March 17, 2023

DiaSorin Molecular LLC
Tara Viviani
Sr. Director, Molecular Regulatory Affairs
11331 Valley View Street
Cypress, California 90630

Re: K220963
Trade/Device Name: Simplexa COVID-19 & Flu A/B Direct
Regulation Number: 21 CFR 866.3981
Regulation Name: Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The SARS-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test
Regulatory Class: Class II
Product Code: QOF, OOI
Dated: March 30, 2022
Received: April 1, 2022

Dear Tara Viviani:

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. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. 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) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

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 https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Himani Bisht -S

Himani Bisht, Ph.D.
Assistant Director
Viral Respiratory and HPV Branch
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Products Evaluation and Quality
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: 06/30/2023
See PRA Statement below.

510(k) Number *(if known)*
K220963

Device Name
Simplexa™ COVID-19 & Flu A/B Direct

Indications for Use *(Describe)*
The DiaSorin Molecular Simplexa™ COVID-19 & Flu A/B Direct is a real-time RT-PCR assay intended for use on the LIAISON® MDX instrument for the in vitro qualitative detection and differentiation of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus and influenza B virus in nasopharyngeal swabs (NPS) from individuals with signs and symptoms of respiratory tract infection.

The Simplexa™ COVID-19 & Flu A/B Direct assay is intended for use as an aid in the differential diagnosis of SARS-CoV-2, influenza A and influenza B infection.

Negative results do not preclude SARS-CoV-2, influenza A or influenza B infection and should not be used as the sole basis for patient management decisions. Positive results do not rule out coinfection with other organisms. Results should be combined with clinical observations, patient history, and epidemiological information.

The Simplexa™ COVID-19 & Flu A/B Direct assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

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	Tara Viviani Sr. Director Molecular Regulatory Affairs Tel. 562.240.6500 Tara.Viviani@DiaSorin.com
Summary Date	March 16, 2023
Proprietary Name	Simplexa™ COVID-19 & Flu A/B Direct
US Product Codes/Names and Regulation Numbers	QOF – Multi-Target Respiratory Specimen Nucleic Acid Test Including Sars-Cov-2 And Other Microbial Agents 21 CFR § 866.3981 OOI - Real Time Nucleic Acid Amplification System 21 CFR § 862.2570
Classification	Class II
Predicate Devices	BioFire Respiratory Panel 2.1 (RP2.1) (DEN200031)

Intended Use

Simplexa COVID-19 & Flu A/B Direct (Catalog Number: MOL4250):

The DiaSorin Molecular Simplexa™ COVID-19 & Flu A/B Direct is a real-time RT-PCR assay intended for use on the LIAISON® MDX instrument for the in vitro qualitative detection and differentiation of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus and influenza B virus in nasopharyngeal swabs (NPS) from individuals with signs and symptoms of respiratory tract infection.

The Simplexa™ COVID-19 & Flu A/B Direct assay is intended for use as an aid in the differential diagnosis of SARS-CoV-2, influenza A and influenza B infection.

Negative results do not preclude SARS-CoV-2, influenza A or influenza B infection and should not be used as the sole basis for patient management decisions. Positive results do not rule out coinfection with other organisms. Results should be combined with clinical observations, patient history, and epidemiological information.

The Simplexa™ COVID-19 & Flu A/B Direct assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Device Description

The Simplexa™ COVID-19 & Flu A/B Direct assay system is a real-time RT-PCR system that enables the direct amplification, detection and differentiation of SARS-CoV-2 RNA, human influenza A (Flu A) virus RNA and human influenza B (Flu B) virus RNA from unprocessed nasopharyngeal swabs (NPS) that have

not undergone nucleic acid extraction. The system consists of the Simplexa™ COVID-19 & Flu A/B Direct assay, the LIAISON® MDX (with LIAISON® MDX Studio Software), the Direct Amplification Disc and associated accessories.

In the Simplexa™ COVID-19 & Flu A/B Direct assay, fluorescent probes are used together with corresponding forward and reverse primers to amplify SARS-CoV-2, Flu A, Flu B and internal control RNA targets. For COVID-19 detection, the assay targets two different regions specific to the SARS-CoV-2 genome; the S gene which encodes the spike glycoprotein and the ORF1ab region which encodes well-conserved non-structural proteins and therefore is less susceptible to recombination. For Flu detection the assay targets conserved regions of influenza A viruses (matrix gene) and influenza B viruses (matrix gene). The assay provides three results; COVID-19 (ORF1ab and/or S gene detection), influenza A viruses (matrix gene detection) and influenza B viruses (matrix gene detection). An RNA internal control is used to detect RT-PCR failure and/or inhibition.

Simplexa™ COVID-19 &Flu A/B Direct REF MOL4250

Component Name	REF	EC Symbol on Label		Abbreviated Name	Cap Color	Number of Vials	Reactions per Vial/Kit	Volume per Vial
Simplexa™ COVID-19 & Flu A/B Direct Reaction Mix	MOL4251	REAG	C	RM	Blue	24	1/24	50 µL

Simplexa™ COVID-19 &Flu A/B Direct Components and Descriptions

Kit Component	Contents				
Simplexa™ COVID-19 & Flu A/B Direct Reaction Mix (RM)	DNA polymerase, Reverse Transcriptase, RNase inhibitor, buffer and dNTPs, encapsulated RNA Template, primers and dye-labeled fluorescent probes specific for detection of SARS-CoV-2, influenza A, influenza B and for the RNA Internal Control				
	Target	**Channel**	**Excitation**	**Emission**	**Targeted Gene**
	Flu A	520	445-505	507-533	matrix
	Flu B	560	505-543	547-573	matrix
	SARS-CoV-2 "COVID"	610	502-596	597-623	S and ORF1ab
	Internal Control "RNA IC"	690	622-658	652-708	N/A
Simplexa™ COVID-19 & Flu A/B Direct Barcode Card	Assay specific parameters, lot number, expiration date				

Materials Supplied Separately

Direct Amplification Disc Kit (REF MOL1455) Direct Amplification Discs for use on the LIAISON® MDX
Simplexa™ COVID-19 & Flu A/B Positive Control Pack (REF MOL2260)

