



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT**

I Background Information:

A 510(k) Number

K252387

B Applicant

Diasorin Molecular, LLC

C Proprietary and Established Names

Simplexa COVID-19/ Flu A/B & RSV Direct (MOL4450); Simplexa COVID-19/ Flu A/B & RSV Positive Control Pack (MOL4460)

D Regulatory Information

| Product Code(s) | Classification | Regulation Section | Panel |
|-----------------|----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|
| QOF | Class II | 21 CFR 866.3981 - 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 | MI - Microbiology |

II Submission/Device Overview:

A Purpose for Submission:

The purpose of this submission is to show that the Simplexa COVID-19/ Flu A/B & RSV Direct (MOL4450) test is substantially equivalent to the FDA cleared Panther Fusion SARS-CoV-2/Flu A/B/RSV assay (K242465).

B Measurand:

- Influenza A RNA
- Influenza B RNA
- Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) RNA
- Respiratory Syncytial Virus (RSV) RNA

C Type of Test:

Qualitative RT-PCR

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) 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 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.

C Special Conditions for Use Statement(s):

Rx - For prescription use only

For in vitro diagnostic use only

D Special Instrument Requirements:

For use with LIAISON MDX Instrument.

IV Device/System Characteristics:

A 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 (ANS) 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 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.

B Principle of Operation:

The Simplexa COVID-19 / Flu A/B & RSV Direct assay targets specific genomic regions for each virus: the S and ORF7a regions of SARS-CoV-2, the matrix gene of Influenza A, the matrix and nucleoprotein genes of Influenza B, and the M and G genes of RSV. An encapsulated RNA internal control (RNA IC) derived from *bacteriophage MS2* is included in each reaction to monitor for RT-PCR inhibition or failure. The RNA IC is co-amplified with the assay targets and detected

independently using post-amplification melting curve analysis. It is assigned to a dedicated fluorescent channel and does not interfere with target detection. Its presence 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. The assay employs fluorescent dye-labeled probes and primers specific to each target, and the LIAISON MDX software automates signal detection, amplification curve analysis, and qualitative result interpretation.

C Instrument Description Information:

1. Instrument Name:

The LIAISON MDX instrument using LIAISON MDX Studio Software version 2.4.4.0 or higher

2. Specimen Identification:

Barcodes on the reagent kit and the barcode card contain the assay definition protocol and parameters for identification for the test and specimen type.

3. Specimen Sampling and Handling:

Nasopharyngeal and anterior nasal swab specimens collected in Universal Transport Medium (UTM), BD Universal Viral Transport (UVT) or M4RT.

4. Calibration:

The user does not perform calibration of the LIAISON MDX instrument.

5. Quality Control:

The RNA Internal Control (RNA-IC) is derived from bacteriophage MS2. The RNA-IC is included in each reaction to monitor for RT-PCR inhibition or failure. In addition, the test utilizes external controls – the Simplexa COVID-19 / Flu A/B & RSV Positive Control Pack (REF MOL4460) and No Template Control, provided separately.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Panther Fusion SARS-CoV-2/Flu A/B/RSV assay

B Predicate 510(k) Number(s):

K242465

C Comparison with Predicate(s):

| Device & Predicate Device(s): | <u>K252387</u> | <u>K242465</u> |
|---------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Device Trade Name | Simplexa COVID-19 & Flu A/B & RSV Direct | Panther Fusion SARS-CoV-2/Flu A/B/RSV |
| General Device Characteristic Similarities | <u>K252387</u> | <u>K242465</u> |
| Product Code | Same | QOF |
| Regulation | Same | 21 CFR 866.3981 |
| Organisms Detected | Same | Influenza A, Influenza B, Sars-CoV-2, Respiratory Syncytial Virus |
| Measurand | Same | Nucleic acid from Organisms detected |
| Intended Use/Indications For Use | <p>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.</p> <p>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</p> | <p>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 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</p> |

| | | |
|------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| | <p>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.</p> <p>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.</p> | <p>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 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.</p> |
| Specimen Types | NPS/ANS swabs in UVT/UTM/M4RT | NP/AN swabs in VTM/UTM/eSTM |
| Internal Control | MS2 phage (bacteriophage) | Internal Control-S (IC-S) synthetic RNA |
| Positive Control | Manual single-use vial | PRD-07401, system-integrated control |

| General Device Characteristic Differences | <u>K252387</u> | <u>K242465</u> |
|--------------------------------------------------|-------------------------------|-----------------------------------------------|
| Technology | RT-PCR (direct amplification) | RT-PCR (automated extraction + amplification) |

VI Standards/Guidance Documents Referenced:

