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

A. 510(k) Number:

K123197

B. Purpose for Submission:

Substantial equivalence determination of the Verigene® *Clostridium difficile* Nucleic Acid Test (CDF) on the Verigene® System.

C. Measurand:

Targets DNA sequences of the toxin A (*tcdA*), toxin B (*tcdB*) and *tcdC* genes within the PaLoc of toxigenic strains of *C. difficile*; presumptive identification of the PCR ribotype 027 strain of *C. difficile* is via detection of the binary toxin (*cdt*) gene sequence and the single base pair deletion at nucleotide 117 in the *tcdC* gene (which encodes a negative regulator of toxin production).

D. Type of Test:

Qualitative, *in vitro* diagnostic test using polymerase chain reaction (PCR) amplification of *tcdA*, *tcdB*, *tcdC*, and *cdt* gene sequences, as well as detection of the single base pair deletion at nucleotide 117 in the *tcdC* gene combined with a nanoparticle-based array hybridization detection assay.

E. Applicant:

Nanosphere, Inc

F. Proprietary and Established Names:

Verigene® *Clostridium difficile* Nucleic Acid Test (CDF)

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3130 - *C. Difficile* Nucleic Acid Amplification Test Assay

2. Classification:

   Class II

3. Product code:

   OZN - Amplification assay for the detection of *Clostridium difficile* toxin genes from stool specimens of symptomatic patients

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The Verigene® *Clostridium difficile* Nucleic Acid Test (CDF) is a qualitative, multiplexed *in vitro* diagnostic test for the rapid detection of toxin A (*tcdA*), toxin B (*tcdB*), and *tcdC* gene sequences of toxigenic strains *Clostridium difficile* and for presumptive identification of PCR ribotype 027 strains from unformed (liquid or soft) stool specimens collected from patients suspected of having *C. difficile* infection (CDI). Presumptive identification of the PCR ribotype 027 strain of *C. difficile* is by detection of the binary toxin (*cdt*) gene sequence and the single base pair deletion at nucleotide 117 in the *tcdC* gene. The *tcdC* gene encodes for a negative regulator in *C. difficile* toxin production. The test is performed on the Verigene System and utilizes automated specimen preparation and polymerase chain reaction (PCR) amplification, combined with a nanoparticle-based array hybridization assay to detect the toxin gene sequences associated with toxin-producing *C. difficile*.

   The CDF Test is indicated for use as an aid in the diagnosis of CDI. Detection of PCR ribotype 027 strains of *C. difficile* by the CDF Test is solely for epidemiological purposes and is not intended to guide or monitor treatment for *C. difficile* infections. Concomitant culture is necessary only if further typing or organism recovery is required.

2. Indication(s) for use:

   The Verigene® *Clostridium difficile* Nucleic Acid Test (CDF) is a qualitative, multiplexed *in vitro* diagnostic test for the rapid detection of toxin A (*tcdA*), toxin B (*tcdB*), and *tcdC* gene sequences of toxigenic strains *Clostridium difficile* and for presumptive identification of PCR ribotype 027 strains from unformed (liquid or soft) stool specimens collected from patients suspected of having *C. difficile* infection (CDI). Presumptive identification of the PCR ribotype 027 strain of *C. difficile* is by detection of the binary toxin (*cdt*) gene sequence and the single base pair deletion at nucleotide 117 in the *tcdC* gene. The *tcdC* gene encodes for a negative regulator in *C. difficile* toxin production. The test is performed on the Verigene System and utilizes automated specimen preparation and polymerase chain reaction (PCR) amplification, combined with a nanoparticle-based array hybridization assay to detect the toxin gene sequences associated with toxin-producing *C. difficile*.

The CDF Test is indicated for use as an aid in the diagnosis of CDI. Detection of PCR ribotype 027 strains of *C. difficile* by the CDF Test is solely for epidemiological purposes and is not intended to guide or monitor treatment for *C. difficile* infections. Concomitant culture is necessary only if further typing or organism recovery is required.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   To be used with the Verigene® System

I. Device Description:

The Verigene® *C. difficile* Nucleic Acid Test (CDF) is a molecular assay which relies on detection of specific nucleic acid targets in a microarray format. For each of the bacterial nucleic acid sequences detected by the CDF test, the assay utilizes unique Capture and Mediator oligonucleotides, followed by gold nanoparticle probe-based endpoint detection. The Capture oligonucleotides are covalently bound to the microarray substrate and hybridize to a specific portion of the nucleic acid targets. The Mediator oligonucleotides have a region which bind to a different portion of the same nucleic acid targets and also have a sequence which allows binding of a gold nanoparticle probe. Specific silver enhancement of the bound gold nanoparticle probes at the capture sites results in gold-silver aggregates that scatter light with high efficiency and provide accurate detection of target capture.

The CDF Test is performed on the Verigene System, a "sample-to-result," fully automated, bench-top molecular diagnostics workstation. The System enables automated nucleic acid extraction from unformed stool specimens (liquid or soft) and detection of specific bacterial target DNA. The Verigene System consists of two components: the Verigene Reader and the Verigene Processor *SP*.

The Reader is the Verigene System's user interface, which serves as the central control unit for all aspects of test processing, automated imaging, and result generation using a touchscreen control panel and a barcode scanner. The Verigene Processor *SP* executes the test procedure, automating the steps of (1) Sample Preparation and Target Amplification (i.e., cell lysis and magnetic bead-based bacterial DNA isolation and nucleic acid amplification), and (2) Hybridization (i.e., detection and identification of bacterial-specific DNA in a microarray format by using gold nanoparticle probe-based technology). Once the specimen is loaded by the operator, all other fluid transfer steps are performed by an automated pipette that transfers reagents between wells of the trays and finally loads the specimen into the Test Cartridge for hybridization. Single-use disposable test consumables and a self-contained Verigene Test Cartridge are utilized for each sample tested with the CDF assay.

To obtain the test results after test processing is complete the user removes the Test Cartridge from the Processor *SP* and inserts the substrate holder into the Reader for analysis. Light scatter from the capture spots is imaged by the Reader and intensities from the microarray spots are used to make a determination regarding the presence (Detected) or absence (Not Detected) of a bacterial nucleic acid sequence/analyte. This determination is made by means of software-based decision algorithm resident in the Verigene Reader.

To prevent reagent dispensing errors, all reagents are prepackaged in single-use disposables, including Stool Lysis Buffer (SLB) Tubes, reagent trays, and cartridges. Several layers of controls built into the CDF test ensure that failures at any step are identified during the procedure or in the end-point image analysis of the Test Cartridge.

An artificial DNA construct serves as an amplification or PCR control (i.e., Internal Processing Control 1 [IC1]) and is included within the Amplification Tray. *Bacillus subtilis* serves as a specimen preparation & amplification control (i.e., Internal Processing Control 2 [IC2]) and is automatically added by the Verigene SP to each specimen prior to the extraction step. Additional positive controls are immobilized on the Test Slide and are used to determine that hybridization was performed correctly. The CDF algorithm requires that these controls be valid before decisions regarding the presence or absence of any other target on the panel can be determined.