Comparison to Predicate Device

Comparison to Predicate Device	Predicate Device BioFire Respiratory Panel 2.1 (RP2.1) (DEN200031)	Candidate Device: -
Product Code	QOF	QOF
Regulation Number	21 CFR 866.3981	21 CFR 866.3981
Organism Detected	Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2), Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, including subtypes H1, H1-2009, and H3, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus, Bordetella parapertussis (IS1001), Bordetella pertussis (ptxP), Chlamydia pneumoniae, and Mycoplasma pneumoniae	Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2), Influenza A (Flu A) and Influenza B (Flu B)
Measurand	DNA/RNA	RNA
Target	FilmArray Torch Technology SARS-CoV-1 Well conserved region of the S gene SARS-CoV-2 Well conserved region of the M gene	SARS-CoV-2 Well conserved regions of the S and ORF genes Flu A and B Targets Well conserved regions of the matrix gene (TAQ Man Technology)
Intended Use Kit	The BioFire Respiratory Panel 2.1 (RP2.1) is a PCR-based multiplexed nucleic acid test intended for use with the BioFire FilmArray 2.0 or BioFire FilmArray Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections, including COVID-19. The following organism types and subtypes are identified using the BioFire RP2.1: • Adenovirus, • Coronavirus 229E, • Coronavirus HKU1, • Coronavirus NL63, • Coronavirus OC43, • Severe Acute Respiratory Syndrome • Coronavirus (SARS-CoV-2), • Human Metapneumovirus, • Human Rhinovirus/Enterovirus, • Influenza A, including subtypes H1, H1-2009, and H3, • Influenza B, • Parainfluenza Virus 1, • Parainfluenza Virus 2, • Parainfluenza Virus 3, • Parainfluenza Virus 4, • Respiratory Syncytial Virus,	The DiaSorin Molecular Simplexa™ COVID-19 & Flu A/B Direct is a real-time RT-PCR assay intended for use on the LIAISON® MDX instrument for the *in vitro* qualitative detection and differentiation of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus and influenza B virus in nasopharyngeal swabs (NPS) from individuals with signs and symptoms of respiratory tract infection. The Simplexa™ COVID-19 & Flu A/B Direct assay is intended for use as an aid in the differential diagnosis of SARS-CoV-2, influenza A and influenza B infection. Negative results do not preclude SARS-CoV-2, influenza A or influenza B infection and should not be used as the sole basis for patient management decisions. Positive results do not rule out coinfection with other organisms; Results should be combined with clinical observations, patient history, and epidemiological information. The Simplexa™ COVID-19 & Flu A/B Direct assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.

Comparison to Predicate Device	Predicate Device BioFire Respiratory Panel 2.1 (RP2.1) (DEN200031)	Candidate Device: -
	• *Bordetella parapertussis* (IS1001), • *Bordetella pertussis* (ptxP), • *Chlamydia pneumoniae*, and • *Mycoplasma pneumoniae* Nucleic acids from the respiratory viral and bacterial organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection is indicative of the presence of the identified microorganism and aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the BioFire RP2.1 may not be the definite cause of disease. Additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.	
Automated System (Sample to Answer)	Automated	Same
Instrumentation	BioFire® FilmArray® 2.0 or BioFire® FilmArray® Torch Systems	LIAISON® MDX
Sample Types	Nasopharyngeal Swab (NPS)	Same

CLINICAL PERFORMANCE

The performance of Simplexa™ COVID-19 & Flu A/B Direct was evaluated using prospective, retrospective, archived positive and negative nasopharyngeal swab (NPS) specimens from human patients with signs and symptoms of respiratory tract infection. The clinical study was conducted using a total of over 1400 prospective and archived specimens in transport media. Prospective specimens from pediatric and adult patients (0 – 99 years) were collected from six (6) geographically diverse clinical sites within the United States between August 2021 and March 2022. The Simplexa™ COVID-19 & Flu A/B Direct clinical agreement testing was performed at two (2) external clinical sites and one (1) internal site. Additionally, retrospective archived specimens consisted of 82 positive influenza A, 114 influenza B, and 62 negative specimens blinded and randomized for the study. The comparator for influenza A and B targets was an FDA cleared NAAT. For the SARS-CoV-2, target three COVID-19 Emergency Use Authorized NAAT assays were used to establish a composite reference method (CRM). Two out of three positive results determined “Detected” CRM and two out of three negative results determined “Not Detected” CRM.

Table 2 shows the results of the Simplexa™ COVID-19 & Flu A/B Direct assay and comparator assay results in the prospective study analysis and Table 3 shows the results of the retrospective study analysis. The positive percent agreement (PPA) and negative percent agreement (NPA) were calculated for each target.

Table 2. Simplexa™ COVID-19 & Flu A/B Direct Prospective Study Analysis – Aug. 2021 to Mar. 2022 Collection

Simplexa™ COVID-19 & Flu A/B Direct Target	Positive Percent Agreement			Negative Percent Agreement		
	TP / (TP+FN)	PPA (%)	95% CI	TN / (TN+FP)	NPA (%)	95% CI
Influenza A	57/62[a]	91.9%	82.5% - 96.5%	1104/1106[b]	99.8%	99.3% - 100%
Influenza B	0/0	N/A	N/A	1165/1165	100%	99.7% - 100%
SARS-CoV-2	67/68[c]	98.5%	92.1% - 99.7%	417/428[d]	97.4%	95.5% - 98.6%

[a] Five (5) specimens were negative by an additional FDA cleared NAAT.
[b] Two (2) specimens were positive by an additional FDA cleared NAAT. One of the two specimens (1/2) was tested with PCR followed by BDS and was positive.
[c] One specimen was positive by an additional FDA cleared NAAT.
[d] Nine of the eleven specimens (9/11) were positive by an additional FDA cleared NAAT and four (4) were positive by PCR followed by BDS.
N/A = Not Applicable, PPA = Positive Percent Agreement, NPA = Negative Percent Agreement, CI = Confidence Interval. The 95% confidence intervals (CI) were calculated following the Wilson Score method.

