510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K232092

B Applicant

Vela Operations USA

C Proprietary and Established Names

Great Basin Toxigenic *C. difficile* Direct Test (CDF2)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
OZN	Class II	21 CFR 866.3130 - *Clostridium Difficile* Toxin Gene Amplification Assay	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:
To obtain substantial equivalence for the Great Basin Toxigenic *C. difficile* Direct Test (CDF2).

B Measurand:
tcdB gene of toxigenic *Clostridioides difficile*

C Type of Test:
The Toxigenic *C. difficile* Direct Test (CDF2) is a polymerase chain reaction (PCR) which amplifies specific sequences that are hybridized onto probes immobilized on a silicon chip surface.

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

B Indication(s) for Use:

The Great Basin Toxigenic *C. difficile* Direct Test, performed on the Great Basin PA500 Analyzer, is a qualitative *in vitro* diagnostic test for the detection of toxigenic *Clostridioides difficile* in unformed (liquid or soft) stool samples collected from patients suspected of having *C. difficile* infection (CDI). The automated assay utilizes polymerase chain reaction (PCR) to detect a conserved region of the toxin gene (*tcdB*) associated with toxin-producing *C. difficile*.

The Toxigenic *C. difficile* Direct Test is intended for use as an aid in the diagnosis of CDI in conjunction with clinical and epidemiological risk factors

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Great Basin PA500 Analyzer

IV Device/System Characteristics:

A Device Description:

The Great Basin Toxigenic *C. difficile* Direct Test (CDF2) performed on the PA500 Analyzer utilizes automated, hot-start PCR amplification technology to amplify specific nucleic acid sequences that are then detected using hybridization probes immobilized on a modified silicon chip surface, in a single-use, self-contained test cartridge.

A swab volume of the specimen (raw stool) is first processed using the Sample Preparation Device (SPD). During processing through the SPD, the specimen is infused with a synthetic bacterial sample processing control (SPC). An aliquot of the eluate obtained from the SPD, containing diluted specimen mixed with SPC, is loaded into the sample port of the CDF2 Test Cartridge.

Genomic DNA is extracted from microbial cells and diluted to reduce potential inhibitors of PCR. During the PCR process, biotin-labeled primers direct the amplification of specific nucleic acid sequences within a conserved region of the *C. difficile* Toxin B (*tcdB*) gene.

Following PCR, biotin-labeled, amplified target DNA sequences are hybridized to sequence specific probes immobilized on the silicon chip surface and incubated with anti-biotin antibody conjugated to the horseradish peroxidase enzyme (HRP). The unbound conjugate is washed away, then tetramethylbenzidine (TMB) is added to produce a colored precipitate at the location of the probe/target sequence complex.

The resulting signal is detected by the automated PA500 Optical Reader within the PA500 Analyzer System. The SPC undergoes the same extraction, amplification, and detection steps as the sample in order to monitor for inhibitory substances, as well as process inefficiency due to instrument or reagent failure. No operator intervention is required once the sample is loaded into

the sample port, and the Toxigenic *C. difficile* Direct Test cartridge is loaded into the PA500 Analyzer.

B Principle of Operation:

The Great Basin System is a fully automated in vitro diagnostic system that includes: the PA500 Analyzer device, single-use SPDs, single-use Toxigenic *C. difficile* Direct Test Cartridges, and the PA500 Data Analysis Software Program.

The PA500 Analyzer is designed to perform automated sample preparation, target amplification via PCR, chip-based (optical) target detection, and integrated data analysis in less than two hours.

V Substantial Equivalence Information:

A Predicate Device Name(s):
GenePOC CDiff

B Predicate 510(k) Number(s):
K172569

C Comparison with Predicate(s):

Device & Predicate Device(s):	**Toxigenic *C. difficile* Direct Test (CDF2) K232092**	**GenePOC CDiff K172569**
Device Trade Name	Great Basin Toxigenic *C. difficile* Direct Test	GenePOC CDiff Assay
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Great Basin Toxigenic *C. difficile* Direct Test, performed on the Great Basin PA500 Analyzer, is a qualitative in vitro diagnostic test for the detection of toxigenic *Clostridioides difficile* in unformed (liquid or soft) stool samples collected from patients suspected of having *C. difficile* infection (CDI). The automated assay utilizes polymerase chain reaction (PCR) to	The GenePOC CDiff assay performed on the revogene instrument is a qualitative in vitro diagnostic test that utilizes automated sample processing and real-time polymerase chain reaction (PCR) to detect the toxin B (*tcdB*) gene of toxigenic *Clostridium difficile* (*C. difficile*) in unformed (liquid or soft) stool specimens obtained from patients suspected of having *C. difficile*

