October 30, 2025

Diasorin Molecular, LLC
Michael Treas
Senior Regulatory Affairs Associate
11331 Valley View Street
Cypress, California 90630

Re: K252387
Trade/Device Name: Simplexa COVID-19/ Flu A/B & RSV Direct (MOL4450); Simplexa COVID-19/ Flu A/B & RSV Positive Control Pack (MOL4460)
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
Dated: July 30, 2025
Received: July 31, 2025

Dear Michael Treas:

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 (the 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 available 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.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device" (https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

Your device is also subject to, among other requirements, the Quality System (QS) regulation (21 CFR Part 820), which includes, but is not limited to, 21 CFR 820.30, Design controls; 21 CFR 820.90, Nonconforming product; and 21 CFR 820.100, Corrective and preventive action. Please note that regardless of whether a change requires premarket review, the QS regulation requires device manufacturers to review and approve changes to device design and production (21 CFR 820.30 and 21 CFR 820.70) and document changes and approvals in the device master record (21 CFR 820.181).

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 Part 803) for devices or postmarketing safety reporting (21 CFR Part 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 Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

All medical devices, including Class I and unclassified devices and combination product device constituent parts are required to be in compliance with the final Unique Device Identification System rule ("UDI Rule"). The UDI Rule requires, among other things, that a device bear a unique device identifier (UDI) on its label and package (21 CFR 801.20(a)) unless an exception or alternative applies (21 CFR 801.20(b)) and that the dates on the device label be formatted in accordance with 21 CFR 801.18. The UDI Rule (21 CFR 830.300(a) and 830.320(b)) also requires that certain information be submitted to the Global Unique Device Identification Database (GUDID) (21 CFR Part 830 Subpart E). For additional information on these requirements, please see the UDI System webpage at https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/unique-device-identification-system-udi-system.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 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,

JOSEPH BRIGGS -S

Joseph Briggs, Ph.D.
Deputy Director
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product 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: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K252387

Device Name

Simplexa COVID-19 / Flu A/B & RSV Direct

Indications for Use *(Describe)*

The Simplexa COVID-19 / Flu A/B & RSV Direct is a real-time RT-PCR assay intended for use on the LIAISON MDX instrument for the simultaneous in vitro qualitative detection and differentiation of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A (Flu A) virus, influenza B (Flu B) virus and respiratory syncytial virus (RSV) in nasopharyngeal swab and anterior nasal swab specimens from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2, influenza A, influenza B, and RSV can be similar.

The Simplexa COVID-19 / Flu A/B & RSV Direct assay is intended for use as an aid in the differential diagnosis of SARS-CoV-2, influenza A, influenza B and RSV infections if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A, influenza B, and RSV viral RNA are generally detectable in nasopharyngeal swab and anterior nasal swab specimens during the acute phase of infection. This test is not intended to detect influenza C virus infections.

Positive results are indicative of the presence of the identified virus, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test. The agent(s) detected by the Simplexa COVID-19 / Flu A/B & RSV Direct real-time RT-PCR assay may not be the definite cause of the disease. Negative results do not preclude SARS-CoV-2, influenza A, influenza B, or RSV infection and should not be used as the sole basis for patient management decisions.

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.

510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CRF 807.92.

A. 510(k) Number:

K252387

B. Purpose of Submission:

Traditional 510(k), New Device

C. Measurand:

The assay detects and differentiates RNA targets specific to SARS-CoV-2, Influenza A (including specific novel influenza A virus subtypes), Influenza B, and Respiratory Syncytial Virus (RSV) in human respiratory specimens using a multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) method.

D. Type of Test:

This is a qualitative, multiplex nucleic acid amplification test (NAAT) that utilizes real-time reverse transcription polymerase chain reaction (RT-PCR) technology for the simultaneous detection and differentiation of multiple respiratory viral RNA targets.

E. Applicant:

Michael Treas
DiaSorin Molecular LLC
11331 Valley View Street
Cypress, CA 90630

F. Proprietary and Established Names:

SIMPLEXA™ COVID-19 / FLU A/B & RSV DIRECT
SIMPLEXA™ COVID-19 / FLU A/B & RSV POSITIVE CONTROL PACK

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
QOF	II	21 CFR 866.3981 – Multi Target Respiratory Specimen Nucleic Acid Test Including SARS-CoV-2 and Other Microbial Agents	Microbiology

510(k) Summary

H. Intended Use(s):

a. SIMPLEXA™ COVID-19 / FLU A/B & RSV DIRECT —

The Simplexa™ COVID-19 / Flu A/B & RSV Direct is a real-time RT-PCR assay intended for use on the LIAISON® MDX instrument for the simultaneous in vitro qualitative detection and differentiation of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A (Flu A) virus, influenza B (Flu B) virus and respiratory syncytial virus (RSV) in nasopharyngeal swab and anterior nasal swab specimens from individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2, influenza A, influenza B, and RSV can be similar.

The Simplexa™ COVID-19 / Flu A/B & RSV Direct assay is intended for use as an aid in the differential diagnosis of SARS-CoV-2, influenza A, influenza B and RSV infections if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A, influenza B, and RSV viral RNA are generally detectable in nasopharyngeal swab and anterior nasal swab specimens during the acute phase of infection. This test is not intended to detect influenza C virus infections.

Positive results are indicative of the presence of the identified virus, but do not rule out bacterial infection or co-infection with other pathogens not detected by the test. The agent(s) detected by the Simplexa™ COVID-19 / Flu A/B & RSV Direct real-time RT-PCR assay may not be the definite cause of the disease. Negative results do not preclude SARS-CoV-2, influenza A, influenza B, or RSV infection and should not be used as the sole basis for patient management decisions.

b. Indication(s) for use:

Same as intended use.

c. Special conditions for use statement(s):

For prescription use only.

d. Special instrument requirements:

For use with LIAISON® MDX Instrument.

510(k) Summary

Device Description:

The Simplexa™ COVID-19 & Flu A/B & RSV Direct assay is a qualitative, multiplex real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the simultaneous detection and differentiation of RNA from SARS-CoV-2, Influenza A, Influenza B, and Respiratory Syncytial Virus (RSV) in nasopharyngeal swabs (NPS) and anterior nasal swabs (NS) in UTM/UVT and M4RT specimen transport media. The assay is performed on the LIAISON® MDX Instrument using a Direct Amplification Disc (DAD) format, enabling sample-to-answer processing without separate nucleic acid extraction.

The LIAISON® MDX Instrument is a benchtop real-time PCR thermocycler that utilizes a self-contained, single-use direct amplification disc (DAD) to process samples. It performs thermal cycling and real-time fluorescence detection using optical detection modules, each with specific excitation and emission wavelengths. The instrument includes a laser enclosed in a laser product housing, with integrated hardware and software interlocks to ensure user safety. It is operated via a USB connection to a dedicated computer running the LIAISON® MDX Studio software.

The LIAISON® MDX Studio software controls the instrument and provides a user interface for assay setup, execution, and result analysis. The software automatically interprets results for in vitro diagnostic (IVD) assays using pre-defined assay definitions encoded in barcode inserts included with the assay kits. It performs spectral compensation, verifies internal control amplification, and checks for sufficient sample volume prior to amplification. The software also includes user authentication, audit logging, laboratory information system (LIS) connectivity, and cybersecurity features.

The assay kit includes single-use reaction mix vials, a positive control pack with inactivated viral particles in transport media, and the Direct Amplification Disc consumable, which supports up to eight simultaneous reactions.

The assay format is designed for direct amplification, with 24 single-use reaction mix vials per kit. The required sample volume input is 50 µL. The reaction mix is provided in single-use vials and includes DNA polymerase, reverse transcriptase, RNase inhibitor, primers, probes, and encapsulated RNA templates. The buffer component in the reaction mix maintains optimal pH and ionic strength to support enzyme activity and amplification efficiency throughout the RT-PCR process.

The assay includes an encapsulated RNA internal control (RNA IC) in each reaction to monitor for potential RT-PCR inhibition or process failure. The RNA IC is derived from *bacteriophage MS2.* This non-target RNA is co-amplified with the assay's viral targets and detected independently using post-amplification melting curve analysis. The presence of the RNA IC in a negative specimen confirms that the amplification process functioned as expected, while its absence—along with no target detection—results in an invalid outcome. Detection of the RNA IC is not required in the Positive Control but is expected in the No Template Control (NTC) to verify assay validity.

510(k) Summary

The assay is intended for use by trained laboratory personnel in moderate to high complexity clinical laboratories. The system is validated for stability under various conditions and verified transport and shipping stability for both domestic and international distribution.