The Verigene® CDF Test Kit contains sufficient reagents to process 20 specimens or quality control samples. The kit contains the following:

Verigene® CDF Test Kit

- 20 Verigene® CDF Test Cartridges: Each Test Cartridge comes preloaded with all required reaction solutions, including wash solutions, oligonucleotide probe solutions and signal amplification solutions required to generate a test result.
- 20 Verigene® CDF Extraction Trays (with Tip Holder Assemblies): Each Extraction Tray comes preloaded with all required solutions, including lysis/binding buffer, wash solutions, and buffer solutions necessary to extract nucleic acids and generate a test result.
- 20 Verigene® CDF Stool Lysis Buffer (SLB) tubes and sterile swabs.

Verigene® CDF Test Amplification Kit

- 20 Verigene® CDF Amplification Trays: Each Amplification Tray comes preloaded with all required solutions, including enzymes and buffers necessary to amplify nucleic acids and generate a test result.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Portrait Toxigenic *C. Difficile* Assay - Great Basin Scientific
   Xpert *C. difficile*/Epi Assay - Cepheid
   Verigene RVNATSP Test - Nanosphere

2. Predicate 510(k) number(s):

   Portrait Toxigenic *C. Difficile* Assay - K113358
   Xpert *C. difficile*/Epi Assay - K110203
   Verigene RVNATSP Test - K092566

3. Comparison with predicate:

Similarities			
Item	**New Device: Nanosphere Verigene *C. difficile* Assay K123197**	**Predicate 1: Great Basin Portrait Toxigenic *C. difficile Assay* K113358**	**Predicate 2: Cepheid Xpert *C. difficile/Epi* Assay K110203**
Intended Use	The Verigene® *Clostridium difficile* Nucleic Acid Test (CDF) is a qualitative, multiplexed *in vitro* diagnostic test for the rapid detection of toxin A (*tcdA*), toxin B (*tcdB*), and *tcdC* gene sequences of toxigenic strains *Clostridium difficile* and for presumptive identification of PCR ribotype 027 strains from unformed (liquid or soft) stool specimens collected from patients suspected of having *C. difficile* infection (CDI). Presumptive identification of the PCR ribotype 027 strain of *C. difficile* is by detection of the binary toxin (*cdt*) gene sequence and the single base pair deletion at nucleotide 117 in the *tcdC* gene. The *tcdC* gene encodes for a negative regulator in *C. difficile* toxin production. The test is performed on the Verigene System and utilizes automated specimen preparation and polymerase chain reaction (PCR) amplification, combined with a nanoparticle-based array hybridization assay to detect the toxin gene sequences associated with toxin-producing *C. difficile*. The CDF Test is indicated for use as an aid in the diagnosis of CDI. Detection of PCR ribotype 027 strains of *C. difficile* by the CDF Test is solely for epidemiological purposes and is not intended to guide or monitor treatment for *C. difficile* infections. Concomitant culture is necessary only if further typing or organism recovery is required.	Portrait Toxigenic *C. difficile* Assay, a prescription device under 21 CFR Part 801.109 that is indicated for the detection of toxigenic *Clostridium difficile* in human fecal samples collected from patients suspected of having *Clostridium difficile* infection (CDI). The test utilizes automated blocked primer enabled helicase-dependent amplification (bpHDA) to detect toxin gene sequences associated with toxin producing *C. difficile*. The Portrait Toxigenic *C. difficile* Assay is intended as an aid in the diagnosis of CDI.	The Cepheid Xpert® *C. difficile/Epi* Assay is a qualitative *in vitro* diagnostic test for rapid detection of toxin B gene sequences and for presumptive identification of 027/NAP1/BI strains of toxigenic *Clostridium difficile* from unformed (liquid or soft) stool specimens collected from patients suspected of having *C. difficile* infection (CDI). Presumptive identification of 027/NAP1/BI strains of *C. difficile* is by detection of binary toxin (CDT) gene sequences and the single base pair deletion at nucleotide 117 in the *tcdC* gene. The *tcdC* gene encodes for a negative regulator in *C. difficile* toxin production. The test is performed on the Cepheid GeneXpert® Dx System and utilizes automated real-time polymerase chain reaction (PCR) to detect toxin gene sequences associated with toxin producing *C. difficile*. The Xpert *C. difficile/Epi* Assay is intended as an aid in the diagnosis of CDI. Detection of 027/NAP1/BI strains of *C. difficile* by the Xpert *C. difficile/Epi* Assay is presumptive and is solely for epidemiological purposes and is not intended to guide or monitor treatment for *C. difficile* infections. Concomitant culture is necessary only if further typing or organism recovery is required.
Techno-logical	Fully-automated nucleic acid amplification	Same	Same

Test Cartridge	Disposable single-use, multi-chambered fluidic test cartridge.	Same	Same
Specimen Type	Unformed (liquid or soft) Stool	Same	Same

Differences			
Item	**New Device: Nanosphere Verigene *C. difficile* Assay K123197**	**Predicate 1: Great Basin Portrait Toxigenic *C. difficile Assay* K113358**	**Predicate 2: Cepheid Xpert *C. difficile/Epi* Assay K110203**
Principle	DNA: PCR	DNA: Isothermal, helicase-dependent nucleic acid amplification	DNA: real-time PCR
DNA Target Sequences	*C. difficile* toxin A (*tcdA*), toxin B (*tcdB*), *tcdC*, binary toxin (*cdt*) and the *tcdC* deletion at nt 117 (tcdCΔ117)	*C. difficile* toxin B (*tcdB*)	*C. difficile* toxin B (*tcdB*), binary toxin and the *tcdC* deletion nt 117 (tcdCΔ117)
Instrument System	Verigene Reader Verigene Processor SP	Great Basin Portrait Analyzer	Cepheid GeneXpert Dx System
Sample Extraction	Self-contained cartridge with magnetic bead-based bacterial DNA extraction after elution from prepared stool specimens.	Self-contained and automated lysis and extraction after swab elution and filtration.	Self-contained and automated after swab elution and two single-dose reagent additions.
Probes	Capture/Mediator oligonucleotides, followed by gold nanoparticle probe-based endpoint detection. Specific silver enhancement of the bound gold nanoparticle probes at the capture sites results in gold-silver aggregates that scatter light with high efficiency. Light scattering is detected by the Verigene reader.	Amplification primers are biotin-labeled primers and hybridized to an array of probes immobilized on the silicon chip. Incubation with anti-biotin antibody conjugated to the horseradish peroxidase with tetramethylbenzidine development is visualized by Portrait Analyzer	TaqMan® Probes – real-time fluorescence detection by GeneXpert Dx System

K. Standard/Guidance Document Referenced (if applicable):

Establishing the Performance Characteristics of *In Vitro* Diagnostic Devices for the Detection of *Clostridium difficile* – Draft Guidance for Industry and FDA Staff (FDA document 1715, issued on November 29, 2010)

L. Test Principle:

The Verigene® *Clostridium difficile* Nucleic Acid Test (CDF Test) is a rapid, automated *in vitro* diagnostic test for the qualitative detection of *C. difficile* DNA directly from unformed (liquid or soft) stool specimens of patients suspected of having *C. difficile* infection (CDI).

