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

A. 510(k) Number:

K163085

B. Purpose for Submission:

To obtain a substantial equivalence determination for a new device

C. Measurand:

tcdB gene of toxigenic *Clostridium difficile*

D. Type of Test:

Qualitative real-time polymerase chain reaction (PCR) assay

E. Applicant:

Focus Diagnostics, Inc.: DBA DiaSorin Molecular LLC

F. Proprietary and Established Names:

Simplexa™ *C. difficile* Direct

Simplexa™ *C. difficile* Positive Control Pack

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3130, *Clostridium difficile* toxin gene amplification assay

   21 CFR 862.2570 - Instrumentation for clinical multiplex test systems

2. Classification:

   II

3. Product code:

   OZN, OOI

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   Simplexa™ *C. difficile* Direct
   The Focus Diagnostics Simplexa™ *C. difficile* Direct assay is intended for use on the Integrated Cycler instrument for the detection of *Clostridium difficile* toxin B gene (*tcdB*) present in liquid or unformed stool samples from individuals suspected of *C. difficile* infection (CDI). This test aids in the diagnosis of illness resulting from CDI.
   The assay is for professional and prescription use only.

   Simplexa™ *C. difficile* Positive Control Pack
   The Simplexa™ *C. difficile* Positive Control Pack is intended to be used as a control with the Simplexa™ *C. difficile* Direct kit.
   This control is not intended for use with other assays or systems.

2. Indication(s) for use:

   Same as Intended Use.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   3M Integrated Cycler with Integrated Cycler Studio Software

I. Device Description:

The Simplexa *C. difficile* Direct assay system is a real-time PCR system that enables the direct amplification and detection of toxigenic *C. difficile* DNA from unprocessed liquid or unformed stool specimens that have not undergone nucleic acid extraction. The system consists of the Simplexa *C. difficile* Direct assay, the Integrated Cycler (with Integrated Cycler Studio Software), the Direct Amplification Disc and associated accessories.

Materials provided:

- Simplexa *C. difficile* Direct Reaction Mix

Materials provided separately:

- Direct Amplification Disc Kit
- Simplexa *C. difficile* Sample Prep Kit

Materials required but not supplied:

- Integrated Cycler with Integrated Cycler Studio Software version 6.0 or higher
- Simplexa *C. difficile* Positive Control Pack
- 50 µL fixed volume pipette
- Sterile, nuclease-free disposable pipette tips with filters
- Freezer (manual defrost) at -10 to -30 °C (for kit component)
- Refrigerator at 2 to 8 °C (for specimens)
- Disposable, powder-free gloves

J. Substantial Equivalence Information:

1. Predicate device name(s):

   BD MAX *Cdiff*

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

   K130470

3. Comparison with predicate:

Similarities		
Item	Device **Simplexa *C. difficile* Direct**	Predicate **BD MAX *Cdiff* (K130470)**
Intended use	Detection of *C. difficile* toxin gene sequences in liquid or unformed stool samples from individuals suspected of *C. difficile* infection (CDI). Intended to aid in the diagnosis of CDI.	Same
Assay target	*C. difficile* toxin B gene (*tcdB)*	Same
Sample type	Liquid or unformed stool	Same
Assay technology	Real-time PCR	Same
Sample extraction	Automated	Same
Test interpretation	Automated	Same
Qualitative/Quantitative	Qualitative	Same

Differences		
Item	Device **Simplexa *C. difficile* Direct**	Predicate **BD MAX *Cdiff* (K130470)**
Detection	Real-time PCR with bifunctional fluorescent primer-probes	Real-time PCR using fluorogenic target-specific hybridization probes
Instrument platform	3M Integrated Cycler	BD MAX System
Samples/controls per run	Eight	24
Positive control	Positive Control Pack provided separately	User provided

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

Guidance for Industry and FDA Staff: Administrative Procedures for CLIA Categorization, March 12, 2014

Guidance for Industry and FDA Staff, Format for Traditional and Abbreviated 510(k), August 12, 2005

Off-The-Shelf Software Use in Medical Devices, September 9, 1999

Cybersecurity for Networked Medical Devices Containing Off-The-Shelf (OTS) Software, January 14, 2005

Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, May 11, 2005

General Principles of Software Validation, January 11, 2002

L. Test Principle:

The Simplexa *C. difficile* Direct assay system is a real-time PCR system that enables the direct amplification and detection of toxigenic *C. difficile* DNA from unprocessed liquid or unformed stool specimens that have not undergone nucleic acid extraction. In the Simplexa *C. difficile* Direct assay, bi-functional fluorescent probe primers are used together with corresponding reverse primers to amplify *C. difficile* and DNA internal control targets. The assay targets a sequence which is in a well conserved region of *C. difficile* toxin B gene (tcdB). A DNA internal control is used to detect PCR failure and/or inhibition.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Reproducibility

   Reproducibility testing for the Simplexa *C. difficile* Direct was performed at three sites with a coded and randomized sample panel that included a positive control, a negative control (TE-stool matrix without analyte) and four contrived samples spiked into a negative stool - TE buffer matrix. As shown in Table 1, the four contrived positive samples consisted of a low positive (LP) and a medium positive (MP) for each of two strains of toxigenic *C. difficile* (ATCC 43255 and NAP1A). Each contrived sample was prepared by spiking a specific organism concentration into negative stool - TE matrix. Both positive and negative controls were also tested during this study.

   Table 1. Reproducibility Sample Panel

Sample Panel Member	Strain at Spiked Concentration
ATCC 43255 – LP	ATCC 43255 at 0.95 CFU/mL (1 X LoD)
ATCC 43255 – MP	ATCC 43255 at 3.8 CFU/mL (4 X LoD)
NAP1A – LP	NAP1A at 0.43 CFU/mL (1 X LoD)
NAP1A – MP	NAP1A at 1.7 CFU/mL (4 X LoD)
Negative	None
Positive Control	n/a

   Each sample panel member was coded, randomized, and tested in triplicate per run for two runs per day for a total of five non-consecutive days per site. Two runs per day were performed by each operator. Therefore, a total of 90 replicates (three replicates x two runs x five days x three sites) were tested for each sample panel member. Two Integrated Cycler instruments were used at each site for a total of six instruments overall.

   One negative control replicate and one NAP1A sample replicate produced invalid results with the test device due to insufficient specimen volume upon initial testing. Both samples were retested according to the labelled instructions for use, and both samples produced the expected result upon retest.

   Test results were acceptable and did not vary between sites, operators, days, runs, or instruments. Combined results for all sites are presented in the Table 2.

Table 2. Site-to-Site Reproducibility Results

Sample	Site – 1			Site – 2			Site – 3			Total % Agreement with Expected Results	95% CI
	% Agreement with Expected Results	Avg Ct	Total %CV	% Agreement with Expected Results	Avg Ct	Total %CV	% Agreement with Expected Results	Avg Ct	Total %CV		
ATCC 43255 – LP	100% (30/30)	38.0	1.5	100.0% (30/30)	38.1	1.4	100.0% (30/30)	37.5	1.4	100.0% (90/90)	95.9 - 100%
ATCC 43255 – MP	100% (30/30)	37.3	1.7	100% (30/30)	37.1	1.3	100% (30/30)	37.0	1.5	100% (90/90)	95.9 - 100%
NAP1A - LP	100% (30/30)	39.6	2.6	100% (30/30)	39.1	2.0	96.7% (29/30)	39.4	1.9	98.9% (89/90)	94.0 - 99.8%
NAP1A - MP	100% (30/30)	36.4	1.3	100% (30/30)	36.3	1.0	100% (30/30)	36.6	1.4	100% (90/90)	95.9 - 100%
Positive Control	100% (30/30)	31.7	1.7	100% (30/30)	31.4	1.6	100% (30/30)	30.9	1.4	100% (90/90)	95.9 - 100%
Negative[1]	100% (30/30)	N/A	N/A	100% (30/30)	N/A	N/A	100% (30/30)	N/A	N/A	100% (90/90)	95.9 - 100%

[1]Expected results of negative sample is "Negative" for *C. difficile*.

b. Linearity/assay reportable range:

Not applicable.

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

Controls:

The Simplexa *C. difficile* Positive Control Pack consists of inactivated *C. difficile*, and is intended to be used as an external control for the Simplexa *C. difficile* Direct kit. The positive control pack is provided separately. Focus recommends testing external controls once per day.