1. 21 CFR 866.3981 - Special Controls
2. AAMI. Principles for medical device security – Risk Management. AAMI document TIR57:2016. Association for the Advancement of Medical Instrumentation; 2016.
3. AAMI. Principles for medical device security – Postmarket risk management for device manufacturers. AAMI document TIR97:2019. Association for the Advancement of Medical Instrumentation; 2019.
4. 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.
5. CLSI. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition. CLSI document EP05-A3. Wayne, PA: Clinical Laboratory Standards Institute; 2019.
6. CLSI. Interference Testing in Clinical Chemistry. 3rd Ed. CLSI Document EP07. Wayne, PA: Clinical Laboratory Standards Institute; 2018.
7. CLSI. Evaluation of Qualitative, Binary Output Examination Performance; Approved Guideline – Third Edition. CLSI document EP12. Wayne, PA: Clinical Laboratory Standards Institute; 2023.
8. 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.
9. 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.
10. CLSI. Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI document EP25-A. Wayne, PA: Clinical Laboratory Standards Institute; 2009.
11. CLSI. Collection Transport Preparation and Storage of Specimens for Molecular Methods. 2nd Edition. CLSI Document MM13. Wayne, PA: Clinical Laboratory Standards Institute; 2020.
12. CLSI. Verification and Validation of Multiplex Nucleic Acid Assays. 2nd Edition. CLSI Document MM17. Wayne, PA: Clinical Laboratory Standards Institute; 2018.
13. ISTA. Packaged-Products for Parcel Delivery System Shipment 70 kg (150 lb) or Less. ISTA Document 3A. International Safe Transit Association. 2018.
14. IEC 62366-1 Edition 1.1 2020-06 Consolidated Version; Medical devices – Part 1: Application of usability engineering to medical devices
15. 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
16. IEC 60601-1-2 Edition 4.1 2020-09 Consolidated Version; Medical electrical equipment – Part 1-2: General requirements for basic safety and essential performance – Collateral Standard: Electromagnetic disturbances – Requirements and tests

17. IEC 61326-1 Edition 3.0 2020-10; Electrical equipment for measurement, control and laboratory use – EMC requirements – Part 1: General requirements
18. 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
19. IEC 62304 Edition 1.1 2015-06 Consolidated Version; Medical device software – Software life cycle processes
20. IEC TR 60878 Ed. 4.0 2022-11; Graphical symbols for electrical equipment in medical practice [Including: Corrigendum 1 (2023)]
21. 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
22. 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
23. ISO 14971:2019 Medical Devices – Application of risk management to medical devices
24. ISO 15223-1: 2021-07 – Medical Devices- Symbols to be used with information to be supplied by the manufacturer – Part 1: General requirements
25. UL ANSI 2900-1 First Edition 2017; Standard for Safety, Standard for Software Cybersecurity Network-Connectable Products, Part 1: General Requirements
26. 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

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

a) Within-Laboratory Precision

Within-laboratory precision of the Simplexa COVID-19/FLUA/B & RSV Direct assay was evaluated at a single site by testing a contrived panel (**Table 1**) in duplicate each day across six non-consecutive days using a single instrument. The panel consisted of ten members (live virus for influenza A, influenza B and RSV, and inactivated virus for SARS-CoV-2) prepared by spiking organisms in negative pooled NPS matrix. Three reagent lots were used in this study with 8 replicates of each panel member tested with each reagent lot. Results were generated from a total of 24 replicates of each panel member (2 runs/day x 2 replicate/run x 6 days).

Table 1. Precision/Reproducibility Study Sample Panel

| Target | Concentration | Concentration (cps/mL) |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------|-----------------------------------------------------------------------------------|
| Positive Control (PC): β-PL inactivated Flu A Strain A/California/7/2009, β-PL inactivated Flu B Strain B/Malaysia/2506/2004, SARS-nCoV-2 USA/WA1/2020, β-PL Inactivated RSV Strain A2 | 5x LoD | Influenza A, Influenza B, SARS-CoV-2: 2500 cps/mL RSV: 5000 cps/mL |
| No Template Control (NTC) – Pooled Negative Human Nasopharyngeal Matrix | N/A | N/A |
| Influenza A/Victoria/4897/2022 (H1N1) | Low (2x LoD) | 1000 cps/mL |
| Influenza A/Victoria/4897/2022 (H1N1) | Moderate (5x LoD) | 2500 cps/mL |
| Influenza B/Austria/1359417/2021 | Low (2x LoD) | 1000 cps/mL |
| Influenza B/Austria/1359417/2021 | Moderate (5x LoD) | 2500 cps/mL |
| RSV, B CH93(18)-18 | Low (2x LoD) | 2000 cps/mL |
| RSV, B CH93(18)-18 | Moderate (5x LoD) | 5000 cps/mL |
| SARS-CoV-2, Lineage BA.2.3; Omicron Variant | Low (2x LoD) | 1000 cps/mL |
| SARS-CoV-2, Lineage BA.2.3; Omicron Variant | Moderate (5x LoD) | 2500 cps/mL |

The qualitative results (i.e., % agreement with expected results) from the study are illustrated in **Table 2**.

