DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center - WO66-G609
Silver Spring, MD 20993-0002

August 13, 2018

DiaSorin Molecular LLC
Sharon Young
Senior Regulatory Affairs Specialist
11331 Valley View Street
Cypress, CA 90630

Re: K173498
Trade/Device Name: Simplexa Bordetella Direct MOL2750, Simplexa Bordetella Positive Control Pack MOL2760
Regulation Number: 21 CFR 866.3980
Regulation Name: Respiratory Viral Panel Multiplex Nucleic Acid Assay
Regulatory Class: Class II
Product Code: OZZ
Dated: November 16, 2017
Received: November 17, 2017

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. 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 Part 801 and Part 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 regulation (21 CFR Part 801 and Part 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,

Kristian M. Roth -S

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)*

Device Name
Simplexa™ Bordetella Direct MOL2750 and Simplexa™ Bordetella Positive Control Pack MOL 2760

Indications for Use *(Describe)*

Simplexa™ Bordetella Direct MOL2750

The DiaSorin Molecular Simplexa™ Bordetella Direct MOL2750 assay is an in vitro diagnostic test intended for use on the LIAISON® MDX instrument for the qualitative detection and differentiation of *Bordetella pertussis* and *Bordetella parapertussis* nucleic acids from frozen nasopharyngeal (NPS) specimens from patients with signs and symptoms of Bordetella infection of the respiratory tract.

The Simplexa™ Bordetella Direct MOL2750 assay is performed on the LIAISON® MDX instrument and utilizes real-time PCR amplification to detect *B. pertussis* by targeting the IS481 insertional element of the *B. pertussis* genome and to detect B. parapertussis by targeting the IS1001 insertional element of the *B. parapertussis* genome. The IS481 insertional element can also be present in *B. holmesii* and *B. bronchiseptica*. Specimens collected from patients with respiratory infection caused by *B. pertussis*, *B. holmesii* or *B. bronchiseptica* may yield positive test results in IS481 assays. *B. holmesii* infection may cause clinical illness similar to *B. pertussis*, and mixed outbreaks involving both *B. pertussis* and *B. holmesii* infection have been reported. Additional testing should be performed if necessary to differentiate *B. holmesii* and *B. pertussis*. *B. bronchiseptica* is a rare cause of infection in humans. When clinical factors suggest that *B. pertussis* may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines.

Negative results for the Simplexa™ Bordetella Direct MOL2750 assay do not preclude Bordetella infection and positive results do not rule out co-infection with other respiratory pathogens. Results from the Simplexa™ Bordetella Direct MOL2750 assay should be used with other clinical findings and epidemiological information as an aid in diagnosis of Bordetella infection. Test results should not be used as the sole basis for treatment or other patient management decisions.

Simplexa™ Bordetella Positive Control Pack MOL 2760

The Simplexa™ Bordetella Positive Control Pack MOL2760 is intended to be used as a control with the Simplexa™ Bordetella 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	DiaSorin Molecular LLC. 11331 Valley View Street Cypress, California 90630 USA
Establishment Registration No.	2023365
Contact Person	Sharon Young tel 562.240.6680 fax 562.240.6529 Sharon.Young@DiaSorin.com
Summary Date	August 7, 2018
Proprietary Name	Simplexa™ Bordetella Direct and Simplexa™ Bordetella Positive Control Pack
Generic Name	Bordetella nucleic acid
Classification	Class II
Predicate Devices	ARIES® Bordetella Assay for use with the ARIES® Systems

Intended Use

Simplexa™ Bordetella Direct
The DiaSorin Molecular Simplexa™ Bordetella Direct assay is an *in vitro* diagnostic test intended for use on the LIAISON® MDX instrument for the qualitative detection and differentiation of *Bordetella pertussis* and *Bordetella parapertussis* nucleic acids from frozen nasopharyngeal (NPS) specimens from patients with signs and symptoms of Bordetella infection of the respiratory tract.

The Simplexa™ Bordetella Direct assay is performed on the LIAISON® MDX instrument and utilizes real-time PCR amplification to detect *B. pertussis* by targeting the IS481 insertional element of the *B. pertussis* genome and to detect *B. parapertussis* by targeting the IS1001 insertional element of the *B. parapertussis* genome. The IS481 insertional element can also be present in *B. holmesii* and *B. bronchiseptica*. Specimens collected from patients with respiratory infection caused by *B. pertussis*, *B. holmesii* or *B. bronchiseptica* may yield positive test results in IS481 assays. *B. holmesii* infection may cause clinical illness similar to *B. pertussis*, and mixed outbreaks involving both *B. pertussis* and *B. holmesii* infection have been reported. Additional testing should be performed if necessary to differentiate *B. holmesii* and *B. pertussis*. *B. bronchiseptica* is a rare cause of infection in humans. When clinical factors suggest that *B. pertussis* may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines.

Negative results for the Simplexa™ Bordetella Direct assay do not preclude Bordetella infection and positive results do not rule out co-infection with other respiratory pathogens. Results from the Simplexa™ Bordetella Direct assay should be used with other clinical findings and epidemiological information as an aid in diagnosis of Bordetella infection. Test results should not be used as the sole basis for treatment or other patient management decisions.