	detect a conserved region of the toxin gene (*tcdB*) associated with toxin-producing *C. difficile*. The Toxigenic *C. difficile* Direct Test is intended for use as an aid in the diagnosis of CDI in conjunction with clinical and epidemiological risk factors.	infection (CDI). The GenePOC CDiff assay is intended to aid in the diagnosis of CDI.
Specimen Type	Unformed (liquid or soft) stool specimens	Same
Assay Target	toxin B gene (*tcdB*)	Same
Sample Lysis and DNA Extraction	Automated (in cartridge)	Same
Amplification Technology	Multiplex PCR	Same
Result Interpretation	Automated	Same
General Device Characteristic Differences		
Detection Probes	Hybridization Probes	TaqMan Probes
Detection Technology	Colorimetric target specific hybridization to probe on a chip surface, optical reader, automated software with built-in result interpretation.	Real-time PCR (fluorescent signals) and embedded detection algorithm
Sample Volume	1 swab	5 ul
Sample per Run	One	Up to 8
Time to Result	2 hours	70 min

VI Standards/Guidance Documents Referenced:

1. Guidance for Clinical Investigators, Industry and FDA Staff: Financial Disclosure by Clinical Investigators. February 2013
2. Guidance on Informed Consent for In Vitro Diagnostics Device Studies Using Leftover Human Specimens that are not Individually Identifiable. April 2006
3. Class II Special Controls Guideline Document: Toxin Gene Amplification Assays for the Detection of Clostridium difficile – Guideline for Industry and Food and Drug Administration Staff. August 2015

4. CLSI MM13-A: Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods: Approved Guideline.
5. CLSI MM3-A2: Molecular Diagnostic Methods for Infectious Diseases: Approved Guideline

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

A between-site reproducibility study was performed at three sites (two external and one internal) by two operators per site, over five non-consecutive days using two cartridge lots and 24 analyzers. A total of 90 negative sample replicates and 360 positive sample replicates were tested. Two toxigenic *C. difficile* strains were tested: ATCC 43255 (toxinotype 0, ribotype 087) and ATCC BAA-1805 (toxinotype IIIb, NAP1/ribotype 027).

A five-member panel, tested in triplicate, was contrived using a pool of *C. difficile*-negative clinical stool matrix at the following target concentrations:

- Low positive at 1.5X LoD
- Moderate positive at 3X LoD
- True negative (TN) samples without toxigenic *C. difficile*

The low positive (1.5X LoD) concentrations tested were 3.9×10^4 CFU/mL and 1.1×10^5 CFU/mL for ATCC 43255 and ATCC BAA-1805, respectively. The moderate positive (3X LoD) concentrations tested were 7.8×10^4 CFU/ml and 2.2×10^5 CFU/mL for ATCC 43255 and ATCC BAA-1805, respectively.

Results are shown by site and overall, in Table 1. For the between-site reproducibility, the overall percent agreement was 97.8% for the low positive (1.5X) samples and 100% for moderate positive (3X) samples for ATCC strain 43255. The overall percent agreement was 100% for the low positive (1.5X) and moderate positive (3X) samples of strain ATCC BAA-1805. The overall percent agreement was 98.9% for the negative samples. The two false negative results from Site 02 were attributed to assay variability and sampling error. The false positive result from Site 02 was attributed to unintentional sample contamination by the operator. There was no variability in results by operator or by lot.

Table 1. Reproducibility Results by Site and Overall

Panel	Strain	Assay Results/Expected				Agreement Total [95% CI]
		Site 01	Site 02	Site 03	All sites	
Low Positive (1.5X LoD)	ATCC 43255	30/30	28/30	30/30	88/90	97.8% [92.2%-99.7%]
	ATCC BAA-1805	30/30	30/30	30/30	90/90	100% [96%-100%]
Moderate Positive (3X LoD)	ATCC 43255	30/30	30/30	30/30	90/90	100%% [96%-100%]
	ATCC BAA-1805	30/30	30/30	30/30	90/90	100% [96%-100%]

Negative	**N/A**	30/30	29/30	30/30	**89/90**	**98.9% [94%-100%]**

2. Linearity:

   Not applicable

3. Analytical Specificity/Interference:

   a. *Analytical inclusivity*:

   The analytical inclusivity of the Toxigenic *C. difficile* Direct Test (CDF2) assay was evaluated by testing 22 strains of toxigenic *C. difficile* representing six different toxinotypes (Summarized in Table 2). Quantitated cultures of each *C. difficile* strain were diluted to 9.7 x 10^4 CFU/ml in clinical negative stool matrix, and three replicates per strain were tested using six cartridge lots. All strains were detected, which is acceptable.