Table 2. Within-Laboratory Precision Study – Qualitative Results

| Target | Level | Positive Results | Total Results | % Expected Result | Two-sided 95% CI Lower Bound | Two-sided 95% CI Upper Bound |
|-------------|--------|------------------|---------------|-------------------|------------------------------|------------------------------|
| Influenza A | 2x LoD | 24 | 24 | 100 | 86.2 | 100 |
| | 5x LoD | 24 | 24 | 100 | 86.2 | 100 |
| Influenza B | 2x LoD | 24 | 24 | 100 | 86.2 | 100 |

| Target | Level | Positive Results | Total Results | % Expected Result | Two-sided 95% CI Lower Bound | Two-sided 95% CI Upper Bound |
|---------------------|--------|------------------|---------------|-------------------|------------------------------|------------------------------|
| | 5x LoD | 24 | 24 | 100 | 86.2 | 100 |
| RSV A | 2x LoD | 24 | 24 | 100 | 86.2 | 100 |
| | 5x LoD | 24 | 24 | 100 | 86.2 | 100 |
| SARS-CoV-2 | 2x LoD | 24 | 24 | 100 | 86.2 | 100 |
| | 5x LoD | 24 | 24 | 100 | 86.2 | 100 |
| PC | 5x LoD | 96 | 96 | 100 | 86.2 | 100 |
| No Template Control | Blank | 0 | 24 | 100 | 86.2 | 100 |

All positive panel members exhibited a detection rate of 100%. The negative panel was negative 100% of the time. There was no lot-to-lot variability observed in the study. The results of the study demonstrate acceptable assay variability.

The means, standard deviations, and coefficients of variation (%) for cycle threshold (Ct) values by target analyte and expected concentration (Positive Panel Members) are shown in **Table 3**.

Table 3. Precision - standard deviations and coefficients of variation of Ct values

| Target | Level | Hit rate % | Mean Ct | Inter-Day | | Inter-Lot | | Inter-Run | | Total | |
|-------------|--------|------------|---------|-----------|-----|-----------|-----|-----------|-----|-------|-----|
| | | | | SD | CV% | SD | CV% | SD | CV% | SD | CV% |
| Influenza A | 2x LoD | 100 | 31.1 | 0.4 | 1.4 | 0.4 | 1.4 | 0.7 | 2.1 | 0.7 | 2.3 |
| | 5x LoD | 100 | 30.4 | 0.5 | 1.6 | 0.5 | 1.6 | 0.4 | 1.3 | 0.6 | 1.9 |
| Influenza B | 2x LoD | 100 | 32.3 | 0.6 | 1.8 | 0.6 | 1.8 | 0.4 | 1.2 | 0.7 | 2.1 |
| | 5x LoD | 100 | 31.7 | 0.3 | 0.8 | 0.3 | 0.8 | 0.4 | 1.4 | 0.4 | 1.4 |
| RSV A | 2x LoD | 100 | 30.6 | 0.3 | 1.0 | 0.3 | 1.0 | 0.5 | 1.7 | 0.5 | 1.7 |
| | 5x LoD | 100 | 29.7 | 0.5 | 1.5 | 0.5 | 1.6 | 1.1 | 3.7 | 1.0 | 3.5 |
| SARS-CoV-2 | 2x LoD | 100 | 29.9 | 0.2 | 0.6 | 0.2 | 0.6 | 0.4 | 1.2 | 0.3 | 1.2 |
| | 5x LoD | 100 | 29.4 | 0.3 | 1.1 | 0.4 | 1.3 | 0.4 | 1.4 | 0.5 | 1.6 |

Note: SD= standard deviation, CV= coefficient of variation, Ct= cycle threshold. LoD limit of detection

b) Reproducibility

A reproducibility study was conducted with the Simplexa COVID-19/FLUA/B & RSV Direct assay at three clinical sites by a total of six operators (two operators at each site). Each operator tested the contrived panel used to evaluate precision (**Table 1**) in triplicate each day across five non-consecutive days. The panel consisted of ten members (live

virus for influenza A, influenza B and RSV, and inactivated virus for SARS-CoV-2) prepared by spiking organisms in negative pooled NPS matrix. Six instruments (two per site) and one reagent lot were used in this study. Results were generated from a total of 90 replicates of each panel member (3 sites x 5 days x 2 operators/site x 3 replicates/operator/day). The qualitative results of the study are illustrated in **Table 4**.

