DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
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February 14, 2017

FOCUS DIAGNOSTICS, INC.: DBA DIASORIN MOLECULAR LLC
IRENE M. GUZMAN
SENIOR REGULATORY AFFAIRS SPECIALIST
11331 VALLEY VIEW STREET
CYPRESS CA 90630

Re: K163085
Trade/Device Name: Simplexa *C. difficile* Direct;
Simplexa *C. difficile* Positive Control Pack
Regulation Number: 21 CFR 866.3130
Regulation Name: *Clostridium difficile* toxin gene amplification assay
Regulatory Class: II
Product Code: OZN
Dated: January 12, 2017
Received: January 17, 2017

Dear Ms. Guzman:

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. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. 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); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. 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 Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Ribhi Shawar -A

For Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K163085

Device Name
Simplexa C. difficile Direct
Simplexa C. difficile Positive Control Pack

Indications for Use *(Describe)*

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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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Applicant	Focus Diagnostics, Inc. DBA DiaSorin Molecular LLC 11331 Valley View Street Cypress, California 90630 USA
Establishment Registration No.	2023365
Contact Person	Irene M. Guzman tel 562.240.6133 fax 562.240.6529 irene.guzman@diasorin.com
Summary Date	February 10, 2017
Proprietary Name	Simplexa™ *C. difficile* Direct and Simplexa™ *C. difficile* Positive Control Pack
Generic Name	*C. difficile* RT PCR
Classification	21 CFR 866.3130 *Clostridium difficile* toxin gene amplification assay Class II Special Controls
Predicate Device	BD MAX *CDIFF* ASSAY (K130470)

Intended Use

Simplexa™ *C. difficile* Direct (MOL2950)

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 (MOL2960)

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.

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.

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

Predicate Device Information

Item	Device	Predicate
Name	Simplexa™ *C. difficile* Direct	BD MAX™ *Cdiff* (K130470)
Intended Use	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 BD MAX™ Cdiff Assay performed on the BD MAX™ System is an automated in vitro diagnostic test for the direct, qualitative detection of the Clostridium difficile toxin B gene (tcdB) in human liquid or soft stool specimens from patients suspected of having C. difficile infection (CDI). The test, performed directly on the specimen, utilizes real-time polymerase chain reaction (PCR) for the amplification of C. difficile toxin B gene DNA and fluorogenic target-specific hybridization probes for the detection of the amplified DNA. The BD MAX™ Cdiff Assay is intended to aid in the diagnosis of CDI.
Assay Targets	*C. difficile* toxin B gene (*tcdB*)	*C. difficile* toxin B gene (*tcdB*)
Sample Types	Liquid or unformed stool	Liquid or soft stool specimen
Extraction Methods	Automated	Automated
Assay Methodology	The Simplexa *C. difficile* Direct assay incorporates direct, qualitative detection of toxigenic *C. difficile* DNA from clinical specimens in human stool specimens using the Integrated Cycler. The assay utilizes real-time PCR technology with fluorescently labeled, bifunctional primer-probes that amplify and detect a conserved region of the toxin B (*tcdB*) gene.	The BD MAX™ Cdiff Assay performed on the BD MAX™ System is an automated in vitro diagnostic test for the direct, qualitative detection of the *Clostridium difficile* toxin B gene (*tcdB*) in human liquid or soft stool specimens from patients suspected of having C. difficile infection (CDI). The test, performed directly on the specimen, utilizes real-time polymerase chain reaction (PCR) for the amplification of *C. difficile* toxin B gene DNA and fluorogenic target-specific hybridization probes for the detection of the amplified DNA. The BD MAX™ Cdiff Assay is intended to aid in the diagnosis of CDI.
Detection Techniques	Real time PCR with bifunctional fluorescent primer-probes using the Integrated Cycler.	The test, performed directly on the specimen, utilizes real-time polymerase chain reaction (PCR) for the amplification of C. difficile toxin B gene DNA and fluorogenic target- specific hybridization probes for the detection of the amplified DNA.
Limit of Detection	C. difficile Bacterial Strain — LoD Concentration (CFU/mL) ATCC 43255 — 0.95 NAP1A — 0.43	*C. difficile* Strain — Toxinotype — LoD in CFU per-loop ATCC® 43255 — 0 — 265 (95% CI: 140, 502) ATCC® 9689 — 0 — 156 (95% CI: 82. 298) ATCC® BAA-i1805 — III — 205 (95% CI: 102, 412) ATCC® 43598 — VIII — 125 (95% CI: 66, 235)

