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K113433

510(k) Summary

Simplexa™ *C. difficile* Universal Direct Catalog No. MOL2975

Prepared Date: April 5, 2012

Applicant	Focus Diagnostics, Inc. 11331 Valley View Street Cypress, California 90630 USA
Establishment Registration No.	2023365
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Summary Date	April 5, 2012
Proprietary Name	Simplexa™ *C. difficile* Universal Direct
Generic Name	*C.difficile* nucleic acid
Classification	Class I
Predicate Devices	illumigene™ *C. difficile* (K110012) BD GeneOhm™ Cdiff (K081920)

Intended Use

The Focus Diagnostics Simplexa™ C. difficile Universal Direct is a real-time polymerase chain reaction (PCR) assay and is intended for use on the 3M Integrated Cycler instrument for the detection of toxigenic *Clostridium difficile* toxin B gene (*tcdB*) in liquid or unformed stool samples from individuals suspected of *C. difficile* infection. This test aids in the diagnosis of *Clostridium difficile* associated disease (CDAD).

Device Description

The test is a real-time polymerase chain reaction (PCR) amplification and detection system that utilizes bi-functional fluorescent probe-primers for the detection of *C. difficile* in liquid or unformed stool. The Simplexa™ *C. difficile* Universal Direct kit contains primers, enzymes, buffers and controls. The assay is composed of two principal steps: (1) Heat treatment of stool samples, (2) Amplification of the *C. difficile* DNA and internal control DNA using bi-functional fluorescent probe-primers together with reverse primers. The DNA internal control is used to monitor potential presence of PCR inhibitors. The assay targets a sequence which is in a well conserved region of *C. difficile* toxin B gene (*tcdB*).

Predicate Device Information

Trade Name / Method	510(k) submitter	510(k) number	Decision Date	Panel	Product Code(s)
illumigene™ *C. difficile*	MERIDIAN BIOSCIENCE, INC	K110012	02/24/2011	Microbiology	OMN
BD GeneOhm™ Cdiff Assay	BD DIAGNOSTICS (GENEOHM SCIENCES, INC.)	K081920	12/19/2008	Microbiology	LLH

Comparison to Predicate

Item	Device	Predicate 1	Predicate 2
Name	Simplexa™ *C. difficile* Universal Direct	illumigene™ *C. difficile*	BD GeneOhm™ Cdiff
Intended Use	The Focus Diagnostics Simplexa™ *C. difficile* Universal Direct is a real-time polymerase chain reaction (PCR) assay and is intended for use on the 3M Integrated Cycler instrument for the detection of toxigenic *Clostridium difficile* toxin B gene (*tcdB*) in liquid or unformed stool samples from individuals suspected of *C. difficile* infection. This test aids in the diagnosis of *Clostridium difficile* associated disease (CDAD).	The Illumigene™ *C. difficile* DNA amplification assay, performed on the illumipro-10, is a qualitative *in vitro* diagnostic test for the direct detection of toxigenic *C. difficile* in human stool specimens from pediatric and adult patients suspected of having *Clostridium difficile*-associated disease (CDAD). The Illumigene™ *C. difficile* assay utilizes loop-mediated isothermal DNA amplification (LAMP) technology to detect the pathogenicity locus (PaLoc) of toxigenic *Clostridium difficile*. The *Clostridium difficile* PaLoc is a gene segment present in all known toxigenic *C. difficile* strains. The *C. difficile* PaLoc codes for both the Toxin A gene (*tcdA*) and the Toxin B gene (*tcdB*), has conserved border regions, and is found at the same site on the *C. difficile* genome for all toxigenic strains. The Illumigene™ *C. difficile* assay detects the PaLoc by targeting a partial DNA fragment on the Toxin A gene. The *tcdA* target region was selected as an intact region remaining in all known A+B+ and A-B+ toxinotypes. Illumigene™ *C. difficile* is intended for use in hospital, reference or state laboratory settings. The device is not intended for point-of-care use.	The BD GeneOhm™ C diff Assay is a rapid *in vitro* diagnostic test for the direct, qualitative detection of *C. difficile* toxin B gene (*tcdB*) in human liquid or soft stool specimens from patients suspected of having *Clostridium difficile*-associated disease (CDAD). The test, based on real-time PCR, is intended for use as an aid in diagnosis of CDAD. The test is performed directly on the specimen, utilizing polymerase chain reaction (PCR) for the amplification of specific targets and fluorogenic target-specific hybridization probes for the detection of the amplified DNA.
Assay Targets	*C. difficile* toxin B gene (*tcdB*)	PaLoc region (encoding *tcdA* and *tcdB*)	*C. difficile* toxin B gene (*tcdB*)