Table 4. Reproducibility Study- Qualitative Results

| Target | Level | % Agreement with Expected Results (n Detected/ N Tested) | | | |
|-------------|----------|-------------------------------------------------------------|-----------------|------------------|-----------------------------|
| | | Site 1 | Site 2 | Site 3 | Overall (95% CI) |
| NA | Negative | 100% (30/30) | 100% (30/30) | 100% (30/300) | 100% (90/90) (95.9-100%) |
| PC | 5x LoD | 100% (30/30) | 100% (30/30) | 100% (30/30) | 100% (90/90) (95.9-100%) |
| Influenza A | 2x LoD | 100% (30/30) | 100% (30/30) | 100% (30/30) | 100% (90/90) (95.9-100%) |
| Influenza B | | 100% (30/30) | 100% (30/30) | 100% (30/30) | 100% (90/90) (95.9-100%) |
| RSV | | 100% (30/30) | 100% (30/30) | 100% (30/30) | 100% (90/90) (95.9-100%) |
| SARS-CoV-2 | | 100% (30/30) | 100% (30/30) | 100% (30/30) | 100% (90/90) (95.9-100%) |
| Influenza A | 5x LoD | 100% (30/30) | 100% (30/30) | 100% (30/30) | 100% (90/90) (95.9-100%) |
| Influenza B | | 100% (30/30) | 100% (30/30) | 100% (29/30) | 98.9% (89/90) (94-99.8%) |
| RSV | | 100% (30/30) | 100% (30/30) | 100% (30/30) | 100% (90/90) (95.9-100%) |
| SARS-CoV-2 | | 100% (30/30) | 100% (30/30) | 100% (30/30) | 100% (90/90) (95.9-100%) |

The Simplexa COVID-19/FLUA/B & RSV Direct assay results demonstrated acceptable site-to-site, instrument-to-instrument, day-to-day, and between operator variation for the ~2x LoD, and ~5x LoD panel members (**Table 5**).

Table 5. Reproducibility - Standard Deviations and Coefficients of Variation of Ct Values

| Target | Level | (n/N) ^a | Mean Ct | Within Run (Repeatability) | | Site | | Operator | | Day | | Instrument | | Total | |
|-------------|--------|--------------------|---------|-------------------------------|--------|------|--------|----------|--------|------|--------|-----------------|-----------------|-------|--------|
| | | | | SD | CV (%) | SD | CV (%) | SD | CV (%) | SD | CV (%) | SD | CV (%) | SD | CV (%) |
| Influenza A | 2x LoD | 90/90 | 33.2 | 0.76 | 2.3 | 0.50 | 1.5 | 0.51 | 1.5 | 0.85 | 2.6 | 0.6 | 1.8 | 0.99 | 3.0 |
| | 5x LoD | 90/90 | 32.5 | 0.45 | 1.4 | 0.65 | 2.0 | 0.59 | 1.8 | 0.65 | 2.0 | nc ^b | nc ^b | 0.69 | 2.1 |
| Influenza B | 2x LoD | 90/90 | 33.0 | 0.94 | 2.9 | 0.51 | 1.5 | 0.47 | 1.4 | 0.78 | 2.4 | 0.55 | 1.7 | 1.12 | 3.4 |

| Target | Level | (n/N) ^a | Mean Ct | Within Run (Repeatability) | | Site | | Operator | | Day | | Instrument | | Total | |
|------------|--------|--------------------|---------|----------------------------|--------|------|--------|----------|--------|------|--------|------------|--------|-------|--------|
| | | | | SD | CV (%) | SD | CV (%) | SD | CV (%) | SD | CV (%) | SD | CV (%) | SD | CV (%) |
| | 5x LoD | 89/90 | 31.9 | 0.61 | 1.9 | 0.10 | 0.3 | 0.11 | 0.4 | 0.59 | 1.8 | 0.41 | 1.3 | 0.75 | 2.4 |
| RSV | 2x LoD | 90/90 | 29.7 | 0.84 | 2.8 | 0.16 | 0.5 | 0.22 | 0.7 | 0.58 | 1.9 | 0.40 | 1.4 | 0.94 | 3.1 |
| | 5x LoD | 90/90 | 28.8 | 0.78 | 2.7 | 0.21 | 0.7 | 0.23 | 0.8 | 0.51 | 1.8 | 0.35 | 1.2 | 0.87 | 3.0 |
| SARS-CoV-2 | 2x LoD | 90/90 | 30.7 | 0.44 | 1.4 | 0.42 | 1.4 | 0.38 | 1.2 | 0.49 | 1.6 | 0.34 | 1.1 | 0.59 | 1.9 |
| | 5x LoD | 90/90 | 30.0 | 0.41 | 1.4 | 0.45 | 1.5 | 0.43 | 1.4 | 0.49 | 1.6 | 0.35 | 1.2 | 0.56 | 1.9 |

Ct = cycle threshold, LoD = limit of detection, SD = standard deviation, CV (%) = percent coefficient of variation, nc = not calculable.

^a n is the number of tests which matched the expected results. N is the total number of valid tests for the panel member.

^b Between instrument SD and %CV could not be calculated for Influenza A (~5x LoD) as all replicates were tested on a single instrument per site per operator

2. Linearity:

Not applicable; this is a qualitative assay.

3. Analytical Specificity/Interference:

The inclusivity of the Simplexa COVID-19/FLUA/B & RSV Direct assay was evaluated using a combination of *in silico* analysis of publicly available sequence information and laboratory testing of contrived specimens containing viral isolates that were selected to represent phylogenetic, geographic, and temporal diversity.