The CDF Test detects toxin A (*tcdA*), toxin B (*tcdB*), and *tcdC* gene sequences of toxigenic strains of *C. difficile*. Presumptive identification of the PCR ribotype 027 strain of *C. difficile* is by detection of the binary toxin (*cdt*) gene sequence and the single base pair deletion at nucleotide 117 in the *tcdC* gene. The *tcdC* gene encodes for a negative regulator in *C. difficile* toxin production. The test is performed on the Verigene® System and utilizes automated specimen preparation and polymerase chain reaction (PCR) amplification, combined with a nanoparticle-based array hybridization assay to detect the toxin gene sequences associated with toxin-producing *C. difficile*.

Prior to initiating a test on the Verigene Processor SP, the Extraction Tray, the Amplification Tray and Tip Holder are loaded into the Verigene Processor SP. The barcode located on the CDF Test Cartridge is entered via the scanner attached to the Reader, and the associated sample information is entered either using the barcode-scanner or the Reader touch-screen interface (this links specific patient information to a specific Test Cartridge number). The CDF Test Cartridge is then inserted into the Processor SP. Once the consumables are loaded, 150 µL of liquid stool sample is added to Stool Lysis Buffer, vortexed, and then centrifuged. A 100 µL aliquot of this Stool Lysis Buffer is then pipetted into the designated Sample Well within the Extraction Tray and the Drawer Assembly is closed to initiate the test. The Processor SP identifies the Test Cartridge via an internal barcode scanner and communicates with the Reader to receive test instructions. Once the Processor SP module completes processing (approximately 2.0 hours), the CDF Test Cartridge is removed and inserted into the Reader for automated identification of the gene-specific DNA amplification products by nanoparticle-based array hybridization detection.

M. Performance Characteristics:

1. Analytical performance:

   a. Precision/Reproducibility:

   The inter-laboratory reproducibility of the CDF Test was determined by conducting a reproducibility study at three external sites. A seven sample panel for the reproducibility study was comprised of two different strains at three different

concentrations (six positive samples) and one (1) negative sample. Each of the seven samples in the panel was tested daily in triplicate by two operators for five non-consecutive days at three sites for a total of 90 tests per sample (3 sites x 2 operators/site x 3 replicates/operator x 5 days = 90 tests per sample). The study tested a total of 630 samples. Positive and negative controls were run by each site once per combination of consumable lots received

The results of the Reproducibility Study are provided in the table below. The results indicate the Verigene CDF Test was reproducible in this multi-center study.

Panel Member	***Strain***	***Level***	***Expected Occurrence***	***Total Agreement with Expected Result* (95% CI)***			
				Site 1	*Site 2*	*Site 3*	*Total*
1	CDF Negative Stool Matrix	Negative	~100% Negative	100% 30/30 (88.4–100)	100% 30/30 (88.4–100)	100% 30/30 (88.4–100)	100% 90/90 (96.0–100)
2	Toxigenic Wild Type *C. difficile*	Moderate Positive (MP)	~100% Positive	100% 30/30 (88.4–100)	100% 30/30 (88.4–100)	100% 30/30 (88.4–100)	100% 90/90 (96.0–100)
3		Low Positive (LP)	~95% Positive	100% 30/30 (88.4–100)	100% 30/30 (88.4–100)	100% 30/30 (88.4–100)	100% 90/90 (96.0–100)
4		High Negative (HN)	~20-80% Negative	30% 9/30 (14.7–49.4)	33.30% 10/30 (17.3–52.8)	16.70% 5/30 (5.6–34.7)	26.70% 24/90 (17.9–37.0)
5	Toxigenic Hypervirulent *C. difficile*	Moderate Positive (MP)	~100% Positive	100% 30/30 (88.4–100)	100% 30/30 (88.4–100)	100% 30/30 (88.4–100)	100% 90/90 (96.0–100)
6		Low Positive (LP)	~95% Positive	96.70% 29/30 (82.8–99.9)	96.70% 29/30 (82.8–99.9)	100% 30/30 (88.4–100)	97.80% 88/90 (92.2–99.7)
7		High Negative (HN)	~20-80% Negative	36.70% 11/30 (19.9–56.1)	40.00% 12/30 (22.7–59.4)	30.00% 9/30 (14.7–49.4)	35.60% 32/90 (25.7–46.4)

b. Linearity/assay reportable range:

Not applicable.

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

Internal Control: An artificial DNA construct serves as an amplification or PCR control and is referred to as the Internal Processing Control 1 (IC1). This control

material along with the primers and detection oligonucleotides are included within the Amplification Tray. If the process control is not valid a No Call result will be obtained and the test should be repeated.

Processing Control*:* *Bacillus subtilis* serves as a specimen preparation & amplification control and is referred to as the Internal Processing Control 2 (IC2). This control is automatically added by the Verigene SP to each specimen prior to the extraction step. If the process control is not valid a No Call result will be obtained and the test should be repeated.

Additional positive controls are immobilized on the Test Slide. These are used to determine that hybridization was performed correctly. CDF algorithm requires that these controls be valid before decisions regarding the presence or absence of any other target on the panel can be determined. If these controls are not detected a no call result will be obtained and the test should be repeated.

d. Detection limit:

Analytical sensitivity (LoD) of the CDF Test was determined for seven strains of *C. difficile*, representing all major toxinotypes found in North America and including two PCR Ribotype 027 strains. The LoD was defined as the concentration at which the test produces a positive result greater than 95% of the time. Serial dilutions of the strains were tested and the putative LoD confirmed with 20 replicates. The LoDs for the seven strains are shown in the table below and ranged from 63 to 1250 CFU/ml of stool. This study established the overall limit of detection of the CDF Test to be 1250 CFU/ml of organism present in stool.

Strain Designation (Source ID)	***Toxinotype***	***Calculated CFU/ml Stool at LoD***	***CFU per CDF Test at LoD***	***LoD Confirmation Results***
ATCC BAA-1805	III	250	5	20/20
ATCC 43255 (VPI 10463)	0	63	1.25	20/20
ATCC BAA-1875 (5325)	V	500	10	20/20
CDC 2007858	IX/XXIII	1250	25	20/20
CDC 2009087	0	1250	25	20/20
CDC 2009292	III	1250	25	20/20
ATCC 43598 (1470)	VIII	250	5	20/20

e. Analytical Reactivity:

Analytical reactivity of the CDF Test was demonstrated with a comprehensive panel of 63 *C. difficile* strains, tested in triplicate at three times the LoD (i.e. 3,750 CFU/mL). The panel was comprised of a wide range of toxinotypes, including

toxinotypes 0, I, IV, V, VIII, IX, X, XI, XII, XXI, XXII, IX/XXIII, XIV/XV, and six PCR ribotype 027 strains (toxinotype III).

