overall mean Ct values with variance components (SD and %CV) are shown in Table 10. As expected, the more dilute panel member (100-fold below the LOD) containing lower levels of target, demonstrates a higher percent agreement for negative test results than the less dilute panel member (10-fold below the LOD) which contains higher levels of target. Although high negative panel members are below the analytical LOD of the assay, positive test results may still be observed due to the presence of target in these specimens.

Table 8: Site-To-Site Reproducibility Study Results using One Lot

Category	SITE						Overall Percent Agreement		Ct Values		
	Site 1		Site 2		Site 3						
	Percent Agreement		Percent Agreement		Percent Agreement				Overall Mean	SD	%CV
NEG	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90	100.0%	36.2†	0.3†	0.8%†
LOW POS	28/30	93.3%	29/30	96.7%	30/30	100.0%	87/90	96.7%	38.8	0.9	2.3%
MOD POS	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90	100.0%	38.3	1.0	2.7%

† Data represent values from the internal control.

Table 9: Lot-To-Lot Reproducibility Study Results using Three Lots

Category	LOT						Overall Percent Agreement		Ct Values		
	Lot 1		Lot 2		Lot 3						
	Percent Agreement		Percent Agreement		Percent Agreement				Overall Mean	SD	%CV
NEG	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90	100.0%	36.1‡	0.3‡	0.8%‡
LOW POS	30/30	100.0%	30/30	100.0%	30/30	100.0%	90/90	100.0%	38.6	1.0	2.5%
MOD POS	29/30	96.7%	29/30	96.7%	30/30	100.0%	88/90	97.8%	37.8	1.1	2.8%

‡ Data represent values from the internal control.

Table 10: Additional Reproducibility Study Using a High Negative Sample Panel

High Negative Panel Member	Site 1		Site 2		Site 3		Overall Percent Agreement*		Ct Values		
	Percent Agreement*		Percent Agreement*		Percent Agreement*				Overall Mean	SD	%CV
1:100 dilution	25/30	83.3%	21/30	70.0%	26/30	86.7%	72/90	80.0%	41.3	0.9	2.1%
1:10 dilution	11/30	36.7%	5/30	16.7%	5/30	16.7%	21/90	23.3%	40.2	1.4	3.4%

*Percent agreement for a negative result.

b. Precision:

Within-laboratory precision was evaluated for the BD GeneOhm Cdiff Assay at one (1) site. The study was performed over 12 days, with two (2) runs per day and two (2) sample replicates per run. Samples included simulated specimens representing low and moderate positive *C. difficile* as well as negative *C. difficile*. One (1) out of 24 runs was excluded due to failure of the positive control (PC). One (1) moderate positive sample produced an unresolved result. All remaining samples and controls produced reportable results for a total of 46 replicates. Precision study results for low and moderate positive samples demonstrated agreement for (46/46) and (45/46) replicates, respectively; negative sample results demonstrated agreement for (46/46) replicates.

b. Linearity/assay reportable range:

N/A

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Controls

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.
For specimen processing controls, 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. A culture of a non toxigenic C. difficile strain (e.g. ATCC 700057) may be used as a negative specimen processing control.

Stability

The stability of *C. difficile* (carrying the *tcdB* gene) crude DNA from the sample prep procedure was determined with spiked fresh negative stool as well as fresh positive characterized stool specimens. Tests were performed after storage for up to 2 hrs at 25°C, 4 and 24 hrs at 4°C and after 24 hrs at -20°C (one freeze and thaw cycle). Results were adequate.

d. Detection limit:

Quantitated culture and purified genomic DNA diluted in 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[16]), V (SE881[16]), VII (57267[16]) and VIII (1470[16]) *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.

e. Analytical specificity:

Cross Reactivity
Genomic DNA from one non toxigenic *C. difficile* strain, two strains of Toxinotype XI lacking *tcdB* gene[17] and 29 other-*Clostridium* strains 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.