Table 2. *C. difficile* Strain Evaluated for Inclusivity

Toxigenic *C. difficile* Strain	**Toxinotype, Toxin**
ATCC 9689	Toxinotype 0, A+, B+
ATCC 700792	
ATCC 17858	
ATCC BAA-1382	
ATCC BAA-1808	
ATCC BAA-1871	
ATCC BAA-1872	
ATCC BAA-1874	
ATCC BAA-2156	
ATCC 51695	
ATCC 43600	
ATCC 43599	
ATCC 43596	
ATCC 43594	
ATCC 17857	
ATCC BAA-1804	
ATCC 43598	Toxinotype VIII, A-, B+
ATCC BAA-1812	Toxinotype XII, A+, B+
ATCC BAA-1803	Toxinotype IIIc, NAP1, A+, B+
ATCC BAA-1814	Toxinotype XXII, A+, B+
ATCC BAA-1875	Toxinotype V, A+, B+
ATCC BAA-2155	Toxinotype XXII, A+, B+

   b. *Analytical specificity (exclusivity):*

   The potential cross-reactivity of the assay was assessed by testing concentrations of 82 non-targeted organisms (69 bacteria, one yeast, three parasites, nine viruses) and human DNA preparations that may be found in unformed stool specimens (see Table 3, Table 4, and Table 5). The test panel included commensal and pathogenic microorganisms from the intestinal tract, species phylogenetically related to *C.*

difficile, non-pathogenic strains of *C. difficile*, and human DNA. Bacteria and yeast were tested at a load of $\geq 10^6$ CFU/mL, except for a single strain of *Clostridium butyricun* ATCC 19398 which was tested at 1.0×10^5 CFU/mL. Nucleic acids from viruses and human DNA were tested at a load of $\geq 10^6$ copies/mL or $\geq 10^6$ $TCID_{50}$/mL. The test panel was contrived by spiking quantitated cell cultures or nucleic acid solutions into *C. difficile*-negative clinical stool matrix. Each organism was tested in triplicate.

Under the conditions of the study, *Salmonella enterica subsp. Enterica serovar Newport* gave a false positive result by the assay for one out of three replicates. However, no false positives were observed when the culture stock was regrown, and an additional three replicates were tested. All results were acceptable.

Table 3. Cross Reactivity Panel - Non-toxigenic *C. difficile*, other *Clostridium sp.*

Name	ID
Clostridium difficile (non-toxigenic)	ATCC 43593
Clostridium difficile (non-toxigenic)	ATCC 43601
Clostridium bifermentans	ATCC 638
Clostridium butyricum	ATCC 19398
Clostridium novyi	ATCC 19402
Clostridium perfringens	ATCC 13124
Clostridium scindens	ATCC 35704
Clostridium septicum	ATCC 12464
Clostridium sporogenes	ATCC 15579
Flavonifractor plautii (formerly *Clostridium orbiscindens*)	ATCC 49531
Hathewaya histolytics (formerly *Clostridium histolyticum*)	ATCC 19401
Paeniclostridium (Clostridium) sordellii	ATCC 9714

Table 4. Cross Reactivity Panel - Other Bacteria

Name	ID	Name	ID
Abiotrophia defective	ATCC 49176	*Peptostreptococcus anaerobius*	ATCC 27337
Acinetobacter baumannii	ATCC 19606	*Plesiomonas shigelloides*	ATCC 51903
Aeromonas hydrophila	ATCC 35654	*Porphyromonas asaccharolytica*	ATCC 27908
Alcaligenes faecalis	ATCC 15554	*Prevotella melaninogenica*	ATCC 700524
Bacillus cereus	ATCC 14579	*Proteus mirabilis*	ATCC 25933
Bacteroides fragilis	ATCC 23745	*Proteus vulgaris*	ATCC 6896
Campylobacter jejuni	ATCC 49943	*Providencia alcalifaciens*	ATCC 9886
Campylobacter coli	ATCC 49941	*Providencia rettgeri*	ATCC 9250
Campylobacter fetus	ATCC 25936	*Pseudomonas aeruginosa*	ATCC 10145
Citrobacter freundii	ATCC 8090	*Pseudomonas fluorescens*	ATCC 13525