No.	*Strain*	*Toxinotype*	*No.*	*Strain*	*Toxinotype*
1	ATCC 9689	0	33	CDC 2005088	V
2	ATCC 17857	0	34	CDC 2005283	0
3	ATCC 17858	0	35	CDC 2005325	V
4	ATCC 43255	0	36	CDC 2005359	III
5	ATCC 43594	0	37	CDC 2006017	0
6	ATCC 43596	0	38	CDC 2006062	I
7	ATCC 43597	0	39	CDC 2006376	VIII
8	ATCC 43598	VIII	40	CDC 2007070	0
9	ATCC 43599	0	41	CDC 2007217	V
10	ATCC 43600	0	42	CDC 2007302	0
11	ATCC 51695	0	43	CDC 2007435	XII
12	ATCC 700792	0	44	CDC 2007816	V
13	ATCC BAA-1382	0	45	CDC 2007838	V
14	ATCC BAA-1803	III	46	CDC 2007858	IX/XXIII
15	ATCC BAA-1804	0	47	CDC 2007886	IX/XXIII
16	ATCC BAA-1805	III	48	CDC 2008222	0
17	ATCC BAA-1806	0	49	CDC 2009048	XIV/XV
18	ATCC BAA-1808	0	50	CDC 2009078	0
19	ATCC BAA-1811	0	51	CDC 2009087	0
20	ATCC BAA-1812	XII	52	CDC 2009141	0
21	ATCC BAA-1813	0	53	CDC 2009287	XXI
22	ATCC BAA-1814	XXII	54	CDC 2009292	III
23	ATCC BAA-1815	0	55	CDC 2009363	XXII
24	ATCC BAA-1874	0	56	CDC 20100276	VIII
25	ATCC BAA-2155	XXII	57	CDC 20100286*	XI
26	ATCC BAA-2156	0	58	CDC 20100304	IV
27	CDC 2004013	III	59	CDC 20100307	IV
28	CDC 2004111	0	60	CDC 20100375	IX
29	CDC 2004118	III	61	CDC 20100378	IX
30	CDC 2004205	0	62	CDC 20100381	IX
31	CDC 2004206	0	63	CCUG 8864/20309	X
32	CDC 2005022	0			

*Per information provided by the CDC, this strain was tentatively identified as toxinotype XI

All tests correctly reported the expected results for the detection of gene sequences for toxigenic *C. difficile* and for presumptive PCR ribotype 027, with one exception: Strain **CDC 2009048 strain**, classified by the CDC as Toxinotype XIV/XV, is associated with non-027 strains (Ribotypes 111/122). However, the Verigene CDF Test reported detection of the *tcdA*, *tcdB*, binary and *tcdC*-MUT targets as would be expected for a PCR ribotype 027 strain. Subsequent sequencing of the *tcdC* gene verified the presence of the Δ117 deletion.

f. Analytical specificity:

Ninety-four (94) microorganisms, including two (2) non-toxigenic *C. difficile* strains and fourteen (14) non *C. difficile Clostridium* species, seventy-seven (77) other microorganisms, and one (1) human cell line, were tested with the CDF Test to determine analytical specificity. In addition, the cross-reactivity of *Clostridium botulinum* was evaluated by in silico analysis.

Each bacterial strain was prepared in Negative Stool Matrix and tested in triplicate in concentrations of 5×10^6 CFU/mL stool. Two (2) organisms, Cryptosporidium parvum and Giardia lamblia, were tested using genomic DNA at a concentration of 1×10^6 copies of gDNA. For the viruses, Echovirus 11 and Coxsackievirus were tested at 5×10^5 PFU/mL stool. Adenovirus, Enterovirus, Cytomegalovirus and Rotavirus were also tested using genomic DNA or RNA at a concentration of 1×10^6 copies per reaction. Noroviruses were tested as clinical samples.

No.	***Organism/Cross-Reactant***	***Strain ID***	***No.***	***Organism/Cross-Reactant***	***Strain ID***
1	*Clostridium difficile (non-toxigenic)*	43601	49	*Klebsiella oxytoca*	13182
2	*Clostridium difficile (non-toxigenic)*	43593	50	*Klebsiella pneumonia ssp. pneumonia*	13883
3	*Abiotrophia defective*	49176	51	*Lactobacillus acidophilus*	4356
4	*Acinetobacter baumanii*	19606	52	*Lactobacillus reuteri*	23272
5	*Aeromonas hydrophila*	7966	53	*Lactobacillus rhamnosus*	53103
6	*Alcaligenes faecalis ssp. faecalis*	15554	54	*Lactococcus lactis*	11454
7	*Bacillus cereus*	10702	55	*Leminorella grimontii*	33999
8	*Bacteroides caccae*	43185	56	*Listeria grayi*	19120
9	*Bacteroides fragilis*	25285	57	*Listeria monocytogenes*	7644
10	*Bacteroides merdae*	43184	58	*Peptostreptococcus anaerobius*	27337
11	*Bacteroides stercoris*	43183	59	*Plesiomonas shigelloides*	14029
12	*Campylobacter coli*	43479	60	*Porphyromonas asaccharolyticus*	25260
13	*Campylobacter jejuni ssp. jejuni*	33292	61	*Prevotella melaninogenica*	25845
14	*Candida albicans*	10231	62	*Proteus mirabilis*	43071
15	*Cedecea davisae*	33431	63	*Proteus penneri*	35198
16	*Citrobacter amalonaticus*	25407	64	*Providencia alcalifaciens*	9886
17	*Citrobacter freundii*	8090	65	*Providencia rettgeri*	9250
18	*Citrobacter sedlakii*	51115	66	*Pseudomonas aeruginosa*	27853
19	*Clostridium bifermentans*	638	67	*Pseudomonas aeruginosa*	35554
20	*Clostridium bolteae*	BAA-613	68	*Ruminococcus bromii*	27255
21	*Clostridium butyricum*	19398	69	*Salmonella choleraesuis (typhimurium)*	14028
22	*Clostridium haemolyticum*	9650	70	*Salmonella enterica ssp. arizonae*	13314
23	*Clostridium methylpentosum*	43829	71	*Salmonella enterica ssp. enterica*	7001
24	*Clostridium nexile*	27757	72	*Serratia liquefacians*	27592
25	*Clostridium noyvi*	19402	73	*Serratia marcescens*	13880
26	*Clostridium orbiscindens*	49531	74	*Shigella boydii*	9207
27	*Clostridium perfringens*	13124	75	*Shigella dysenteriae*	11835
28	*Clostridium scindens*	35704	76	*Shigella sonnei*	29531
29	*Clostridium septicum*	12464	77	*Staphylococcus aureus*	43300
30	*Clostridium sordellii*	9714	78	*Staphylococcus epidermidis*	14990
31	*Clostridium spiroforme*	29899	79	*Streptococcus agalactiae*	49446
32	*Clostridium sporogenes*	15579	80	*Streptococcus agalactiae*	12386
33	*Collinsella aerofaciens*	25986	81	*Vibrio Cholerae*	25870
34	*Cryptosporidium parvum*	PRA-67-D	82	*Vibrio parahaemolyticus*	17802