Sample Prep Buffer should be used as a No Template Control (NTC). A NTC should be tested for every control run in which a positive control is tested.

A DNA internal control (DNA IC) template with a specific fluorescent primers/probe set is included in each reaction mix and is used to detect PCR failure and/or inhibition. Failure of the DNA IC will cause the software to report an invalid result when toxigenic *C. difficile* DNA has not been detected in the sample. In case of an invalid result, the sample should be re-prepared and the assay repeated. A new Sample Prep Swab and Sample Prep Buffer should be used.

Expected control results are listed in Table 3. If the controls are not within these parameters, patient results should be considered invalid and the assay repeated.

Table 3. Expected Control Results

Control Type	*C. difficile tcd*B Gene Expected Result	DNA IC
Simplexa *C. difficile* Direct Positive Control[1]	Detected	Not applicable[2]
No Template Control	Not Detected	Valid

[1]Typical Ct values for the Positive Control range between 28 to < 34.

[2] Detection of the Simplexa DNA IC is not required for a valid result when toxigenic *C. difficile* DNA is detected.

Positive Controls and No Template Controls were tested daily at each site during the prospective clinical study. A total of 458 Positive controls and 459 No Template Controls were tested across all sites and produced valid expected initial results for 456 (99.6%) Positive Controls and 451 (98.3%) No Template Controls. After QC retesting, 100% of the unexpected QC results resolved to the expected result.

d. *Detection limit:*

Analytical sensitivity/ Limit of detection

The Limit of Detection (LoD) for the Simplexa *C. difficile* Direct was determined using a panel consisting of negative stool-TE matrix spiked with two strains of toxigenic *C. difficile* (ATCC 43255 and NAP1A). The bacterial stocks were serially diluted, and a total of 32 replicates were tested at each dilution. The lowest concentration of *C. difficile* where at least 95% of the replicates were detected was determined to be the LoD for each strain. The results are shown in Table 4 below.

Table 4. Simplexa *C. difficile* Direct LoD

Toxigenic *C. difficile* Strain	LoD Concentration (CFU/mL)
ATCC 43255	0.95
NAP1A	0.43

Analytical reactivity

The Simplexa *C. difficile* Direct assay was evaluated for analytical reactivity to 32 strains of toxigenic *C. difficile* from a variety of toxinotypes.

Analytical reactivity samples were prepared by spiking regrown and retitered strains into negative stool-TE matrix at 4X LoD of the ATCC 43255 strain (approximately 4 CFU/mL). Each sample was tested in triplicate. If at least one of three replicates produced a negative assay result, higher concentrations were tested until all three replicates were detected. Of the 32 tested strains, 22 strains were detected at 4 CFU/mL, eight strains were detected at 8 – 32 CFU/mL, and two strains were

detected at 256 – 512 CFU/mL. The results are shown in Table 5.

Table 5. Inclusivity/Reactivity

Strain	Conc (CFU/mL)	Toxinotype	Toxin	Ribotype
Clostridium difficile ATCC 43596	4	0	A+B+	012
Clostridium difficile ATCC 43598	4	VIII	A-B+	017
Clostridium difficile ATCC 43600	4	0	A+B+	014
Clostridium difficile ATCC 51695	4	0	A+B+	001
Clostridium difficile ATCC 9689	4	0	A+B+	001
Clostridium difficile BAA-1382	4	0	A+B+	012
Clostridium difficile BAA-1805	8	IIIb	A+B+	027
Clostridium difficile BAA-1870	4	IIIb	A+B+	027
Clostridium difficile BAA-1871	4	0	A+B+	001
Clostridium difficile BAA-1873	4	0	A+B+	053
Clostridium difficile CCUG 8864 (CCUG 20309)	4	X	A-B+	Unknown
Clostridium difficile IS81	4	III	A+B+	034
Clostridium difficile R1880	4	I	A+B+	086
Clostridium difficile R7771	4	VIII	A-B+	110
Clostridium difficile R8366	4	0	A+B+	001
Clostridium difficile R8637	4	IX	A+B+	019
Clostridium difficile R9367	4	XIII	A+B+	070
Clostridium difficile R9385	4	XV	A+B+	122
Clostridium difficile R10456	4	IV	A+B+	058
Clostridium difficile R10725	4	V	A+B+	078
Clostridium difficile R10842	4	VI	A+B+	045
Clostridium difficile R10870	4	XIV	A+B+	111
Clostridium difficile R12425	4	II	A+B+	103
Clostridium difficile ATCC 17858	8	0	A+B+	054
Clostridium difficile ATCC 43594	16	0	A+B+	005
Clostridium difficile ATCC 17857	16	0	A+B+	001
Clostridium difficile ATCC 700792	16	0	A+B+	005
Clostridium difficile BAA-1875	16	V	A+B+	078
Clostridium difficile ATCC 43599	32	0	A+B+	001
Clostridium difficile BAA-1872	32	0	A+B+	207
Clostridium difficile BAA-1814	256	XXII	A+B+	251
Clostridium difficile BAA-1874	512	0	A+B+	002