Substantial Equivalence Information:
This document presents a comprehensive comparative analysis between the Simplexa™ COVID-19 & Flu A/B & RSV Direct assay and the Panther Fusion® SARS-CoV-2/Flu A/B/RSV assay (FDA 510(k) K242465). The analysis is structured to fulfill the predicate device comparison requirements of 21 CFR 807.92(a)(3). Table 1 contains a side-by-side technical comparison and rationale for each difference to ensure clarity, traceability, and regulatory alignment.

Table 1. Comparative Summary Table

Comparison to Predicate Device	Candidate device Simplexa™ COVID-19 & Flu A/B & RSV Direct	Predicate Device Panther Fusion® SARS-CoV-2/Flu A/B/RSV (K242465)	Equivalent
Product Code	QOF	QOF	Yes
Regulation Number	21 CFR 866.3981	21 CFR 866.3981	Yes
Organisms Detected	Influenza A, Influenza B, Sars-CoV-2, Respiratory Syncytial Virus	Influenza A, Influenza B, Sars-CoV-2, Respiratory Syncytial Virus	Yes
Measurand	Nucleic acid from Organisms detected	Nucleic acid from Organisms detected	Yes
Intended Use/ Indications for Use	The Simplexa™ COVID-19 / Flu A/B & RSV Direct is a real-time RT-PCR assay intended for use on the LIAISON® MDX instrument for the simultaneous in vitro qualitative detection and differentiation of nucleic acid from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A (Flu A) virus, influenza B (Flu B) virus and respiratory syncytial virus (RSV) in nasopharyngeal swabs and anterior nasal swabs specimens from	The Panther Fusion® SARS-CoV-2/Flu A/B/RSV Assay is a fully automated multiplexed real-time polymerase chain reaction (RT-PCR) in vitro diagnostic test intended for the qualitative detection and differentiation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), influenza A virus (Flu A), influenza B virus (Flu B), and respiratory syncytial virus (RSV). Nucleic	Negligible differences

510(k) Summary

Comparison to Predicate Device	Candidate device Simplexa™ COVID-19 & Flu A/B & RSV Direct	Predicate Device Panther Fusion® SARS-CoV-2/Flu A/B/RSV (K242465)	Equivalent
	individuals with signs and symptoms of respiratory tract infection. Clinical signs and symptoms of respiratory tract infection due to SARS-CoV-2, influenza A, influenza B, and RSV can be similar. The Simplexa™ COVID-19 / Flu A/B & RSV Direct assay is intended for use as an aid in the differential diagnosis of SARS-CoV-2, influenza A, influenza B and RSV infections if used in conjunction with other clinical and epidemiological information, and laboratory findings. SARS-CoV-2, influenza A, influenza B, and RSV viral RNA are generally detectable in nasopharyngeal swab and anterior nasal swab specimens during the acute phase of infection. This test is not intended to detect influenza C virus infections. Positive results are indicative of the presence of the identified virus, but do not rule out bacterial infection or co-	acids are isolated and purified from nasopharyngeal (NP) swab specimens and anterior nasal (AN) swab specimens obtained from individuals exhibiting signs and symptoms of a respiratory tract infection. Clinical signs and symptoms of respiratory viral infection due to SARS-CoV-2, influenza, and RSV can be similar. This assay is intended to aid in the differential diagnosis of SARS-CoV-2, Flu A, Flu B, and RSV infections in humans and is not intended to detect influenza C virus infections. Nucleic acids from the viral organisms identified by this test are generally detectable in NP and AN swab specimens during the acute phase of infection. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory tract infection are indicative of the presence	

510(k) Summary

Comparison to Predicate Device	Candidate device Simplexa™ COVID-19 & Flu A/B & RSV Direct	Predicate Device Panther Fusion® SARS-CoV-2/Flu A/B/RSV (K242465)	Equivalent
	infection with other pathogens not detected by the test. The agent(s) detected by the Simplexa COVID-19 / Flu A/B & RSV Direct real-time RT PCR assay may not be the definite cause of the disease. Negative results do not preclude SARS-CoV-2, influenza A, influenza B, or RSV infection and should not be used as the sole basis for patient management decisions.	of the identified virus and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Positive results do not rule out coinfection with other organisms. The organism(s) detected by the Panther Fusion SARS-CoV-2/Flu A/B/RSV Assay may not be the definite cause of disease. Negative results do not preclude SARS-CoV-2, influenza A virus, influenza B virus, or RSV infections. This assay is designed for use on the Panther Fusion System.	
Multiplexing	4-plex (SARS-CoV-2, Flu A, Flu B, RSV)	4-plex (SARS-CoV-2, Flu A, Flu B, RSV)	Same
Technology	RT-PCR (direct amplification)	RT-PCR (automated extraction + amplification)	Negligible difference. Simplexa does not require extraction.
Amplification Chemistry	TaqMan probes	TaqMan probes	Same
Gene Targets	SARS-CoV-2 (S Gene, ORF1ab), FluA (Matrix	SARS-CoV-2 (ORF1ab), FluA (Matrix), FluB	Same

510(k) Summary

Comparison to Predicate Device	Candidate device Simplexa™ COVID-19 & Flu A/B & RSV Direct	Predicate Device Panther Fusion® SARS-CoV-2/Flu A/B/RSV (K242465)	Equivalent
	gene), FluB (Matrix gene, nucleoprotein gene), RSV (M and G genes)	(Matrix), RSV (Matrix) genes	
Detection Channels	FAM, HEX, CFR610, Quasar 670	FAM, HEX, ROX, RED647, RED677	Negligible difference.
Transport Media	UVT/UTM and M4RT	VTM/UTM and eSTM (RespDirect)	Negligible difference.
Internal Control	MS2 phage (bacteriophage)	Internal Control-S (IC-S) synthetic RNA	Negligible difference.
Positive Control	Manual single-use vial	PRD-07401, system-integrated control	Negligible difference
Specimen Types	NP/AN swabs in UVT/UTM and M4RT	NP/AN swabs in VTM/UTM/eSTM	Negligible difference
Sample Preparation	None	None	Same
Time to Result	~50 minutes	~2.5 hours	Negligible difference.
Workflow Complexity	Minimal hands-on time	Sample transfer or RespDirect tube setup	Negligible difference.
Instrumentation	LIAISON® MDX	Panther Fusion System	Negligible difference.
Throughput	Moderate	High	Negligible difference
Analytical Specificity	No cross-reactivity	No cross-reactivity	Same
Competitive Interference	No competitive interference	Observed at high viral loads	Negligible difference.
Interfering Substances	No Interference	Minimal	Same
Carryover Contamination	0%	0%	Same
Precision	CV ≤3.4% (Total)	CV ≤10.92% (Total)	Negligible difference
Clinical Performance	PPA/NPA 91.3-99.8% across all targets (prospective)	PPA/NPA 84.6–100% across all targets (prospective)	Negligible difference.
Control Strategy	Manual control per lot	Automated control tracking and enforcement	Negligible difference.

510(k) Summary

Standards/Guidance Documents Referenced:

FDA Guidance – Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay.

AAMI. Principles for medical device security – Risk Management. AAMI document TIR57:2016. Association for the Advancement of Medical Instrumentation; 2016.

AAMI. Principles for medical device security – Postmarket risk management for device manufacturers. AAMI document TIR97:2019. Association for the Advancement of Medical Instrumentation; 2019.

CLSI. Information Technology Security of In Vitro Diagnostic Instruments and Software Systems; Approved Standard – Second Edition. CLSI document AUTO11-A2. Wayne, PA: Clinical Laboratory Standards Institute; 2014.

CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical Laboratory Standards Institute; 2019.

CLSI. Interference Testing in Clinical Chemistry. 3rd Ed. CLSI Document EP07. Wayne, PA: Clinical Laboratory Standards Institute; 2018.

CLSI. Evaluation of Qualitative, Binary Output Examination Performance; Approved Guideline – Third Edition. CLSI document EP12. Wayne, PA: Clinical Laboratory Standards Institute; 2023.

CLSI. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition. CLSI document EP17-A2. Wayne, PA: Clinical Laboratory Standards Institute; 2012.

CLSI. Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline – Second Edition. CLSI document EP24-A2. Wayne, PA: Clinical Laboratory Standards Institute; 2011.

CLSI. Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI document EP25-A. Wayne, PA: Clinical Laboratory Standards Institute; 2009.

CLSI. Collection Transport Preparation and Storage of Specimens for Molecular Methods. 2nd Edition. CLSI Document MM13. Wayne, PA: Clinical Laboratory Standards Institute; 2020.

CLSI. Verification and Validation of Multiplex Nucleic Acid Assays. 2nd Edition. CLSI Document MM17. Wayne, PA: Clinical Laboratory Standards Institute; 2018.

ISTA. Packaged-Products for Parcel Delivery System Shipment 70 kg (150 lb) or Less. ISTA Document 3A. International Safe Transit Association. 2018.