Item	Device	Predicate
Name	Simplexa™ *C. difficile* Direct	BD MAX™ *Cdiff* (K130470)
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) ATCC 43255 – LP* 54.4% (49/90) Positive 95% CI: 44.2 to 64.3% ATCC 43255 – LP** 100.0% (90/90) Positive 95% CI: 95.9 to 100% ATCC 43255 – MP 100.0% (90/90) Positive 95% CI: 95.9 to 100.0% Negative 100% 100.0% (90/90) Negative 95% CI: 95.9 to 100.0% Positive Control 100.0% (90/90) Positive 95% CI: 95.9 to 100.0% NAP1A – LP 98.9% (89/90) Positive 95% CI: 94.0 to 99.8% NAP1A – MP 100.0% (90/90) Positive 95% CI: 95.9 to 100.0% *Lower percent detection of C. difficile is related to qualified underperformance of matrix during original build as determined in LoD study. **Reformulated with a different matrix	For Site-to-Site Reproducibility, the overall percent agreement was 100% for MP, LP and Neg categories, with 92.2% and 50.0% negative agreement for HN1:100 and HN1:10 categories, respectively. For Lot-to- Lot Reproducibility, the overall percent agreement was 100% for MP, LP and Neg categories, with 96.7% and 64.4% negative agreement for HN1:100 and HN1:10 categories, respectively. Second Derivative Peak Abscissa (SDPA), an internal criteria used to determine a final assay result, was selected as an additional means of assessing assay reproducibility.

METHOD COMPARISON

Two thousand three hundred and fifty one (2351) samples were prospectively collected from five (5) geographically diverse sites between December 3, 2015 and June 10, 2016 from patients with signs and symptoms of *C. difficile* infection. Of the 2351 specimens, 2330 were evaluable on Simplexa™ *C. difficile* Direct & the Direct Culture method and 2336 were evaluable on Simplexa™ *C. difficile* Direct & the Combination of Direct/Enriched Culture methods. Of the combination culture evaluable specimens, 1232 (52.7%) were collected from females and 1104 (47.3%) were collected from males. The patient ages ranged from less than one year old to 106 years old, and 2198 (94.1%) were 22 years old or older.

Samples were tested on Simplexa™ *C. difficile* Direct at the collection sites and the comparator culture method was performed at one (1) central laboratory. 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). Discrepant analysis was performed using FDA cleared nucleic acid amplification test (NAAT) results provided by the sites.

Positive Controls and No Template Controls were tested daily at each site during the clinical studies. 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 re-testing, 100% of the unexpected QC results were resolved by repeat.

Simplexa™ *C. difficile* Direct versus Direct Toxigenic Culture Method

	Direct Culture + Toxin Assay		
Simplexa Results	**Detected**	**Not Detected**	**Total**
Detected	265	163[a]	428
Not Detected	12[b]	1890	1902
Total	277	2053	2330[c]
Positive Agreement	95.7% (265/277) 95% CI: 92.6% to 97.5%	Negative Agreement	92.1% (1890/2053) 95% CI: 90.8% to 93.2%

[a] 116/163 discrepant samples were positive, 46/163 were negative, 1/163 was indeterminate for *C. difficile* Toxin B gene DNA when tested using a FDA cleared molecular test.
[b] 8/12 discrepant samples were negative and 4/12 were positive for *C. difficile* Toxin B gene DNA when tested using a 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 the 6 samples and are tabulated in the Combined Culture.

Simplexa™ *C. difficile* Direct versus Combined Direct and Enriched Toxigenic Culture Methods

	Combined Culture (Direct & Enriched) + Toxin Assay		
Simplexa	**Detected**	**Not Detected**	**Total**
Detected	336	96[a]	432
Not Detected	55[b]	1849	1904
Total	391	1945	2336
Sensitivity	85.9%(336/391) 95% CI: 82.1% to 89.0%	Specificity	95.1%(1849/1945) 95% CI: 94.0% to 95.9%
PPV	77.8%(336/432) 95% CI: 73.6% to 81.4%	NPV	97.1%(1849/1904) 95% CI: 96.3% to 97.8%

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

In addition, samples from the multi-site investigational study were also tested with three (3) commercially available FDA cleared molecular NAAT assays, and the performance of the Simplexa™ C. difficile Direct assay was compared to the cleared assays. A total of 2326 specimens were tested across 5 external sites. The Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) was calculated for the Simplexa™ C. difficile Direct versus each of the NAAT comparators.

Simplexa™ *C. difficile* Direct versus Nucleic Acid Amplification Test 1 (NAAT-1)

	NAAT1		
Simplexa™	**Detected**	**Not Detected**	**Total**
Detected	114	24	138
Not Detected	8	683	691
Total	122	707	829
Positive Agreement	93.4% (114/122) 95% CI: 87.6% to 96.6%	Negative Agreement	96.6% (683/707) 95% CI: 95.0% to 97.7%

Simplexa™ *C. difficile* Direct versus Nucleic Acid Amplification Test 2 (NAAT-2)

	NAAT-2		
Simplexa™	**Detected**	**Not Detected**	**Total**
Detected	138	38	176
Not Detected	9	591	600
Total	147	629	776
Positive Agreement	93.9% (138/147) 95% CI: 88.8% to 96.7%	Negative Agreement	94.0% (591/629) 95% CI: 91.8% to 95.6%

Simplexa™ *C. difficile* Direct versus Nucleic Acid Amplification Test 3 (NAAT-3)

	NAAT-3		
Simplexa™	**Detected**	**Not Detected**	**Total**
Detected	112	5	117
Not Detected	20	584	604
Total	132	589	721
Positive Agreement	84.8% (112/132) 95% CI: 77.8% to 90.0%	Negative Agreement	99.2% (584/589) 95% CI: 98.0% to 99.6%

REPRODUCIBILITY

Reproducibility for Simplexa™ *C. difficile* Direct was performed at three sites on at least five non-consecutive days per site. A total of seven sample panel members were tested to evaluate reproducibility of the assay.