Item	Device	Predicate 1	Predicate 2
Name	Simplexa™ *C. difficile* Universal Direct	illumigene™ *C. difficile*	BD GeneOhm™ Cdiff
Sample Types	Liquid or unformed stool	Unformed human stool	Liquid or soft stool specimen
Extraction Methods	Off-board 10 minute preheating step.	Off-board 10 minute preheating step, followed by vortexing.	5 minutes of vortexing, sample centrifugation, followed by a 5 minute heating step.
Assay Methodology	The Simplexa *C. difficile* Universal Direct assay incorporates direct, qualitative detection of toxigenic *C. difficile* DNA from clinical specimens in human stool specimens using the 3M Integrated Cycler. The assay utilizes real-time PCR technology with fluorescently labeled, bi-functional probe-primer that amplify and detect a conserved region of the toxin B (*tcdB*) gene.	The assay is performed on the *illumipro-10*, and is a qualitative assay for direct detection of toxigenic *C. difficile* in human stool specimens. It utilizes loop-mediated isothermal DNA amplification technology to detect the pathogenicity locus (PaLoc) of toxigenic *C. difficile* . The PaLoc is a gene segment present in all known toxigenic *C. difficile* strains, and it codes for both the Toxin A gene (*tcdA*) and the Toxin B gene (*tcdB*).	The BD GeneOhm Cdiff assay is used for qualitative detection of *C. difficile* toxin B gene (*tcdB*) in human liquid or soft stool, using the Cepheid SmartCycler. The test uses real-time PCR for the amplification of specific targets which are detected by fluorogenic target-specific hybridization probes (molecular beacons). The amplification, detection and interpretation of the signals are done automatically by the Cepheid SmartCycler software.
Detection Techniques	Real time PCR with bi-functional fluorescent probe-primers using the 3M Integrated Cycler.	Isothermal loop-mediated amplification technology, with detection of light transmission change based on magnesium pyrophosphate precipitation.	Real time PCR with molecular beacons using the Cepheid SmartCycler.
Reference Method	Toxigenic Culture	Cytotoxigenic Bacterial Culture	Cytotoxicity Assay
Limit of Detection	ATCC 43255 560.7 CFU/mL or 1.12 CFU/PCR NAP 1A 76.3 CFU/mL or 0.15 CFU/PCR	VPI 10463, 4CFU/test 2007431, 32 CFU/test CFI, 64 CFU/test 2006240, 32 CFU/test BI8, 64 CFU/test 2007858, 32 CFU/test 8864, 64 CFU/test	ATCC 43255 10 DNA copies/reaction, 4 CFU/reaction
Reproducibility	Low Positive 100% (90/90) Medium Positive 100% (89/89) High Negative 98.9% (89/90) No Template Control (NTC) 98.9% (89/90)	Low Positive 100% (90/90) Positive 100% (60/60) High Negative 91% (82/90) Negative 100% (59/59)	Low positive 96.7% (87/90) Moderate Positive 100% (90/90) Negative 100% (90/90) Additional reproducibility using dilutions of high negative at 1:100 dilution 80% (72/90) and 1:10 dilution 23.3% (21/90)

REPRODUCIBILITY

Three investigators assessed the device's inter-laboratory reproducibility and inter/intra-assay reproducibility. Each of the three sites used the same panel, which consisted of contrived samples in stool-TE buffer matrix spiked with *C. difficile* bacterial stock. The panel included high negative, low positive, and medium positive samples. Each site utilized at least two testing operators and one lot of Simplexa™ *C.difficile* Universal Direct kit across five days. On each day two runs were performed, one by each operator. A summary of the results are shown in Table 3.