Interfering Substances

Twenty-six (26) biological and chemical substances occasionally used or found in perianal, rectal and/or stool specimens were evaluated for interference with the BD GeneOhm™ Cdiff Assay. 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. The remaining twenty-four (24) substances illustrated in the table below showed no detectable interference with the BD GeneOhm™ Cdiff Assay.

Endogenous and Commercial Exogenous Substances Tested with the BD GeneOhm™ Cdiff Assay

Substance	Result
Anusol MC Plus *	NI**
Atlas Ihle's Paste * Zinc oxide 25 % w/w paste (Laboratoire Atlas Inc.)	NI
Barium sulfate Fresh solution from powder form (LabMat)	NI
Exact™ Hydrocortisone acetate * Cream USP 0.5 % (Taro Pharmaceuticals Inc.)	NI
Exact™ stomach relief Bismuth subsalicylate liquid (Perrigo®)	NI
Fecal fat	NI
Fresh control® Moist towelettes pH 5,5 (Blue Skin)	NI
Gyne Moistrin®Vaginal moisturizing gel (Schering)	NI
Imodium AD® * Loperamide hydrochloride oral solution (McNeil)	NI
Kaopectate® Oral attaplugite suspension (Pharmacia & Upjohn)	NI

Substance	Result
Monistat™ Derm Miconazole nitrate cream USP 2% (McNeil)	NI
Palmitic acid * Fresh solution from powder (LabMat)	NI
Preparation H® with Bio-Dyne® * Cream (Wyeth)	NI
Preparation H® with Bio-Dyne® * Ointment (Wyeth)	NI
Rougier Neo-Laryngobis *Suppositories (Rougier Pharma)	NI
SAB-Dimenhydrinate® * Suppositories (SABEX®)	NI
Steric acid * Fresh solution from powder (LabMat)	NI
Trojan® latex condoms (with nonoxynol-9) Spermicidal lubricant (Church & Dwight Co., Inc.)	NI
Tucks MC personal cleansing pads Moist, soft cloth pads (Pfizer)	NI
Vagisil® Anti-itch cream (Combe Incorporated)	NI

Substance	Result
K-Y® Jelly (Johnson & Johnson Inc.)	NI
Metronidazole Fresh solution from powder form (Acros Organics)	NI

Substance	Result
Vancomycin Liquid (MP Biomedicals, LLC)	NI
Vaseline™ * White petroleum jelly U.S.P. (Lever Pond's)	NI

* Substance tested with two strains of C. difficile (Tox 0 and Tox VIII)
** NI: No detectable interference with the BD GeneOhm™ Cdiff Assay

f. Assay cut-off:

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

2. Comparison studies:

a. Method comparison with predicate device:

Predicate device is the tissue culture cytotoxicity assay which is the reference method. See results below under Clinical Studies

b. Matrix comparison:

N/A

3. Clinical studies:

a. Clinical Sensitivity:

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. Specimens were collected from individuals for whom *Clostridium difficile* testing was indicated and/or ordered. A tissue culture cytotoxicity assay – the reference method – was performed on the liquid or soft stool specimens within 48 hours of collection.

A total of 1108 specimens were tested with both the cytotoxicity assay mentioned above and the BD GeneOhm™ Cdiff Assay, producing 1090 reportable results. The first dataset includes 835 fresh specimens tested at 3 of the 4 clinical sites (Table 1). Overall clinical sensitivity was 93.8% and clinical specificity was 95.5% .(Table 2). For the population tested this resulted in a Negative Predictive Value (NPV) of 99.1% and a Positive Predictive Value (PPV) of 67.3%.

At the fourth clinical site, frozen stool aliquots were tested with both the cytotoxicity reference assay and the BD GeneOhm™ Cdiff Assay. The second dataset included results from 255 frozen stool specimens (Table 3). In comparison to the cytotoxicity assay, the BD GeneOhm™ Cdiff Assay showed a clinical sensitivity of 100% and clinical specificity of 97.7% in the frozen dataset;

resulting in a NPV of 99.2% and PPV of 81.5% (Table 4).