The panel included a positive control (PC), a negative control (TE-stool matrix without analyte) and four contrived samples spiked into a negative stool - TE matrix. The four contrived samples consisted of a low positive (LP) and a medium positive (MP) for *C. difficile*. Each contrived sample was prepared by spiking a specific concentration of the strain(s) into negative stool - TE matrix. Combined results for all sites are presented in the table below. The invalid rate for the reproducibility study using Simplexa™ *C. difficile* Direct was 0.24% (2/819 samples). After repeat testing, 0/2 samples remained invalid, thus resulting in a final invalid rate of 0.0% (0/819 samples).

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	100.0% (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% to 100.0%
ATCC 43255 – MP	100.0% (30/30)	37.3	1.7	100.0% (30/30)	37.1	1.3	100.0% (30/30)	37.0	1.5	100.0% (90/90)	95.9% to 100.0%
Negative[1]	100.0% (30/30)	N/A	N/A	100.0% (3030)	N/A	N/A	100.0% (3030)	N/A	N/A	100.0% (90/90)	95.9% to 100.0%
Positive Control	100.0% (30/30)	31.7	1.7	100.0% (30/30)	31.4	1.6	100.0% (30/30)	30.9	1.4	100.0% (90/90)	95.9% to 100.0%
NAP1A - LP	100.0% (30/30)	39.6	2.6	100.0% (30/30)	39.1	2.0	96.7% (29/30)	39.4	1.9	98.9% (89/90)	94.0% to 99.8%
NAP1A - MP	100.0% (30/30)	36.4	1.3	100.0% (30/30)	36.3	1.0	100.0% (30/30)	36.6	1.4	100.0% (90/90)	95.9% to 100.0%

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

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The Limit of Detection (LoD) for Simplexa™ *C. difficile* Direct was determined using a panel consisting of negative stool-TE matrix spiked with 2 strains of *C. difficile* bacterial stock. The stock was serially diluted. A total of 32 replicates were tested. The lowest concentration level of *C. difficile* where at least 95% of the replicates were detected as "*C. difficile*" was determined to be the LoD of the strain.

The following table shows Limit of Detection (LoD) value in terms of CFU/mL for two *C. difficile* bacterial strains.

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

ANALYTICAL REACTIVITY

Simplexa™ *C. difficile* Direct was evaluated for analytical reactivity to 32 *C. difficile* strains not tested in the Limit of Detection (LoD) study.

Analytical reactivity samples were prepared by spiking one of the 32 regrown and re-titered strains into negative stool-TE matrix at 2-4 X LoD. Each sample was tested in triplicate. The strain was initially spiked at a 2-4 X LoD. If at least one of three replicates was negative for *C. difficile*, a higher concentration was tested until all three replicates were detected.

All 32 strains were detected positive for *C. difficile* at or below 512 CFU/mL

Strain	**Concentration (CFU/mL)**	**Toxinotype**	**Toxin**	**Ribotype**
Clostridium difficile ATCC 17857	16	0	A+B+	001
Clostridium difficile ATCC 17858	8	0	A+B+	054
Clostridium difficile ATCC 43594	16	0	A+B+	005
Clostridium difficile ATCC 43596	4	0	A+B+	012
Clostridium difficile ATCC 43598	4	VIII	A-B+	017
Clostridium difficile ATCC 43599	32	0	A+B+	001
Clostridium difficile ATCC 43600	4	0	A+B+	014
Clostridium difficile ATCC 51695	4	0	A+B+	001
Clostridium difficile ATCC 700792	16	0	A+B+	005
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-1814	256	XXII	A+B+	251
Clostridium difficile BAA-1870	4	IIIb	A+B+	027
Clostridium difficile BAA-1871	4	0	A+B+	001
Clostridium difficile BAA-1872	32	0	A+B+	207

Strain	Concentration (CFU/mL)	Toxinotype	Toxin	Ribotype
Clostridium difficile BAA-1873	4	0	A+B+	053
Clostridium difficile BAA-1874	512	0	A+B+	002
Clostridium difficile BAA-1875	16	V	A+B+	078
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

Cross Reactivity (Analytical Specificity)

The cross-reactivity study for Simplexa™ *C. difficile* Direct was evaluated using 127 organisms for potential cross reactivity.

The samples were prepared at one internal site. Cross-reactivity samples were prepared by spiking each of the 127 organisms at clinically relevant concentrations into negative stool-TE matrix. Clinically relevant concentration is defined as ≥10^6 CFU/mL for bacteria and fungus and ≥10^5 $TCID_{50}$/mL (PFU/mL) for viruses. If $TCID_{50}$/mL (PFU/mL) or CFU/mL were not available, clinically relevant concentrations were prepared using industry accepted units. After preparation, all the samples were stored at -70 °C or below until testing. Each cross-reactivity sample was tested at least in triplicate. If at least one of the three signals was detected, an additional five replicates of the same cross reactant was tested. Baseline samples were tested in five replicates per instrument with four baseline references based on batch testing of organisms.