Table 3. Simplexa™ COVID-19 & Flu A/B Direct Retrospective (Archived) Study Analysis

Simplexa™ COVID-19 & Flu A/B Direct Target	Positive Percent Agreement			Negative Percent Agreement		
	TP / (TP+FN)	PPA (%)	95% CI	TN / (TN+FP)	NPA (%)	95% CI
Influenza A	80/82	97.6%	91.5% - 99.3%	176/176	100%	97.9% - 100%
Influenza B	112/114	98.2%	93.8% - 99.5%	144/144	100%	97.4% - 100%
SARS-CoV-2	0/0	N/A	N/A	252/252	100%	98.5% -100%

N/A = Not Applicable, PPA = Positive Percent Agreement, NPA = Negative Percent Agreement, CI = Confidence Interval. The 95% confidence intervals (CI) were calculated following the Wilson Score method.

REPRODUCIBILITY

The reproducibility study was performed by one (1) internal site and two (2) external sites. The panel consisted of eight (8) reproducibility panel members, including six (6) contrived samples,one (1) negative sample [UTM as No Template Control (NTC)] and one (1) positive sample, Positive Control (PC). The contrived panel members were prepared by spiking each analyte at approximately two times the Limit of Detection (2x LoD, low positive) and approximately four times (4x) LoD (medium positive) into native negative nasopharyngeal swab matrix in UTM. Each panel member was tested in triplicate for five (5) days. Each site had two (2) operators who each assayed the entire panel once per day, for a total of two (2) sets of data per day. Agreement with expected results are presented in Table 4 with average Cts, standard deviation (SD) and coefficient of variation (%CV).

Table 4. Simplexa™ COVID-19 & Flu A/B Direct Reproducibility

Sample	Site 1		Site 2		Site 3		All Sites		
	Agreement with expected results	Avg. Ct ± SD (%CV)	Agreement with expected results	Avg. Ct ± SD (%CV)	Agreement with expected results	Avg. Ct ± SD (%CV)	Agreement with expected results	Avg. Ct ± SD (%CV)	95% CI
Flu A Hong Kong - LP	100.0% (30/30)	32.3 ± 0.54 (1.7%)	100.0% (30/30)	32.9 ± 1.22 (3.7%)	100.0% (30/30)	32.8 ± 0.55 (1.7%)	100.0% (90/90)	32.7 ± 0.86 (2.6%)	95.9% to 100.0%
Flu A Hong Kong - MP	100.0% (30/30)	31.7 ± 0.49 (1.5%)	100.0% (30/30)	31.5 ± 0.43 (1.4%)	100.0% (30/30)	31.9 ± 0.44 (1.4%)	100.0% (90/90)	31.7 ± 0.47 (1.5%)	95.9% to 100.0%
Flu B Malaysia - LP	100.0% (30/30)	30.8 ± 0.69 (2.2%)	100.0% (30/30)	30.9 ± 0.60 (1.9%)	100.0% (30/30)	31.5 ± 0.62 (2.0%)	100.0% (90/90)	31.1 ± 0.70 (2.2%)	95.9% to 100.0%
Flu B Malaysia - MP	100.0% (30/30)	30.0 ± 0.35 (1.2%)	100.0% (30/30)	29.9 ± 0.44 (1.5%)	100.0% (30/30)	30.4 ± 0.48 (1.6%)	100.0% (90/90)	30.1 ± 0.48 (1.6%)	95.9% to 100.0%

Sample	Site 1		Site 2		Site 3		All Sites		
	Agreement with expected results	Avg. Ct ± SD (%CV)	Agreement with expected results	Avg. Ct ± SD (%CV)	Agreement with expected results	Avg. Ct ± SD (%CV)	Agreement with expected results	Avg. Ct ± SD (%CV)	95% CI
COVID-19 USA-WA1 - LP	100.0% (30/30)	30.2 ± 0.41 (1.4%)	100.0% (30/30)	29.5 ± 0.40 (1.4%)	100.0% (30/30)	30.2 ± 0.67 (2.2%)	100.0% (90/90)	30.0 ± 0.59 (2.0%)	95.9% to 100.0%
COVID-19 USA-WA1 - MP	100.0% (30/30)	29.0 ± 0.46 (1.6%)	100.0% (30/30)	28.8 ± 0.45 (1.6%)	100.0% (30/30)	29.4 ± 0.61 (2.1%)	100.0% (90/90)	29.1 ± 0.56 (1.9%)	95.9% to 100.0%
Positive Control (PC)	100.0% (30/30)	26.2 ± 0.32 (1.2%)	100.0% (30/30)	26.1 ± 0.21 (0.8%)	100.0% (30/30)	26.0 ± 0.20 (0.8%)	100.0% (90/90)	26.1 ± 0.26 (1.0%)	95.9% to 100.0%
		27.6 ± 0.87 (3.2%)		28.2 ± 0.39 (1.4%)		27.1 ± 0.22 (0.8%)		27.6 ± 0.71 (2.6%)	
		27.8 ± 1.18 (4.2%)		27.2 ± 0.23 (0.9%)		26.8 ± 0.22 (0.8%)		27.3 ± 0.80 (2.9%)	
Negative (UTM)	100.0% (30/30)	0.0 ± 0.00 (N/A%)	100.0% (30/30)	0.0 ± 0.00 (N/A%)	100.0% (30/30)	0.0 ± 0.00 (N/A%)	100.0% (90/90)	0.0 ± 0.00 (N/A%)	95.9% to 100.0%
		0.0 ± 0.00 (N/A%)		0.0 ± 0.00 (N/A%)		0.0 ± 0.00 (N/A%)		0.0 ± 0.00 (N/A%)	
		0.0 ± 0.00 (N/A%)		0.0 ± 0.00 (N/A%)		0.0 ± 0.00 (N/A%)		0.0 ± 0.00 (N/A%)	
Total	100.0% (240/240)		100.0% (240/240)		100.0% (240/240)		100.0% (720/720)		99.5% to 100.0%

Ct. = Cycle threshold
SD = Standard Deviation
%CV = Percent Coefficient of Variation
CI = Confidence Interval
LP = Low Positive
MP = Medium Positive

ANALYTICAL SENSITIVITY/LIMIT OF DETECTION

The Limit of Detection (LoD) of the Simplexa™ COVID-19 & Flu A/B Direct assay in nasopharyngeal swabs (NPS) was determined to be the lowest detectable concentration of quantitated inactivated titered viral stocks (copies/mL or International Units/mL) at which ≥ 95% of all replicates were detected. Two (2) strains of influenza A, two (2) strains of influenza B and two (2) strains SARS-CoV-2 serially diluted in negative nasopharyngeal swab (NPS) matrix were used to determine the LoD. The LoD results are shown in Table 5.