IEC 62366-1 Edition 1.1 2020-06 Consolidated Version; Medical devices – Part 1: Application of usability engineering to medical devices

IEC 61010-1 Edition 3.1 2017-01 Consolidated Version; Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements

IEC 60601-1-2 Edition 4.1 2020-09 Consolidated Version; Medical electrical equipment – Part 1-2:

510(k) Summary

General requirements for basic safety and essential performance – Collateral Standard: Electromagnetic disturbances – Requirements and tests

IEC 61326-1 Edition 3.0 2020-10; Electrical equipment for measurement, control and laboratory use – EMC requirements – Part 1: General requirements IEC 61326-2 Edition 3.0 2020-10; Electrical equipment for measurement, control and laboratory use – EMC requirements – Part 2-6: Particular requirements – In vitro diagnostic (IVD) medical equipment

IEC 62304 Edition 1.1 2015-06 Consolidated Version; Medical device software – Software life cycle processes

IEC TR 60878 Ed. 4.0 2022-11; Graphical symbols for electrical equipment in medical practice [Including: Corrigendum 1 (2023)]

IEC TR 80001-2-2:2012. Application of risk management for IT Networks incorporating medical devices – Part 2-2: Guidance for the disclosure and communication of medical device security needs, risks and controls

IEC TR 80001-2-8 Edition 1.0 206-05; Application of risk management for IT – networks incorporating medical devices – Part 2-8: Application guidance – Guidance on standards for establishing the security capabilities identified in IEC TR 80001-2-2

ISO 14971:2019 Medical Devices – Application of risk management to medical devices

ISO 15223-1: 2021-07 – Medical Devices- Symbols to be used with information to be supplied by the manufacturer – Part 1: General requirements

UL ANSI 2900-1 First Edition 2017; Standard for Safety, Standard for Software Cybersecurity Network-Connectable Products, Part 1: General Requirements

UL ANSI 2900-2-1 First Edition 2017; Standard for Safety, Software Cybersecurity for Network-Connectable Products, Part 2-1: Particular Requirements for Network Connectable Components of Healthcare and Wellness Systems

Test Principle:

The Simplexa™ COVID-19 / Flu A/B & RSV Direct assay is a qualitative, multiplex real-time RT-PCR test designed for the simultaneous detection and differentiation of:

- SARS-CoV-2
- Influenza A
- Influenza B
- Respiratory Syncytial Virus (RSV)

The assay is performed on the LIAISON® MDX instrument using the Direct Amplification Disc (DAD), which enables direct amplification which enables direct amplification without separate nucleic acid extraction. The system uses fluorescent probes and melting curve analysis for detection. The assay includes an RNA internal control to monitor for inhibition and reagent integrity.

510(k) Summary

CLINICAL AGREEMENT

Clinical Performance Summary

The performance of the Simplexa™ COVID-19/ Flu A/B & RSV Direct assay was evaluated using prospective nasopharyngeal swab (NPS) and anterior nasal swab (NS) specimens from individuals with signs and symptoms of respiratory tract infection. The prospective samples were collected from nine (9) external sites. Collection sites included five (5) reference laboratories and four (4) outpatient clinics, across seven (7) different geographical locations.

Testing was performed from December 2024 to March 2025. The comparator for all targets was an FDA-cleared molecular assay. A second FDA-cleared molecular assay and/or PCR/bidirectional sequencing assay was used for discordant result analysis.

A total of 1,401 NPS specimens and 978 NS specimens were enrolled for the prospective clinical study and tested with the Simplexa™ COVID-19/ Flu A/B & RSV Direct assay and the comparator method. 526 NPS specimens and NS specimens were collected in pair from the same subject. Forty (40) NPS specimens and forty-six (46) NS specimens were not evaluable due to protocol deviations, sample handling issues and/or invalid reference testing result. The Simplexa™ COVID-19/ Flu A/B & RSV Direct assay demonstrated adequate clinical performance, the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) point estimates between the Simplexa™ COVID-19/ Flu A/B & RSV Direct assay and the comparator for the different target pathogens in NS, NPS samples are summarized in Table 2 and 3. The performance of the Simplex COVID-19/ Flu A/B & RSV Direct assay in unpaired NS and NPS samples is summarized in Table 4.

Both the Simplexa™ COVID-19 / Flu A/B & RSV Direct assay and the comparator assay testing were conducted at four (4) external sites and one (1) internal testing facility. After a single repeat, a valid result for the SARS-CoV-2, Influenza A and Influenza B targets was obtained for 2283 specimens (8 invalid NPS and 2 invalid NS) and 2270 specimens for the RSV target (15 invalid NPS and 8 invalid NS).

Table 2: Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of Prospective Data Set (NPS)

Pathogen Target	Positive Percent Agreement			Negative Percent Agreement		
	TP / (TP+FN)	PPA (%)	95% CI	TN / (TN+FP)	NPA (%)	95% CI
Flu A	362/375[a]	96.5%	94.2%-98.0%	971/978[b]	99.3%	98.5%-99.7%
Flu B	45/46[c]	97.8%	88.7%-99.6%	1305/1307[d]	99.8%	99.4%-100.0%
RSV	95/102[e]	93.1%	86.5%-96.6%	1238/1244[f]	99.5%	99.0%-99.8%
COVID-19	61/65[g]	93.8%	85.2%-97.6%	1281/1288[h]	99.5%	98.9%-99.7%

PPA = Positive Percent Agreement, NPA = Negative Percent Agreement, CI = Confidence Interval. The 95% confidence intervals (CI) were calculated following the Wilson Score method.

[a]Five (5) of the thirteen (13) Flu A False Negative specimens were negative by PCR/BDS. One (1) additional specimen was negative by Standard of Care.

[b]Three (3) of the seven (7) Flu A False Positive specimens were positive by PCR/BDS. Two (2) additional specimens were positive by Standard of Care.

[c]The one (1) Flu B False Negative specimen was negative by PCR/BDS.

[d]Both of the two (2) Flu B False Positive specimens were positive by PCR/BDS and one (1) specimen was also positive by Standard of Care.

[e]Three (3) of the seven (7) RSV False Negative specimens were negative by PCR/BDS. Three (3) additional specimens were negative by Standard of Care.

510(k) Summary

[f]Five (5) of the six (6) RSV False Positive specimens were positive by PCR/BDS.
[g]Two (2) of the four (4) COVID-19 False Negative specimens were negative by PCR/BDS.
[h]Four (4) of the seven (7) COVID-19 False Positive specimens were positive by PCR/BDS.

Table 3: Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of Prospective Data Set (NS)

Pathogen Target	Positive Percent Agreement			Negative Percent Agreement		
	TP / (TP+FN)	PPA (%)	95% CI	TN / (TN+FP)	NPA (%)	95% CI
Flu A	250/255[a]	98.0%	95.5%-99.2%	662/675[b]	98.1%	96.7%-98.9%
Flu B	28/29[c]	96.6%	82.8%-99.4%	899/901[d]	99.8%	99.2%-99.9%
RSV	52/59[e]	88.1%	77.5%-94.1%	863/865[f]	99.8%	99.2%-99.9%
COVID-19	42/43[g]	97.7%	87.9%-99.6%	882/887[h]	99.4%	98.7%-99.8%

PPA = Positive Percent Agreement, NPA = Negative Percent Agreement, CI = Confidence Interval. The 95% confidence intervals (CI) were calculated following the Wilson Score method.
[a]Four (4) of the five (5) Flu A False Negative specimens were negative by PCR/BDS. The other specimen was negative by Standard of Care.
[b]All thirteen (13) Flu A False Positive specimens were positive by PCR/BDS.
[c]The one (1) Flu B False Negative specimen was negative by Standard of Care.
[d]The two (2) Flu B False Positive specimens were negative by PCR/BDS.
[e]Two (2) of the seven (7) RSV False Negative specimens were negative by PCR/BDS.
[f]One (1) of the two (2) RSV False Positive specimens was positive by PCR/BDS.
[g]The COVID-19 False Negative specimen was negative by PCR/BDS.
[h]Two (2) of the five (5) COVID-19 False Positive specimens were positive by PCR/BDS.