Organism	Tested Concentration	*C. difficile* Qualitative Results: % Detection (# Detected/#Tested)
Abiotrophia defectiva	1.00 X 10^6 CFU/mL	0.0% (0/3)
Acinetobacter baumannii	1.00 X 10^6 CFU/mL	0.0% (0/3)
Acinetobacter lwoffi	1.00 X 10^6 CFU/mL	0.0% (0/3)
Adenovirus 1	1.00 X 10^5 $TCID_{50}$/mL	0.0% (0/3)
Aeromonas hydrophila	1.00 X 10^6 CFU/mL	0.0% (0/3)
Alcaligenes faecalis subsp. *Faecalis*	1.00 X 10^6 CFU/mL	0.0% (0/3)
Anaerococcus tetradius	1.00 X 10^6 CFU/mL	0.0% (0/3)
Bacillus cereus ATCC 11778	1.00 X 10^6 CFU/mL	0.0% (0/3)
Bacillus cereus ATCC 13472	1.00 X 10^6 CFU/mL	0.0% (0/3)
Bacteroides caccae	1.00 X 10^6 CFU/mL	0.0% (0/3)

Organism	Tested Concentration	*C. difficile* Qualitative Results: % Detection (# Detected/#Tested)
Bacteroides fragilis	1.00 X 10^6 CFU/mL	0.0% (0/3)
Bacteroides merdae	1.00 X 10^6 CFU/mL	0.0% (0/3)
Bacteroides stercoris	1.00 X 10^6 CFU/mL	0.0% (0/3)
Bifidobacterium adolescentis	1.00 X 10^6 CFU/mL	0.0% (0/3)
Bifidobacterium longum	1.00 X 10^6 CFU/mL	0.0% (0/3)
Campylobacter coli	1.00 X 10^6 CFU/mL	0.0% (0/3)
Campylobacter jejuni[1]	1.00 X 10^6 CFU/mL	0.0% (0/3)
Candida albicans	1.00 X 10^6 CFU/mL	0.0% (0/3)
Candida catenulate	1.00 X 10^6 CFU/mL	0.0% (0/3)
Cedecea davisae	1.00 X 10^6 CFU/mL	0.0% (0/3)
Chlamydia trachomatis	1.00 X 10^6 IFU/mL	0.0%(0/3)
Citrobacter amalonaticus	1.00 X 10^6 CFU/mL	0.0% (0/3)
Citrobacter freundii	1.00 X 10^6 CFU/mL	0.0% (0/3)
Citrobacter koseri	1.00 X 10^6 CFU/mL	0.0% (0/3)
Citrobacter sedlakii	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium beijerinckii	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium bifermentans	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium bolteae	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium butyricum	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium chauvoei	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium difficile ATCC 43593	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium difficile ATCC 43601	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium fallax	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium haemolyticum	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium histolyticum	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium innocuum	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium methylpentosum	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium nexile	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium novyi	1.00 X 10^6 CFU/mL	12.5% (1/8)
Clostridium paraputrificum	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium perfringens	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium ramosum	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium scindens	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium sepiticum	1.00 X 10^6 cells/mL	0.0% (0/3)
Clostridium sordellii	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium sphenoides	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium spiroforme	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium sporogenes	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium symbiosum	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium tertium	1.00 X 10^6 CFU/mL	0.0% (0/3)
Clostridium tetani	1.00 X 10^6 CFU/mL	0.0% (0/3)
Collinsella aerofaciens	1.00 X 10^6 CFU/mL	0.0% (0/3)