Table 5. Simplexa™ COVID-19 & Flu A/B Direct Limit of Detection

Virus Strain	LoD (copies/mL)
Influenza A/Hong Kong/8/68	500
Influenza A/Michigan/45/2015	500
Influenza B/Phuket/3073/2013	750
Influenza B/Malaysia/2506/2004	250
SARS-CoV-2 (USA-WA1/2020)	500
Virus Strain	**IU/mL**
WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146)	651

ANALYTICAL REACTIVITY

Analytical Reactivity – Influenza A and B and SARS-CoV-2 (Wet-testing)

Analytical reactivity was evaluated with nasopharyngeal swab (NPS) matrix for the Simplexa™ COVID-19 & Flu A/B Direct assay. A total of 63 Flu A strains, 21 Flu B strains and five (5) SARS-CoV-2 strains were tested. Quantified viral material was spiked into negative NPS matrix at the concentrations listed in Tables 6-8 below and assayed in triplicate. The results are shown in Tables 6-8. Additional testing of all influenza strains in the CDC panels for 2018-2021 was performed. The results are also shown in Tables 6-7 and the CDC panels tested highlighted in Tables 9 - 11. All strains and subtypes were 100% detected with the Simplexa™ COVID-19 & Flu A/B Direct assay.

Table 6. Simplexa™ COVID-19 & FLU A/B Direct Analytical Reactivity Results - Flu A

Organism	Tested Concentration*	% Detected
A/Anhui/1/2013	1:100,000 Dilution	100.0% (3/3)
A/black-legged kittiwake/Quebec/02838-1/2009	100 $CEID_{50}$/mL	100.0% (3/3)
A/Brisbane/02/2018	100 EID_{50}/mL	100.0% (3/3)
A/Brisbane/10/07	100 $TCID_{50}$/mL	100.0% (3/3)
A/Brisbane/59/07	100 $TCID_{50}$/mL	100.0% (3/3)
A/American green-winged teal/Mississippi/300/2010	100 $CEID_{50}$/mL	100.0% (3/3)
A/California/02/2014	100 $TCID_{50}$/mL	100.0% (3/3)
A/California/4/2009	100 $TCID_{50}$/mL	100.0% (3/3)
A/California/7/2009	100 $TCID_{50}$/mL	100.0% (3/3)
A/chicken/Germany/N/49	100 $CEID_{50}$/mL	100.0% (3/3)
A/chicken/Vietnam/NCVD-016/2008(H5N1)-PR8-IDCDC-RG12	1:100,000 Dilution	100.0% (3/3)
A/Christ Church/16/2010	1,000 EID_{50}/mL	100.0% (3/3)
A/duck/Chabarovsk/1610/1972	100 $CEID_{50}$/mL	100.0% (3/3)

Organism	Tested Concentration*	% Detected
A/duck/Czechoslovakia/1956	5,000 $CEID_{50}$/mL	100.0% (3/3)
A/duck/Wisconsin/480/1979	100 $CEID_{50}$/mL	100.0% (3/3)
A/Egypt/N03072/2010(H5N1)-PR8-IDCDC-RG29	1:100,000 Dilution	100.0% (3/3)
A/Guangdong-Maonan/1536/2019	100 EID_{50}/mL	100.0% (3/3)
A/Hawaii/15/2001	100 $CEID_{50}$/mL	100.0% (3/3)
A/Hong Kong/2671/2019	100 EID_{50}/mL	100.0% (3/3)
A/Hong Kong/4801/2014	100 TCID50/mL	100.0% (3/3)
A/Hong Kong/33982/2009(H9N2)-PR8-IDCDC_RG26	100 $CEID_{50}$/mL	100.0% (3/3)
A/Hubei/1/2010(H5N1)-PR8-IDCDC-RG30	1:100,000 Dilution	100.0% (3/3)
A/India/NIV/2006(H5N1)-PR8-IBCDC-RG7	1:100,000 Dilution	100.0% (3/3)
A/Indiana/08/2011	100 TCID50/mL	100.0% (3/3)
A/Kansas/14/2017	100 EID_{50}/mL	100.0% (3/3)
A/mallard/Netherlands/12/2000(H7N7)/PR8-IBCDC-1	1:100,000 Dilution	100.0% (3/3)
A/mallard/Illinois/10OS4334/2010	100 $CEID_{50}$/mL	100.0% (3/3)
A/mallard/Wisconsin/4218/2009	100 $CEID_{50}$/mL	100.0% (3/3)
A/mallard/Wisconsin/4230/2009	100 $CEID_{50}$/mL	100.0% (3/3)
A/Massachusetts/15/2013	100 $CEID_{50}$/mL	100.0% (3/3)
A/Mexico/4108/2009	100 $CEID_{50}$/mL	100.0% (3/3)
A/Minnesota/11/2010	100 $CEID_{50}$/mL	100.0% (3/3)
A/Minnesota/19/2011	100 $CEID_{50}$/mL	100.0% (3/3)
A/New Caledonia/20/99	100 $TCID_{50}$/mL	100.0% (3/3)
A/New York/18/2009	100 $CEID_{50}$/mL	100.0% (3/3)
A/New York/55/2004	100 $CEID_{50}$/mL	100.0% (3/3)
A/NY/02/09	100 $TCID_{50}$/mL	100.0% (3/3)
A/Ohio/02/2012	100 $CEID_{50}$/mL	100.0% (3/3)
A/Perth/16/2009	100 EID_{50}/mL	100.0% (3/3)
A/pheasant/New Jersey/1355/1998(H5N2)-PR8-IBCDC-4	1:100,000 Dilution	100.0% (3/3)
A/Port Chalmers/1/1973	100 $TCID_{50}$/mL	100.0% (3/3)
A/PR/8/34	100 $TCID_{50}$/mL	100.0% (3/3)
A/quail/Italy/1117/1965	100 $CEID_{50}$/mL	100.0% (3/3)
A/red knot/Delaware Bay/240/1994	100 $CEID_{50}$/mL	100.0% (3/3)
A/red knot/Delaware/541/1988	1,000 $CEID_{50}$/mL	100.0% (3/3)
A/redhead/Alberta/192/2002	100 $CEID_{50}$/mL	100.0% (3/3)
A/Rhode Island/01/2010	100 $CEID_{50}$/mL	100.0% (3/3)