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

Device Description

The Simplexa™ Bordetella Direct assay system is a real-time PCR assay that enables the direct amplification, detection and differentiation of *Bordetella pertussis* and *Bordetella parapertussis* DNA from unprocessed nasopharyngeal swabs (NPS) without nucleic acid extraction. The system consists of the

Simplexa™ Bordetella Direct assay, the LIAISON® MDX (with LIAISON® MDX Studio Software), the Direct Amplification Disc and associated accessories.

In the Simplexa™ Bordetella Direct assay, primers and fluorescent probes are used together to amplify and detect *Bordetella pertussis*, *Bordetella parapertussis* and internal control targets. Insertion sequences IS481 and IS1001 are targeted to identify *Bordetella pertussis* and *Bordetella parapertussis* DNA respectively in the specimen. An internal control is used to detect PCR failure and/or inhibition.

The DiaSorin Molecular Simplexa™ Bordetella Direct kit contains sufficient reagents for 24 reactions. Upon receipt, store at -10 to -30ºC (do not use a frost-free freezer). Each vial contains sufficient material for a single reaction. Use within 30 minutes of thawing.

Kit Description

Component Name	REF	EC SYMBOL ON LABEL		Abbreviated Name	Cap Color	Number of Vials	Reactions per Vial/Kit	Volume per Vial
Simplexa™ Bordetella Direct Reaction Mix	MOL2751	REAG	A	RM	Brown	24	1/24	50 µL

Component Description

Kit Component	Contents
Simplexa™ Bordetella Direct Reaction Mix (RM)	**DNA polymerase, buffer, dNTPs, Internal Control template DNA, dye-labeled fluorescent probes and primers specific for detection of *Bordetella pertussis*, *Bordetella parapertussis* and DNA Internal Control**

Target	Probe Fluorophore (Dye)	Excitation (nm)	Emission (nm)	Targeted Gene
Bordetella pertussis	FAM	495	520	IS481
Bordetella parapertussis	CFR610	590	610	IS1001
DNA Internal Control	Q670	644	670	DNA IC

Simplexa™ Bordetella Kit Barcode Card	Assay specific parameters

MATERIALS SUPPLIED SEPARATELY

1. Direct Amplification Disc Kit (REF MOL1455)
   a) Direct Amplification Discs for use on the LIAISON® MDX

Predicate Device Information

Item	Device	
Name	**ARIES® Bordetella Assay for use with the ARIES® Systems (K163626)**	**Simplexa™ Bordetella Direct REF MOL2750 And Simplexa™ Bordetella Positive Control Pack REF MOL2760**
Intended Use	The ARIES® Bordetella Assay is a real-time polymerase chain reaction (PCR) based qualitative in vitro diagnostic test for the direct detection and identification of *Bordetella pertussis* (*B. pertussis*) and *Bordetella parapertussis* (*B. parapertussis*) nucleic acid in nasopharyngeal swab (NPS) specimens obtained from individuals suspected of having a respiratory tract infection attributable to *B. pertussis* or *B. parapertussis*. The ARIES® Bordetella Assay targets the *B. pertussis* toxin promoter and the *B. parapertussis* IS1001 insertion element in the genomes. When clinical factors suggest that *B. pertussis* or *B. parapertussis* may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines. Negative results for the ARIES® Bordetella Assay do not preclude *B. pertussis* or *B. parapertussis* infection and positive results do not rule out co-infections with other respiratory pathogens. The direct detection and identification of *B. pertussis* and *B. parapertussis* nucleic acids from symptomatic patients aids in the diagnosis of *B. pertussis* and *B. parapertussis* respiratory infection in conjunction with other clinical findings and epidemiological information. The ARIES® Bordetella Assay is indicated for use with the ARIES® Systems.	Simplexa™ Bordetella Direct The DiaSorin Molecular Simplexa™ Bordetella Direct assay is an *in vitro* diagnostic test intended for use on the LIAISON® MDX instrument for the qualitative detection and differentiation of *Bordetella pertussis* and *Bordetella parapertussis* nucleic acids from frozen nasopharyngeal (NPS) specimens from patients with signs and symptoms of Bordetella infection of the respiratory tract. The Simplexa™ Bordetella Direct assay is performed on the LIAISON® MDX instrument and utilizes real-time PCR amplification to detect *B. pertussis* by targeting the IS481 insertional element of the *B. pertussis* genome and to detect *B. parapertussis* by targeting the IS1001 insertional element of the *B. parapertussis* genome. The IS481 insertional element can also be present in *B. holmesii* and *B. bronchiseptica*. Specimens collected from patients with respiratory infection caused by *B. pertussis*, *B. holmesii* or *B. bronchiseptica* may yield positive test results in IS481 assays. *B. holmesii* infection may cause clinical illness similar to *B. pertussis*, and mixed outbreaks involving both *B. pertussis* and *B. holmesii* infection have been reported. Additional testing should be performed if necessary to differentiate *B. holmesii* and *B. pertussis*. *B. bronchiseptica* is a rare cause of infection in humans. When clinical factors suggest that *B. pertussis* may not be the cause of respiratory infection, other clinically appropriate investigation(s) should be carried out in accordance with published guidelines. Negative results for the Simplexa™ Bordetella Direct assay do not preclude Bordetella infection and positive results do not rule out co-infection with other respiratory pathogens. Results from the Simplexa™