Organism	Tested Concentration	*C. difficile* Qualitative Results: % Detection (# Detected/#Tested)
Corynebacterium genitalium	1.00×10^6 CFU/mL	0.0% (0/3)
Coxsackievirus A10	1.00×10^5 $TCID_{50}$/mL	0.0% (0/3)
Cytomegalovirus AD-169	1.00×10^5 $TCID_{50}$/mL	0.0% (0/3)
Echovirus 11	1.00×10^5 $TCID_{50}$/mL	0.0% (0/3)
Edwardsiella tarda	1.00×10^6 CFU/mL	0.0% (0/3)
Eggerthella lenta	1.00×10^6 CFU/mL	0.0% (0/3)
Enterobacter aerogenes	1.00×10^6 CFU/mL	0.0% (0/3)
Enterobacter cloacae	1.00×10^6 CFU/mL	0.0% (0/3)
Enterococcus casseliflavus	1.00×10^6 CFU/mL	0.0% (0/3)
Enterococcus cecorum	1.00×10^6 CFU/mL	0.0% (0/3)
Enterococcus dispar	1.00×10^6 CFU/mL	0.0% (0/3)
Enterococcus faecalis vanB	1.00×10^6 CFU/mL	0.0% (0/3)
Enterococcus faecium vanA	1.00×10^6 CFU/mL	0.0% (0/3)
Enterococcus gallinarum vanC	1.00×10^6 CFU/mL	0.0% (0/3)
Enterococcus hirae	1.00×10^6 CFU/mL	0.0% (0/3)
Enterococcus raffinosus	1.00×10^6 CFU/mL	0.0% (0/3)
Enterovirus 71	1.00×10^6 $TCID_{50}$/mL	0.0% (0/3)
Escherichia coli ATCC 11775	1.00×10^6 CFU/mL	0.0% (0/3)
Escherichia coli ATCC 23511	1.00×10^6 CFU/mL	0.0% (0/3)
Escherichia coli ATCC 25922	1.00×10^6 CFU/mL	0.0% (0/3)
Escherichia coli O157:H7 (ATCC 700927)	1.00×10^6 CFU/mL	0.0% (0/3)
Escherichia fergusonii	1.00×10^6 CFU/mL	0.0% (0/3)
Escherichia hermannii	1.00×10^6 CFU/mL	0.0% (0/3)
Flavonifractor plautii (deposited as *Clostridium orbiscindens*)	1.00×10^6 CFU/mL	0.0% (0/3)
Fusobacterium varium	1.00×10^6 CFU/mL	0.0% (0/3)
Gardnerella vaginalis	1.00×10^6 CFU/mL	0.0% (0/3)
Gemella morbillorum	1.00×10^6 CFU/mL	0.0% (0/3)
Hafnia alvei	1.00×10^6 CFU/mL	0.0% (0/3)
Helicobacter fennelliae	1.00×10^6 CFU/mL	0.0% (0/3)
Helicobacter pylori	1.00×10^6 CFU/mL	0.0% (0/3)
Klebsiella oxytoca	1.00×10^6 CFU/mL	0.0% (0/3)
Klebsiella pneumoniae subsp. *Pneumoniae*	1.00×10^6 CFU/mL	0.0% (0/3)
Lactobacillus acidophilus	1.00×10^6 CFU/mL	0.0% (0/3)
Lactobacillus reuteri	1.00×10^6 CFU/mL	0.0% (0/3)
Lactococcus lactis	1.00×10^6 CFU/mL	0.0% (0/3)
Leminorella grimontii	1.00×10^6 CFU/mL	0.0% (0/3)
Listeria grayi	1.00×10^6 CFU/mL	0.0% (0/3)
Listeria innocua	1.00×10^6 CFU/mL	0.0% (0/3)
Listeria monocytogenes	1.00×10^6 CFU/mL	0.0% (0/3)
Norovirus G2	1.00×10^5 copies/mL	0.0% (0/3)
Peptoniphilus asaccharolyticus	1.00×10^6 CFU/mL	0.0% (0/3)
Peptostreptococcus anaerobius	1.00×10^6 CFU/mL	0.0% (0/3)

Organism	Tested Concentration	*C. difficile* Qualitative Results: % Detection (# Detected/#Tested)
Plesiomonas shigelloides	1.00×10^6 CFU/mL	0.0% (0/3)
Porphyromonas asaccharolytica	1.00×10^6 CFU/mL	0.0% (0/3)
Prevotella melaninogenica	1.00×10^6 CFU/mL	0.0% (0/3)
Proteus mirabilis	1.00×10^6 CFU/mL	0.0% (0/3)
Proteus penneri	1.00×10^6 CFU/mL	0.0% (0/3)
Providencia alcalifaciens	1.00×10^6 CFU/mL	0.0% (0/3)
Providencia rettgeri	1.00×10^6 CFU/mL	0.0% (0/3)
Providencia stuartii	1.00×10^6 CFU/mL	0.0% (0/3)
Pseudomonas aeruginosa	1.00×10^6 CFU/mL	0.0% (0/3)
Pseudomonas putida	1.00×10^6 CFU/mL	0.0% (0/3)
Rotavirus, strain Wa	1.00×10^5 $TCID_{50}$/mL	0.0% (0/3)
Salmonella enterica subsp. *Arizonae*	1.00×10^6 CFU/mL	0.0% (0/3)
Salmonella enterica subsp. *Enterica* serovar *Choleraesuis*	1.00×10^6 CFU/mL	0.0% (0/3)
Salmonella enterica subsp. *Enterica* serovar *Typhimimurium*	1.00×10^6 CFU/mL	0.0% (0/3)
Serratia liquefaciens	1.00×10^6 CFU/mL	0.0% (0/3)
Serratia marcescens subsp. *marcescens*	1.00×10^6 CFU/mL	0.0% (0/3)
Shigella boydii	1.00×10^6 CFU/mL	0.0% (0/3)
Shigella dysenteriae	1.00×10^6 CFU/mL	0.0% (0/3)
Shigella sonnei	1.00×10^6 CFU/mL	0.0% (0/3)
Staphylococcus aureus	1.00×10^6 CFU/mL	0.0% (0/3)
Staphylococcus epidermidis	1.00×10^6 CFU/mL	0.0% (0/3)
Stenotrophomonas maltophilia	1.00×10^6 CFU/mL	0.0% (0/3)
Streptococcus agalactiae	1.00×10^6 CFU/mL	0.0% (0/3)
Streptococcus dysgalactiae	1.00×10^6 CFU/mL	0.0% (0/3)
Streptococcus intermedius	1.00×10^6 CFU/mL	0.0% (0/3)
Streptococcus uberis	1.00×10^6 CFU/mL	0.0% (0/3)
Trabulsiella guamensis	1.00×10^6 CFU/mL	0.0% (0/3)
Veillonella parvula	1.00×10^6 CFU/mL	0.0% (0/3)
Vibrio cholerae	1.00×10^6 CFU/mL	0.0% (0/3)
Vibrio parahaemolyticus	1.00×10^6 CFU/mL	0.0% (0/3)
White Blood Cells (Human)	1.00×10^6 WBC/mL	0.0% (0/3)
Yersinia bercovieri	1.00×10^6 CFU/mL	0.0% (0/3)
Yersinia rohdei	1.00×10^6 CFU/mL	0.0% (0/3)