Organism	Tested Concentration*	% Detected
A/Santiago/7981/2006	100 $CEID_{50}$/mL	100.0% (3/3)
A/shorebird/Delaware Bay/211/1994	1,000 $CEID_{50}$/mL	100.0% (3/3)
A/shorebird/Delaware/172/2006	1,000 $CEID_{50}$/mL	100.0% (3/3)
A/Singapore/INFIMH-16-0019/2016	100 $TCID_{50}$/mL	100.0% (3/3)
A/Solomon Island/3/2006	100 $TCID_{50}$/mL	100.0% (3/3)
A/Swine/1976/31	100 $TCID_{50}$/mL	100.0% (3/3)
A/Swine/Iowa/15/30	100 $TCID_{50}$/mL	100.0% (3/3)
A/swine/Ohio/09SW1477/2009	100 $TCID_{50}$/mL	100.0% (3/3)
A/swine/Ohio/09SW83E/2009	100 $CEID_{50}$/mL	100.0% (3/3)
A/Switzerland/9715293/2013	100 $CEID_{50}$/mL	100.0% (3/3)
A/Taiwan/42/06	100 $TCID_{50}$/mL	100.0% (3/3)
A/turkey/Massachusetts/3740/1965	2,000 $CEID_{50}$/mL	100.0% (3/3)
A/turkey/Virginia/4529/2002 (H7N2)xPR8-IBCDC-5	1:100,000 Dilution	100.0% (3/3)
A/Texas/50/2012	100 $TCID_{50}$/mL	100.0% (3/3)
A/Wisconsin/67/05	100 $TCID_{50}$/mL	100.0% (3/3)
A/WS/33	100 $TCID_{50}$/mL	100.0% (3/3)

*$TCID_{50}$/mL = Tissue Culture Infectious Dose
$CEID_{50}$/mL = Chicken Embryo Infectious Dose
EID_{50}/mL = Egg Infectious Dose

Table 7. Simplexa™ COVID-19 & Flu A/B Direct Analytical Reactivity Results - Flu B

Organism	Tested Concentration*	% Detection
B/Brisbane/33/2008	100 $CEID_{50}$/mL	100% (3/3)
B/Brisbane/60/2008	100 $TCID_{50}$/mL	100% (3/3)
B/Christchurch/33/2004	100 $TCID_{50}$/mL	100% (3/3)
B/Colorado/06/2017	100 $TCID_{50}$/mL	100% (3/3)
B/Florida/02/2006	100 $TCID_{50}$/mL	100% (3/3)
B/Florida/04/2006	100 $TCID_{50}$/mL	100% (3/3)
B/Florida/07/04	100 $TCID_{50}$/mL	100% (3/3)
B/Great Lakes/1739/54	100 $TCID_{50}$/mL	100% (3/3)
B/Guangdong-Liwan/1133/2014	1,000 $CEID_{50}$/mL	100% (3/3)
B/Maryland/1/59	100 $TCID_{50}$/mL	100% (3/3)

Organism	Tested Concentration*	% Detection
B/Massachusetts/02/2012	100 $TCID_{50}$/mL	100% (3/3)
B/Michigan/09/2011	100 EID_{50}/mL	100% (3/3)
B/Nevada/03/2011	100 $CEID_{50}$/mL	100% (3/3)
B/New Hampshire/01/2016	100 EID_{50}/mL	100% (3/3)
B/Panama/45/90	100 $TCID_{50}$/mL	100% (3/3)
B/Texas/02/2013	100 $TCID_{50}$/mL	100% (3/3)
B/Texas/81/2016	100 EID_{50}/mL	100% (3/3)
B/Utah/09/2014	100 $CEID_{50}$/mL	100% (3/3)
B/Victoria/304/2006	100 $CEID_{50}$/mL	100% (3/3)
B/Washington/02/2019	100 EID_{50}/mL	100% (3/3)
B/Wisconsin/01/2010	100 $CEID_{50}$/mL	100% (3/3)

*$TCID_{50}$/mL = Tissue Culture Infectious Dose
$CEID_{50}$/mL = Chicken Embryo Infectious Dose
EID_{50}/mL = Egg Infectious Dose

Table 8. Simplexa™ COVID-19 & Flu A/B Direct Analytical Reactivity Results – SARS-CoV-2

Organism	Tested Concentration*	% Detection
England/204820464/2020	1000 copies/mL	100% (3/3)
hCoV19/USA/PHC658/2021	1500 copies/mL	100% (3/3)
HongKong/VM200001061/2020	1000 copies/mL	100% (3/3)
Japan/TY7-503/2021	1000 copies/mL	100% (3/3)
South Africa/KRISP-EC-K005325/2020	1000 copies/mL	100% (3/3)

Table 9 2018-2019 CDC panel Flu A and Flu B Strains Tested with Simplexa™ COVID-19 & Flu A/B Direct