Item	**Device**	
Name	**ARIES® Bordetella Assay for use with the ARIES® Systems (K163626)**	**Simplexa™ Bordetella Direct REF MOL2750 And Simplexa™ Bordetella Positive Control Pack REF MOL2760**
		Bordetella Direct assay should be used with other clinical findings and epidemiological information as an aid in diagnosis of Bordetella infection. Test results should not be used as the sole basis for treatment or other patient management decisions. Simplexa™ Bordetella Positive Control Pack The Simplexa™ Bordetella Positive Control Pack is intended to be used as a control with the Simplexa™ Bordetella Direct kit. This control is not intended for use with other assays or systems.
Assay Targets	*B. pertussis* Target *B. pertussis* and *B. parapertussis* toxin promoter (ptxA-pr), *B. parapertussis* Target IS1001 insertion element .	The Simplexa™ Bordetella Direct assay detects the following multi-copy insertion sequence targets: *Bordetella pertussis* target IS481 and *Bordetella parapertussis* target IS1001.
Sample Types	Nasopharyngeal swabs in UTM™, M5® , M6™ and ESwab.	Nasopharyngeal swabs in Remel M4, Remel M4RT, Remel M5, Remel M6, UTM, VTM and Liquid Aimes (ESwab).
Instrument	ARIES® System, ARIES® M1 System.	LIAISON® MDX.
Extraction Methods	None.	None.
Assay Methodology	Real-time system for detecting the presence / absence of *Bordetella pertussis* and *Bordetella parapertussis* DNA in clinical specimens.	PCR-based system for detecting the presence / absence of *Bordetella pertussis* and *Bordetella parapertussis* DNA in clinical specimens.
Detection Techniques	Different fluorescent reporter dyes for each target and melt analysis.	Multiplex assay using different reporter dyes for each target.

Item	Device	
Name	**ARIES® Bordetella Assay for use with the ARIES® Systems (K163626)**	**Simplexa™ Bordetella Direct REF MOL2750 And Simplexa™ Bordetella Positive Control Pack REF MOL2760**
Optical Detection	Fluorescence Emissions and Detection.	Fluorescence.
Time to result	Less than 2 hours.	Approximately 1 hour
Test Interpretation	Automated test interpretation and report generation.	Automated test interpretation and report generation.

METHOD COMPARISON

One thousand one hundred and forty-two (1142) samples were prospectively collected and frozen from five (5) geographically diverse sites between January 2017 and August 2017, from patients with signs and symptoms of Bordetella infections. Of the one thousand one hundred and forty-two (1142) samples, one thousand one hundred and thirteen (1113) samples were evaluable on Simplexa™ Bordetella Direct and a composite reference method. In addition, fifty-six (56) samples were contrived for *Bordetella parapertussis* at various concentrations across the clinical range of the assay (2-50 X LoD) and randomized among fifty-six (56) negatives for a total of one hundred and twelve (112) additional samples, which were evaluable on Simplexa™ Bordetella Direct and a composite reference method. The composite reference method consisted of two well-characterized real-time PCR assays followed by confirmation of positive PCR amplification products with bi-directional sequencing, per target. Samples were characterized as positive if one or both composite reference methods were positive and confirmed by bi-directional sequencing. Samples were characterized as negative if both composite reference methods were negative. Samples were tested on Simplexa™ Bordetella Direct at the collection sites and the composite reference method was performed at DiaSorin Molecular. The *Bordetella pertussis* prospectively banked frozen sample results are shown in Table 1. The *Bordetella parapertussis* prospectively banked frozen sample results are shown in Table 2 and the *Bordetella parapertussis* contrived sample results are shown in Table 3.

Table 1. Simplexa™ Bordetella Direct *Bordetella pertussis* Results Versus PCR/Bi-Directional Sequencing Method Prospectively Banked Frozen Samples

Simplexa™ Bordetella Direct *B. pertussis*	PCR/Bi-directional Sequencing		
	Detected	**Not Detected**	**Total**
Detected	68	13	81
Not Detected	6	1026	1032
Total	74	1039	1113
	%PPA 91.9%(68/74) 95% CI: 83.4% to 96.2%	**%NPA** 98.7%(1026/1039) 95% CI: 97.9% to 99.3%	

Table 2. Simplexa™ Bordetella Direct *Bordetella parapertussis* Results versus PCR/Bi-Directional Sequencing Method Prospectively Collected Frozen Samples