Analytical Reactivity (Inclusivity)

a) Wet-Testing

This study was performed to determine the analytical reactivity of the Simplexa COVID-19/FLUA/B & RSV Direct test with clinically relevant strains, serotypes, or subtypes of the target specie. The inclusivity panel was prepared by spiking various target microorganisms into pooled negative NPS matrix. Each strain was tested with 3 replicates near LoD starting at 3x LoD. The concentration which showed a 100% hit rate is shown in **Table 6** through **Table 9**.

Table 6. Strains of Influenza A Evaluated for Inclusivity

| Subtype | Strain/Isolate | Source | Catalogue No. | Concentration | | % Detected (# Detected/#Tested) |
|---------|-----------------------|----------------|------------------------|---------------|------|---------------------------------|
| | | | | Cp/mL | xLoD | |
| H1N1 | A/New Caledonia/20/99 | Microbiologics | Custom growth - J2019F | 1500 | 3x | 100% (3/3) |
| | A/Puerto Rico/8/34 | Virapur | Custom growth - I1508A | 1500 | 3x | |
| | A/Brisbane/59/07 | ZeptoMetrix | 0810244CF | 1500 | 3x | 100% (3/3) |
| | A/California/07/09 | ZeptoMetrix | 0810165CF | 1500 | 3x | 100% (3/3) |

| Subtype | Strain/Isolate | Source | Catalogue No. | Concentration | | % Detected (# Detected/#Tested) |
|---------------|----------------------------------------------------|----------------|---------------|---------------|------|------------------------------------|
| | | | | Cp/mL | xLoD | |
| H1N1 pdm09 | A/Guangdong-Maonan/SWL1536/2019 (H1N1) | Microbiologics | Lot: B2214X | 1500 | 3x | 100% (3/3) |
| | A/Brisbane/02/18 (H1N1) (CDC Panel) | Microbiologics | Lot: D1929A | 1500 | 3x | 100% (3/3) |
| | A/Nebraska/14/2018 (H1N1) | Microbiologics | Lot: D2013A | 1500 | 3x | 100% (3/3) |
| | A/Victoria/2570/2019 (H1N1) | Microbiologics | Lot: B2214P | 2000 | 4x | 100% (3/3) |
| | A/Mexico/4108/2009 (H1N1) | ZeptoMetrix | 0810166CF | 1500 | 3x | 100% (3/3) |
| | A/Sydney/05/2021 (H1N1) | Microbiologics | Lot: E2305A | 1500 | 3x | 100% (3/3) |
| | A/New York/02/09 (H1N1) | ZeptoMetrix | 0810109CF N | 1500 | 3x | 100% (3/3) |
| H3N2 | A/Perth/16/2009 (H3N2) (CDC Panel) | Virapur | L1909A | 2250 | 3x | 100% (3/3) |
| | A/Hong Kong/2671/2019 (H3N2) (CDC Panel) | Microbiologics | Lot: B2214V | 2250 | 3x | 100% (3/3) |
| | A/Kansas/14/2017 (H3N2) (CDC Panel) | Microbiologics | Lot: D1929B | 2250 | 3x | 100% (3/3) |
| | A/Cambodia/e0826360/2020 (H3N2) | Microbiologics | Lot: I2107G | 2250 | 3x | 100% (3/3) |
| | A/Darwin/6/2021 (H3N2) | Microbiologics | Lot: L2216B | 2250 | 3x | 100% (3/3) |
| | A/Tasmania/503/2020 (H3N2) | Microbiologics | Lot: B2221J | 2250 | 3x | 100% (3/3) |
| | A/Switzerland/9715293/13 (H3N2) | ZeptoMetrix | 0810511CF | 2250 | 3x | 100% (3/3) |
| | A/Texas/50/2012 (H3N2) | Microbiologics | Lot: B2322D | 2250 | 3x | 100% (3/3) |
| | A/Thailand/08/2022 (H3N2) | Microbiologics | Lot: C2419C | 2250 | 3x | 100% (3/3) |
| | A/Singapore/INFIMH-16-0019/2016 (H3N2) (CDC Panel) | Microbiologics | Lot: B1913A | 2250 | 3x | 100% (3/3) |
| | A/Hong Kong/4801/14 (H3N2) | Microbiologics | Lot: B1913E | 2250 | 3x | 100% (3/3) |
| | A/Massachusetts/18/2022 (H3N2) | Microbiologics | Lot: C2429E | 2250 | 3x | 100% (3/3) |
| | A/Hong Kong/8/1968 (H3N2) | Virapur | Lot: L1503A | 2250 | 3x | 100% (3/3) |
| | A/Port Chalmers 1/1973 (H3N2) | Microbiologics | Lot: K1516D | 2250 | 3x | 100% (3/3) |
| H5 | Influenza A H5N1 Gentaaur | AffiGen | AFG-CHK-0591 | 1500 | 3x | 100% (3/3) |
| | A/Anhui/01/2005 (H5N1) | IRR | FR-735 | 1500 | 3x | 100% (3/3) |
| | A/Egypt/N03072/2010 (H5N1) | IRR | FR-1065 | 1500 | 3x | 100% (3/3) |