e. Analytical specificity:

Cross reactivity

The cross reactivity of the Simplexa *C. difficile* Direct assay was evaluated by wet

testing 127 organisms that are closely related to *C. difficile*, or that cause similar clinical symptoms, or that are present as normal flora in the specimen type of interest. Samples were prepared by spiking each of the 127 organisms at clinically relevant concentrations into TE-stool matrix. Clinically relevant concentrations were defined as $\geq 10^6$ CFU/mL for bacteria and fungi and $\geq 10^5$ $TCID_{50}$/mL (PFU/mL) for viruses, or other industry accepted units. Each sample was tested in triplicate. If at least one of the three replicates was detected as positive with the assay, an additional five replicates of the same cross reactant was tested. The organisms are listed in Table 6.

Cross reactivity was not observed. One replicate of *Clostridium noyii* produced a positive result; however, testing with an additional five replicates produced negative results.

Campylobacter jejuni was tested during validation. However, it is unknown if subspecies *jejuni* was tested. *In silico* NCBI BLAST analysis was performed for *C. jejuni* subsp *jejuni* did not predict cross reactivity with the Simplexa *C. difficile* Direct kit.

The organisms *Clostridium botulinum, Desulfovibrio piger,* and *Ruminococcus bromii* were not available for wet testing. These organisms were evaluated for potential cross reactivity with the assay primers and probes by *in silico* NCBI BLAST analysis of genomic DNA sequences. The *in silico* analysis did not predict cross reactivity with the Simplexa *C. difficile* Direct kit for these species.

Table 6. Cross reactivity

Organisms	
Abiotrophia defectiva	*Enterococcus faecium vanA*
Acinetobacter baumannii	*Enterococcus gallinarum vanC*
Acinetobacter lwoffi	*Enterococcus hirae*
Adenovirus 1	*Enterococcus raffinosus*
Aeromonas hydrophila	*Enterovirus 71*
Alcaligenes faecalis subsp. *Faecalis*	*Escherichia coli ATCC 11775*
Anaerococcus tetradius	*Escherichia coli ATCC 23511*
Bacillus cereus ATCC 11778	*Escherichia coli ATCC 25922*
Bacillus cereus ATCC 13472	*Escherichia coli O157:H7 (ATCC 700927)*
Bacteroides caccae	*Escherichia fergusonii*
Bacteroides fragilis	*Escherichia hermannii*
Bacteroides merdae	*Flavonifractor plautii (deposited as Clostridium orbiscindens)*
Bacteroides stercoris	*Fusobacterium varium*
Bifidobacterium adolescentis	*Gardnerella vaginalis*
Bifidobacterium longum	*Gemella morbillorum*
Campylobacter coli	*Hafnia alvei*
Campylobacter jejuni	*Helicobacter fennelliae*
Candida albicans	*Helicobacter pylori*