Table 3. 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		
Low Positive	30/30	35.20	1.64	30/30	35.26	1.30	30/30	35.30	2.04	100% (90/90)	95.9% - 100.0%
Medium Positive[1]	29/29	32.71	0.82	30/30	32.60	1.07	30/30	32.65	0.77	100% (89/89)	95.9% - 100.0%
Positive Control (PC)[2]	30/30	32.55	1.11	29/29	32.13	0.87	31/31	32.33	0.63	100% (90/90)	95.9% - 100.0%
High Negative	29/30			30/30			30/30			98.9% (89/90)	94.0% - 99.8%
No Template Control (NTC)[3]	30/30			30/30			29/30			98.9% (89/90)	94.0% - 99.8%
Total Agreement	148/149 (99.3%)			149/149 (100.0%)			150/151 (99.3%)			447/449 (99.6%)	98.4% - 99.9%

[1]One replicate was declared "invalid" based on the site operator discretion. It was "Not Detected".
[2]One replicate was "Invalid" at Site 2 and additional replicate was tested in Run-1, Day-1 at Site 3 because the site had thought that one of the three replicates had a 'bubble' and therefore as a precaution loaded an additional replicate at the end of the run.
[3]One replicate of the NTC is "Detected" and may be attributed to possible contamination due to handling.
Note: Two samples – "NTC" and "High Negative" were excluded from reporting Quantitative Reproducibility Results.

LIMIT OF DETECTION

The Limit of Detection (LoD) was determined for the Simplexa™ *C. difficile* Universal Direct assay by performing limiting dilution studies using bacterial stocks for two *C. difficile* bacterial strains. The strains (ATCC 43255 and NAP 1A) were cultured and quantified. The LoD was determined using one lot of the Simplexa™ *C. difficile* Universal Direct Kit. Tentative LoD was determined using three replicates in screening followed by confirmation using twenty replicates. LoD was determined to be 560.7 CFU/mL or 1.12 CFU/PCR for strain ATCC 43255 and 76.3 CFU/mL or 0.15 CFU/PCR for strain NAP 1A.

ANALYTICAL REACTIVITY

Analytical reactivity of additional strains of *C. difficile* was evaluated in negative stool-TE buffer matrix. Quantified bacterial material was spiked into the negative stool-TE buffer matrix at a single dilution. A total of 20 different strains were tested in triplicate. All of the tested strains were detected (Table 4).

Table 4. Analytical Reactivity Results for *C. difficile* strains

No.	Strain	Concentration (cfu/mL)	Toxinotype	*C.difficile* Result #Detected / #Total
1	ATCC 17857 (870) A+B+	1.12×10^3	0	3/3
2	ATCC 43594 (W1194) A+B+	1.12×10^3	0	3/3
3	ATCC 43596 (545) A+B+	1.12×10^3	0	3/3
4	ATCC 43597 A+B+	1.12×10^3		3/3
5	ATCC 43598 (1470) A-B+	1.12×10^3	VIII	3/3
6	ATCC 43599 (2022) A+B+	1.12×10^3	0	3/3
7	ATCC 43600 (2149) A+B+	1.12×10^3	0	3/3
8	ATCC 51695 (BDMS 18 AN) A+B+	1.12×10^3	0	3/3
9	ATCC 700792 (14797-2) A+B+	1.12×10^3	0	3/3
10	ATCC 9689 (90556-M6S) A+B+	1.12×10^3	0	3/3
11	ATCC BAA-1382 (630) A+B+	1.12×10^3	0	3/3
12	ATCC BAA-1805 A+B+	1.12×10^3	III	3/3
13	BAA-1814 A+B+	1.12×10^3	XXII	3/3
14	BAA-1870 A+B+	1.12×10^3	III	3/3
15	BAA-1871 A+B+	1.12×10^3	0	3/3
16	BAA-1872 A+B+	1.12×10^3	0	3/3
17	BAA-1873 A+B+	1.12×10^3	0	3/3
18	BAA-1874 A+B+	1.12×10^3	0	3/3
19	BAA-1875 A+B+	1.12×10^3	V	3/3
20	CCUG 8864 A-B+	1.12×10^3	X	3/3