Simplexa™ Bordetella Direct *B. parapertussis*	PCR/Bi-directional Sequencing		
	Detected	Not Detected	Total
Detected	13	4	17
Not Detected	0	1096	1096
Total	13	1100	1113
	%PPA 100.0%(13/13) 95% CI: 77.2% to 100.0%	**%NPA** 99.6%(1096/1100) 95% CI: 99.1% to 99.9%	

Table 3. Simplexa™ Bordetella Direct *Bordetella parapertussis* results versus PCR/Bi-Directional Sequencing Method Contrived Frozen Samples

Simplexa™ Bordetella Direct *B. parapertussis*	PCR/Bi-directional Sequencing		
	Detected	Not Detected	Total
Detected	56	0	56
Not Detected	0	56	56
Total	56	56	112
	%PPA 100.0%(56/56) 95% CI: 93.6% to 100.0%	**%NPA** 100.0%(56/56) 95% CI: 93.6% to 100.0%	

REPRODUCIBILITY

Three (3) investigative laboratory testing sites assessed the device's inter-site, inter-day and inter/intra-assay reproducibility. Each of the laboratories tested a panel of six (6) members that included contrived *Bordetella pertussis* A639, and *Bordetella parapertussis* A747 samples at the following concentrations; low positive (LP) approximately 1-2 X LoD and a medium positive sample (MP) approximately 3-4 X LoD. A positive and negative control was also included in the panel. Each sample panel member was tested in triplicate per run for two (2) runs per day for five (5) non-consecutive days per site. Two (2) runs per day were each performed by a different operator. In total ninety (90) replicates (3 replicates X 2 runs X 5 days X 3 sites) were tested for each sample panel member. Combined results for all sites are presented in Table 4. The data in Table 4 show that the Simplexa™ Bordetella Direct is 100% in agreement with the expected results demonstrating the reproducibility of the assay for both *Bordetella pertussis* and *Bordetella parapertussis* targets.

Table 4. Simplexa™ Bordetella Direct Reproducibility

Sample Panel Member	Site 1			Site 2			Site 3			Overall	
	% Agreement with Expected Results	Avg. Ct	%CV	% Agreement with Expected Results	Avg. Ct	%CV	% Agreement with Expected Results	Avg. Ct	%CV	Total %	95% CI
Bordetella pertussis A639 – LP (FAM)	100.0% (30/30)	33.9	3.4	100.0% (30/30)	34.2	3.3	100.0% (30/30)	34.9	2.6	100.0% (90/90)	95.9% to 100.0%
Bordetella pertussis A639 – MP (FAM)	100.0% (30/30)	32.9	2.5	100.0% (30/30)	33.8	2.9	100.0% (30/30)	33.9	2.7	100.0% (90/90)	95.9% to 100.0%
Bordetella para-pertussis A747 – LP (CFR 610)	100.0% (30/30)	33.9	3.9	100.0% (30/30)	34.3	2.7	100.0% (30/30)	35.4	3.5	100.0% (90/90)	95.9% to 100.0%
Bordetella para-pertussis A747 – MP (CFR 610)	100.0% (30/30)	33.0	2.7	100.0% (30/30)	33.5	2.6	100.0% (30/30)	33.7	2.0	100.0% (90/90)	95.9% to 100.0%
Native negative naso-pharyngeal swab (UTM)	100.0% (30/30)	N/A	N/A	100.0% (30/30)	N/A	N/A	100.0% (30/30)	N/A	N/A	100.0% (90/90)	95.9% to 100.0%
Positive Control (FAM)	100.0% (30/30)	24.0	0.8	100.0% (30/30)	22.0	1.1	100.0% (30/30)	23.3	0.7	100.0% (90/90)	95.9% to 100.0%
Positive Control (CFR 610)	100.0% (30/30)	23.6	0.9	100.0% (30/30)	20.8	1.0	100.0% (30/30)	22.4	0.7	100.0% (90/90)	95.9% to 100.0%
Total Agreement	**100.0% (180/180)**			**100.0% (180/180)**			**100.0% (180/180)**			**100.0% (540/540) 99.3% to 100.0%**	

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The Limit of Detection (LoD) was determined for the Simplexa™ Bordetella Direct assay using quantified stocks of two (2) strains of *Bordetella pertussis (*A639 & BAA-589) and *Bordetella parapertussis* (A747 & E595) serially diluted into native negative nasopharyngeal swab matrix in Universal Transport Media (UTM). LoD is the lowest concentration that could be detected as positive $\geq$ 95% of the time. The LoD concentrations for the Bordetella strains are summarized in Table 5.