35	*Desulfovibrio piger*	29098	83	*Yersinia bercovieri*	43970
36	*Edwardsiella tarda*	15947	84	*Yersinia enterocolitica*	9610
37	*Enterobacter aerogenes*	13048	85	*Yersinia rohdei*	43380
38	*Enterobacter cloacae*	29006		Non-bacterial Organisms	
39	*Enterococcus faecalis vanB*	51299	86	Adenovirus Type 40	VR-931
40	*Enterococcus faecium vanA*	700221	87	Adenovirus Type 41	VR-930
41	*Escherichia coli*	23511	88	Coxsackievirus B4	VR-184
42	*Escherichia coli O157:H7*	43894	89	Cytomegalovirus	0810003-CF
43	*Escherichia fergusonii*	35469	90	Echovirus 11	0810023-CF
44	*Escherichia hermannii*	33650	91	Enterovirus 68	VR-213
45	*Fusobacterium varium*	8501	92	Homo sapiens	CCL-218
46	*Giardia lamblia*	50803-D	93	Norovirus Group I	n/a
47	*Helicobacter fennelliae*	35683	94	Norovirus Group II	n/a
48	*Helicobacter pylori*	43504	95	Rotavirus	VR-2272

In addition, the cross-reactivity of *Clostridium botulinum* was evaluated by *in silico* analysis, which assessed the specificity of the CDF Capture, Mediator and primer oligos to GenBank sequence information for *C. botulinum*. Analytical specificity was observed to be 100%, including that determined by *in silico* analysis.

g. Microbial Interference:

The CDF Test was tested against the same ninety-five (95) organisms/cell line that were used for analytical specificity, at the same medically relevant concentrations, using two strains of toxigenic *C. difficile* (ATCC BAA-1805 [toxinotype III] and ATCC 43255 [toxinotype 0]) at 1.5x LoD and 3x LoD, respectively, to evaluate the potential for microbial interference. No interference was observed with the CDF Test for any of the samples tested.

h. Interfering Substances:

Thirty-four products/exogenous substances (shown in the table below) that are possibly encountered in stool samples were evaluated for potential inhibitory effects. Each interfering substance was evaluated at its "worst case" concentration, against two *C. difficile* strains (ATCC BAA-1805, ATCC 43255). Additionally, Cary-Blair media was tested. None of the 34 substances or the Cary-Blair media tested in this study showed any inhibitory effect on the detection of *C. difficile* when using the CDF Test.

No.	Product	Active Ingredient	Concentration (w/w %)
1.	Stearic Acid, Grade I	Steric Acid	40%
2.	Palmitic Acid Free Acid Sigma Grade	Palmitic Acid	40%
3.	Whole Blood	Glucose, Hormones, Enzymes, Ions, Iron, etc	40%
4.	Nasopharyngeal Swab Sample in Universal Transport Media (UTM)	Immunoglobulins, Lysozyme, Polymers, etc	40%
5.	Nystatin Suspension	Nystatin	30%
6.	Monistat® 3	Miconazole	30%
7.	Preparation H® Medicated Wipes	Witch Hazel	30%
8.	Vagisil Anti-Itch Crème Maximum Strength	Benzocaine, Resorcinol	30%

9.	Preparation H® Anti-Itch Hydrocortisone 1%	Hydrocortisone	30%
10.	Desitin Maximum Strength Original Paste	Zinc Oxide	30%
11.	Sarna Anti-Itch Lotion, Sensitive	Pramoxine HCl	30%
12.	Preparation H® Hemorrhoidal Ointment	Phenylephrine	30%
13.	Walgreens Ready to Use Enema Mineral Oil Laxative	Mineral Oil	30%
14.	Options Conceptrol® Vaginal Contraceptive Gel	Nonoxynol-9	30%
15.	Dulcolax® Laxative Suppositories	Bisacodyl	30%
16.	Dimenhydrinate	Dimenhydrinate	30%
17.	Neosporin® First Aid Antibiotic Ointment	Bacitracin, Neomycin, Polymyxin	30%
18.	Wet Ones® Antibacterial Hand Wipes	Benzalkonium Chloride, Ethanol	30%
19.	K-Y® Personal Lubricant Jelly	Glycerin	30%
20.	Vaseline Original 100% Pure Petroleum Jelly	Petroleum	30%
21.	Bile, bovine, dried, unfractioned	Bile	20%
22.	Tums® Antacid with Calcium Extra Strength 750	Calcium Carbonate	10%
23.	Gaviscon® Extra Strength Liquid Antacid	Aluminum Hydroxide, Magnesium Hydroxide	10%
24.	Phillips'® Genuine Milk of Magnesia Saline Laxative	Magnesium Hydroxide	10%
25.	Aluminum Hydroxide, Reagent Grade	Aluminum Hydroxide	10%
26.	Mesalazine	Mesalazine	10%
27.	Immodium® AD Anti-Diarrheal	Loperamide Hydrochloride	10%
28.	Pepto-Bismol Max Strength	Bismuth Subsalicylate	10%
29.	Ex·lax® Maximum Strength Stimulant Laxative	Sennosides	10%
30.	Vancomycin	Vancomycin	10%
31.	Metronidazole Topical Cream (0.75%)	Metronidazole	10%
32.	Naproxen Sodium	Naproxen Sodium	10%
33.	Mucin from bovine submaxillary glands, Type I-S (Dehydrated)	Mucin	10%
34.	Barium Sulfate	Barium Sulfate	10%
35.	Cary-Blair Medium	Salts, Agar, thioglycollate	300%

i. Assay cut-off:

The presence or absence of each target analyte was determined by the mean intensity of target capture spots relative to the Signal Detection Threshold (filter 1). With PCR amplification of the extracted sample DNA, a significant difference was expected in the signal intensity from toxigenic *C. difficile* bacteria-containing samples versus that obtained from negative samples. The capture, mediator, and PCR primer oligonucleotides in the CDF Test were designed to eliminate sequence-related cross-reactivity, thereby ensuring that the non-specific target signal intensities at capture spots are similar to the microarray background signal. In contrast, amplicon hybridization to complementary capture and mediator probes were expected to give high positive signals, well-separated from negative capture spots. When reading a test slide, multiple images of each array were taken at increasing exposures times and the final target group mean intensity value for an analyte was assigned at the shortest exposure at which the value exceeds the Signal Detection Threshold. If the target signal did not exceed the threshold for any exposure, the mean spot intensity was

evaluated at the longest exposure taken.

The *tcdC* target was identified by unique captures that detect the *tcdC*-wild type (WT) and *tcdC*-mutant (Δ117) (MUT) genotypes. If one of the captures exceeded the Signal Detection Threshold then *tcdC* was "Detected." A second filter, the *tcdC* Genotype Threshold, then distinguished between the *tcdC*-wild type and *tcdC*-mutant (Δ117). A *tcdC* genotype ratio was calculated from the individual tcdC-WT and -MUT group mean intensities.