Name	ID	Name	ID
Edwardsiella tarda	ATCC 15947	*Salmonella enterica subsp. Arizonae*	ATCC 13314
Enterobacter aerogenes	ATCC 15038	*Salmonella enterica subsp. Enterica serovar Choleraesuis*	ATCC 13312
Enterobacter cloacae	ATCC 13047	*Salmonella enterica subsp. Enterica serovar Newington*	ATCC 29628
Enterococcus faecalis	ATCC 29212	*Salmonella enterica subsp. Enterica serovar Newport*	ATCC 6962
Enterococcus faecalis (vanB)	ATCC 51299	*Salmonella enterica subsp. Enterica serovar Typhi*	ATCC 6539
Enterococcus faecium	ATCC19434	*Salmonella enterica subsp. Enterica serovar Typhimurium*	ATCC 13311
Escherichia coli	ATCC 4157	*Serratia liquefaciens*	ATCC 35551
Escherichia coli O157:H7	ATCC 43895	*Serratia marcescens*	ATCC 13880
Escherichia coli O121:H19	ATCC BAA-2221	*Shigella boydii*	ATCC 29928
Escherichia coli O104:H4	ATCC BAA-2326	*Shigella dysenteriae*	ATCC 9361
Escherichia fergusonii	ATCC 35469	*Shigella flexneri*	ATCC 25929
Escherichia hermanii	ATCC 33650	*Shigella sonnei*	ATCC 25931
Helicobacter pylori	ATCC 43504	*Staphylococcus aureus*	ATCC 43300
Klebsiella oxytoca	ATCC 49131	*Staphylococcus aureus*	ATCC 12598
Klebsiella pneumoniae	ATCC 13883	*Staphylococcus epidermidis*	ATCC 12228
Lactobacillus acidophilus	ATCC 4356	*Streptococcus agalactiae*	ATCC 13813
Listeria monocytogenes	ATCC 19115	*Streptococcus dysalactiae*	ATCC 43078
		Vibrio parahaemolyticus	ATCC 17802
		Yersinia enterocolitica	ATCC 49397

Table 5. Cross Reactivity Panel - Viruses and Other Organisms

Name	ID	Name	ID
Adenovirus type 40	ATCC VR-931D	Human genomic DNA	Promega G147A
Adenovirus type 41	ATCC VR-930D	Enterovirus 71	ATCC VR-1775DQ
Cryptosporidium parvum	ATCC PRA-67D	*Entamoeba histolytica*	ATCC 30459DQ
Rotavirus A	ATCC VR-2018DQ	Echovirus 4	ATCC VR-1734D

Norovirus GI	ATCC VR-3234SD	Human Herpesvirus 5 (Cytomegalovirus) (DNA)	ATCC VR-538D
Norovirus GII	ATCC VR-3235SD	*Giardia intestinalis*	ATCC 50803D
Coxsackievirus B2	ATCC VR-29		

c. *Microbial Interference*

A microbial interference study was conducted to assess the potential inhibitory effect of 82 non-targeted microorganisms (See Table 6) that may be found in stool specimens. Pools of three to five organisms were prepared in *C. difficile* negative clinical stool matrix and tested in three replicates each in the presence of toxigenic *C. difficile* strain ATCC 43255. The bacteria/fungi, DNA/RNA, and viruses were spiked at ≥1 x 10^6 CFU/mL, ≥1 x 10^6 copies/mL, and 1x10^6 TCID50/mL, respectively. The toxigenic *C. difficile* was spiked at 2X LoD of this strain (5.2 x 10^4 CFU/mL).

No interference was observed for the detection of *C. difficile* strain ATCC 43255.