| Subtype | Strain/Isolate | Source | Catalogue No. | Concentration | | % Detected (# Detected/#Tested) |
|---------|-----------------------------------------------------|--------|---------------|---------------|------|------------------------------------|
| | | | | Cp/mL | xLoD | |
| | A/Hubei/1/2010 (H5N1) | IRR | FR-1066 | 1500 | 3x | 100% (3/3) |
| | A/bovine/Ohio/B24 OSU-439/2024 (H5N1) - Genomic RNA | BEI | NR-59885 | 1500 | 3x | 100% (3/3) |
| H7 | A/Mallard/Netherlands/12/2000 (H7N7) | IRR | FR-773 | 1500 | 3x | 100% (3/3) |
| H9 | A/Hong Kong/33982/2009 (H9N2) | IRR | FR-1068 | 1500 | 3x | 100% (3/3) |

Table 7. Strains of Influenza B Evaluated for Inclusivity

| Lineage | Strain/Isolate | Source | Catalogue No. | Concentration | | % Detected (# Detected/#Tested) |
|----------|---------------------------------------------|----------------|---------------|---------------|------|------------------------------------|
| | | | | Cp/mL | xLoD | |
| Victoria | B/Brisbane/60/2008 (Victoria) (CDC Panel) | Virapur | Lot: E1703B | 1500 | 3x | 100% (3/3) |
| | B/Colorado/06/2017 (Victoria) (CDC Panel) | Microbiologics | Lot: L2212B | 1500 | 3x | 100% (3/3) |
| | B/Brisbane/33/08 (Victoria) | ZeptoMetrix | 0810253 CF | 1500 | 3x | 100% (3/3) |
| | B/Texas/2/13 (Victoria) (CDC Panel) | ZeptoMetrix | 0810527 CF | 1500 | 3x | 100% (3/3) |
| | B/Florida/02/06 (Victoria) | ZeptoMetrix | 0810037 CF | 1500 | 3x | 100% (3/3) |
| | B/Malaysia/2506/2004 (Victoria) | Virapur | Lot: G1626A | 1500 | 3x | 100% (3/3) |
| | B/Alabama/2/17 (Victoria) | ZeptoMetrix | 0810572 CF | 2500 | 5x | 100% (3/3) |
| | B/Maryland/01/1959 (Victoria) | Microbiologics | Lot: D1915A | 1500 | 3x | 100% (3/3) |
| | B/Washington 02/2019 (Victoria) (CDC Panel) | Microbiologics | Lot: D2013B | 1500 | 3x | 100% (3/3) |
| Yamagata | B/Florida/07/04 (Yamagata) | ZeptoMetrix | 0810256 CF | 1500 | 3x | 100% (3/3) |
| | B/Massachusetts/2/2012 (Yamagata) | Microbiologics | Lot: C2207E | 1500 | 3x | 100% (3/3) |
| | B/Utah/9/14 (Yamagata) | ZeptoMetrix | 0810516 CF | 1500 | 3x | 100% (3/3) |
| | B/Florida/04/06 (Yamagata) | ZeptoMetrix | 0810255 CF | 1500 | 3x | 100% (3/3) |
| | B/Panama/45/90 (Yamagata) | ZeptoMetrix | 0810259 CF | 1500 | 3x | 100% (3/3) |

Table 8. Strains of RSV Evaluated for Inclusivity

| Subtype | Strain/Isolate | Source | Catalogue No. | Concentration | | % Detected (# Detected/#Tested) |
|---------|------------------|----------------|---------------|---------------|------|------------------------------------|
| | | | | Cp/mL | xLoD | |
| A | 2006 Isolate | ZeptoMetrix | 0810040ACF | 3000 | 3x | 100% (3/3) |
| | 3/2015 Isolate 3 | ZeptoMetrix | 0810482CF | 4000 | 4x | 100% (3/3) |
| | A2 | Microbiologics | Lot: K2115I | 3000 | 3x | 100% (3/3) |

| | | | | | | |
|---|--------------------|----------------|-------------|------|----|------------|
| | 12/2014 Isolate 12 | ZeptoMetrix | 0810462CF | 3000 | 3x | 100% (3/3) |
| B | B1 | Microbiologics | Lot: A2320F | 3000 | 3x | 100% (3/3) |
| | 12/2014 Isolate 1 | ZeptoMetrix | 0810450CF | 3000 | 3x | 100% (3/3) |
| | 3/2015 Isolate 2 | ZeptoMetrix | 0810480CF | 3000 | 3x | 100% (3/3) |
| | 3/2015 Isolate 1 | ZeptoMetrix | 0810479CF | 3000 | 3x | 100% (3/3) |
| | Washington | Microbiologics | Lot: B2228B | 3000 | 3x | 100% (3/3) |