Table 4: Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of Prospective Data Set (Unpaired)

		Positive Percent Agreement			Negative Percent Agreement		
Pathogen Target	Sample Type	TP /(TP+FN)	PPA	95%CI	TN/(TN+FP)	NPA	95%CI
Flu A	NS	94/96	97.90%	92.7%-99.4%	301/308	97.70%	95.4%-98.9%
	NPS	215/224	96.00%	92.5%-97.9%	620/626	99.00%	97.9%-99.6%
	Total	309/320[a]	96.60%	94.0%-98.1%	921/934[b]	98.60%	97.6%-99.2%
Flu B	NS	8/8	100.00%	67.6%-100.0%	396/396	100.00%	99.0%-100.0%
	NPS	26/27	96.30%	81.7%-99.3%	823/823	100.00%	99.5%-100.0%
	Total	34/35[c]	97.10%	85.5%-99.5%	1219/1219	100.00%	99.7%-100.0%
RSV	NS	27/31	87.10%	71.1%-94.9%	372/372	100.00%	99.0%-100.0%
	NPS	72/77	93.50%	85.7%-97.2%	765/771	99.20%	98.3%-99.6%
	Total	99/108[d]	91.70%	84.9%-95.6%	1137/1143[e]	99.50%	98.9%-99.8%
COVID-19	NS	24/25	96.00%	80.5%-99.3%	376/379	99.20%	97.7%-99.7%
	NPS	47/50	94.00%	83.8%-97.9%	799/800	99.90%	99.3%-100.0%
	Total	71/75[f]	94.70%	87.1%-97.9%	1175/1179[g]	99.70%	99.1%-99.9%

PPA = Positive Percent Agreement, NPA = Negative Percent Agreement, CI = Confidence Interval. The 95% confidence intervals (CI) were calculated following the Wilson Score method.
[a]Five (5) of the eleven (11) Flu A False Negative specimens were negative by PCR/BDS.
[b]Nine (9) of the thirteen (13) Flu A False Positive specimens were positive by PCR/BDS. Three (3) additional specimens were positive by Standard of Care.
[c]The one (1) Flu B False Negative specimen was negative by PCR/BDS.
[d]Four (4) of the nine (9) RSV False Negative specimens were negative by PCR/BDS. One (1) additional specimen was negative by Standard of Care.
[e]Five (5) of the six (6) RSV False Positive specimens were positive by PCR/BDS.

510(k) Summary

[f]Two (2) of the four (4) COVID-19 False Negative specimens were negative by PCR/BDS.
[g]One (1) of the four (4) COVID-19 False Positive specimens was positive by PCR/BDS.

Table 5 provides a summary of the general demographic information of the 1361 prospectively collected NPS specimens and 932 prospectively collected NS specimens that were included in the prospective analysis.

Table 5: General Demographic Details of the Prospective Study Population

	NPS (N=1361)	NS (N=932)
Gender		
Male	669 (49.2%)	440 (47.2%)
Female	691 (50.8%)	492 (52.8%)
Unknown	1 (0.1%)	0 (0.0%)
Total	1361 (100%)	932 (100%)
Age		
<=5	508 (37.3%)	242 (26.0%)
6-18	486 (35.7%)	417 (44.7%)
19-40	153 (11.2%)	137 (14.7%)
41-60	102 (7.5%)	94 (10.1%)
61+	106 (7.8%)	42 (4.5%)
Unknown	6 (0.4%)	0 (0.0%)
Total	1361 (100%)	932 (100%)
Subject Location		
ER	692 (50.8%)	176 (18.9%)
ICU	1 (0.1%)	754 (80.9%)
Hospitalized	84 (6.2%)	0 (0.0%)
Outpatient	584 (42.9%)	0 (0.0%)
Unknown	0	2

510(k) Summary

	NPS (N=1361)	NS (N=932)
	(0.0%)	(0.2%)
Total	1361 (100%)	932 (100%)

REPRODUCIBILITY

Reproducibility of the Simplexa™ COVID-19 / Flu A/B & RSV Direct assay was assessed across one (1) internal site and two (2) external sites. The reproducibility panel consisted of ten (10) members, including eight (8) contrived samples, one (1) negative sample [pooled negative NPS matrix] and one (1) positive control sample (PC used "as is"). Panel member details are presented in
Table 6. The contrived panel members were prepared by spiking each analyte at approximately two times (2X) the Limit of Detection (LoD, low positive) and approximately five times (5X) LoD (medium positive) into pooled negative nasopharyngeal swab matrix in UTM. Each panel member was tested in triplicate for five (5) non-consecutive days. Each site had two (2) operators who each tested the entire panel once per day, for a total of two (2) runs per day. Agreements with expected results are presented in Table 7 for the panel members and Table 8 for the RNA Internal Control with average Cts, standard deviation (SD) and coefficient of variation (%CV).

Table 6. Reproducibility Sample Panel Member Details

Sample Panel Member	Spiked Concentration (copies/mL)
Influenza A Victoria/4897/2022 Low Positive	1000 (2X LoD)
Influenza A Victoria/4897/2022 Medium Positive	2500 (5X LoD)
Influenza B/Austria /1359417/2021 Low Positive	1000 (2X LoD)
Influenza B/Austria /1359417/2021 Medium Positive	2500 (5X LoD)
SARS-CoV-2 Lineage BA.2.3 (Omicron Variant) Low Positive	1000 (2X LoD)
SARS-CoV-2 Lineage BA.2.3 (Omicron Variant) Medium Positive	2500 (5X LoD)
RSV B CH93(18)-18 Low Positive	2000 (2X LoD)
RSV B CH93(18)-18 Medium Positive	5000 (5X LoD)
Pooled negative nasopharyngeal swab (NPS) matrix	N/A
Positive Control	N/A

510(k) Summary

Table 7. Simplexa™ COVID-19 / Flu A/B & RSV 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)	Total % Agreement with Expected Results	Avg. Ct ± SD (%CV)	95% CI
Influenza A LP	100% (30/30)	33.1 ± 1.25 (3.8%)	100% (30/30)	33.7 ± 0.95 (2.8%)	100% (30/30)	32.7 ± 0.65 (2.0%)	100% (90/90)	33.2 ± 1.05 (3.2%)	95.9% - 100%
Influenza A MP	100% (30/30)	32.5 ± 0.58 (1.8%)	100% (30/30)	33.2 ± 0.56 (1.7%)	100% (30/30)	31.9 ± 0.38 (1.2%)	100% (90/90)	32.5 ± 0.74 (2.3%)	95.9% - 100%
Influenza B LP	100% (30/30)	33.1 ± 0.74 (2.2%)	100% (30/30)	32.4 ± 0.90 (2.8%)	100% (30/30)	33.3 ± 1.33 (4.0%)	100% (90/90)	33.0 ± 1.09 (3.3%)	95.9% - 100%
Influenza B MP	100% (30/30)	32.0 ± 0.67 (2.1%)	100% (30/30)	31.9 ± 0.87 (2.7%)	96.7% (29/30)[a]	31.8 ± 0.73 (2.3%)	98.9% (89/90)	31.9 ± 0.76 (2.4%)	94% - 99.8%
SARS-CoV-2 LP	100% (30/30)	30.4 ± 0.54 (1.8%)	100% (30/30)	31.2 ± 0.52 (1.7%)	100% (30/30)	30.6 ± 0.44 (1.4%)	100% (90/90)	30.7 ± 0.60 (2.0%)	95.9% - 100%
SARS-CoV-2 MP	100% (30/30)	29.7 ± 0.41 (1.4%)	100% (30/30)	30.5 ± 0.60 (2.0%)	100% (30/30)	29.8 ± 0.37 (1.2%)	100% (90/90)	30.0 ± 0.59 (2.0%)	95.9% - 100%
RSV LP	100% (30/30)	29.7 ± 0.42 (1.4%)	100% (30/30)	29.9 ± 0.56 (1.9%)	100% (30/30)	29.6 ± 1.38 (4.7%)	100% (90/90)	29.7 ± 0.89 (3.0%)	95.9% - 100%
RSV MP	100% (30/30)	29.0 ± 0.59 (2.0%)	100% (30/30)	28.6 ± 1.05 (3.7%)	100% (30/30)	28.9 ± 0.70 (2.4%)	100% (90/90)	28.8 ± 0.81 (2.8%)	95.9% - 100%
Positive Control (PC) Flu A	100% (30/30)	32.1 ± 0.61 (1.9%)	100% (30/30)	32.4 ± 0.83 (2.6%)	100% (30/30)	31.9 ± 0.54 (1.7%)	100% (90/90)	32.1 ± 0.69 (2.1%)	95.9% - 100%
Positive Control (PC) Flu B	100% (30/30)	32.9 ± 1.37 (4.2%)	100% (30/30)	32.0 ± 0.60 (1.9%)	100% (30/30)	32.3 ± 1.20 (3.7%)	100% (90/90)	32.4 ± 1.16 (3.6%)	95.9% - 100%
Positive Control (PC) SARS-CoV-2	100% (30/30)	30.9 ± 0.37 (1.2%)	100% (30/30)	31.6 ± 0.47 (1.5%)	100% (30/30)	31.2 ± 0.43 (1.4%)	100% (90/90)	31.2 ± 0.52 (1.7%)	95.9% - 100%
Positive Control PC RSV	100% (30/30)	31.4 ± 0.70 (2.2%)	100% (30/30)	31.3 ± 0.78 (2.5%)	100% (30/30)	31.6 ± 1.02 (3.2%)	100% (90/90)	31.4 ± 0.84 (2.7%)	95.9% - 100%
Negative (pooled NPS)	100% (30/30)	NA	100% (30/30)	NA	100% (30/30)	NA	100% (90/90)	NA	95.9% - 100%
Total	**100.0% (300/300)**		**100.0% (300/300)**		**99.7% (299/300)**		**99.9% (899/900)**		**99.4% - 100%**