[1] *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* and it did not show cross reactivity with the Simplexa™ *C. difficile* Direct kit based on *in silico* analysis.

Note: *Clostridium botulinum, Desulfovibrio piger, and Ruminococcus bromii* were not available for testing. *In silico* NCBI BLAST analysis was performed for all three organisms. *Clostridium botulinum, Desulfovibrio piger,* and *Ruminococcus bromii* do not show cross reactivity with the Simplexa™ *C. difficile* Direct kit based on *in silico* analysis.

INTERFERENCE
A total of 33 substances (endogenous & exogenous) were tested for potential interference with Simplexa™ *C. difficile* Direct using two bacterial strains of *C. difficile* – ATCC 43255 & NAP1A.

The following table summarizes the qualitative results for *C. difficile* for all 33 potentially interfering substances.

Interfering Substance	Active Ingredient	Interfering Substance Concentration	*C. difficile* Qualitative Detection: %Detection (#Detected/#Tested)	
			ATCC 43255	NAP1A
Afrin	Oxymetazoline hydrochloride 0.05%	10% (w/v)	100.0%(3/3)	100.0%(3/3)
Antacid and Anti-gas generic	Aluminum hydroxide/Magnesium hydroxide	0.1 mg/mL	100.0%(3/3)	100.0%(3/3)
Anusol Plus (Pramoxine HCL)	Pramoxine hydrochloride 1% and zinc sulfate monohydrate 0.5%	10% (w/v)	100.0%(3/3)	100.0%(3/3)
Barium Sulfate	Barium sulfate (98% for suspension)	5 mg/mL	100.0% (3/3)	100.0% (3/3)
Benzalkonium (Towlettes)	Benzalkonium chloride, Ethanol	10% (v/v)	100.0% (3/3)	100.0% (3/3)
Blood	Glucose, Hormones, Enzymes, Ions, Iron, etc.	5% (v/v)	100.0% (3/3)	100.0% (3/3)
Colace[1]	Ducosate sodium USP 100 mg	2.5% (w/v)	62.5% (5/8)	87.5% (7/8)
Dramamine	Dimenhydrinate	10% (w/v)	100.0% (3/3)	100.0% (3/3)
Dulcolax[2]	Bisacodyl USP 5 mg	10% (w/v) for ATCC 43255 & 0.3125% (w/v) for NAP1A	100.0% (3/3)	100.0% (3/3)
Gynol II, Nonoxynol 9	Nonoxynol 9 (4.0%)	10% (w/v)	100.0% (3/3)	100.0% (3/3)
Hydrocortisone cream	Hydrocortisone	2% (w/v)	100.0% (3/3)	100.0% (3/3)
Imodium	Loperamide hydrochloride	0.005 mg/mL	100.0% (3/3)	100.0% (3/3)
KY Jelly	Glycerin	2% (w/v)	100.0% (3/3)	100.0% (3/3)
Mesalazine	Mesalazine rectal suspension (4 g/60 mL)	10% (w/v)	100.0% (3/3)	100.0% (3/3)
Metronidazole	Metronidazole	14 mg/mL	100.0% (3/3)	100.0% (3/3)
Milk of Magnesia	Magnesium hydroxide	0.2 mg/mL	100.0% (3/3)	62.5% (5/8)
Mineral Oil	Mineral oil	2% (w/v)	100.0% (3/3)	100.0% (3/3)