Virus	Subtype	Organism
Flu A	A (H3N2)	A/Perth/16/2009
		A/Singapore/INFIMH-16-0019/2016*
	A (H1N1) pdm09	A/California/07/2009
		A/Michigan/45/2015*
Flu B	B (Victoria lineage)	B/Brisbane/60/2008
		B/Colorado/06/2017*
	B (Yamagata lineage)	B/Wisconsin/01/2010
		B/Phuket/3073/2013*

*WHO recommended vaccine strains

Table10. 2019-2020 CDC panel Flu A and Flu B Strains Tested with Simplexa™ COVID-19 & Flu A/B Direct

Virus	Subtype	Organism
Flu A	A (H3N2)	A/Perth/16/2009
		A/Kansas/14/2017*
	A (H1N1) pdm09	A/Christ Church/16/2010
		A/Brisbane/02/2018*
Flu B	B (Victoria lineage)	B/Michigan/09/2011
		B/Colorado/06/2017*
	B (Yamagata lineage)	B/New Hampshire/01/2016
		B/Phuket/3073/2013*

*WHO recommended vaccine strains

Table 11. 2020-2021 CDC panel Flu A and Flu B Strains Tested with Simplexa™ COVID-19 & Flu A/B Direct

Virus	Subtype	Organism
Flu A	A (H3N2)	A/Perth/16/2009
		A/Hong Kong/2671/2019*
	A (H1N1) pdm09	A/Christ Church/16/2010
		A/Guangdong-Maonan/1536/2019*
Flu B	B (Victoria lineage)	B/Michigan/09/2011
		B/Washington/02/2019*
	B (Yamagata lineage)	B/Texas/81/2016
		B/Phuket/3073/2013*

*WHO recommended vaccine strain

Analytical Reactivity/Inclusivity – SARS-CoV-2

An *in silico* inclusivity analysis of the SARS-CoV-2 target primers and probes in the Simplexa™ COVID-19 & Flu A/B Direct assay was performed. All primer and probe sets designed for detection of the ORF1ab and S gene were tested against the complete SARS-CoV-2 genome sequences available in the GISAID database submitted from November 01, 2021 to January 31, 2022. The analysis included 2,170,584 sequences in the amplicon regions of the ORF1ab and S gene primer/probe regions. Only target sequences with full coverage of all three ORF1ab and S gene forward and reverse primer as well as probe region were included in the analyses. The analysis showed that the Simplexa™ COVID-19 & Flu A/B Direct target regions had no mismatch to 2,170,382 sequences (~99.99%) and those sequences were predicted to be detected by the assay based on sequence homology. There were 202 sequences (~0.01%) with mismatches in at least one primer or probe binding region, region in either ORF1ab or S gene target region. A melting temperature (Tm) analysis was conducted for those sequences with mismatches in the binding sites of both gene assay target regions. A Tm calculation was performed with assay-specific conditions using a Tm Mismatch Bioinformatics Tool. Tm values observed above their respective annealing temperature had mismatches that were not located at the 3' end for the primers; as such, detection of these sequences are not affected by the mismatches.

In addition, an analysis was conducted for the oligonucleotide (oligo) sequences for the SARS-CoV-2 ORF1ab and S gene sets against all SARS-CoV-2 sequences available in the GISAID database submitted from May 01, 2022 to July 31, 2022. In this sequence set, there were 211,184 sequences (~99.98%) with no mismatches in at least one gene oligo set and 40 (~0.02%) sequences with mismatches in at least one oligo binding region for both gene oligo sets. Based on *in silico* analysis of the percent homology between assay oligos and target sequences, potential impact of location of the mismatches on extension and/or binding, and the mismatch Tm values of the oligo sequence to its binding region on each analyzed SARS-CoV-2 sequence, it is predicted that the assay will also detect 100% of theses 211,224 SARS-CoV-2 sequences available in the database from May 01, 2022 to July 31, 2022. A more recent *in silico* inclusivity analysis of the oligonucleotide (oligo) sequences for the SARS-CoV-2 ORF1ab and S gene sets was performed against all SARS-CoV-2 sequences submitted to the GISAID EpiCoV database from November 01, 2022 to February 08, 2023. The analysis as described above in previous studies, showed that the Simplexa™ COVID-19 & Flu A/B Direct target is predicted to detect the additional 12,378 sequences (100%) analyzed during this period. Table 12 below summarizes the Tm analysis results from the studies.

Table 12. Summary of Tm Analysis Results

No. seq. where at least one gene oligo set meets Tm criteria	2,394,186
No. seq. where no gene oligo set meets Tm criteria	0

Cross-Reactivity (Analytical Specificity)

Cross-reactivity of the Simplexa™ COVID-19 & Flu A/B Direct assay was evaluated by testing whole organisms or purified nucleic acid from other organisms. Specimens for laboratory testing were prepared by spiking cultured isolates/inactivated organisms/purified nucleic acids (whole genome) into negative matrix (NPS) and determining cross reactivity based on three replicates. Results from cross-reactivity testing are summarized in Table 13.

Table 13. Simplexa™ COVID-19 & Flu A/B Direct Cross-Reactivity Results

Organism	Tested Concentration[1]	SARS-CoV-2	Flu A	Flu B
Adenovirus Type 1	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Adenovirus Type 7A	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Bordetella pertussis	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Candida albicans	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Chlamydia pneumoniae	1 x 10^6 IFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)

Organism	Tested Concentration[1]	SARS-CoV-2	Flu A	Flu B
Corynebacterium diphtheriae	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Coxiella burnetii	1 x 10^6 genome copies/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Cytomegalovirus	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Enterovirus Type 68	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Enterovirus Type 71	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Epstein-Barr Virus	1 x 10^5 copies/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Escherichia coli O157:H7	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Haemophilus influenzae	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Human Coronavirus 229E*	1 x 10^4 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Human Coronavirus NL63*	1 x 10^4 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Human Coronavirus OC43	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Human Metapneumovirus 9*	1 x 10^4 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Lactobacillus plantarum,17-5	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Legionella longbeachae	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Legionella pneumophila	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Leptospira interrogans	1 x 10^6 copies/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Measles	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
MERS-Coronavirus	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Moraxella catarrhalis	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Mumps	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Mycobacterium tuberculosis Genomic DNA	1 x 10^6 copies/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Mycoplasma pneumoniae	1 x 10^6 CCU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Neisseria elongata	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Neisseria meningitidis	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)