CROSS REACTIVITY

Analytical specificity for various possible cross-reactants was performed. A total of 119 potential cross-reactants were tested. No cross reactivity was observed (Table 5).

Table 5. Cross Reactivity Results

Tested Cross-Reactants			
No.	Cross Reactant	Concentration	Result
1	*Abiotrophia defective*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
2	*Acinetobacter baumanii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
3	*Acinetobacter lwofii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
4	Adenovirus 40	1.00×10^5 $TCID_{50}$/mL	No Cross Reactivity Observed

Tested Cross-Reactants			
No.	Cross Reactant	Concentration	Result
5	*Aeromonas hydrophila*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
6	*Alcaligenes faecalis* subsp. *Faecalis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
7	*Anaerococcus tetradius*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
8	*Bacillus cereus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
9	*Bacteroides caccae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
10	*Bacteroides merdae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
11	*Bacteroides stercoris*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
12	*Bifidobacterium adolescentis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
13	*Bifidobacterium longum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
14	*Campylobacter coli*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
15	*Campylobacter jejuni*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
16	*Candida albicans*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
17	*Candida catenulate*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
18	*Cedecea davisae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
19	*Chlamydia trachomatis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
20	*Citrobacter amalonaticus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
21	*Citrobacter freundii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
22	*Citrobacter koseri*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
23	*Citrobacter sedlakii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
24	*Clostridium beijerinckii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
25	*Clostridium bifermentans*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
26	*Clostridium bolteae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
27	*Clostridium butyricum*	6.80×10^5 cfu/mL	No Cross Reactivity Observed
28	*Clostridium chauvoei*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
29	*Clostridium difficile* non-toxigenic ATCC 43593	1.00×10^6 cfu/mL	No Cross Reactivity Observed
30	*Clostridium difficile* non-toxigenic ATCC43601	1.00×10^6 cfu/mL	No Cross Reactivity Observed
31	*Clostridium fallax*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
32	*Clostridium histolyticum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
33	*Clostridium innocuum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
34	*Clostridium methylpentosum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
35	*Clostridium nexile*	6.90×10^5 cfu/mL	No Cross Reactivity Observed
36	*Clostridium novyi*	8.90×10^5 cfu/mL	No Cross Reactivity Observed
37	*Clostridium paraputrificum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
38	*Clostridium perfringens*	6.70×10^5 cfu/mL	No Cross Reactivity Observed
39	*Clostridium ramosum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
40	*Clostridium scindens*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
41	*Clostridium sepiticum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed

Tested Cross-Reactants			
No.	Cross Reactant	Concentration	Result
42	*Clostridium sordellii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
43	*Clostridium sphenoides*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
44	*Clostridium sporogenes*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
45	*Clostridium symbiosum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
46	*Clostridium terdium*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
47	*Clostridium tetani*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
48	*Collinsella aerofaciens*	8.60×10^5 cfu/mL	No Cross Reactivity Observed
49	*Corynebacterium genitalium*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
50	Coxsackie virus A16	1.00×10^5 $TCID_{50}$/mL	No Cross Reactivity Observed
51	Cytomegalovirus AD-169	1.00×10^5 $TCID_{50}$/mL	No Cross Reactivity Observed
52	*Desulfovibrio piger*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
53	Echovirus 9	1.00×10^5 $TCID_{50}$/mL	No Cross Reactivity Observed
54	*Edwardsiella tarda*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
55	*Eggerthellalenta*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
56	*Enterobacter aerogenes*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
57	*Enterobacter cloacae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
58	*Enterococcu raffinosus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
59	*Enterococcus casseliflavus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
60	*Enterococcus cecorum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
61	*Enterococcus dispar*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
62	*Enterococcus hirae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
63	*Enterococcusfaecalis* vanB	1.00×10^6 cfu/mL	No Cross Reactivity Observed
64	*Enterococcusfaecium* vanA	1.00×10^6 cfu/mL	No Cross Reactivity Observed
65	*Enterococcusgallinarum* vanC	1.00×10^6 cfu/mL	No Cross Reactivity Observed
66	Enterovirus 71	5.01×10^4 $TCID_{50}$/mL	No Cross Reactivity Observed
67	*Escherichia coli*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
68	*Escherichia fergusonii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
69	*Escherichia hermannii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
70	*Fusobacterium varium*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
71	*Gardnerella vaginalis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
72	*Gemella morbillorum*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
73	*Hafnia alvei*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
74	*Helicobacter pylori*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
75	*Homo sapiens*	3.07 pg/mL	No Cross Reactivity Observed
76	*Klebsiella oxytoca*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
77	*Klebsiella pneumoniae* subsp. *Pneumoniae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
78	*Lactobacillus acidophilus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
79	*Lactobacillus reuteri*	1.00×10^6 cfu/mL	No Cross Reactivity Observed

Tested Cross-Reactants			
No.	Cross Reactant	Concentration	Result
80	*Lactococcus lactis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
81	*Leminorela grimontii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
82	*Listeria grayi*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
83	*Listeria innocua*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
84	*Listeria monocytogenes*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
85	Norovirus Group I (recombinant)	8.13×10^4 $TCID_{50}$/mL	No Cross Reactivity Observed
86	*Peptoniphilus asaccharolyticus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
87	*Peptostreptococcus anaerobius*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
88	*Plesiomonas shigelloides*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
89	*Porphyromaonas asaccharolytica*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
90	*Prevotella melaninogenica*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
91	*Proteus mirabilis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
92	*Proteus penneri*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
93	*Providencia alcalifaciens*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
94	*Providencia rettgeri*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
95	*Providencia stuartii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
96	*Pseudomonas aeruginosa*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
97	*Pseudomonas putida*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
98	Rotavirus, Strain Wa	1.00×10^5 $TCID_{50}$/mL	No Cross Reactivity Observed
99	*Salmonella enterica* subsp. *Arizonae* (formerly *Choleraesuis arizonae)*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
100	*Salmonella enterica* subsp. *Choleraesuis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
101	*Salmonella enterica* subsp. *Enterica* serovar *Typhimurium*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
102	*Serratia liquefaciens*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
103	*Serratia marcescens*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
104	*Shigella boydii*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
105	*Shigella dysenteriae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
106	*Shigella sonnei*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
107	*Staphylococcus aureus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
108	*Staphylococcus epidermidis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
109	*Stenotrophomonas maltophilia*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
110	*Streptococcus agalactiae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
111	*Streptococcus dysgalactiae*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
112	*Streptococcus intermedius*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
113	*Streptococcus uberis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
114	*Trabulsiella guamensis*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
115	*Veillonella parvula*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
116	*Vibrio cholerae*	4.10×10^{-3} pg/mL	No Cross Reactivity Observed

Tested Cross-Reactants			
No.	Cross Reactant	Concentration	Result
117	*Vibrio parahaemolyticus*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
118	*Yersinia bercovieri*	1.00×10^6 cfu/mL	No Cross Reactivity Observed
119	*Yersinia rohdei*	1.00×10^6 cfu/mL	No Cross Reactivity Observed

Note:

1. Total 10 runs were performed to test 119 cross reactants in triplicate. Additionally, each run included five replicates of baseline (un-spiked) sample.
2. Each replicate of all 119 cross-reactants and baseline samples were "Not Detected".

INTERFERENCE

The performance of this assay was evaluated with potentially interfering substances that may be present in stool specimens at the concentrations indicated in Table 6 below. A total of 21 potentially interfering substances were spiked into a low positive *C. difficile* stool-TE buffer matrix and tested. No interference was observed.