Interfering Substance	Active Ingredient	Interfering Substance Concentration	*C. difficile* Qualitative Detection: %Detection (#Detected/#Tested)	
			ATCC 43255	NAP1A
Monistat 7	Miconazole nitrate 2% (100 mg)	10% (w/v)	100.0% (3/3)	100.0% (3/3)
Mucin	Immunoglobulins, Lysozyme, Polymers, etc.	3 mg/mL	100.0% (3/3)	100.0% (3/3)
Naproxen[3]	Naproxen sodium	7 mg/mL	100.0% (3/3)	100.0% (3/3)
Nystatin	Nystatin	10000 USP units/ml	100.0% (3/3)	100.0% (3/3)
Palmitic Acid	Palmitic acid	2 mg/mL	100.0% (3/3)	100.0% (3/3)
Pepto-Bismol	Bismuth subsalicylate	0.175 mg/mL	100.0% (3/3)	100.0% (3/3)
Preparation H	Phenylephrine	2% (w/v)	100.0% (3/3)	100.0% (3/3)
Sennosides	Sennosides	0.1 mg/mL	100.0% (3/3)	100.0% (3/3)
SPF 30 Sunscreen	Avobenzone 3%, homosalate 8%, octisalate 5%, octocrylene 4%, and oxybenzone 4%	1% (w/v)	100.0% (3/3)	100.0% (3/3)
SPF 50 Sunscreen	Avobenzone 3%, homosalate 13%, octisalate 5%, octocrylene 7%, and oxybenzone 4%	1% (w/v)	100.0% (3/3)	100.0% (3/3)
Stearic Acid	Stearic acid	4 mg/mL	100.0% (3/3)	100.0% (3/3)
Tums	Calcium Carbonate	0.1 mg/mL	100.0% (3/3)	100.0% (3/3)
Vagisil	Benzocaine USP (20% - External analgesic), Resorcinol (3% - External analgesic)	10% (w/v)	100.0% (3/3)	100.0% (3/3)
Vancomycin	Vancomycin	1.4 mg/mL	100.0% (3/3)	100.0% (3/3)
Vaseline	White petrolatum USP (100%)	10% (w/v)	100.0% (3/3)	100.0% (3/3)
Witch hazel	Witch hazel	Liquid from 1 wipe	100.0% (3/3)	100.0% (3/3)

[1] Colace 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 five 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 seven out of eight replicates of strains NAP1A and ATCC 43255.

INHIBITION BY OTHER MICROORGANISMS
Simplexa™ *C. difficile* Direct was evaluated by testing the ability to identify *C. difficile* when potentially inhibitory organisms are present.

130 organisms were evaluated for potential interference when the concentration of *C. difficile* in the sample was at a low/medium concentration (~2-4 X LoD) using two bacterial strains of *C. difficile* (NAP1A & ATCC 43255). A total of 127 organisms were tested during this study, and *in silico* NCBI BLAST analysis was performed for 3 organisms to determine microbial interference.

Microbial inhibition samples were prepared by spiking each of the 127 organisms at clinically relevant concentrations into negative stool-TE matrix. If $TCID_{50}$/mL (PFU/mL) or CFU/mL were not available, clinically relevant concentrations were prepared using industry accepted units. Each microbial inhibition sample was tested in at least in triplicate. If at least one of the three signals was not detected, an additional five replicates of the same inhibitor was tested.

All replicates of baseline sample and microbial inhibition samples were positive for *C. difficile*. No evidence of microbial inhibition was observed for the 127 organisms at the tested concentrations. Below are the results of the study.

Organism	**Tested Concentration**	***C. difficile* Strain: ATCC 43255**	***C. difficile* Strain NAP1A**
		***C. difficile* Qualitative Results: % Detection (# Detected/#Tested)**	***C. difficile* Qualitative Results: % Detection (# Detected/#Tested)**
Baseline	Not Applicable	100.0% (5/5)	100.0% (5/5)
Abiotrophia defectiva	1.00 X 10^6 CFU/mL	100.0% (3/3)	75.0% (6/8)
Acinetobacter baumannii	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Acinetobacter lwoffi	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Adenovirus 1	1.00 X 10^5 TCID50/mL	100.0% (3/3)	100.0% (3/3)
Aeromonas hydrophila	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Alcaligenes faecalis subsp. *Faecalis*	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Anaerococcus tetradius	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Bacillus cereus ATCC 11778	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Bacillus cereus ATCC 13472	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Bacteroides caccae	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Bacteroides fragilis	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Bacteroides merdae	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Bacteroides stercoris	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)

Organism	Tested Concentration	***C. difficile* Strain: ATCC 43255**	***C. difficile* Strain NAP1A**
		***C. difficile* Qualitative Results: % Detection (# Detected/#Tested)**	***C. difficile* Qualitative Results: % Detection (# Detected/#Tested)**
Bifidobacterium adolescentis	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Bifidobacterium longum	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Campylobacter coli	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Campylobacter jejuni[1]	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Candida albicans	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Candida catenulate	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Cedecea davisae	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Chlamydia trachomatis	1.00 X 10^6 IFU/mL	100.0% (3/3)	100.0% (3/3)
Citrobacter amalonaticus	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Citrobacter freundii	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Citrobacter koseri	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Citrobacter sedlakii	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium beijerinckii	1.00 X 10^6 CFU/mL	100.0%(3/3)	100.0% (3/3)
Clostridium bifermentans	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium bolteae	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium butyricum	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium chauvoei	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium difficile ATCC 43593	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium difficile ATCC 43601	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium fallax	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium haemolyticum	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium histolyticum	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium innocuum	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)