Organism	Tested Concentration[1]	SARS-CoV-2	Flu A	Flu B
Parainfluenza Type 1	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Parainfluenza Type 2	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Parainfluenza Type 3	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Parainfluenza Type 4	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Parechovirus Type 3	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Pseudomonas aeruginosa	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Rhinovirus 1A*	1 x 10^4 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
RSV-A	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
RSV-B	1 x 10^5 $TCID_{50}$/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Staphylococcus aureus	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Staphylococcus epidermidis	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Streptococcus pneumoniae	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Streptococcus pyogenes	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Streptococcus salivarius	1 x 10^6 CFU/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Human Coronavirus RNA HKU1	1 x 10^5 genome copies/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Human Genomic DNA (Leukocytes)	1 x 10^6 cells/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
Pooled Human Nasal Wash	1:1 dilution	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)
SARS-COV1 Synthetic RNA	1 x 10^5 genome copies/mL	0.0% (0/3)	0.0% (0/3)	0.0% (0/3)

*A lower concentration was tested due to inability to obtain stock material with high titer

[1]CCU/mL = Color changing units/milliliter

CFU/mL= Colony forming units/milliliter

IFU/mL = Infectious units/milliliter

$TCID_{50}$/mL = Tissue Culture Infectious Dose

INTERFERING SUBSTANCES

Potentially interfering substances from respiratory specimens were tested for ability to generate false negative results. Samples were prepared by spiking each potentially interfering substance into a baseline sample consisting of pooled negative nasopharyngeal swab specimens and SARS-CoV-2 inactivated viral particles (2019-nCoV/USA-WA1/2020 strain), Influenza A/Hong Kong/8/68 and Influenza B/Malaysia/2506/2004. The test samples contained each of the three

(3) viruses at a concentration of 3X LoD. The results are shown in Table 14. Cold Eeze at a higher concentration of 2.5% (w/v) showed an invalid result in one out of the three replicates for Flu B. The replicates were retested at a lower concentration of 1.25% (w/v) and no interference was observed in any of the targets. No other substances tested in the table below showed any interference with the detection of SARS-CoV-2, influenza A or influenza B at the concentrations tested. The FluMist nasal vaccine was not tested as an interfering substance due to its unavailability at the time of this study.

Table 14. Simplexa™ COVID-19 & Flu A/B Direct – Potentially Interfering Substances Results

Potentially Interfering Substance	Active Ingredient	Tested Concentration*	SARS-CoV-2	Flu A	Flu B
Afrin Nasal spray	Oxymetazoline	15% (v/v)	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Antibacterial, systemic	Tobramycin	4 µg/mL	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Antibiotic, nasal ointment	Mupirocin	6.6 mg/mL	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Whole Blood	N/A	2% (v/v)	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Cold Eeze (Throat lozenges, Oral anesthetic and analgesic)	N/A	2.5% (w/v)	100.0% (4/4)	100.0% (4/4)	100.0% (2/2)*
		1.25% (w/v)	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Nasal corticosteroid (Beconase AQ)	Beclomethasone	5% (v/v)	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Nasal corticosteroid (Flonase)	Fluticasone	5% (v/v)	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Relenza Antiviral Drug	Zanamivir	3.3 mg/mL	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Tamiflu Antiviral drug	Oseltamivir	1 µM	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Zicam Nasal Gel	*Luffa operculata, Galphimia glauca*, histaminum hydrochloricum	5% (w/v)	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Zicam Nasal Spray (Homeopathic allergy relief medicine)	N/A	10% (v/v)	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)
Bovine submaxillary gland mucin, type I-S**	Purified Mucin Protein	5mg/mL	100.0% (3/3)	100.0% (3/3)	100.0% (3/3)

*µg/mL = Micrograms/milliliter
mg/mL = Milligrams/milliliter
µM = Micromolar
v/v = Volume per Volume
w/v = Weight/Volume
*2.5% Cold Eeze Throat lozenges resulted in one out of three replicates with an EC500, invalid, for SARS-CoV-2. An EC500 result was obtained on the repeat. Therefore the concentration was lowered to 1.25% and retested.
**Mucin tested in a separate study using Influenza strains (Influenza A/Michigan/45/2015 and Influenza B/Phuket/3073/2013)

COMPETITIVE INTERFERENCE

Competitive Interference was performed to assess the ability of the assay to detect low concentration of one (1) target analyte in the presence of high concentration of another target analyte. Samples were prepared by spiking one (1) assay target analyte at a low concentration (4X LoD) into negative nasopharyngeal swab (NPS) matrix in the presence of a high concentration (1000X LoD) of one (1) of the other two (2) assay target analytes. All the possible assay target combinations were tested. Each contrived sample was tested in triplicate. The results are shown in Table 15. All of the combinations tested showed no competitive interference for the detection of low concentrations of SARS-CoV-2, Flu A or Flu B in the presence of high concentrations of another assay target analyte.

Table 15. Simplexa™ COVID-19 & Flu A/B Direct – Competitive Interference Results

Low Positive Baseline Sample		Competitive Interferent		% Detection		
Strain	Copies/ mL	Strain	Copies/mL	Flu A	Flu B	SARS-CoV-2
Influenza A/Hong Kong/8/68	2000	Influenza B/ Phuket/3073/2013	7.5×10^5	100%	100%	0.0%
		COVID-19/USA-WA1/2020	5.0×10^5	100%	0.0%	100%
Influenza B/ Phuket/3073/2013	3000	Influenza A/Hong Kong/8/68	5.0×10^5	100%	100%	0.0%
		COVID-19/USA-WA1/2020	5.0×10^5	0.0%	100%	100%
COVID-19/USA-WA1/2020	2000	Influenza A/Hong Kong/8/68	5.0×10^5	100%	0.0%	100%
		Influenza B/ Phuket/3073/2013	7.5×10^5	0.0%	100%	100%