Table 5. Simplexa™ Bordetella Direct Limit of Detection

Bordetella species	Bordetella strain	LoD Concentration (CFU/mL)
Bordetella pertussis	A639	14.7
	BAA-589	20.9
Bordetella parapertussis	A747	347.3
	E595	239.0

ANALYTICAL REACTIVITY / CROSS REACTIVITY

Analytical Reactivity

Analytical Reactivity for Simplexa™ Bordetella Direct was assessed using eighteen (18) Bordetella strains including twelve (12) *Bordetella pertussis* strains and six (6) *Bordetella parapertussis* strains that were not tested as a part of the Limit of Detection (LoD) study. All eighteen (18) strains were detected as positive for *Bordetella pertussis* at or below 80 CFU/mL or for *Bordetella parapertussis* at or below 590 CFU/mL. In addition to the strains that were tested, *in silico* BLAST analysis demonstrated that the assay should detect at least two hundred and ninety-four (294) additional *Bordetella pertussis* and five (5) additional *Bordetella parapertussis* strains. The results of the testing are summarized in Table 6 for *Bordetella pertussis* and in Table 7 for *Bordetella parapertussis.*

Table 6. Simplexa™ Bordetella Direct Analytical Reactivity – *Bordetella pertussis*

Bordetella Strain	Concentration	***Bordetella pertussis*** **(IS481) Result (# detected/# tested)**
Bordetella pertussis BAA-1335	35.6 CFU/mL	3/3
Bordetella pertussis ATCC 8467	35.6 CFU/mL	3/3
Bordetella pertussis ATCC 9306	79.7 CFU/mL	3/3
Bordetella pertussis ATCC 12742	35.6 CFU/mL	3/3
Bordetella pertussis ATCC 51445	35.6 CFU/mL	3/3
Bordetella pertussis ATCC 53894	79.7 CFU/mL	3/3
Bordetella pertussis ATCC 8478	35.6 CFU/mL	3/3
Bordetella pertussis ATCC 12743	35.6 CFU/mL	3/3
Bordetella pertussis ATCC 9340	35.6 CFU/mL	3/3
Bordetella pertussis ATCC 9797	35.6 CFU/mL	3/3
Bordetella pertussis ATCC 10380	35.6 CFU/mL	3/3
Bordetella pertussis E431	35.6 CFU/mL	3/3

Table 7. Simplexa™ Bordetella Direct Analytical Reactivity – *Bordetella parapertussis*

Bordetella Strain	Concentration	*Bordetella parapertussis* (IS1001) Result (# detected/# tested)
Bordetella parapertussis ATCC 15311	586.3 CFU/mL	3/3
Bordetella parapertussis ATCC 15237	586.3 CFU/mL	3/3
Bordetella parapertussis ATCC 15989	586.3 CFU/mL	3/3
Bordetella parapertussis BAA-587	586.3 CFU/mL	3/3
Bordetella parapertussis C510	586.3 CFU/mL	3/3
Bordetella parapertussis E838	586.3 CFU/mL	3/3

Cross Reactivity (Analytical Specificity)

Analytical specificity was evaluated for the Simplexa™ Bordetella Direct by testing cross reactivity to organisms that are closely related, or cause similar clinical symptoms, or are present as normal flora in the nasopharynx. Negative specimens were spiked with potentially cross reactive organisms at the concentrations indicated in Table 8. The spiked specimens were examined for reactivity with Simplexa™ Bordetella Direct. Ninety-seven (97) organisms were tested. No cross reactivity was found with the exception of *Bordetella holmseii* which was expected due to the presence of the IS481 element in *Bordetella holmesii*. The results are shown in Table 8.

Table 8. Simplexa™ Bordetella Direct Cross Reactivity

Cross Reactant	Concentration	*Bordetella pertussis* (IS481) % Detection (# Detected/# Tested)	*Bordetella parapertussis* (IS1001) % Detection (# Detected/# Tested)
Acinetobacter baumannii	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Acinetobacter lwoffi	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Adenovirus 1	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Adenovirus 31	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Arcanobacterium haemolyticum	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Bacillus cereus	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Bacteroides fragilis	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Bordetella avium	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Bordetella bronchiseptica RB50	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Bordetella hinzi	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Bordetella holmseii F061	1 x 10^6 CFU/mL	100% (8/8)	0% (0/3)
Bordetella petri	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Bordetella trematum	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Burkholderia cenocepacia	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Burkholderia cepacia	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Burkholderia multivorans	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Burkholderia thailandensis	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)

Cross Reactant	Concentration	*Bordetella pertussis* (IS481) % Detection (# Detected/# Tested)	*Bordetella parapertussis* (IS1001) % Detection (# Detected/# Tested)
Candida albicans	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Candida glabrata	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Chlamydia pneumoniae	1 x 10^6 IFU/mL	0% (0/3)	0% (0/3)
Chlamydia trachmomatis	1 x 10^6 IFU/mL	0% (0/3)	0% (0/3)
Citrobacter freundii	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Clostridium difficile	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Coronavirus 229E	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Coronavirus NL63*	1 x 10^4 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Coronavirus OC43	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Corynebacterium diptheriae	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Coxsackievirus A16	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Coxsackievirus B4	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Cytomegalovirus	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Echovirus 6	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Echovirus 7	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Echovirus 9	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Echovirus 11	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Enterobacter aerogenes Z052	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Enterobacter cloacae	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Enterococcus faecalis vanB	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Enterovirus 70	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Enterovirus 71	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Epstein-Barr Virus	1 x 10^5 copies/mL	0% (0/3)	0% (0/3)
Escherichia coli	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Fusobacterium necrophorum	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Haemophilus influenzae	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Haemophilus parainfluenzae	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
HSV-1 (MacIntyre)	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
HSV-2 (G)	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Influenza A/Swine/Iowa/15/30 H1N1*	1 x 10^4 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Influenza B/Malaysia/2506/04*	1 x 10^4 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Klebsiella oxytoca	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Klebsiella pneumoniae	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Lactobacillus acidophilus	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Lactobacillus plantarum 17-5	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Legionella longbeachae	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)