Table 6. Microbial Interference Panel

Pool	Potentially Interfering Microorganisms
1	*Aeromonas hydrophila*
	Bacteroides fragilis
	Campylobacter fetus
	Campylobacter jejuni
	Campylobacter coli
2	*Citrobacter freundii*
	Clostridium difficile (non-toxigenic)
	Clostridium difficile (non-toxigenic)
	Clostridium perfringens
	Paeniclostridium (Clostridium) sordellii
3	*Enterobacter cloacae*
	Enterococcus faecium
	Enterococcus faecalis
	Escherichia coli O157:H7
	Escherichia coli
4	*Escherichia fergusonii*
	Escherichia coli hermanii
	Helicobacter pylori
	Klebsiella pneumoniae
	Peptostreptococcus anerobius
5	*Salmonella enterica serovar Choleraesuis*
	Salmonella enterica serovar Newington
	Salmonella enterica serovar Newport

Pool	Potentially Interfering Microorganisms
	Salmonella enterica serovar Typhi
	Salmonella enterica serovar Typhimurium
6	*Serratia liquefaciens*
	Serratia marcescens
	Shigella boydii
	Shigella flexneri
	Shigella sonnei
7	*Candida albicans*
	Staphylococcus aureus
	Staphylococcus aureus
	Staphylococcus epidermidis
	Yersinia entercolitica
8	*Klebsiella oxytoca*
	Proteus mirabilis
	Salmonella enterica subsp. Arizonae
	Shigella dysenteriae
	Streptococcus agalactiae
9	*Acinetobacter baumannii*
	Bacillus cereus
	Edwardsiella tarda
	Enterobacter aerogenes
	Pseudomonas fluorescens
10	*Abiotrophia defective*
	Alcaligenes faecalis
	Clostridium bifermentans
	Clostridium butyricum
	Clostridium novyi
11	*Hathewaya histolytica (Clostridium histolyticum)*
	Lactobacillus lactis
	Listeria monocytogenes
	Pseudomonas aeruginosa
	Vibrio paraharmolyticus
12	*Clostridium sporogenes*
	Lactobacillus acidophilus
	Plesiomonas shigelloides
	Proteus vulgaris
	Providencia rettgeri
13	*Flavonifractor plautii* (formerly *Clostridium orbiscindens*)
	Providencia alcalifaciens
	Salmonella enterica serovar Newport
	Escherichia coli
	Escherichia coli
14	Adenovirus type 40

Pool	Potentially Interfering Microorganisms
	Enterovirus 71
	Norovirus GI
	Norovirius GII
	Rotavirus A
15	*Clostridium scindens*
	Clostridium septicum
	Enterococcus faecalis (van B)
	Porphyromonas asaccharolytica
	Prevotella melaninogenica
16	Adenovirus type 41
	Coxsackievirus B2
	Herpesvirus 5 (Cytomegalovirus)
	Echovirus 4
	Giardia intestinalis
17	*Cryptosporidium parvum*
	Entamoeba histolytica
	Human gDNA

d. *Interfering Substances*

An interfering substances study was conducted to assess the potentially inhibitory effects of 30 substances (Table 7) that may be present in stool specimens. The substances were tested in triplicate in the presence of two strains of toxigenic *C. difficile* (ATCC 43255 and ATCC BAA-1805) in *C. difficile* negative clinical stool matrix. Substances were spiked at higher concentrations than expected in stool specimens, and *C. difficile* strains were spiked at 2X LoD (ATCC 43255 at 5.2 x 10^4 CFU/mL, and ATCC BAA-1805 at 1.4 x 10^5 CFU/mL).

No interference was observed for the 30 substances at the tested concentrations.

Table 7. Substance Interference Panel

Substance (commercial name)	Active Ingredient(s)	Concentration
Vaginal antifungal / anti-itch (Nystatin)	Nystatin	10% w/v
Creams / ointments (Cortizone-10)	Hydrocortisone	30% w/v
Anti-hemorrhoidal creams / ointments (Preparation H)	Phenylephrine HCI	20% w/v
Antacids (Tums)	Calcium Carbonate	38% w/v
Mineral Oil (Fisher Bioreagents Cat# B2629-1)	Mineral Oil	50% v/v
Enemas (Mesalazine Sigma Aldrich Cat# Y0000297)	Mesalazine	2.5% w/v
Condom with spermicidal lubricant (Trojan)	Nonoxynol-9	40% w/v