[a]One Influenza B Medium Positive replicate tested at Site 3 had an unexpected false negative ("Not Detected") result.
Avg = Average
Ct = Cycle threshold
SD = Standard deviation
%CV = Coefficient of variation CI = Confidence Interval
LP = Low Positive
MP = Medium Positive

510(k) Summary

Table 8. Simplexa™ COVID-19 / Flu A/B & RSV Direct Reproducibility – RNA Internal Control

Sample	Site 1		Site 2		Site 3		All Sites		
	% Agreement with Expected Results	Avg. Tm ± SD (%CV)	% Agreement with Expected Results	Avg. Tm ± SD (%CV)	% Agreement with Expected Results	Avg. Tm ± SD	Total % Agreement with Expected Results	Avg. Tm ± SD (%CV)	95% CI
Influenza A LP	100% (30/30)	56.8 ± 0.33 (0.6%)	100% (30/30)	57.0 ± 0.49 (0.9%)	100% (30/30)	56.7 ± 0.21 (0.4%)	100% (90/90)	56.8 ± 0.37 (0.7%)	95.9% - 100%
Influenza A MP	100% (30/30)	56.7 ± 0.29 (0.5%)	100% (30/30)	57.2 ± 0.61 (1.1%)	100% (30/30)	56.7 ± 0.23 (0.4%)	100% (90/90)	56.9 ± 0.48 (0.8%)	95.9% - 100%
Influenza B LP	100% (30/30)	56.8 ± 0.30 (0.5%)	100% (30/30)	56.9 ± 0.47 (0.8%)	100% (30/30)	56.8 ± 0.28 (0.5%)	100% (90/90)	56.8 ± 0.36 (0.6%)	95.9% - 100%
Influenza B MP	100% (30/30)	56.8 ± 0.32 (0.6%)	100% (30/30)	56.8 ± 0.43 (0.8%)	100% (30/30)	56.7 ± 0.07 (0.1%)	100% (90/90)	56.8 ± 0.31 (0.5%)	95.9% - 100%
SARS-CoV-2 LP	100% (30/30)	56.7 ± 0.10 (0.2%)	100% (30/30)	56.9 ± 0.56 (1.0%)	100% (30/30)	56.6 ± 0.18 (0.3%)	100% (90/90)	56.7 ± 0.36 (0.6%)	95.9% - 100%
SARS-CoV-2 MP	100% (30/30)	56.7 ± 0.24 (0.4%)	100% (30/30)	57.1 ± 0.60 (1.1%)	100% (30/30)	56.8 ± 0.33 (0.6%)	100% (90/90)	56.9 ± 0.44 (0.8%)	95.9% - 100%
SARS-CoV-2 MP	100% (30/30)	56.7 ± 0.24 (0.4%)	100% (30/30)	57.1 ± 0.60 (1.1%)	100% (30/30)	56.8 ± 0.33 (0.6%)	100% (90/90)	56.9 ± 0.44 (0.8%)	95.9% - 100%
RSV LP	100% (30/30)	56.9 ± 0.38 (0.7%)	100% (30/30)	57.2 ± 0.60 (1.0%)	100% (30/30)	56.8 ± 0.44 (0.8%)	100% (90/90)	57.0 ± 0.51 (0.9%)	95.9% - 100%
RSV MP	100% (30/30)	56.9 ± 0.47 (0.8%)	100% (30/30)	57.1 ± 0.52 (0.9%)	100% (30/30)	56.8 ± 0.37 (0.7%)	100% (90/90)	56.9 ± 0.48 (0.8%)	95.9% - 100%
Positive Control (PC)	100% (30/30)	56.6 ± 0.15 (0.3%)	100% (30/30)	56.7 ± 0.42 (0.7%)	100% (30/30)	56.7 ± 0.27 (0.5%)	100% (90/90)	56.7 ± 0.30 (0.5%)	95.9% - 100%
Negative (pooled NPS)	100% (30/30)	56.7 ± 0.24 (0.4%)	100% (30/30)	57.2 ± 0.58 (1.0%)	100% (30/30)	56.9 ± 0.35 (0.6%)	100% (90/90)	56.9 ± 0.47 (0.8%)	95.9% - 100%
Total	**100% (300/300)**		**100% (300/300)**		**100% (300/300)**		**100% (900/900)**		**99.6% - 100%**

Avg = Average
Tm = Melting temperature
SD = Standard deviation
%CV = 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 & RSV Direct assay in Nasopharyngeal Swabs (NPS) was determined to be the lowest detectable concentration of quantitated titered viral stocks (copies/mL) at which ≥ 95% of all replicates were detected. Two (2) strains of influenza A, two (2) strains of influenza B, two (2) strains of SARS-CoV-2 and two (2) strains of RSV serially diluted in negative nasopharyngeal swab (NPS) matrix were used to determine the LoD. The LoD results are shown in Table 9.

510(k) Summary

Table 9. Simplexa™ COVID-19 / Flu A/B & RSV Direct Limit of Detection in Nasopharyngeal Swabs (NPS)

Virus strain	LoD (copies/mL)
Influenza A Victoria/4897/2022 (H1N1)	500
Influenza A Darwin/9/21 (H3N2)	750
Influenza B/Austria /1359417/2021 (Victoria)	500
Influenza B Phuket/3073/2013 (Yamagata)	500
SARS-CoV-2 USA/WA 1/2020	500
SARS-CoV-2 Lineage BA.2.3 (Omicron Variant)	500
RSV A 1/2015 Isolate #1	1000
RSV B CH93(18)-18	1000

The Limit of Detection (LoD) of the Simplexa™ COVID-19 / Flu A/B & RSV Direct assay in anterior nasal swabs (NS) was determined to be the lowest detectable concentration of quantitated titered viral stocks (copies/mL) at which ≥ 95% of all replicates were detected. Two (2) strains of influenza A, two (2) strains of influenza B, two (2) strains of SARS-CoV-2 and two (2) strains of RSV serially diluted in negative anterior nasal swab (NS) matrix were used to determine the LoD. The LoD results are shown in Table 10.

Table 10. Simplexa™ COVID-19 / Flu A/B & RSV Direct Limit of Detection in Anterior Nasal Swabs (NS)

Virus strain	LoD (copies/mL)
Influenza A Victoria/4897/2022 (HN1)	750
Influenza A Darwin/9/21 (H3N2)	750
Influenza B/Austria /1359417/2021 (Victoria)	500
Influenza B Phuket/3073/2013 (Yamagata)	500
SARS-CoV-2 USA/WA 1/2020	500
SARS-CoV-2 Lineage BA.2.3 (Omicron Variant)	500
RSV A 1/2015 Isolate #1	2000
RSV B CH93(18)-18	1000

510(k) Summary

The Limit of Detection (LoD) of the Simplexa™ COVID-19 / Flu A/B & RSV Direct assay using inactivated SARS-CoV-2 WHO International Standard viral particles in nasopharyngeal swabs (NPS) was determined to be the lowest detectable concentration of quantitated viral stock (IU/mL) at which ≥ 95% of all replicates were detected. The Second WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 22/252) diluted in negative nasopharyngeal swab (NPS) matrix was used to determine the LoD. The LoD results are shown in Table 11.

Table 11. Simplexa™ COVID-19 / Flu A/B & RSV Direct Limit of Detection for WHO International Standard for SARS-CoV-2 RNA in Nasopharyngeal Swabs (NPS)

Virus strain	LoD (IU/mL)
Second WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 22/252)	1000

ANALYTICAL REACTIVITY/CROSS REACTIVITY

Analytical reactivity was evaluated with nasopharyngeal swab (NPS) matrix for the Simplexa™ COVID-19 / Flu A/B & RSV Direct assay. A total of thirty-three (33) Flu A strains, fourteen (14) Flu B strains, nineteen (19) SARS-CoV-2 strains and nine (9) RSV strains were tested. Quantified viral material was spiked into negative NPS matrix at the concentrations listed in Tables 12-15 (corresponding to 3X LoD) and tested in triplicate. The results are shown in Tables 12-15. All strains and subtypes were 100% detected with the Simplexa™ COVID-19 & Flu A/B Direct assay at 3X LoD except for Influenza A/Victoria/2570/2019 and RSV-A (3/2015 Isolate 3) which were detected at 4X LoD, and Influenza B/Alabama/2/17 which was detected at 5X LoD.