Cross Reactant	Concentration	*Bordetella pertussis* (IS481) % Detection (# Detected/# Tested)	*Bordetella parapertussis* (IS1001) % Detection (# Detected/# Tested)
Legionella pneumophila (Philadelphia)	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Listeria monocytogenes	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Measles	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Metapneumovirus-9	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Moraxella catarrhalis Ne 11	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Morganella morganii	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Mumps	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Mycobacterium avium	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Mycobacterium tuberculosis (genomic DNA)	1 x 10^6 genome copies/mL	0% (0/3)	0% (0/3)
Mycoplasma hominis	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Mycoplasma pneumoniae Strain M129	1 x 10^6 CCU/mL	0% (0/3)	0% (0/3)
Neisseria elongata	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Neisseria gonorrhoeae	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Neisseria meningitidis	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Neisseria mucosa	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Parainfluenza 1	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Parainfluenza 2	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Parainfluenza 3*	1 x 10^4 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Parainfluenza 4*	1 x 10^4 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Parvimonas micra	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Peptostreptococcus anaerobius	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Proteus mirabilis Z050	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Proteus vulgaris	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Pseudomonas aeruginosa	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Pseudomonas fluorescens	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Rhinovirus 1A*	1 x 10^4 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
RSV A	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
RSV B WV/14617/85	1 x 10^5 $TCID_{50}$/mL	0% (0/3)	0% (0/3)
Serratia liquefaciens	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Serratia marcescens	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Staphylococcus aureus (MRSA)	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Staphylococcus epidermidis (MRSE)	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Stenotrophomonas maltophilia	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Streptococcus anginosus	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Streptococcus canis	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)

Cross Reactant	Concentration	*Bordetella pertussis* (IS481) % Detection (# Detected/# Tested)	*Bordetella parapertussis* (IS1001) % Detection (# Detected/# Tested)
Streptococcus dysgalactiae	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Streptococcus intermedius	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Streptococcus mitis	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Streptococcus mutans	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Streptococcus pneumoniae	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Streptococcus pyogenes M1	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Streptococcus salivarius	1 x 10^6 CFU/mL	0% (0/3)	0% (0/3)
Ureaplasma urealyticum	1 x 10^6 CCU/mL	0% (0/3)	0% (0/3)
Varicella Zoster Virus*	1 x 10^4 $TCID_{50}$/mL	0% (0/3)	0% (0/3)

* The testing concentration of these viruses is lower due to the lack of a high titer stock.

INTERFERENCE

The performance of Simplexa™ Bordetella Direct was evaluated with potentially interfering substances that may be present in the nasopharynx. The potentially interfering substances were evaluated in a contrived sample that contained *Bordetella pertussis* and *Bordetella parapertussis* at approximately 2-4 X LoD. There was no evidence of interference caused by the substances at the concentrations listed in Table 9 (*Bordetella pertussis*) and Table 10 (*Bordetella parapertussis*).

Table 9. Simplexa™ Bordetella Direct Interference – *Bordetella pertussis*

Potentially Interfering Substance	Active Ingredient	Interferent Concentration	B. pertussis (IS481)
			% Detection
Albuterol sulfate	Albuterol sulfate	10 mg/mL	100% (3/3)
Ampicillin powder	Ampicillin	10 mg/mL	100% (3/3)
Azithromycin powder	Azithromycin	10 mg/mL	100% (3/3)
Beclomethasone dipropionate	Beclomethasone dipropionate	10 mg/mL	100% (3/3)
Blood	NA	10% v/v	100% (3/3)
Chloraseptic sore throat spray	Phenol	10% v/v	100% (3/3)
Ciprofloxacin	Ciprofloxacin	1.25 mg/mL	100% (3/3)
Erythromycin	Erythromycin	10 mg/mL	100% (3/3)
Flonase Nasal Spray	Fluticasone propionate-corticosteroid	10% v/v	100% (3/3)
Mucin	Mucin	10 mg/mL	100% (3/3)
Mupirocin	Mupirocin	10 mg/mL	100% (3/3)
Rifampicin	Rifampicin	2.5 mg/mL	100% (3/3)
Robitussin DM	Robitussin DM	10% v/v	100% (3/3)
Saline Nasal spray-Sodium chloride	Sodium chloride	10% v/v	100% (3/3)

Potentially Interfering Substance	Active Ingredient	Interferent Concentration	B. pertussis (IS481)
			% Detection
Sudafed PE	Phenylephrine	10 mg/mL	100% (3/3)
Zicam 12 hrs spray	Oxymetazoline HCl	10% v/v	100% (3/3)