Substance (commercial name)	Active Ingredient(s)	Concentration
Anti-diarrheal medication (Pepto Bismol)	Bismuth Subsalicylate	50% v/v
Anti-diarrheal medication (Imodium)	Loperamide Hydrochloride	50% v/v
Laxatives (Senna Glycoside Tablet)	Sennosides	17% w/v
Antibiotics (Vancomycin 100mg/mL in DMSO – Sigma Cat# SBR00001)	Vancomycin	5% w/v
Antibiotics (Metronidazole – MP Biomedicals Cat# 155710)	Metronidazole	0.5% w/v
Non-steroidal anti-inflammatory (Aleve)	Naproxen Sodium	10% w/v
Moist towelettes (Wet Ones)	Benzalkonium Chloride	50% v/v
Suppository (Fleet)	Liquid Glycerin	50% v/v
Personal lubricant (KY)	Glycerin	50% v/v
Personal lubricant (Astroglide)	Silicone	50% v/v
Aloe leaf juice flushable wipes (Generic)	Butoxy PEG-4 PG-Amodimethicone	50% v/v
Anti-fungal cream (Miconazole-3)	Miconazole Nitrate	30% w/v
Petroleum Jelly (Vaseline)	Petroleum	30% w/v
Biotin Supplement (Nature Made, 2500 mcg/capsule)	Biotin	51% w/v[a]
Contrast agent	Barium Sulfate	25% w/v
Stearic Acid (ScienceLab Cat# SLS3742	Stearic Acid	1.1% w/v
Palmitic Acid (Sigma Cat# P0500)	Palmitic acid	1.5% w/v
Whole Blood (Fisher Scientific Cat# NC0088224)	Blood components	40% v/v[b]
Biotin (Sigma Cat# B4501)	Biotin	0.5% w/v
Nonoxynol-9 (Sigma Aldrich Cat# PHR2731)	Nonoxynol-9	48% v/v
Mucin from Porcine Stomach (Sigma Cat# M1778)	Mucin	2.5% w/v
Topical antibiotic (Neosporin)	Bacitracin, Neomycin, Polymyxin B	50% w/v
Nystatin (Sigma Cat# N6261)	Nystatin	10% w/v

Substance (commercial name)	Active Ingredient(s)	Concentration
Erythromycin (Alfa Aesar Cat# J62279)	Erythromycin	10% w/v

a Input listed is the total amount of the gel-capsule biotin supplement tested and equates to approximately 1.3 mg of biotin per swab.
b Raw stool was formulated to 40% whole blood by volume (v/v). One (1) swab volume of the mixture was evaluated in the CDF2 test.

4. Assay Reportable Range:

Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

e. *Quality Control:*

The Great Basin Toxigenic *C. difficile* Direct Test (CDF2) assay uses three internal controls: a sample processing control (SPC), a hybridization control (HC), and a detection control (DC). The SPC is included in the Sample Preparation Device supplied with each kit. The SPC is extracted, amplified, and detected along with each specimen tested, and it verifies the efficacy of the sample preparation, PCR amplification, and detection processes. The HC is a control oligonucleotide attached to the silicon chip surface. A biotinylated oligonucleotide with complementary sequence to the HC is added to the hybridization buffer and the oligonucleotide pair provides verification that probe hybridization is functioning properly. The DC is a biotinylated oligonucleotide probe attached to the silicon chip surface that provides a control for the performance of the conjugate and detection reagents for proper signal development.

The use of two external controls is recommended: a positive external control (PEC), and negative external control (NEC). The PEC monitors for substantial reagent failure, and the NEC monitors for environmental contamination or carryover. Each laboratory must establish the number, type and frequency of testing external control materials per applicable regulations or accrediting agencies. External Control materials are recommended but not provided. External Controls are treated as if they are specimens.

Optional PEC materials include:

- ZeptoMetrix *Clostridium difficile* Positive Control IVD CE (Catalog No. NATCDI-6MC-IVD).
- Remnants of a known *C. diff*icile positive clinical stool sample or
- Culture of a known C. difficile strain (e.g., Microbiologics KWIK-STIK *C. difficile* ATCC 9689, catalog no. 0329)

Optional NEC materials include:

- ZeptoMetrix *Clostridium difficile* Negative Control IVD CE (Catalog No. NATCSO-6MC-IVD) or
- Remnants of a known *C. difficile* negative clinical stool sample.