Table 12. Simplexa™ COVID-19 / Flu A/B & RSV Direct Analytical Reactivity – Flu A

Influenza A Strain	Tested Concentration (copies/mL)	Agreement with Expected Results (#Detected/#Total)
A/California/7/2009 (H1N1)	1500	100% (3/3)
A/Guangdong- Maonan/SWL1536/2019 (H1N1)	1500	100% (3/3)
A/Brisbane/02/18 (H1N1)	1500	100% (3/3)
A/Nebraska/14/2018 (H1N1)	1500	100% (3/3)
A/Victoria/2570/2019 (H1N1)	1500 (3X LoD)	33% (1/3)
	2000 (4X LoD)	100% (3/3)
A/New Caledonia/20/1999 (H1N1)	1500	100% (3/3)
A/Puerto Rico/8/34 (H1N1)	1500	100% (3/3)
A/Brisbane/59/2007 (H1N1)	1500	100% (3/3)
A/Mexico/4108/2009 (H1N1)	1500	100% (3/3)
A/New York/02/09 (H1N1)	1500	100% (3/3)
A/Sydney/05/2021 (H1N1)	1500	100% (3/3)
A Michigan /45/2015 (H1N1)	1500	100% (3/3)

510(k) Summary

Influenza A Strain	Tested Concentration (copies/mL)	Agreement with Expected Results (#Detected/#Total)
A/Perth/16/2009 (H3N2)	2250	100% (3/3)
A/Hong Kong/2671/2019 (H3N2)	2250	100% (3/3)
A/Kansas/14/2017 (H3N2)	2250	100% (3/3)
A/Cambodia/e0826360/2020 (H3N2)	2250	100% (3/3)
A/Darwin/6/2021 (H3N2)	2250	100% (3/3)
A/Tasmania/503/2020 (H3N2)	2250	100% (3/3)
A/Switzerland/9715293/13 (H3N2)	2250	100% (3/3)
A/Texas/50/2012 (H3N2)	2250	100% (3/3)
A/Thailand/08/2022 (H3N2)	2250	100% (3/3)
A/Singapore/INFIMH-16-0019/2016 (H3N2)	2250	100% (3/3)
A/Hong Kong/4801/14 (H3N2)	2250	100% (3/3)
A/Massachusetts/18/2022 (H3N2)	2250	100% (3/3)
A/Hong Kong/8/1968 (H3N2)	2250	100% (3/3)
A/Port Chalmers 1/1973 (H3N2)	2250	100% (3/3)
A/H5N1 (H5N1)	1500	100% (3/3)
A/Anhui/01/2005 (H5N1)	1500	100% (3/3)
A/Egypt/N03072/2010 (H5N1)	1500	100% (3/3)
A/Hubei/1/2010 (H5N1)	1500	100% (3/3)
A/bovine/Ohio/B24OSU-439/2024 (H5N1)	1500	100% (3/3)
A/Hong Kong/33982/2009 (H9N2)	1500	100% (3/3)
A/Mallard/Netherlands/12/2000 (H7N7)	1500	100% (3/3)

Table 13. Simplexa™ COVID-19 / Flu A/B & RSV Direct Analytical Reactivity – Flu B

Influenza B Strain	Tested Concentration (copies/mL)	Agreement with Expected Results (#Detected/#Total)
B/Brisbane/60/2008 (Victoria)	1500	100% (3/3)
B/Maryland/01/1959 (Unknown)	1500	100% (3/3)
B/Colorado/06/2017 (Victoria)	1500	100% (3/3)
B/Brisbane/33/08 (Victoria)	1500	100% (3/3)
B/Texas/2/13 (Victoria)	1500	100% (3/3)
B/Florida/02/06 (Victoria)	1500	100% (3/3)
B/Malaysia/2506/2004 (Victoria)	1500	100% (3/3)
B/Alabama/2/17 (Victoria)	1500 (3X LoD)	33% (1/3)
	2000 (4X LoD)	66% (2/3)

510(k) Summary

Influenza B Strain	Tested Concentration (copies/mL)	Agreement with Expected Results (#Detected/#Total)
	2500 (5X LoD)	100% (3/3)
B/Washington 02/2019 (Victoria)	1500	100% (3/3)
B/Florida/07/04 (Yamagata)	1500	100% (3/3)
B/Massachusetts/2/2012 (Yamagata)	1500	100% (3/3)
B/Utah/9/14 (Yamagata)	1500	100% (3/3)
B/Florida/04/06 (Yamagata)	1500	100% (3/3)
B/Panama/45/90 (Yamagata)	1500	100% (3/3)

Table 14. Simplexa™ COVID-19 / Flu A/B & RSV Direct Analytical Reactivity – SARS-CoV-2

SARS-CoV-2 Strain	Tested Concentration (copies/mL)	Agreement with Expected Results (#Detected/#Total)
SARS-CoV-2 Isolate Hong Kong/ VM20001061/2020	1500	100% (3/3)
SARS-CoV-2 Isolate England/204820464/2020	1500	100% (3/3)
SARS-CoV-2 Isolate South Africa/KRISP-EC-K005325/2020	1500	100% (3/3)
SARS-CoV-2 Isolate hCoV-19/Japan/TY7-503/2021	1500	100% (3/3)
SARS-CoV-2 Isolate hCoV-19/USAIPHC 658/2021 Delta	1500	100% (3/3)
SARS-CoV-2 Omicron B.1.1.529	1500	100% (3/3)
SARS-CoV-2 USA-WI1/2020	1500	100% (3/3)
Kappa, (B.1.617.1) USA/CA-Stanford-15_S02/2021	1500	100% (3/3)
Iota, (B.1.526_2021) NY-Wadsworth-21025952-01/2021	1500	100% (3/3)
Zeta, (P2_2021) NY-Wadsworth-21006055-01/2021	1500	100% (3/3)
SARS-CoV-2 Isolate USA-CA3/2020	1500	100% (3/3)
SARS-CoV-2 Lineage B Isolate Germany/BavPat1/2020	1500	100% (3/3)
SARS-CoV-2 Epsilon, (B.1.429) USA/CA/VRLC014/2021	1500	100% (3/3)
SARS-CoV-2 Delta (AY4.2) hCoV-19/USA/VA-FBCH_675/2021	1500	100% (3/3)
SARS-CoV-2 Lambda (C.37) Isolate Peru/un-CDC-2-4069945/2021	1500	100% (3/3)
SARS-CoV-2 Omicron B.1.1.529 Lineage BQ.1.1 Isolate hCoV-19/USA/MDHP38861/2022	1500	100% (3/3)
SARS-CoV-2 Omicron XBB hCoV19/USA/CA-Standford-109_S21/2022	1500	100% (3/3)
SARS-CoV-2 Omicron Lineage JN.1 Isolate hCoV-19/USA/NewYork/PV96109/2023	1500	100% (3/3)
SARS-CoV-2 Lin. JN.1.4; Omicron Var (USA/NY-Wadsworth-230681 07-01/2023)	1500	100% (3/3)

510(k) Summary

Table 15. Simplexa™ COVID-19 / Flu A/B & RSV Direct Analytical Reactivity – RSV

RSV Strain	Tested Concentration (copies/mL)	Agreement with Expected Results (#Detected/#Total)
RSV-A (Isolate: 2006 Isolate)	3000	100% (3/3)
RSV-A (3/2015 Isolate 3)	3000 (3X LoD)	66% (2/3)
	4000 (4X LoD)	100% (3/3)
RSV A2	3000	100% (3/3)
RSV A (12/2014 Isolate 12)	3000	100% (3/3)
RSV B1	3000	100% (3/3)
RSV-B (12/2014 Isolate 1)	3000	100% (3/3)
RSV-B (3/2015 Isolate 2)	3000	100% (3/3)
RSV-B (3/2015 Isolate 1	3000	100% (3/3)
RSV B Washington	3000	100% (3/3)

Analytical reactivity – *In silico* inclusivity

An *in silico* analysis of the assay oligo sequences in the Simplexa™ COVID-19/Flu A/B & RSV Direct assay was performed. All primer and probe sets were aligned against sequences available in the GISAID EpiCoV (as of May 5, 2025), EpiFlu (between January 1, 2012, and May 4, 2025) or EpiRSV (as of May 4, 2025) databases depending on the target. Specifically, 5,641,034 sequences of SARS-CoV-2 (including available sequences from variants of concern or variants of interest defined as of May 5, 2025), 230093 sequences of influenza A, 61232 sequences of influenza B and 22970 sequences of RSV were aligned.

Based on the *in silico* analysis, the Simplexa™ COVID-19/Flu A/B & RSV Direct assay exhibits ~100% inclusivity to SARS-CoV-2 sequences available in the GISAID EpiCoV database as of May 5, 2025, with predicted detection by oligos of the ORF7a and/or the S gene. The assay oligos for influenza A and influenza B are predicted to have ~100% inclusivity to influenza A sequences and ~100% inclusivity to influenza B sequences available in the GISAID EpiFlu database between January 1, 2012, to May 4, 2025. The RSV assay oligos are predicted to have ~99% inclusivity to RSV sequences available in the GISAID EpiRSV database as of May 4, 2025.