Organisms	
Candida catenulate	*Klebsiella oxytoca*
Cedecea davisae	*Klebsiella pneumoniae subsp. Pneumoniae*
Chlamydia trachomatis	*Lactobacillus acidophilus*
Citrobacter amalonaticus	*Lactobacillus reuteri*
Citrobacter freundii	*Lactococcus lactis*
Citrobacter koseri	*Leminorella grimontii*
Citrobacter sedlakii	*Listeria grayi*
Clostridium beijerinckii	*Listeria innocua*
Clostridium bifermentans	*Listeria monocytogenes*
Clostridium bolteae	*Norovirus G2*
Clostridium butyricum	*Peptoniphilus asaccharolyticus*
Clostridium chauvoei	*Peptostreptococcus anaerobius*
Clostridium difficile ATCC 43593	*Plesiomonas shigelloides*
Clostridium difficile ATCC 43601	*Porphyromonas asaccharolytica*
Clostridium fallax	*Prevotella melaninogenica*
Clostridium haemolyticum	*Proteus mirabilis*
Clostridium histolyticum	*Proteus penneri*
Clostridium innocuum	*Providencia alcalifaciens*
Clostridium methylpentosum	*Providencia rettgeri*
Clostridium nexile	*Providencia stuartii*
Clostridium novyi	*Pseudomonas aeruginosa*
Clostridium paraputrificum	*Pseudomonas putida*
Clostridium perfringens	*Rotavirus, strain Wa*
Clostridium ramosum	*Salmonella enterica subsp. Arizonae*
Clostridium scindens	*Salmonella enterica subsp. Enterica serovar Choleraesuis*
Clostridium sepiticum	*Salmonella enterica subsp. Enterica serovar Typhimimurium*
Clostridium sordellii	*Serratia liquefaciens*
Clostridium sphenoides	*Serratia marcescens subsp. marcescens*
Clostridium spiroforme	*Shigella boydii*
Clostridium sporogenes	*Shigella dysenteriae*
Clostridium symbiosum	*Shigella sonnei*
Clostridium tertium	*Staphylococcus aureus*
Clostridium tetani	*Staphylococcus epidermidis*
Collinsella aerofaciens	*Stenotrophomonas maltophilia*
Corynebacterium genitalium	*Streptococcus agalactiae*
Coxsackievirus A10	*Streptococcus dysgalactiae*
Cytomegalovirus AD-169	*Streptococcus intermedius*
Echovirus 11	*Streptococcus uberis*
Edwardsiella tarda	*Trabulsiella guamensis*
Eggerthella lenta	*Veillonella parvula*

Organisms	
Enterobacter aerogenes	*Vibrio cholerae*
Enterobacter cloacae	*Vibrio parahaemolyticus*
Enterococcus casseliflavus	*White Blood Cells (Human)*
Enterococcus cecorum	*Yersinia bercovieri*
Enterococcus dispar	*Yersinia rohdei*
Enterococcus faecalis vanB	

Microbial interference:

The same 127 potentially interfering organisms that were tested for cross reactivity were also evaluated for potential interference with the detection of toxigenic *C. difficile* DNA. Two strains of toxigenic *C. difficile* (ATCC 42355 and NAP1A) were tested at two to four times LoD in triplicate in the presence of potentially interfering organisms. If at least one replicate was not detected, an additional five replicates were tested.

The Simplexa *C. difficile* Direct kit successfully detected toxigenic *C. difficile* DNA in the presence of these organisms at the concentrations tested. Strain NAP1A was detected in seven out of eight replicates of *Clostridium septicum* and *Clostridium sordellii,* and six out of eight replicates of *Abiotrophia defective*. All replicates of both strains NAP1A and ATCC 43255 were detected successfully in the presence of all other organisms tested.

Interference:

A study was conducted to evaluate the effects of exogenous and endogenous substances that could potentially interfere with assay performance. A total of 33 potentially interfering substances were co-spiked into TE-stool matrix with toxigenic *C. difficile* strains ATCC 43255 or NAP1A spiked at two to four times the LoD. Endogenous interferents were spiked at the highest possible endogenous level, and exogenous interferents were spiked at approximately 10% of the recommended dose. The samples were tested in triplicate. If at least one replicate showed evidence of interference, an additional five replicates were tested. For substances that showed evidence of interference, successively lower concentrations were tested to determine the minimally interfering level. The results are summarized in Table 7 below.

Four substances produced evidence of interference at the highest concentrations tested. Colace tested at 10% w/v and 5% w/v produced invalid results in all replicates. When the Colace concentration was reduced to 2.5% w/v, five out of eight (62.5%) replicates of *C. difficile* strain ATCC 42355 and seven out of eight (87.5%) replicates of *C. difficile* strain NAP1A were detected successfully. The remaining replicates were not detected.