INTERFERENCE BY OTHER MICROORGANISMS

The Simplexa™ COVID-19 & Flu A/B Direct assay was evaluated by testing the ability to identify SARS-CoV-2, influenza A virus, and influenza B virus, when other potentially interfering organisms were present. Specimens were prepared by spiking cultured isolates/inactivated organisms/purified nucleic acids (whole genome) at a minimum of 10^6 CFU/mL (or higher) for bacteria, and 10^5 $TCID_{50}$/mL or PFU/mL (or higher) for viruses into negative nasopharyngeal swab (NPS) matrix in the presence of a low concentration (2X LoD) of the three (3) targets (SARS-CoV-2, Flu A and Flu B viral particles) and determining microbial interference based on three (3) replicates. For organisms not titered in CFU/mL or $TCID_{50}$/mL, other industry acceptable units were used as indicated. The panel of forty seven (47) potentially inhibitory organisms was individually spiked into a pool with a low concentration influenza A (Influenza A/Hong Kong/8/68), influenza B (Influenza B/Malaysia/2506/2004), and SARS-CoV-2 (2019-nCoV/USA-WA1/2020). No interference by other organisms was observed for SARS-CoV-2, influenza A or influenza B at the concentrations indicated in Table 16.

Table 16. Simplexa™ COVID-19 & Flu A/B Direct – Microbial Interference Results

Organism	Tested Concentration[1]	% Detection		
		SARS-CoV-2	Flu A	Flu B
Adenovirus Type 1	1×10^5 $TCID_{50}$/mL	100%	100%	100%
Adenovirus Type 7A	1×10^5 $TCID_{50}$/mL	100%	100%	100%
Bordetella pertussis	1×10^6 CFU/mL	100%	100%	100%
Candida albicans	1×10^6 CFU/mL	100%	100%	100%
Chlamydia pneumoniae	1×10^6 IFU/mL	100%	100%	100%
Corynebacterium diphtheriae	1×10^6 CFU/mL	100%	100%	100%
Coxiella burnetii	1×10^6 genome copies/mL	100%	100%	100%
Cytomegalovirus	1×10^5 $TCID_{50}$/mL	100%	100%	100%
Enterovirus Type 68	1×10^5 $TCID_{50}$/mL	100%	100%	100%

Organism	Tested Concentration[1]	% Detection		
		SARS-CoV-2	Flu A	Flu B
Enterovirus Type 71	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Epstein-Barr Virus	1 x 10^5 copies/mL	100%	100%	100%
Escherichia coli O157:H7	1 x 10^6 CFU/mL	100%	95%	100%
Haemophilus influenzae	1 x 10^6 CFU/mL	100%	100%	100%
Human Coronavirus 229E*	1 x 10^4 $TCID_{50}$/mL	100%	100%	100%
Human Coronavirus NL63*	1 x 10^4 $TCID_{50}$/mL	100%	100%	100%
Human Coronavirus OC43	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Human Metapneumovirus 9*	1 x 10^4 $TCID_{50}$/mL	100%	100%	100%
Lactobacillus plantarum,17-5	1 x 10^6 CFU/mL	100%	100%	100%
Legionella longbeachae	1 x 10^6 CFU/mL	100%	100%	100%
Legionella pneumophila	1 x 10^6 CFU/mL	100%	100%	100%
Leptospira interrogans	1 x 10^6 copies/mL	100%	100%	100%
Measles	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
MERS-Coronavirus	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Moraxella catarrhalis	1 x 10^6 CFU/mL	100%	100%	100%
Mumps	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Mycobacterium tuberculosis Genomic DNA	1 x 10^6 copies/mL	100%	100%	100%
Mycoplasma pneumoniae	1 x 10^6 CCU/mL	100%	100%	100%
Neisseria elongata	1 x 10^6 CFU/mL	100%	100%	100%
Neisseria meningitidis	1 x 10^6 CFU/mL	100%	100%	100%
Parainfluenza Type 1	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Parainfluenza Type 2	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Parainfluenza Type 3	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Parainfluenza Type 4	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Parechovirus Type 3	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
Pseudomonas aeruginosa	1 x 10^6 CFU/mL	100%	100%	100%
Rhinovirus 1A*	1 x 10^4 $TCID_{50}$/mL	95%	100%	100%
RSV-A	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%
RSV-B	1 x 10^5 $TCID_{50}$/mL	100%	100%	100%

Organism	Tested Concentration[1]	% Detection		
		SARS-CoV-2	Flu A	Flu B
Staphylococcus aureus	1 x 10^6 CFU/mL	100%	100%	100%
Staphylococcus epidermidis	1 x 10^6 CFU/mL	100%	100%	100%
Streptococcus pneumoniae	1 x 10^6 CFU/mL	100%	100%	100%
Streptococcus pyogenes	1 x 10^6 CFU/mL	100%	100%	100%
Streptococcus salivarius	1 x 10^6 CFU/mL	100%	100%	100%
Human Coronavirus RNA HKU1	1 x 10^5 genome copies/mL	100%	100%	100%
Human Genomic DNA (Leukocytes)	1 x 10^6 cells/mL	100%	100%	100%
Pooled Human Nasal Wash	1:1 dilution	100%	100%	100%
SARS-COV1 Synthetic RNA	1 x 10^5 genome copies/mL	100%	100%	100%

*A lower concentration was tested due to inability to obtain stock material with high titer
[1]CCU/mL = Color Changing Units/milliliter
CFU/mL = Colony Forming Units/milliliter
IFU/mL = Infectious units/milliliter

$TCID_{50}$/mL = Tissue Culture Infectious Dose/milliliter

CARRY-OVER CONTAMINATION

Amplification carry-over for the Simplexa™ assays has been assessed against existing assays that use the same sample matrices, workflow and specimen type, and therefore no carry-over is anticipated. The study was designed by alternately placing high positive and negative samples on each disc. No evidence of carry-over contamination was observed.

CONCLUSION

The analytical and method comparison studies have demonstrated that the Simplexa™ COVID-19 & Flu A/B Direct is substantially Equivalent to the predicate device (DEN200031). The device labeling is compliant with 21 CFR § 809.10.