Table 9. Strains of SARS-CoV-2 Evaluated for Inclusivity

| Strain/Isolate | Source | Catalogue No. | Concentration | | % Detected (# Detected/#Tested) |
|----------------------------------------------------------------------|----------------|---------------|---------------|------|------------------------------------|
| | | | Cp/mL | xLoD | |
| Hong Kong/VM20001061/2020 | Virapur | Lot: F2016D | 1500 | 3x | 100% (3/3) |
| England/204820464/2020 | Microbiologics | Lot: D2112H | 1500 | 3x | 100% (3/3) |
| South Africa/KRISP-EC-K005325/2020 | Microbiologics | Lot: D2126L | 1500 | 3x | 100% (3/3) |
| hCoV-19/Japan/TY7-503/2021 | Microbiologics | Lot: G2126B | 1500 | 3x | 100% (3/3) |
| hCoV-19/USAIPHC 658/2021 Delta | Microbiologics | Lot: G2126D | 1500 | 3x | 100% (3/3) |
| Omicron B.1.1.529 | Microbiologics | Lot: C2330F | 1500 | 3x | 100% (3/3) |
| USA-WI1/2020 | Microbiologics | Lot: J2026G | 1500 | 3x | 100% (3/3) |
| B.1.617.1; Kappa Variant USA/CA-Stanford-15_S02/2021 | ZeptoMetrix | 081 0623CFH1 | 1500 | 3x | 100% (3/3) |
| Iota, (B.1.526_2021) NY-Wadsworth-21025952-01/2021 | ZeptoMetrix | 081 061 9CFHI | 1500 | 3x | 100% (3/3) |
| Zeta, (P2_2021) NY-Wadsworth-21006055-01/2021 | ZeptoMetrix | 0810618CFH1 | 1500 | 3x | 100% (3/3) |
| USA/CA3/2020 | ZeptoMetrix | 0810040ACF | 1500 | 3x | 100% (3/3) |
| Lineage B Isolate Germany/BavPat1/2020 | ZeptoMetrix | 081 0482CF | 1500 | 3x | 100% (3/3) |
| Epsilon, (B.1.429) USA/CA/VRLC014/2021 | Microbiologics | Lot: F2321F | 1500 | 3x | 100% (3/3) |
| Delta (AY4.2) hCoV-19/USA/VAFBCH_675/2021 | Microbiologics | Lot: F2321H | 1500 | 3x | 100% (3/3) |
| Lambda (C.37) Peru/un-CDC-2-4069945/2021 | Microbiologics | Lot: I2120F | 1500 | 3x | 100% (3/3) |
| Omicron B.1.1.529 Lineage BQ.1.1 Isolate hCoV-19/USA/MDHP388 61/2022 | Microbiologics | Lot: C2221L | 1500 | 3x | 100% (3/3) |
| Omicron XBB hCoV19/USA/CASanford-109_S21/2022 | Microbiologics | Lot: D2310F | 1500 | 3x | 100% (3/3) |

| | | | | | |
|--------------------------------------------------------------------------|--------------------|-------------|------|----|------------|
| Omicron Lineage JN.1 Isolate hCoV- 19/USA/NewYork/ PV96109/2023 | Microbiolog ics | Lot: D2403H | 1500 | 3x | 100% (3/3) |
| JN.1.4; Omicron Var (USA/NYWadsworth- 230681 07-01/2023) | ZeptoMetrix | 0810695CHFI | 1500 | 3x | 100% (3/3) |

b) *In Silico*

SARS-CoV-2 In Silico Analysis

The inclusivity of the Simplexa COVID-19/FLUA/B & RSV Direct assay was evaluated using *in silico* analysis of the forward primers, reverse primers, and probes for the SARS-CoV-2 target in relation to all sequences available in the GISAID gene databases. *In silico* analysis on May 5th, 2025, of the dual target SARS- CoV-2 primer and probe binding regions indicate 99.99% detection of all available complete and high-coverage sequences in GISAID (>5.3M sequences, <0.003% predicted failed detections) databases. *In silico* analysis indicated inclusivity to all known SARS- CoV-2 variants.

Cross-Reactivity and Microbial Interference

Cross-reactivity and microbial interference of the Simplexa COVID-19/FLUA/B & RSV Direct assay was evaluated by testing a panel of bacteria, fungi, and viruses commonly found in the respiratory tract for cross-reactivity and interference. The effect of non-target microorganisms on the performance of Simplexa COVID-19/FLUA/B & RSV Direct assay was tested by introducing non-target microorganisms into pooled negative NPS matrix spiked with and without SARS-CoV-2, influenza A, influenza B or RSV viruses at 3x LoD. Three (3) replicates in target positive background and three (3) replicates in target negative background were tested for each non-target microorganism. Bacteria and fungi were spiked at 1.0e+6 units/mL and viruses at 1.0e+5 units/mL, or the highest concentration possible. As summarized in **Table 10**, no cross-reactivity or microbial interference was observed at the concentrations tested, and no invalid results were obtained.