Naproxen tested at 14 mg/mL produced false negative results in four out of five replicates of strains ATCC 42355 and NAP1A. All replicates of both strains were detected when the naproxen concentration was reduced to seven mg/mL.

All replicates of the ATCC 42355 strain were detected at a Dulcolax concentration of 10% w/v. However, Dulcolax at 10% w/v, 5% w/v, 2.5% w/v, and 1.25% w/v produced invalid results for all replicates of strain NAP1A. Dulcolax at 0.625% w/v produced invalid results in four out of eight replicates and a Not Detected result in one replicate of strain NAP1. All replicates of strain NAP1 were detected when Dulcolax concentration was reduced to 0.3125% w/v.

All replicates of the ATCC 42355 strain were detected in the presence of 0.2 mg/mL Milk of Magnesia. Five out of eight replicates of strain NAP1were detected at this concentration of Milk of Magnesia.

Table 7. Interference

Interfering Substance	Interfering Substance Concentration	Active Ingredient	%Detection (#Detected/#Tested)	
			ATCC 43255	NAP1A
Afrin	10% w/v	Oxymetazoline hydrochloride 0 5%	100%(3/3)	100%(3/3)
Antacid and Anti-gas generic	0.1 mg/mL	Aluminum hydroxide/Magnesium hydroxide	100%(3/3)	100%(3/3)
Anusol Plus (Pramoxine HCL)	10% w/v	Pramoxine hydrochloride 1% and zinc sulfate monohydrate 0.5%	100%(3/3)	100%(3/3)
Barium Sulfate	5 mg/mL	Barium sulfate (98% for suspension)	100%(3/3)	100%(3/3)
Benzalkonium (Towlettes)	10% v/v	Benzalkonium chloride, Ethanol	100%(3/3)	100%(3/3)
Blood	5% v/v	Glucose, Hormones, Enzymes, Ions, Iron, etc.	100%(3/3)	100%(3/3)
Colace[1]	2.5 % w/v	Ducosate sodium USP 100 mg	62.5%(5/8)	87.5%(7/8)
Dramamine	10% w/v	Dimenhydrinate	100%(3/3)	100%(3/3)
Dulcolax[2]	10% w/v for ATCC 43255 & 0.3125% w/v for NAP1A	Bisacodyl USP 5 mg	100%(3/3)	100%(3/3)
Gynol II, Nonoxynol 9	10% w/v	Nonoxynol 9 (4 %)	100%(3/3)	100%(3/3)
Hydrocortisone cream	2% w/v	Hydrocortisone	100%(3/3)	100%(3/3)
Imodium	0 05 mg/mL	Loperamide hydrochloride	100%(3/3)	100%(3/3)
KY Jelly	2% w/v	Glycerin	100%(3/3)	100%(3/3)
Mesalazine	10% w/v	Mesalazine rectal suspension (4 g/60 mL)	100%(3/3)	100%(3/3)

Interfering Substance	Interfering Substance Concentration	Active Ingredient	%Detection (#Detected/#Tested)	
			ATCC 43255	NAP1A
Metronidazole	14 mg/mL	Metronidazole	100%(3/3)	100%(3/3)
Milk of Magnesia	0.2 mg/mL	Magnesium hydroxide	100%(3/3)	62.5%(5/8)
Mineral Oil	2% w/v	Mineral oil	100%(3/3)	100%(3/3)
Monistat 7	10% w/v	Miconazole nitrate 2% (100 mg)	100%(3/3)	100%(3/3)
Mucin	3 mg/mL	Immunoglobulins, Lysozyme, Polymers, etc.	100%(3/3)	100%(3/3)
Naproxen[3]	7 mg/mL	Naproxen sodium	100%(3/3)	100%(3/3)
Nystatin	10000 USP units/ml	Nystatin	100%(3/3)	100%(3/3)
Palmitic Acid	2 mg/mL	Palmitic acid	100%(3/3)	100%(3/3)
Pepto-Bismol	0.175 mg/mL	Bismuth subsalicylate	100%(3/3)	100%(3/3)
Preparation H	2% w/v	Phenylephrine	100%(3/3)	100%(3/3)
Sennosides	0.1 mg/mL	Sennosides	100%(3/3)	100%(3/3)
SPF 30 Sunscreen	1% w/v	Avobenzone 3%, homosalate 8%, octisalate 5%, octocrylene 4%, and oxybenzone 4%	100%(3/3)	100%(3/3)
SPF 50 Sunscreen	1% w/v	Avobenzone 3%, homosalate 13%, octisalate 5%, octocrylene 7%, and oxybenzone 4%	100%(3/3)	100%(3/3)
Stearic Acid	4 mg/mL	Stearic acid	100%(3/3)	100%(3/3)
Tums	0.1 mg/mL	Calcium Carbonate	100%(3/3)	100%(3/3)
Vagisil	10% w/v	Benzocaine USP (20% - External analgesic), Resorcinol (3% - External analgesic)	100%(3/3)	100%(3/3)
Vancomycin	1.4 mg/mL	Vancomycin	100%(3/3)	100%(3/3)
Vaseline	10 %w/v	White petrolatum USP (100%)	100%(3/3)	100%(3/3)
Witch hazel	Liquid from 1 wipe	Witch hazel	100%(3/3)	100%(3/3)