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	+	76	34[†]	110
	-	5[‡]	720	725
		81	754	835

[†] Cytotoxicity Assay on isolated strains was positive for 21 out of the 34 samples, verifying the presence of toxigenic *C. difficile*. For the remaining 13 samples, standard PCR with alternative primers followed by bi-directional sequencing revealed that 11 out of the 13 samples contained the expected *tcdB* gene.

[‡] For two (2) of the five (5) false negative specimens, *C. difficile* was recovered by culture, and only one (1) of these two (2) was reported as toxigenic. Of the remaining three (3) false negative PCR specimens, no *C. difficile* was recovered by culture.

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% (40/365)	90.5% (38/42) (77.4% - 97.3%)	95.7% (309/323) (92.8% - 97.6%)
Site 2	6.7% (16/240)	94.4% (17/18) (72.7% - 99.9%)	96.4% (240/249) (93.2% - 98.3%)
Site 3	11.1% (18/162)	100% (21/21) (83.9% - 100%)	94.0% (171/182) (89.4% - 96.9%)
Overall	9.6% (74/767)	93.8% (76/81) (86.2% - 98.0%)	95.5% (720/754) (93.8% - 96.9%)

* CI: Confidence Intervals

Table 3: 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	5	39
	-	0	216	216
		34	221	255

Table 4: 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%)	97.7% (216/221) (94.8% - 99.3%)

* CI: Confidence Intervals

Of 852 fresh specimens tested with the BD GeneOhm™ Cdiff Assay, 39 were initially reported as unresolved (4.6%). Upon repeat testing from the frozen lysates, 22 were resolved and 17 remained unresolved (2.0%). Out of 256 frozen specimens tested with the BD GeneOhm™ Cdiff Assay, only 1 specimen (0.4%) was initially reported unresolved. The specimen remained unresolved upon repeat testing from the frozen lysate (0.4%). One run was reported invalid due to Run Control failure (0.6%). The run was reported valid upon repeat testing of the specimen lysates.

b. Clinical specificity:

See a) above for clinical specificity

c. Other clinical supportive data (when a. and b. are not applicable):

N/A

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

The BD GeneOhm C. diff assay showed a prevalence ranging from 6.7% - 12.7% in the populations tested.

N. Instrument Name:

Cepheid Smart Cycler

O. System Descriptions:

1. Modes of Operation:

The operation of the Smart Cycler instrument is based on the proprietary microprocessor –controlled I-CORE (Intelligent Cooling/Heating Optical Reaction) module. Each Smart Cycler processing block contains 16 independently controlled, programmable I-CORE modules, each with one reaction site. Thermally optimized proprietary reaction tubes combined with the design of the I-CORE modules allow very rapid temperature cycling and rapid amplification. Up to 6 Smart Cycler processing blocks can be daisy-chained together, allowing simultaneous analysis of 96 discrete samples. Information regarding data acquisition, data analysis and diagnostic algorithm was submitted and cleared under K022504 and K042357

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for

this line of product types:

Yes _____ or No __X_____

3. Specimen Identification:

   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.

4. Specimen Sampling and Handling:

   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. Amplified targets are detected with hybridization probes labeled 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 75 to 90 minutes.

5. Calibration:

   N/A

6. 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.

   Specimen Processing Controls

   Additional control strains may be tested according to guidelines or requirements of local, state 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.
Colonies are isolated after 18 to 24 hours of anaerobic incubation on 5% sheep blood agar. Colonies are resuspended in saline to a turbidity of 0.5 McFarland (~1.5 X 10^7 CFU/mL) and diluted with saline to obtain a suspension of ~10^6 CFU/mL. A dry swab is dipped into the bacterial suspension, and excess fluid is pressed out. It is then processed and tested as a clinical specimen, 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.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

N/A

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.