Table 6. Summary of Interfering Substances and Testing Results for two *C. difficile* strains

Interferents	Active Ingredient	Interferent Concentration	Detected/Total	
			C. difficile Strain - ATCC43255	*C. difficile* Strain - NAP 1A
1% Hydrocortisone Cream	Hydrocortisone	2% (w/v)	3/3	3/3
Aleve	Naproxen	14 mg/ml	3/3	3/3
Antacid and Anti-gas generic	Aluminum Hydroxide, Magnesium Hydroxide	0.1 mg/ml	3/3	3/3
Antacid Generic	Calcium Carbonate	0.1 mg/ml	3/3	3/3
Barium sulfate	Barium sulfate	5 mg/ml	3/3	3/3
Fleet	Mineral Oil	2% (v/v)	3/3	3/3
Imodium AD	Loperamide	0.005 mg/ml	3/3	3/3
KY Jelly	Glycerin	2%(w/v)	3/3	3/3
Laxative generic	Sennosides	0.1 mg/ml	5/5*	3/3
Metronidazole	Metronidazole	14 mg/ml	3/3	3/3
Milk of Magnesia	Magnesium Hydroxide	0.2 mg/ml	3/3	3/3
Moist towelettes generic	Benzalkonium Chloride	10%(v/v)	3/3	3/3
Mucin	Mucin	3 mg/ml	3/3	3/3
Nystatin	Nystatin	10000 USP units/ml	3/3	3/3
Palmitic acid	Palmitic acid	2 mg/ml	3/3	3/3
Pepto-Bismol	Bismuth Subsalicylate	0.175 mg/ml	3/3	3/3
Preparation H	Phenylephrine	2% (w/v)	3/3	3/3

Interferents	Active Ingredient	Interferent Concentration	Detected/Total	
			C. difficile Strain - ATCC43255	*C. difficile* Strain - NAP 1A
Stearic acid	Stearic Acid	4 mg/ml	3/3	3/3
Trojan with nonoxynol-9	Nonoxynol-9	1.4 mg/ml	3/3	3/3
Vancomycin	Vancomycin	1.4 mg/ml	5/5*	3/3
Whole blood	Whole blood	3%	3/3	7/8**

*One replicate reported as "Invalid" due to IC failure in initial run of three replicates. All three replicates reported as "Detected" in repeat run.

**One replicate reported as "Not Detected" in initial run of three replicates. However all five replicates reported as "Detected" in repeat run.

CLINICAL STUDIES

Three external testing sites, located on the East Coast of the US, participated in the Clinical Agreement Study. Site 1 prospectively collected fresh specimens and tested them with the Simplexa™ *C. difficile* Universal Direct Kit. A frozen aliquot was sent to Site 2 for toxigenic culture. Site 2 also prospectively collected fresh specimens and tested them with the Simplexa™ *C. difficile* Universal Direct Kit. A frozen aliquot was later set up for toxigenic culture. Site 3 performed Simplexa™ *C. difficile* testing on clinical specimens prospectively collected from the West Coast of the US and Upper Mid-West of the US. A frozen aliquot of each of these specimens was sent to Site 2 for toxigenic culture. Site 2 conducted all direct and enriched toxigenic culture testing for all specimens.

For clinical specimens tested at Site 1, results were also generated using an FDA cleared molecular assay. Similarly, for clinical specimens tested at Site 2, results were generated using an alternative FDA cleared molecular assay.

A total of 970 prospectively collected stool specimens were obtained from patients with signs and symptoms of *C. difficile* infection. Demographic information, including age, gender and the geographic collection location were obtained.

Clinical Agreement summary results are presented in Table 7 and Table 8 below.