In order to demonstrate the appropriateness of the cut-off values for these two thresholds, the target mean intensity values were examined for a panel of 59 *C. difficile* strains in Negative Stool Matrix at a concentration of 3750CFU/mL – 5000 CFU/mL stool. Each strain was tested twice and the six probes on the microarray (*tcdA*, *tcdB*, binary, *tcdC*, and the internal controls IC1, IC2) were compiled to generate a set of 708 data points (118 tests x 6 targets per test).

#	Sample	Expected Result				#	Sample	Expected Result			
		tcdA	*tcdB*	binary	*tcdC*			*tcdA*	*tcdB*	binary	tcdC
1	ATCC 9689	+	+	–	WT	31	CDC 2007217	+	+	+	WT
2	ATCC 17857	+	+	–	WT	32	CDC 2007302	+	+	–	WT
3	ATCC 17858	+	+	–	WT	33	CDC 2007816	+	+	+	WT
4	ATCC 43255	+	+	–	WT	34	CDC 2007838	+	+	+	WT
5	ATCC 43593	–	–	–	–	35	CDC 2007858	+	+	+	WT
6	ATCC 43594	+	+	–	WT	36	CDC 2007886	+	+	+	WT
7	ATCC 43596	+	+	–	WT	37	CDC 2008222	+	+	–	WT
8	ATCC 43597	+	+	–	WT	38	CDC 2009078	+	+	–	WT
9	ATCC 43598	+	+	–	WT	39	CDC 2009087	+	+	–	WT
10	ATCC 43599	+	+	–	WT	40	CDC 2009141	+	+	–	WT
11	ATCC 43600	+	+	–	WT	41	CDC 2009292	+	+	+	MUT
12	ATCC 43601	–	–	–	–	42	ATCC BAA-1382	+	+	–	WT
13	ATCC 43602	–	–	–	–	43	ATCC BAA-1801	–	–	–	–
14	ATCC 43603	–	–	–	–	44	ATCC BAA-1803	+	+	+	MUT
15	ATCC 51695	+	+	–	WT	45	ATCC BAA-1804	+	+	–	WT
16	ATCC 700057	–	–	–	–	46	ATCC BAA-1805	+	+	+	MUT
17	ATCC 700792	+	+	–	WT	47	ATCC BAA-1806	+	+	–	WT
18	CDC 2004013	+	+	+	MUT	48	ATCC BAA-1807	–	–	–	–

19	CDC 2004111	+	+	–	WT	49	ATCC BAA-1808	+	+	–	WT
20	CDC 2004118	+	+	+	MUT	50	ATCC BAA-1809	–	–	–	–
21	CDC 2004205	+	+	–	WT	51	ATCC BAA-1810	–	–	–	–
22	CDC 2004206	+	+	–	WT	52	ATCC BAA-1811	+	+	–	WT
23	CDC 2005022	+	+	–	WT	53	ATCC BAA-1812	+	+	–	WT
24	CDC 2005088	+	+	+	WT	54	ATCC BAA-1813	+	+	–	WT
25	CDC 2005283	+	+	–	WT	55	ATCC BAA-1814	+	+	+	WT
26	CDC 2005325	+	+	+	WT	56	ATCC BAA-1815	+	+	–	WT
27	CDC 2005359	+	+	+	MUT	57	ATCC BAA-1874	+	+	–	WT
28	CDC 2006017	+	+	–	WT	58	ATCC BAA-2155	+	+	+	WT
29	CDC 2006376	+	+	–	WT	59	ATCC BAA-2156	+	+	–	WT
30	CDC 2007070	+	+	–	WT						

Positive signals were well separated from the negative target signals, and the threshold value distinguished the "True Positives" from the "True Negatives."

j. Carry-Over & Cross-Contamination Study:

The potential for carry-over and cross-contamination to occur with the CDF Test on the Verigene system was assessed by testing *C. difficile* negative samples after running high positive (HP) samples. The toxigenic PCR ribotype 027 *C. difficile* strain BAA-1805 was used for the study, and was added at a high titer of $5x10^6$ CFU/mL stool into the Negative Stool Matrix to prepare the high positive sample. The same Negative Stool Matrix was utilized as the *C. difficile* negative sample. In the execution of the study, the high-titer sample was alternated with the negative sample three times on three unique Verigene SP Processors, for a total of eighteen individual tests.

CDF target analytes were not detected by the CDF Test in any of the nine negative samples run immediately following the high-titer sample on three separate SP Processors. Additionally, all nine high-titer samples accurately detected the expected *tcdA*, *tcdB*, binary, and *tcdC* MUT targets. The study demonstrated that the CDF assay does not exhibit carry-over or cross- contamination that could result in a false positive test result.

2. Comparison studies:

   a. *Method comparison with predicate device:*

      Clinical performance was determined by comparing the CDF Test results to reference culture (i.e., direct toxigenic culture or enriched toxigenic culture) followed by cell cytotoxicity testing on the isolates. Subsequent strain typing of the toxigenic strains by PCR Ribotyping and bi-directional sequencing methods was used to confirm toxigenic strains of *C. difficile*.

   b. *Matrix comparison:*

      Not applicable

3. Clinical studies:

   Clinical performance characteristics of the CDF Test were determined in a multi-site prospective investigational study at five U.S. institutions using 1,877 unformed stool specimens from subjects whose routine care called for *C. difficile* testing. A portion of each leftover residual unformed stool specimen was obtained for testing. The clinical evaluation was carried out by comparing the CDF Test results to reference culture followed by cell cytotoxicity testing on the isolates. Subsequent strain typing on toxigenic strains by PCR ribotyping and bi-directional sequencing methods was used to confirm toxigenic strains of *C. difficile.*

   In parallel to Verigene CDF Testing, an aliquot of the same specimen was sent to a reference laboratory for the reference culture and cytotoxin isolate testing. Each stool specimen was inoculated onto pre-reduced CCFA-D (cycloserine-cefoxitin-fructose agar-direct plate; "direct culture") and CCMB-Tal (cycloserine cefoxitin mannitol broth with taurocholate lysozyme cysteine). After 24 hours the CCMB-TAL was sub-cultured onto a second CCFA-E plate (CCFA-Enriched; "enriched culture").

   If *C. difficile* was isolated from the CCFA-D plate and the isolate was positive by the cell cytotoxicity assay, the specimen was classified as "toxigenic *C. difficile* positive" and the CCFA-E plate was not further analyzed. If no *C. difficile* was isolated from the CCFA-D plate or if the isolate was negative by the cell cytoxicity assay, the CCFA-E plate was further analyzed. If CCFA-E was positive for *C. difficile* and the isolate was positive for cell cytotoxicity assay, the specimen was classified as "toxigenic *C. difficile* positive." The specimen was reported as "negative" if CCFA-E was negative for *C. difficile* or the isolate was tested negative by the cell cytotoxicity assay.