Organism	Tested Concentration	*C. difficile* Strain: ATCC 43255	*C. difficile* Strain NAP1A
		C. difficile Qualitative Results: % Detection (# Detected/#Tested)	*C. difficile* Qualitative Results: % Detection (# Detected/#Tested)
Clostridium methylpentosum	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium nexile	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium novyi	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium paraputrificum	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium perfringens	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium ramosum	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium scindens	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium sepiticum	1.00 X 10^6 cells/mL	100.0% (3/3)	87.5% (7/8)
Clostridium sordellii	1.00 X 10^6 CFU/mL	100.0% (3/3)	87.5% (7/8)
Clostridium sphenoides	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium spiroforme	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium sporogenes	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium symbiosum	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium tertium	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Clostridium tetani	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Collinsella aerofaciens	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Corynebacterium genitalium	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Coxsackievirus A10	1.00 X 10^5 TCID50/mL	100.0% (3/3)	100.0% (3/3)
Cytomegalovirus	1.00 X 10^5 TCID50/mL	100.0% (3/3)	100.0% (3/3)
Echovirus 11	1.00 X 10^5 TCID50/mL	100.0% (3/3)	100.0% (3/3)
Edwardsiella tarda	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Eggerthella lenta	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)

Organism	Tested Concentration	*C. difficile* Strain: ATCC 43255	*C. difficile* Strain NAP1A
		C. difficile Qualitative Results: % Detection (# Detected/#Tested)	*C. difficile* Qualitative Results: % Detection (# Detected/#Tested)
Enterobacter aerogenes Z052	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Enterobacter cloacae	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Enterococcus casseliflavus	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Enterococcus cecorum	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Enterococcus dispar	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Enterococcus faecalis	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Enterococcus faecium	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Enterococcus gallinarum	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Enterococcus hirae	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Enterococcus raffinosus	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Enterovirus 71	1.00 X 10^6 TCID50/mL	100.0% (3/3)	100.0% (3/3)
Escherichia coli ATCC 11775	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Escherichia coli ATCC 23511 O16:K1L:NM	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Escherichia coli ATCC 25922	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Escherichia coli O157H7	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Escherichia fergusonii	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Escherichia hermannii	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Flavonifractor plautii	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Fusobacterium varium	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Gardnerella vaginalis	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Gemella morbillorum	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Hafnia alvei	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Helicobacter fennelliae	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)

Organism	Tested Concentration	***C. difficile* Strain: ATCC 43255**	***C. difficile* Strain NAP1A**
		***C. difficile* Qualitative Results: % Detection (# Detected/#Tested)**	***C. difficile* Qualitative Results: % Detection (# Detected/#Tested)**
Helicobacter pylori	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Klebsiella oxytoca	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Klebsiella pneumoniae subsp. *Pneumoniae*	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Lactobacillus acidophilus	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Lactobacillus reuteri	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Lactococcus lactis	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Leminorella grimontii	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Listeria grayi	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Listeria innocua	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Listeria monocytogenes	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Norovirus G2	1.00 X 10^6 copies/mL	100.0% (3/3)	100.0% (3/3)
Peptoniphilus asaccharolyticus	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Peptostreptococcus anaerobius	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Plesiomonas shigelloides	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Porphyromonas asaccharolytica	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Prevotella melaninogenica	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Proteus mirabilis Z050	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Proteus penneri	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Providencia alcalifaciens	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Providencia rettgeri	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Providencia stuartii	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Pseudomonas aeruginosa	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Pseudomonas putida	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)

Organism	Tested Concentration	***C. difficile* Strain: ATCC 43255**	***C. difficile* Strain NAP1A**
		***C. difficile* Qualitative Results: % Detection (# Detected/#Tested)**	***C. difficile* Qualitative Results: % Detection (# Detected/#Tested)**
Rotavirus Strain Wa	1.00 X 10^6 TCID50/mL	100.0% (3/3)	100.0% (3/3)
Salmonella enterica subsp. *Arizonae*	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Salmonella enterica subsp. *Enterica*	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Salmonella enterica subsp. *Enterica serovar Typhim*	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Serratia liquefaciens	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Serratia marcescens subsp. *marcescens*	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Shigella boydii	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Shigella dysenteriae	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Shigella sonnei	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Staphylococcus aureus (MRSA), ATCC 700699	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Staphylococcus epidermidis (MRSE), ATCC 29887	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Stenotrophomonas maltophilia	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Streptococcus agalactiae	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Streptococcus dysgalactiae	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Streptococcus intermedius	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Streptococcus uberis	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Trabulsiella guamensis	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Veillonella parvula	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Vibrio cholerae	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
Vibrio parahaemolyticus	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)
White Blood Cells (Human)	1.00 X 10^6 WBC/mL	100.0% (3/3)	100.0% (3/3)
Yersinia bercovieri	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)

Organism	Tested Concentration	*C. difficile* Strain: ATCC 43255	*C. difficile* Strain NAP1A
		C. difficile Qualitative Results: % Detection (# Detected/#Tested)	*C. difficile* Qualitative Results: % Detection (# Detected/#Tested)
Yersinia rohdei	1.00 X 10^6 CFU/mL	100.0% (3/3)	100.0% (3/3)

[1]*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* and it did not show cross reactivity with the Simplexa™ *C. difficile* Direct kit based on *in silico* analysis.