Table 10. Simplexa™ Bordetella Direct Interference – *Bordetella parapertussis*

Potentially Interfering Substance	Active Ingredient	Interferent Concentration	B. parapertussis (IS1001)
			% Detection
Albuterol sulfate	Albuterol sulfate	10 mg/mL	100% (3/3)
Ampicillin powder	Ampicillin	10 mg/mL	100% (3/3)
Azithromycin powder	Azithromycin	10 mg/mL	100% (3/3)
Beclomethasone dipropionate	Beclomethasone dipropionate	10 mg/mL	100% (3/3)
Blood	NA	10% v/v	100% (3/3)
Chloraseptic sore throat spray	Phenol	10% v/v	100% (3/3)
Ciprofloxacin	Ciprofloxacin	1.25 mg/mL	100% (3/3)
Erythromycin	Erythromycin	10 mg/mL	100% (3/3)
Flonase Nasal Spray	Fluticasone propionate-corticosteroid	10% v/v	100% (3/3)
Mucin	Mucin	10 mg/mL	100% (3/3)
Mupirocin	Mupirocin	10 mg/mL	100% (3/3)
Rifampicin	Rifampicin	5 mg/mL	100% (8/8)*
Robitussin DM	Robitussin DM	10% v/v	100% (3/3)
Saline Nasal spray-Sodium chloride	Sodium chloride	10% v/v	100% (3/3)
Sudafed PE	Phenylephrine	10 mg/mL	100% (3/3)
Zicam 12 hrs spray	Oxymetazoline HCl	10% v/v	100% (3/3)

* The initial three (3) replicates of Rifampicin at 5 mg/mL were valid but not detected for *Bordetella parapertussus*. An additional five (5) replicates were performed with no interference for *Bordetella parapertussis* at 5 mg/mL.

COMPETITIVE INTERFERENCE

The Simplexa™ Bordetella Direct assay was evaluated for competitive interference by testing whether the presence of a clinically relevant high concentration of either *Bordetella pertussis* or *Bordetella parapertussis* could affect the detection of the other Bordetella species when present at a low level. A low positive sample was contrived for each target by spiking *Bordetella pertussis* or *Bordetella parapertussis* separately (at approximately 2 X LoD) into nasopharyngeal swab matrix in Universal Transport Media (UTM) and a baseline Ct was determined for each sample. High concentrations of the other Bordetella strain were then spiked into the low level sample and tested. No competitive interference was observed. The results showed that a low level of *Bordetella pertussis* was detected in the presence of a high level of *Bordetella parapertussis*; similarly, a low level of *Bordetella parapertussis* was detected in the presence of a high level of *Bordetella pertussis*. The results are shown in Table 11.

Table 11. Simplexa™ Bordetella Direct Competitive Interference

Baseline (Low Concentration)		Competitive Interferent (High Concentration)		*Bordetella pertussis* (IS481) (#Detected/#Total)	*Bordetella parapertussis* (IS1001) (#Detected/#Total)
Species	**2 X LoD CFU/mL**	**Species**	**CFU/mL**		
Bordetella pertussis	29.4 CFU/mL	*Bordetella parapertussis*	$2x10^7$ CFU/mL	3/3	3/3
Bordetella parapertussis	694.6 CFU/mL	*Bordetella pertussis*	$1X10^7$ CFU/mL	3/3	3/3

INHIBITION BY OTHER MICROORGANISMS

Simplexa™ Bordetella Direct was tested for the ability to identify Bordetella when potentially inhibitory organisms were present. The panel of ninety-seven (97) potentially inhibitory organisms was individually spiked into a pool with a low concentration (approximately 2X LoD) of *Bordetella pertussis* and *Bordetella parapertussis*. The organisms were spiked into the matrix at the concentrations indicated in Table 12. *Bordetella pertussis* and *Bordetella parapertussis* were tested separately (data not shown) and as a dual positive sample. Below are the results of the dual positive *Bordetella pertussis* and *Bordetella parapertussis* sample in Table 12. No inhibitory effects were observed for Simplexa™ Bordetella Direct at the concentrations tested.

Table 12. Summary of Results for the Microbial Inhibition Study *Bordetella pertussis* and *Bordetella parapertussis* at 2 X LoD

Organism	Concentration	*Bordetella pertussis* (IS481) % Detection (# Detected/# Tested)	*Bordetella parapertussis* (IS1001) % Detection (# Detected/# Tested)
Baseline	N/A	100% (45/45)	100% (45/45)
Acinetobacter baumannii	1×10^6 CFU/mL	100% (3/3)	100% (3/3)
Acinetobacter lwoffi	1×10^6 CFU/mL	100% (3/3)	100% (3/3)
Adenovirus 1	1×10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Adenovirus 31	1×10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Arcanobacterium haemolyticum	1×10^6 CFU/mL	100% (3/3)	100% (3/3)
Bacillus cereus	1×10^6 CFU/mL	100% (3/3)	100% (3/3)
Bacteroides fragilis	1×10^6 CFU/mL	100% (3/3)	100% (3/3)
Bordetella avium	1×10^6 CFU/mL	100% (3/3)	100% (3/3)
Bordetella bronchiseptica	1×10^6 CFU/mL	100% (3/3)	100% (3/3)
Bordetella hinzi	1×10^6 CFU/mL	100% (3/3)	100% (3/3)
Bordetella holmseii F061	1×10^6 CFU/mL	100% (3/3)	100% (3/3)
Bordetella petri	1×10^6 CFU/mL	100% (3/3)	100% (3/3)
Bordetella trematum	1×10^6 CFU/mL	100% (3/3)	100% (3/3)
Burkholderia cenocepacia	1×10^6 CFU/mL	100% (3/3)	100% (3/3)