Cross-reactivity (Analytical specificity)

Cross-reactivity of the Simplexa™ COVID-19 / Flu A/B & RSV Direct assay was evaluated by testing microorganisms that are closely related, cause similar clinical symptoms or may be present in the same sample type. Specimens for laboratory testing were prepared by spiking cultured isolates, inactivated organisms, or purified nucleic acids (whole genome) into negative (NPS) matrix and determining cross reactivity based on three replicates. Results of cross-reactivity testing are shown in Table 16.

510(k) Summary

Table 16. Simplexa™ COVID-19 / Flu A/B & RSV Direct Cross-Reactivity (Analytical Specificity)

Organism	Tested Concentration	Agreement with Expected Results: % Detection (# Detected/#Tested)
Aspergillus fumigatus	1E6 CFU/mL	0% (0/3)
Bordetella pertussis	1E6 CFU/mL	0% (0/3)
Candida albicans	1E6 CFU/mL	0% (0/3)
Chlamydophila pneumoniae	1E6 Cps/mL	0% (0/3)
Corynebacterium diphtheriae	1E6 CFU/mL	0% (0/3)
Escherichia coli	1E6 CFU/mL	0% (0/3)
Haemophilus influenzae	1E6 CFU/mL	0% (0/3)
Lactobacillus plantarum	1E6 CFU/mL	0% (0/3)
Legionella pneumophila	1E6 CFU/mL	0% (0/3)
Moraxella catarrhalis	1E6 CFU/mL	0% (0/3)
Mycobacterium tuberculosis (genomic DNA)	1E6 Cps/mL	0% (0/3)
Mycoplasma pneumoniae	1E6 Cps/mL	0% (0/3)
Neisseria gonorrhoeae	1E6 CFU/mL	0% (0/3)
Neisseria meningitidis	1E6 CFU/mL	0% (0/3)
Pneumocystis jirovecii	1E6 CFU/mL	0% (0/3)
Pseudomonas aeruginosa	1E6 CFU/mL	0% (0/3)
Staphylococcus aureus	1E6 CFU/mL	0% (0/3)
Staphylococcus epidermidis	1E6 CFU/mL	0% (0/3)
Streptococcus pyogenes	1E6 CFU/mL	0% (0/3)
Streptococcus salivarius	1E6 CFU/mL	0% (0/3)
Streptococcus pneumonia	1E6 CFU/mL	0% (0/3)
Adenovirus 1	1E5 $TCID_{50}$/mL	0% (0/3)
Adenovirus 7A	1E5 $TCID_{50}$/mL	0% (0/3)
Coronavirus 229E	1E5 $TCID_{50}$/mL	0% (0/3)
Coronavirus NL63	1E5 $TCID_{50}$/mL	0% (0/3)
Coronavirus OC43	1E5 $TCID_{50}$/mL	0% (0/3)
Coronavirus HKU1	1E5 Cps/mL	0% (0/3)
Cytomegalovirus (CMV)	1E5 $TCID_{50}$/mL	0% (0/3)
Enterovirus	1E5 $TCID_{50}$/mL	0% (0/3)

510(k) Summary

Organism	Tested Concentration	Agreement with Expected Results: % Detection (# Detected/#Tested)
Epstein-Barr Virus (EBV)	1E5 Cps/mL	0% (0/3)
Influenza C	1E5 $TCID_{50}$/mL	0% (0/3)
Measles virus	1E5 $TCID_{50}$/mL	0% (0/3)
MERS-coronavirus	1E5 $TCID_{50}$/mL	0% (0/3)
Human Metapneumovirus 9 type A1	1E5 $TCID_{50}$/mL	0% (0/3)
Mumps virus	1E5 $TCID_{50}$/mL	0% (0/3)
Parainfluenza virus Type 1	1E5 $TCID_{50}$/mL	0% (0/3)
Parainfluenza virus Type 2	1E5 $TCID_{50}$/mL	0% (0/3)
Parainfluenza virus Type 3	1E5 $TCID_{50}$/mL	0% (0/3)
Parainfluenza virus Type 4A	1E5 $TCID_{50}$/mL	0% (0/3)
Parechovirus	1E5 $TCID_{50}$/mL	0% (0/3)
Rhinovirus	1E5 $TCID_{50}$/mL	0% (0/3)
Varicella-zoster virus	1E5 Cps/mL	0% (0/3)
Pooled human nasal wash	N/A	0% (0/3)
*Bacillus anthracis**	N/A	N/A
SARS-Coronavirus-1*	N/A	N/A
Bordetella parapertussis E595	1E6 CFU/mL	0% (0/3)
Fusobacterium necrophorum	1E6 CFU/mL	0% (0/3)
Mycoplasma genitalium	1E6 CCU/mL	0% (0/3)

CFU/mL= Colony forming units/milliliter, $TCID_{50}$/mL = Tissue Culture Infectious Dose/milliliter, Cps/mL = Copies/milliliter, CCU/mL: Color counting unit/milliliter, N/A = not applicable
*Tested *in silico* due to unavailability of the organism

Cross reactivity – *In silico* exclusivity

An *in silico* exclusivity (cross-reactivity) analysis was performed to predict potential cross-reactivity of the assay oligos through a BLAST comparison of the oligo sequences to the human reference genome and the GenBank nt sequence database residing locally on company servers. To ensure that all potential cross-reactivity was assessed, this analysis was performed with all primers and probes in the assay oligo mix, including oligos used to amplify and detect the internal control. This analysis assesses the potential for non-specific amplification and detection of the human genome with sequences from the *Homo sapiens* reference genome GRCh38.p14, as well as other potentially cross-reactive organisms with sequences available in the GenBank nt database as of May 10, 2025. Based on *in silico* exclusivity analysis, the assay oligos are predicted to have no cross reactivity to human genome sequences from the *Homo sapiens* reference genome GRCh38.p14. *In silico*

510(k) Summary

assessment of the analyzed potential cross-reactive organisms, with sequences available in the GenBank nt database as of May 10, 2025, predicts potential cross-reactivity for assay oligos of SARS-CoV-2 to some bat coronavirus and bat SARS-like coronavirus strains.

INTERFERING SUBSTANCES

Potentially interfering substances from respiratory specimens were tested to assess potential interaction with targets and RNA Internal Control detection. Samples were prepared by 1) diluting each potentially interfering substance into a sample consisting of pooled negative nasopharyngeal swab (NPS) matrix and SARS-CoV-2 Lineage BA.2.3 (Omicron Variant) inactivated viral particles, or influenza A Victoria/4897/2022 or influenza B/Austria /1359417/2021 or RSV B CH93(18)-18 active viral particles at 3X LoD; 2) diluting each potentially interfering substance into a sample only consisting of pooled negative nasopharyngeal swab (NPS) matrix and not containing any spiked target. The results are shown in Table 17 and Table 18.

None of the substances tested interfered with the detection of SARS-CoV-2, influenza A, influenza B, RSV or RNA Internal Control at the concentrations tested.

Table 17. Simplexa™ COVID-19 / Flu A/B & RSV Direct Interference for Positive Sample

Potentially Interfering Substance	Active Ingredient	Interferent Concentration*	Flu A	Flu B	SARS-CoV-2	RSV	Internal Control
			% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)
Afrin Nasal spray	Oxymetazoline	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Antibacterial, systemic	Tobramycin	4 µg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Antibiotic, nasal ointment	Mupirocin	6.6 mg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Whole Blood	N/A	2% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Cold Eeze (Throat lozenges, Oral anesthetic and analgesic)	N/A	1.25% (w/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Nasal corticosteroid (Beconase AQ)	Beclomethasone	5% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Nasal corticosteroid (Flonase)	Fluticasone	5% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Relenza Antiviral Drug	Zanamivir	3.3 mg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Tamiflu Antiviral drug	Oseltamivir	1 µM	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

510(k) Summary

Potentially Interfering Substance	Active Ingredient	Interferent Concentration*	Flu A	Flu B	SARS-CoV-2	RSV	Internal Control
			% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)
Zicam Nasal Gel	Luffa operculata, Galphimia glauca, histaminum hydrochloricum	5% (w/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Zicam Nasal Spray (Homeopathic allergy relief medicine)	N/A	10% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Bovine submaxillary gland mucin, type I-S	Purified Mucin Protein	2.5 mg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Antiviral drug	Remdesivir	10 µg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Human Leukocytes	N/A	5% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

*µg/mL = Micrograms/milliliter, mg/mL = Milligrams/milliliter, µM = Micromolar, v/v = Volume per Volume, w/v = Weight/Volume

Table 18. Simplexa™ COVID-19 / Flu A/B & RSV Direct Interference for Negative Sample