One PEC and one NEC were processed each day of analytical and clinical testing. Of the total 361 external controls that were run across all sites in the clinical study, 350 (97.0%)

were valid, eight (2.2%) were canceled by the end-user (test incomplete), and three (0.8%) were invalid due to a failed internal control. All failed results repeated successfully.

f. Sample Stability:

Specimen stability was evaluated analytically by testing pre-characterized positive and negative clinical samples under different storage time and temperature conditions. The results supported the stool specimen storage instructions described in the package insert.

Stool specimens can be stored at refrigerated (2°-8°C) for up to 72 hours (three days), or at room temperature (18°-22°C) for up to 24 hours.

g. Freeze/thaw Stability:

Studies were conducted to examine the stability of stool specimens when subjected to freeze/thaw cycles. Contrived positive stool samples contained 5X LoD and 2X LoD of *C. difficile* (ATCC 43255). The study results supported a recommendation that stool samples stored at -70ºC can undergo no more than one freeze/thaw cycles.

6. Detection Limit:

The analytical sensitivity (Limit of Detection or LoD) of the Toxigenic *C. difficile* Direct Test (CDF2) assay was determined using negative clinical stool specimens spiked with various concentrations of toxigenic *C. difficile* bacterial suspensions. The testing to establish and confirm the LoD included four lots, multiple instruments, and operators. Two strains of toxigenic *C. difficile* (ATCC 43255, Toxinotype 0, *tcdA*+, *tcdB*+, and ATCC BAA-1805, NAP 1/027, Toxinotype IIIb, *tcdA*+, *tcdB*+) were tested in a minimum of 20 replicates per concentration in the confirmation study. The LoD was defined as the lowest concentration at which 95% or more of all replicates tested positive. The LoD was determined at 2.6 X 10^4 CFU/mL for ATCC 43255 and 7.1 X 10^4 CFU/ml for ATCC BAA-1805.

7. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:

The performance of the Toxigenic *C. difficile* Direct Test (CDF2) was assessed with remnants of unpreserved stool specimens prospectively collected from symptomatic individuals suspected of having an *C. difficile* infection. Specimens were collected from three independent clinical sites. Stool specimens were tested with a high-performing FDA-cleared molecular *C. difficile* assay, which served as the basis for performance evaluation of this assay. Specimens with discrepant results were tested with an alternative FDA-cleared molecular C. difficile assay, performance of which is footnoted accordingly, but the original

performance was not changed. Prospective testing consisted of 829 stool specimens that met the inclusion criteria.

The ages of patients ranged from less than 2 years to over 89 years with 53.3% of the specimens coming from patients that were 19-64 years of age. Of the 829 patients tested, 52% were female, 47.4% were male, and 0.6% were undeclared gender.

Of the 829 eligible specimens, ten specimens generated false positive with the Toxigenic *C. difficile* Direct Test (CDF2) and six specimens generated false negative results.

Table 8 and 9 show a summary of the clinical performance of the Toxigenic *C. difficile* Direct Test (CDF2) for all sites individually and combined. The PPA and NPA values for the Toxigenic *C. difficile* Direct Test (CDF2) were 95.8% and 98.5%, respectively. There was no observable difference in performance of the Toxigenic *C. difficile* Direct Test (CDF2) with respect to study site, kit lot, or patient gender or age. These results are acceptable.

Table 8. Clinical Study Results

All Sites		Molecular Comparator		
		Positive	Negative	Total
Toxigenic *C. difficile* Direct Test	Positive	138	10*	148
	Negative	6*	675	681
	Total	144	685	829

* All 16 discrepant results were tested with the alternative FDA-cleared molecular C. difficile assay. None of the 10 false positive samples were positive and five of the six false negatives were negative with the alternative assay.

Table 9. Summary of Clinical Performance

	n	PPA (95% CI)	NPA (95% CI)	PPV (95% CI)	NPV (95% CI)
Toxigenic *C. difficile* Direct Test	829	95.8% (91.2 – 98.5) 138/144	98.5% (97.3 – 99.3) 675/685	93.2% (87.9 - 96.7)	99.1% (98.1 – 99.7)

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Sensitivity:

Not applicable

2. Clinical Specificity:

Not applicable

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

In the clinical studies, a total of 829 specimens were enrolled at three geographically diverse clinical sites, from subjects ranging in age from less than two years old to more than 89 years old. Of these specimens, the overall percentage of positive results observed with the Toxigenic *C. difficile* Direct Test (CDF2) with these specimens was 17.4% [144/829; 95%CI: 14.9 –20.1 %].

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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