Organism	Concentration	*Bordetella pertussis* (IS481) % Detection (# Detected/# Tested)	*Bordetella parapertussis* (IS1001) % Detection (# Detected/# Tested)
Burkholderia cepacia	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Burkholderia multivorans	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Burkholderia thailandensis	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Candida albicans	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Candida glabrata	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Chlamydia pneumoniae	1 x 10^6 IFU/mL	100% (3/3)	100% (3/3)
Chlamydia trachmomatis	1 x 10^6 IFU/mL	100% (3/3)	100% (3/3)
Citrobacter freundii	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Clostridium difficile	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Coronavirus 229E	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Coronavirus NL63*	1 x 10^4 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Coronavirus OC43	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Corynebacterium diptheriae	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Coxsackievirus A16	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Coxsackievirus B4	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Cytomegalovirus	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Echovirus 6	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Echovirus 7	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Echovirus 9	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Echovirus 11	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Enterobacter aerogenes Z052	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Enterobacter cloacae	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Enterococcus faecalis vanB	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Enterovirus 70	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Enterovirus 71	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Epstein-Barr Virus	1 x 10^5 copies/mL	100% (3/3)	100% (3/3)
Escherichia coli	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Fusobacterium necrophorum	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Haemophilus influenzae	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Haemophilus parainfluenzae	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
HSV-1 (MacIntyre)	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
HSV-2 (G)	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Influenza A/Swine/Iowa/15/30 H1N1*	1 x 10^4 $TCID_{50}$/mL	100% (3/3)	100% (3/3)

Organism	Concentration	*Bordetella pertussis* (IS481) % Detection (# Detected/# Tested)	*Bordetella parapertussis* (IS1001) % Detection (# Detected/# Tested)
Influenza B/Malaysia/2506/04*	1 x 10^4 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Klebsiella oxytoca	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Klebsiella pneumoniae	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Lactobacillus acidophilus	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Lactobacillus plantarum 17-5	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Legionella longbeachae	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Legionella pneumophila (Philadelphia)	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Listeria monocytogenes	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Measles	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Metapneumovirus-9	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Moraxella catarrhalis Ne 11	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Morganella morganii	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Mumps	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Mycobacterium avium	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Mycobacterium tuberculosis (genomic DNA)	1 x 10^6 genome copies/mL	100% (3/3)	100% (3/3)
Mycoplasma hominis	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Mycoplasma pneumoniae Strain M129	1 x 10^6 CCU/mL	100% (3/3)	100% (3/3)
Neisseria elongata	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Neisseria gonorrhoeae	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Neisseria meningitidis	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Neisseria mucosa	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Parainfluenza 1	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Parainfluenza 2	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Parainfluenza 3*	1 x 10^4 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Parainfluenza 4*	1 x 10^4 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Parvimonas micra	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Peptostreptococcus anaerobius	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Proteus mirabilis Z050	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Proteus vulgaris	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Pseudomonas aeruginosa	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Pseudomonas fluorescens	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Rhinovirus 1A*	1 x 10^4 $TCID_{50}$/mL	100% (3/3)	100% (3/3)

Organism	Concentration	*Bordetella pertussis* (IS481) % Detection (# Detected/# Tested)	*Bordetella parapertussis* (IS1001) % Detection (# Detected/# Tested)
RSV A	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
RSV B WV/14617/85	1 x 10^5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)
Serratia liquefaciens	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Serratia marcescens	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Staphylococcus aureus (MRSA)	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Staphylococcus epidermidis (MRSE)	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Stenotrophomonas maltophilia	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus anginosus	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus canis	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus dysgalactiae	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus intermedius	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus mitis	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus mutans	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus pneumoniae	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus pyogenes M1	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Streptococcus salivarius	1 x 10^6 CFU/mL	100% (3/3)	100% (3/3)
Ureaplasma urealyticum	1 x 10^6 CCU/mL	100% (3/3)	100% (3/3)
Varicella Zoster Virus*	1 x 10^4 $TCID_{50}$/mL	100% (3/3)	100% (3/3)

* The testing concentration of these viruses is lower due to the lack of a high titer stock.

CARRY-OVER CONTAMINATION

An amplification carry-over for the Simplexa™ assays has been assessed. The study was designed by alternately placing high positive and negative samples on each disc. No evidence of carry-over contamination was observed.