Potentially Interfering Substance	Active Ingredient	Interferent Concentration*	Flu A	Flu B	SARS-CoV-2	RSV	Internal Control
			% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)
Afrin Nasal spray	Oxymetazoline	15% (v/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Antibacterial, systemic	Tobramycin	4 µg/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Antibiotic, nasal ointment	Mupirocin	6.6 mg/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Whole Blood	N/A	2% (v/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Cold Eeze (Throat lozenges, Oral anesthetic and analgesic)	N/A	1.25% (w/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Nasal corticosteroid (Beconase AQ)	Beclomethasone	5% (v/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Nasal corticosteroid (Flonase)	Fluticasone	5% (v/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Relenza Antiviral Drug	Zanamivir	3.3 mg/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Tamiflu Antiviral drug	Oseltamivir	1 µM	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)

510(k) Summary

Potentially Interfering Substance	Active Ingredient	Interferent Concentration*	Flu A	Flu B	SARS-CoV-2	RSV	Internal Control
			% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)	% Detection (#Detected/ #Tested)
Zicam Nasal Gel	Luffa operculata, Galphimia glauca, histaminum hydrochloricum	5% (w/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Zicam Nasal Spray (Homeopathic allergy relief medicine)	N/A	10% (v/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Bovine submaxillary gland mucin, type I-S	Purified Mucin Protein	2.5 mg/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Antiviral drug	Remdesivir	10 µg/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Human Leukocytes	N/A	5% (v/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)

*µg/mL = Micrograms/milliliter, mg/mL = Milligrams/milliliter, µM = Micromolar, v/v = Volume per Volume, w/v = Weight/Volume

COMPETITIVE INTERFERENCE

Competitive interference testing was performed to assess the ability of the assay to detect a low concentration of one (1) target analyte in the presence of a high concentration of another target analyte. Samples were prepared by spiking one (1) assay target analyte at a low concentration (3X LoD) into negative nasopharyngeal swab (NPS) matrix in the presence of a high concentration (≥1E6 cps/mL) of one (1) of the other three (3) assay target analytes. Each contrived sample was tested in triplicate. The results are shown in Table 19. All combinations tested showed no competitive interference for the detection of low concentrations of SARS-CoV-2, influenza A, influenza B, and RSV in the presence of high concentrations of another assay target analyte.

Table 19. Simplexa™ COVID-19 / Flu A/B & RSV Direct Competitive Interference

Low Positive Baseline Sample		Competitive Interferent		Agreement with Expected Results: % Detection (#Detected/#Total)			
Strain	Copies/mL	Strain	Copies/mL	Flu A	Flu B	SARS-CoV-2	RSV
Influenza A Victoria/4897/2022	1500	Influenza B/Austria/ 1359417/2021	1E6	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)
		SARS-CoV-2 USA/WA 1/2020	1E6	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)
		RSV B CH93(18)-18	1E6	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)
Influenza B/Austria/ 1359417/2021	1500	Influenza A Victoria/4897/2022	1E6	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)
		SARS-CoV-2 USA/WA 1/2020	1E6	0% (0/3)	100% (3/3)	100% (3/3)	0% (0/3)

510(k) Summary

Low Positive Baseline Sample		Competitive Interferent		Agreement with Expected Results: % Detection (#Detected/#Total)			
Strain	**Copies/mL**	**Strain**	**Copies/mL**	**Flu A**	**Flu B**	**SARS-CoV-2**	**RSV**
		RSV B CH93(18)-18	1E6	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
SARS-CoV-2 USA/WA 1/2020	1500	Influenza A Victoria/4897/2022	1E6	100% (3/3)	0% (0/3)	100% (3/3)	0% (0/3)
		Influenza B/Austria/ 1359417/2021	1E6	0% (0/3)	100% (3/3)	100% (3/3)	0% (0/3)
		RSV B CH93(18)-18	1E6	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)
RSV B CH93(18)-18	3000	Influenza A Victoria/4897/2022	1E6	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)
		Influenza B/Austria/ 1359417/2021	1E6	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
		SARS-CoV-2 USA/WA 1/2020	1E6	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)

INHIBITION BY OTHER MICROORGANISMS

The Simplexa™ COVID-19 / Flu A/B & RSV Direct assay was evaluated by testing the ability to identify SARS-CoV-2, influenza A, influenza B, and RSV when other potential inhibitory microorganisms were present. Specimens were prepared by spiking cultured isolates, inactivated organisms, or purified nucleic acids (whole genome) at a minimum of 10^6 CFU/ml for bacteria, and 10^5 $TCID_{50}$/mL for viruses into negative nasopharyngeal swab (NPS) matrix in the presence of a low concentration (3X LoD) of either SARS-CoV-2 (SARS-CoV-2 Lineage BA.2.3; Omicron Variant), influenza A (A/Victoria/4897/2022), influenza B (B/Austria/1359417/2021) or RSV (RSV B CH93(18)-18). Forty-six (46) potentially inhibitory microorganisms were individually spiked and tested in triplicate. For organisms not titered in CFU/mL or $TCID_{50}$/mL, other industry acceptable units were used as indicated. No inhibition by other organisms was observed for SARS-CoV-2, influenza A, influenza B or RSV at the concentrations indicated in Table 20.

Table 20. Simplexa™ COVID-19 / Flu A/B & RSV Direct Microbial Inhibition

Organism	Tested Concentration	Agreement with Expected Results: (# Detected/#Tested)			
		Flu A	**Flu B**	**SARS-CoV-2**	**RSV**
Aspergillus fumigatus	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Bordetella pertussis	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Candida albicans	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Chlamydophila pneumoniae	1E6 Cps/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Corynebacterium diphtheriae	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Escherichia coli	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

510(k) Summary

Organism	Tested Concentration	Agreement with Expected Results: (# Detected/#Tested)			
		Flu A	Flu B	SARS-CoV-2	RSV
Haemophilus influenzae	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Lactobacillus plantarum	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Legionella pneumophila	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Moraxella catarrhalis	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Mycobacterium tuberculosis (genomic DNA)	1E6 Cps/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Mycoplasma pneumoniae	1E6 Cps/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Neisseria gonorrhoeae	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Neisseria meningitidis	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Pneumocystis jirovecii	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Pseudomonas aeruginosa	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Staphylococcus aureus	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Staphylococcus epidermidis	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Streptococcus pyogenes	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Streptococcus salivarius	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Streptococcus pneumonia	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Adenovirus 1	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Adenovirus 7A	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Coronavirus 229E	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Coronavirus NL63	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Coronavirus OC43	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Coronavirus HKU1	1E5 Cps/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Cytomegalovirus (CMV)	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Enterovirus	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Epstein-Barr Virus (EBV)	1E5 Cps/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

510(k) Summary

Organism	Tested Concentration	Agreement with Expected Results: (# Detected/#Tested)			
		Flu A	Flu B	SARS-CoV-2	RSV
Influenza C	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Measles virus	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
MERS-coronavirus	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Human Metapneumovirus 9 type A1	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Mumps virus	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parainfluenza virus 1	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parainfluenza virus 2	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parainfluenza virus 3	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parainfluenza virus 4	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parechovirus	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Rhinovirus	1E5 $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Varicella-zoster virus	1E5 Cps/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Pooled human nasal wash	N/A	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Bordetella parapertussis E595	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Fusobacterium necrophorum	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Mycoplasma genitalium	1E6 CCU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

CFU/mL= Colony forming units/milliliter, $TCID_{50}$/mL = Tissue Culture Infectious Dose/milliliter, Cps/mL = Copies/milliliter, CCU/mL: Color counting unit/milliliter; N/A = not applicable

CARRY-OVER CONTAMINATION

Amplification carry-over and cross contamination for the Simplexa™ COVID-19 / Flu A/B & RSV Direct assay has been assessed. The study was designed by alternately placing high positive and negative samples on each disc. High Positive (HP) samples were formulated by spiking 1E6 copies/mL SARS-CoV-2 Lineage BA.2.3 Omicron into pooled negative nasopharyngeal swab (NPS) matrix. Pooled negative nasopharyngeal swab (NPS) matrix was used as negative sample. No evidence of carry-over or cross contamination was observed.

Proposed Labeling:

The labeling provided in the submission satisfies the requirements of 21 CFR 809.10.

510(k) Summary

Conclusion:

The Simplexa™ COVID-19 & Flu A/B & RSV Direct assay is substantially equivalent to the Panther Fusion SARS-CoV-2/Flu A/B/RSV assay (K242465). Despite differences in workflow and instrumentation, both assays share the same intended use, target analytes, RT-PCR amplification and detection chemistry, and demonstrate comparable analytical and clinical performance. Panther Fusion SARS-CoV-2/Flu A/B/RSV assay is the most appropriate predicate for the Simplexa™ COVID-19 & Flu A/B & RSV Direct assay under 21 CFR 807.92.