   After central reference laboratory culture testing, the toxigenic *C. difficile* positive isolates were sent for strain identification by PCR Ribotyping to an external third-party

site. In parallel, Verigene CDF Test extracted DNA from the culture-confirmed *C. difficile* positive isolates were sent for *tcdC* bi-directional sequencing.

a. Clinical Sensitivity:

Assay Performance vs. Direct Culture & PCR Ribotyping

Relative to direct culture with PCR ribotyping, the CDF Test demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 98.7% and 87.6%, respectively. The CDF Test also demonstrated a 97.5% positive agreement and 97.8% agreement for the hypervirulent *C. difficile* strain 027 by PCR ribotyping. The results are summarized in the table below (this data combines all investigational sites).

Verigene CDF Test Performance vs. Direct Culture & PCR Ribotyping

		Direct Culture & PCR Ribotyping			
Verigene CDF Test		**Tox *C. difficile* + 027 +**	**Tox *C. difficile* + 027 -**	**NEG**	**Total[a]**
	Tox *C. difficile* + 027 +	39	2	40	81
	Tox *C. difficile* + 027 –	0	113	173	286
	NEG	1	1	1500	1502
	Total	40	116	1713	1869[b]
		Toxigenic *C. difficile*		Toxigenic *C. difficile*/027	
		Sensitivity: 98.7% (154/156) (95.5%-99.8%)		Pos Agreement: 97.5% (39/40) (86.8%-99.9%)	
		Specificity: 87.6% (1500/1713) (85.9%-89.1%)		Neg Agreement: 97.8% (1787/1828) (97.0%-98.4%)	
		Accuracy: 88.5% (1654/1869) (87.0%-89.9%)		Total Agreement: 97.7% (1827/1869) (96.9%-98.3%)	
		PPV: 42.0% (154/367) (36.9%-47.2%)		PPV: 48.2% (39/81) (36.9%-59.5%)	
		NPV: 99.9% (1500/1502) (99.5%-99.9%)		NPV: 99.9% (1787/1788) (99.7%-100%)	

[a]There were 1,877 evaluable specimens enrolled in the clinical trial; 71 specimens (3.8%) required repeat testing: 46 specimens (2.4%) had an initial "No Call" result due to assay internal control errors; 17 specimens (1.0%) had an initial "Indeterminate" call (No Call-IND), and 8 specimens (0.4%) had pre-analytical errors (four motor stalls, two tip failures, one cracked slide and one cartridge not detected). The eight specimens that experienced pre-analytical errors, and the 46 No Call specimens, were called upon repeat testing; however, two of the No Call specimens required a second repeat test before being called. Repeat testing of the 17 No Call-IND specimens called all but two specimens (five specimens required a second repeat test before calling). Therefore, two specimens had a final "Indeterminate" call and were not included in the clinical data analysis of evaluable results. Thus, 1,875 specimens were analyzed in this clinical evaluation.

[b]Of the 1,875 specimens evaluated, six specimens were culture positive but were not PCR-ribotyped because the isolate was either not sent or the result was inconclusive. These six specimens were not included in the performance characteristics above.

Assay Performance vs. Enriched Culture & PCR Ribotyping

Relative to enriched culture with PCR Ribotyping, the Verigene CDF Test demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 91.8% and 92.5%, respectively. The CDF Test also demonstrated a 91.4% positive agreement and 98.5% agreement for the hypervirulent *C. difficile* strain 027 by PCR Ribotyping. The results are summarized in the table below (this data combines all investigational sites).

Verigene CDF Test Performance vs. Enriched Culture & PCR Ribotyping

		Enriched Culture & PCR Ribotyping			
Verigene CDF Test		**Tox *C. difficile* + 027 +**	**Tox *C. difficile* + 027 -**	**NEG**	**Total[a]**
	Tox *C. difficile* + 027 +	53	5	23	81
	Tox *C. difficile* + 027 –	1	188	97	286
	NEG	4	18	1480	1502
	Total	58	211	1600	1869[b]
		Toxigenic *C. difficile*		Toxigenic *C. difficile*/027	
		Sensitivity: 91.8% (247/269) (87.9%-94.8%) Specificity: 92.5% (1480/1600) (91.1%-93.7%) Accuracy: 92.4% (1727/1869) (91.1%-93.6%) PPV: 67.3% (247/367) (62.2%-72.1%) NPV: 98.5% (1480/1502) (97.8%-99.1%)		Pos Agreement: 91.4% (53/58) (81.0%-97.1%) Neg Agreement: 98.5% (1783/1811) (97.8%-99.0%) Total Agreement: 98.2% (1836/1869) (97.5%-98.8%) PPV: 65.4% (53/81) (54.0%-75.7%) NPV: 99.7% (1783/1788) (99.4%-99.9%)	

[a] There were 1,877 evaluable specimens enrolled in the clinical trial; 71 specimens (3.8%) required repeat testing: 46 specimens (2.4%) had an initial "No Call" result due to assay internal control errors; 17 specimens (1.0%) had an initial "Indeterminate" call (No Call-IND), and 8 specimens (0.4%) had pre-analytical errors (four motor stalls, two tip failures, one cracked slide and one cartridge not detected). The eight specimens that experienced pre-analytical errors, and the 46 No Call specimens, were called upon repeat testing; however, two of the No Call specimens required a second repeat test before being called. Repeat testing of the 17 No Call-IND specimens called all but two specimens (five specimens required a second repeat test before calling). Therefore, two specimens had a final "Indeterminate" call and were not included in the clinical data analysis of evaluable results. Thus, 1,875 specimens were analyzed in this clinical evaluation.

[b] Of the 1,875 specimens evaluated, six specimens were culture positive but were not PCR-ribotyped because the isolate was either not sent or the result was inconclusive. These six specimens were not included in the performance characteristics above.

Assay Performance vs. Direct Culture & Bi-Directional Sequencing

Relative to direct culture with bi-directional sequencing, the Verigene CDF Test demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 98.7% and 87.5%, respectively. The CDF Test also demonstrated a 97.7% positive agreement and 97.8% agreement for the hypervirulent *C. difficile* strain 027 by bi-directional sequencing. The results are summarized in the table below (this data combines all investigational sites).

Verigene CDF Test Performance vs. Direct Culture & Sequencing

		Direct Culture & Sequencing			
Verigene CDF Test		**Tox *C. difficile* + 027 +**	**Tox *C. difficile* + 027 -**	**NEG**	**Total[a]**
	Tox *C. difficile* + 027 +	42	0	40	82
	Tox *C. difficile* + 027 –	0	114	175	289
	NEG	1	1	1500	1502
	Total	43	115	1715	1873[b]
		Toxigenic *C. difficile*		Toxigenic *C. difficile*/027	
		Sensitivity:	98.7% (156/158) (95.5%-99.9%)	Pos Agreement:	97.7% (42/43) (87.7%-99.9%)
		Specificity:	87.5% (1500/1715) (85.8%-89.0%)	Neg Agreement:	97.8% (1790/1830) (97.0%-98.4%)
		Accuracy:	88.4% (1656/1873) (86.9%-89.8%)	Total Agreement:	97.8% (1832/1873) (97.0%-98.4%)
		PPV:	42.1% (156/371) (37.0%-47.3%)	PPV:	51.2% (42/82) (39.9%-62.4%)
		NPV:	99.9% (1500/1502) (99.5%-100%)	NPV:	99.9% (1790/1791) (99.7%-100%)

[a] There were 1,877 evaluable specimens enrolled in the clinical trial; 71 specimens (3.8%) required repeat testing: 46 specimens (2.4%) had an initial "No Call" result due to assay internal control errors; 17 specimens (1.0%) had an initial "Indeterminate" call (No Call-IND), and 8 specimens (0.4%) had pre-analytical errors (four motor stalls, two tip failures, one cracked slide and one cartridge not detected). The eight specimens that experienced pre-analytical errors, and the 46 No Call specimens, were called upon repeat testing; however, two of the No Call specimens required a second repeat test before being called. Repeat testing of the 17 No Call-IND specimens called all but two specimens (five specimens required a second repeat test before calling). Therefore, two specimens had a final "Indeterminate" call and were not included in the clinical data analysis of evaluable results. Thus, 1,875 specimens were analyzed in this clinical evaluation.