Table 7. Simplexa™ *C. difficile* Universal Direct Kit versus Direct Toxigenic Culture Method

	Reference Method: (Direct Culture + Toxin Assay)		
Simplexa™ C. *difficile* Universal Direct Kit	Detected	Not Detected	Total
Detected	118	59	177
Not Detected	13	779	792
Total	131	838	969
Sensitivity	90.1%(118/131) 95% CI:83.8-94.1%		
Specificity	93.0%(779/838) 95% CI:91.0-94.5%		

Table 8. Simplexa™ *C. difficile* Universal Direct Kit versus Enriched Toxigenic Culture Method

	Reference Method: (Enriched Culture + Toxin Assay)		
Simplexa™ *C. difficile* Universal Direct Kit	Detected	Not Detected	Total
Detected	144	33	177
Not Detected	37	755	792
Total	181	788	969
Sensitivity	79.6%(144/181) 95% CI:73.1-84.8%		
Specificity	95.8%(755/788) 95% CI:94.2-97.0%		

Note: One sample was inadvertently missed from being cultured.

In addition to the Simplexa™ *C. difficile* Universal Direct assay the specimens were tested using two different FDA-cleared assays; 402 samples were assayed using one FDA cleared molecular assay, and 305 samples were assayed using another FDA cleared molecular assay. The testing was performed at two different clinical sites. These two FDA cleared molecular assays were compared to direct and enriched toxigenic cultures.

In comparison to direct toxigenic culture, the sensitivity and specificity of the Simplexa™ *C. difficile* Universal Direct Assay were 90.1% (95% CI:83.8-94.1%) and 93% (95% CI:91-94.5%), respectively, as shown above. The sensitivities and specificities of the two FDA cleared molecular tests were 86.1% (95% CI:76.3-92.3%) and 94.8% (95% CI:91.9-96.8%) for the first molecular assay and 81.8% (95% CI:65.6-91.4%) and 93% (95% CI:89.3-95.5%), for the second assay.

In comparison to enriched toxigenic culture, the sensitivity and specificity of the Simplexa™ *C. difficile* Universal Direct Assay were 79.6% (95% CI:73.1-84.8%) and 95.8% (95% CI:94.2-97%), respectively, as shown above. The sensitivities and specificities of the two FDA cleared molecular tests were 78.7% (95% CI:69-85.9%) and 97.1% (95% CI:94.6-98.5%) for the first molecular assay and 69.6% (95% CI:56.7-80.1%) and 97.2% (95% CI:94.3-98.6%), for the second assay.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Food and Drug Administration

10903 New Hampshire Avenue
Silver Spring, MD 20993

FOCUS Diagnostics, Inc.
c/o Ms. Sharon Young
Sr. Regulatory Affairs Specialist
11331 Valley View Street
Cypress, California 90630

APR – 4 2012

Re: K113433
Trade/Device Name: Simplexa™ *C. difficile* Universal Direct
Regulation Number: 21 CFR 866.2660
Regulation Name: Microorganism differentiation and identification device
Regulatory Class: Class I
Product Code: OMN
Dated: April 3, 2012
Received: April 4, 2012

Dear Ms. Young:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into class II (Special Controls), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); and good manufacturing practice

requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820). This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Parts 801 and 809), please contact the Office of *In Vitro* Diagnostic Device Evaluation and Safety at (301) 796-5450. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Sally A. Hojvat, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (if known): K113433

Device Name: Simplexa™ *C. difficile* Universal Direct

Indications for Use:

The Focus Diagnostics Simplexa™ *C. difficile* Universal Direct is a real-time polymerase chain reaction (PCR) assay and is intended for use on the 3M Integrated Cycler instrument for the detection of toxigenic *Clostridium difficile* toxin B gene (*tcdB*) in liquid or unformed stool samples from individuals suspected of *C. difficile* infection. This test aids in the diagnosis of *Clostridium difficile* associated disease (CDAD).

Prescription Use ___X___ (Part 21 CFR 801 Subpart D)	AND/OR	Over-The-Counter Use _____ (21 CFR 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE-CONTINUE ON ANOTHER PAGE OF NEEDED)

Concurrence of CDRH, Office of InVitro Diagnostics (OIVD)

Raquel Beat for F. Poole

Division Sign-Off

Office of In Vitro Diagnostic
Device Evaluation and Safety

510(k) 113433