[1] Colace also tested at two higher concentrations (10 % and 5 %w/v) produced "Invalid" results in all replicates.

[2] Dulcolax also tested at 10 %, 5 %, 2.5 %, and 1.25 %w/v produced "Invalid" results for all replicates of strain NAP1A. Dulcolax at 0.625% w/v produced "Invalid" results in four out of eight replicates and a "Not Detected" result in one replicate of strain NAP1A.

[3] Naproxen also tested at 14 mg/mL produced "Not Detected" results in four out of five replicates of strains NAP1A and ATCC 43255.

f. Assay cut-off:

The initial assay cut-off was established during a period of feasibility testing. The assay cut-off was confirmed by analysis of the results from the limit of detection and the prospective clinical studies.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity:

The clinical performance of the Simplexa *C. difficile* Direct test was established in a prospective multi-site investigation. Two thousand three hundred and fifty one (2351) samples were prospectively collected from five geographically diverse sites between December 3, 2015 and June 10, 2016 from patients with signs and symptoms of *C. difficile* infection. Each site enrolled fresh unformed stool specimens that were left over from routine clinical *C. difficile* infection diagnostic testing. Two thousand three hundred and thirty (2330) were evaluable on the Simplexa *C. difficile* Direct and the Direct Culture method. Two thousand three hundred and thirty six (2336) were evaluable on the Simplexa *C. difficile* Direct and the Combined Direct and Enriched Culture methods.

Of the 2336 samples, 1233 (52.8%) were collected from females and 1103 (47.2%) were collected from males. The age distribution of the subjects is shown in Table 8.

Table 8. Age distribution

Age	n	%
Less than 6	22	0.9
6 Yrs to 21 Yrs	116	5
22 Yrs to 59 Yrs	1073	45.9
More than 60 Yrs	1125	48.2
All	2336	100

Samples were tested with the Simplexa *C. difficile* Direct at the collection sites, and the comparator culture methods were performed at one central laboratory. Discrepant analysis was performed using FDA cleared nucleic acid amplification test (NAAT) results provided by the clinical sites.

The initial invalid rate of the clinical prospective study using Simplexa™ *C. difficile* Direct was 0.64% (15/2351 samples). After repeat testing, 3/15 samples remained invalid, thus resulting in a final invalid rate of 0.13% (3/2351 samples).

Comparison with combined direct and enriched culture

The performance of the Simplexa *C. difficile* Direct test compared with the combined direct and enriched culture result is shown in Table 9.

Table 9. Comparison with combined direct and enriched culture

	Combined Direct & Enriched Culture		
Simplexa *C. difficile* Direct	**Detected**	**Not Detected**	**Total**
Detected	336	96[a]	432
Not Detected	55[b]	1849	1904
Total	391	1945	2336

Sensitivity	85.93%	(336/391)	95% CI	82.1%, 89 %
Specificity	95 6%	(1849/1945)	95% CI	94 %, 95.9%
PPV	77.8%	(336/432)	95% CI	73.6%, 81.4%
NPV	97.1%	(1849/1904)	95% CI	96.3%, 97.8%

[a] 59/96 discrepant samples were positive and 37/96 were negative for *C. difficile* toxin gene DNA when tested with an FDA cleared molecular test.
[b] 43/55 discrepant samples were negative and 12/55 were positive for *C. difficile* toxin gene DNA when tested with an FDA cleared molecular test.