[b] Of the 1,875 specimens evaluated, two specimens were culture positive but were not sequenced because the isolate was either not sent or the result was inconclusive. These two specimens were not included in the performance characteristics above.

Assay Performance vs. Enriched Culture & Bi-Directional Sequencing

Relative to enriched culture with bi-directional sequencing, the Verigene CDF Test demonstrated a sensitivity and specificity for toxigenic *C. difficile* of 91.9% and 92.5%, respectively. The CDF Test also demonstrated a 93.7% positive agreement and 98.7% agreement for the hypervirulent *C. difficile* strain 027 by bi-directional sequencing. The results are summarized in the table below (this data combines all investigational sites).

Verigene CDF Test Performance vs. Enriched Culture & Sequencing

		Enriched Culture & Sequencing			
Verigene CDF Test		**Tox *C. difficile* + 027 +**	**Tox *C. difficile* + 027 -**	**NEG**	**Total[a]**
	Tox *C. difficile* + 027 +	59	0	23	82
	Tox *C. difficile* + 027 –	1	191	97	289
	NEG	3	19	1480	1502
	Total	63	210	1600	1873[b]
		Toxigenic *C. difficile*		Toxigenic *C. difficile*/027	
		Sensitivity: 91.9% (251/273) (88.1%-94.9%)		Pos Agreement: 93.7% (59/63) (84.5%-98.2%)	
		Specificity: 92.5% (1480/1600) (91.1%-93.7%)		Neg Agreement: 98.7% (1787/1810) (98.1%-99.2%)	
		Accuracy: 92.4% (1731/1873) (91.1%-93.6%)		Total Agreement: 98.6% (1846/1873) (97.9%-99.1%)	
		PPV: 67.7% (251/371) (62.6%-72.4%)		PPV: 72.0% (59/82) (60.9%-81.3%)	
		NPV: 98.5% (1480/1502) (97.8%-99.1%)		NPV: 99.8% (1787/1791) (99.4%-99.9%)	

[a] There were 1,877 evaluable specimens enrolled in the clinical trial; 71 specimens (3.8%) required repeat testing: 46 specimens (2.4%) had an initial "No Call" result due to assay internal control errors; 17 specimens (1.0%) had an initial "Indeterminate" call (No Call-IND), and 8 specimens (0.4%) had pre-analytical errors (four motor stalls, two tip failures, one cracked slide and one cartridge not detected). The eight specimens that experienced pre-analytical errors, and the 46 No Call specimens, were called upon repeat testing; however, two of the No Call specimens required a second repeat test before being called. Repeat testing of the 17 No Call-IND specimens called all but two specimens (five specimens required a second repeat test before calling). Therefore, two specimens had a final "Indeterminate" call and were not included in the clinical data analysis of evaluable results. Thus, 1,875 specimens were analyzed in this clinical evaluation.

[b] Of the 1,875 specimens evaluated, two specimens were culture positive but were not sequenced because the isolate was either not sent or the result was inconclusive. These two specimens were not included in the performance characteristics above.

4. Clinical cut-off**:**

Not applicable.

5. Expected values/Reference range:

For the CDF Test clinical study, the prevalence rate for the clinical dataset of 1,875 prospectively-collected unformed stool specimens was determined; this study obtained specimens from five large hospitals geographically distributed across the United States. The number and percentage of toxigenic *C. difficile* positive cases by culture, stratified by age, are presented in the table below.

Age Group (years)	***Number***	***Toxigenic C. difficile Prevalence[a] (includes 027)***	***027 Prevalence[a]***
Infant (<2 years old)	0	0% (0/0)	0% (0/0)
Child (≥2 - <12 years old)	23	13.0% (3/23)	0% (0/23)
Adolescent (≥12 - <18 years old)	14	0% (0/14)	0% (0/0)
Transitional Adolescent (≥18 - ≤21)	42	38.1% (16/42)	4.8% (2/42)
Adults: >21 - 59 Years of Age	913	16.2% (148/913)	2.7% (25/913)
Sr. Adults: ≥60 Years of Age	883	23.1% (204/883)	6.2% (55/883)
All	***1875***	***19.8% (371/1875)***	***4.4% (82/1875)***

[a]Prevalence based on the CDF test results.

N. Instrument Name:

Verigene® System (Verigene Processor SP and Verigene Reader)

O. System Descriptions:

1. Modes of Operation:

The Verigene System allows random access, fully automated 'sample to result' operation. The Verigene System is comprised of two components: a Reader (user interface, central control unit, optics for reading the absence or presence of bacterial DNA on the microarray) and the Processor SP. The CDF test, performed on the Verigene System, involves two steps:

- Sample preparation-Cell lysis and magnetic bead-based bacterial DNA isolation from prepared Stool Lysis Buffer (SLB) samples, and
- Verigene Hybridization Test-Hybridization of bacterial-specific DNA to capture oligonucleotides on a microarray, using gold nanoparticles probe-based technology to aid detection.

Operationally, single use disposables (Extraction Tray, Utility Tray, and Test Cartridge) are loaded into separate modules within the Processor SP. The only 'user-performed' pipetting step required to perform the assay is the addition of the prepared SLB sample into the extraction tray. The sample preparation occurs within the Processor SP's Extraction Tray and is also aided by reagents present in the Utility Tray, while the Verigene Hybridization Test occurs within the Test Cartridge. An automated pipette

using disposable pipette tips delivers and transfers reagents during the assay.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No _____

   The results of the validation and verification testing of the result mask and other general updates were provided for **version 1.9.0** of the Verigene Reader software, and for **version 4.1** of the Verigene Processor *SP* software.

3. Specimen Identification:

   Specimens are labeled with a Barcode. The Processor SP and Reader detect the specimen ID, the results are printed with this specimen identifier.

4. Specimen Sampling and Handling:

   Automated Verigene System

5. Calibration:

   Not required

6. Quality Control:

   A series of internal controls used in conjunction with procedural checks monitors instrument functionality, performance, fluidics, reagent integrity, and result determination, based on a pre-defined decision algorithm.

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

Not applicable.

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.