Comparison with direct culture

The performance of the Simplexa *C.difficile* Direct test compared with direct culture is shown in Table 10 and is reported as positive percent agreement (PPA) and negative percent agreement (NPA).

Table 10. Comparison with direct culture

	Direct Toxigenic Culture		
Simplexa *C. difficile* Direct	**Detected**	**Not Detected**	**Total**
Detected	265	163[a]	428
Not Detected	12[b]	1890[c]	1902
Total	277	2053	2330

PPA	95.7%	(265/277)	95% CI	92.6%, 97.5%
NPA	92.1%	(1890/2053)	95% CI	90.8%, 93.2%

[a] 116/163 discrepant samples were positive, 46/163 were negative, and 1/163 was indeterminate for *C. difficile* toxin gene DNA when tested with an FDA cleared molecular test.
[b] 8/12 discrepant samples were negative and 4/12 were positive for *C. difficile* toxin gene DNA when tested with an FDA cleared molecular test.
[c] Direct culture results were not available from 6 specimens due to plating errors. Overall results were obtained by enriched culture for these 6 samples and so included in the Combined Culture results.

Comparison with FDA cleared nucleic acid amplification tests (NAATs)

Sample subsets from the multi-site investigational study were also tested with three commercially available FDA cleared NAATs as additional information. The percent agreement (PPA, NPA) of the Simplexa *C.difficile* Direct test to each of the three cleared NAATs is shown in Tables 11 through 13.

Table 11. Comparison with cleared NAAT1

	NAAT1		
Simplexa *C. difficile* Direct	**Detected**	**Not Detected**	**Total**
Detected	114	24	138
Not Detected	8	683	691
Total	122	707	829

PPA	93.4%	(114/122)	95% CI	87.6%, 96.6%
NPA	96.6%	(683/707)	95% CI	95 %, 97.7%

Table 12. Comparison with cleared NAAT2

	NAAT2		
Simplexa *C. difficile* Direct	**Detected**	**Not Detected**	**Total**
Detected	138	38	176
Not Detected	9	591	600
Total	147	629	776

PPA	93.9%	(138/147)	95% CI	88.8%, 96.7%
NPA	94 %	(591/629)	95% CI	91.8%, 95.6%

Table 13. Comparison with cleared NAAT3

	NAAT3		
Simplexa *C. difficile* Direct	**Detected**	**Not Detected**	**Total**
Detected	112	5	117
Not Detected	20	584	604
Total	132	589	721

PPA	84.8%	(112/132)	95% CI	77.8%, 90 %
NPA	99.2%	(584/589)	95% CI	98 %, 99.6%

b. Clinical specificity:

See section M3a

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

Not applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

In the prospective clinical study conducted at five geographically diverse sites, 2336 samples were collected from 1233 (52.8%) female and 1103 (47.2%) male patients. Patient ages ranged from less than one years old to 106 years old, and 2198 (94%) were 22 years old or older. Out of the total number of evaluable samples, 391 (16.7%) samples were culture positive by toxigenic *C. difficile* combined direct and enriched culture. The percent positivity with the Simplexa *C. difficile* Direct varied from 14.3% to 21.4% between the clinical sites.

N. Instrument Name:

3M Integrated Cycler

O. System Descriptions:

1. Modes of Operation:

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes _____ or No ___X_____

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes _____ or No ____X____

2. Software:

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

Yes ___X_____ or No _____

3. Specimen Identification:

Specimens are identified by scanning or typing in the sample identifier.

4. Specimen Sampling and Handling:

   The test is intended for use with liquid or unformed human stool specimens. Stool specimens should be collected following standard laboratory procedures and precautions. Specimens may be stored at 2 to 25 °C. Stool samples must be tested within 48 hours of collection.

5. Calibration:

   End-user calibration for the Integrated Cycler is not necessary. Calibration of the optical modules (excitation and emission gain settings) is performed during the manufacturing process and the values are stored in the instrument firmware.

6. Quality Control:

   See section M.1.*c* for information on internal and external controls.

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