Table 10. Cross-reactivity and Microbial Interference Results for Non-Target Microorganisms

| Microorganism | Concentration | Negative Sample (Specificity Test) | | | | Positive Sample (Interference Test) | | | |
|------------------------------|----------------------------|------------------------------------|-------------|-------------|-----|-------------------------------------|-------------|-------------|-----|
| | | Target Result: n Detected/N Tested | | | | Target Result: n Detected/N Tested | | | |
| | | SARS-CoV-2 | Influenza A | Influenza B | RSV | SARS-CoV-2 | Influenza A | Influenza B | RSV |
| Adenovirus 1 | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Adenovirus 7A | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Aspergillus fumigatus</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Bordetella pertussis</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |

| | | | | | | | | | |
|------------------------------------------|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| <i>Bordetella parapertussis</i> E595 | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Candida albicans</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Chlamydophila pneumoniae</i> | 1E6 cps/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Coronavirus 229E | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Coronavirus NL63 | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Coronavirus OC43 | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Coronavirus HKU1 | 1E5 cps/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Corynebacterium diphtheriae</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Cytomegalovirus (CMV) | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Enterovirus | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Epstein-Barr Virus (EBV) | 1E5 cps/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Escherichia coli</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Fusobacterium necrophorum</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Haemophilus influenzae</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Lactobacillus plantarum</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Legionella pneumophila</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Measles | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| MERS-coronavirus | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Metapneumovirus | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Moraxella catarrhalis</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Mumps | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Mycobacterium tuberculosis (genomic DNA) | 1E6 cps/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Mycoplasma pneumoniae</i> | 1E6 cps/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Mycoplasma genitalium</i> | 1E6 CCU/mL | | | | | | | | |
| <i>Neisseria gonorrhoeae</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Neisseria meningitidis</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Parainfluenza virus 1 | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Parainfluenza virus 2 | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Parainfluenza virus 3 | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |

| | | | | | | | | | |
|-----------------------------------|----------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
| Parainfluenza virus 4 | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Parechovirus | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Pneumocystis jirovecii</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Pseudomonas aeruginosa</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Rhinovirus | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Staphylococcus aureus</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Staphylococcus epidermidis</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Streptococcus Pyogenes</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Streptococcus salivarius</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| <i>Streptococcus pneumonia</i> | 1E6 CFU/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Varicella-zoster virus | 1E5 cps/mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Influenza C | 1E5 TCID ₅₀ /mL | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |
| Pooled human nasal wash | n.a. | 0/3 | 0/3 | 0/3 | 0/3 | 3/3 | 3/3 | 3/3 | 3/3 |

CFU = Colony Forming Units; cps = copies; TCID₅₀ = Median Tissue Culture Infectious Dose; CCU = Color Changing Unit; n.a. = not applicable.

Interfering Substances

An analytical study was performed to assess the potential inhibitory effects of endogenous and exogenous substances that may be commonly found in NPS and NS specimens. All substances in **Table 11** were evaluated in the presence and absence of SARS-CoV-2, influenza A, influenza B or RSV viruses at 3x LoD in pooled negative NPS matrix. Testing for each condition was performed in replicates of ten. The results are shown in **Table 11** and **Table 12**.

None of the evaluated substances, at the concentrations tested, interfered with detection of the candidate assay targets.

Table 11. Substances Evaluated for Interference for Negative Samples

| 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) |

| 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) |
| 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) |
| 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

Table 12. Substances Evaluated for Interference for Positive Samples

| 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) |

| 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) |
| 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) |
| 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 ^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

COMPETITIVE INTERFERENCE

Competitive Interference (Co-Infection)

To assess potential competitive interference between the viral targets, Samples were prepared by spiking one (1) assay target analyte at a low concentration (3x LoD) into negative NPS matrix in the presence of a high concentration (up to 2000x LoD) of one (1) of the other three (3) assay target analytes. Three replicates were tested with one viral target at 3x LoD which was mixed with another target at high concentration. As shown in **Table 13**, none of the targets present at high concentration interfered with the detection of other viral targets at low concentration levels.

Table 13. Competitive Interference (Co-infection) Study Results

| Baseline | Competitive Interferent | Flu A Valid Positive Results/ Total # of Valid Results | Flu B Valid Positive Results/ Total # of Valid Results | SARS-CoV-2 Valid Positive Results/ Total # of Valid Results | RSV Valid Positive Results/ Total # of Valid Results |
|---------------------------------------|------------------------------------|--------------------------------------------------------|--------------------------------------------------------|-------------------------------------------------------------|------------------------------------------------------|
| Influenza A/Victoria/4897/2022 (H1N1) | Influenza B 2000x LoD (1E6 cps/mL) | 100% (3/3) | 100% (3/3) | 0% (0/3) | 0% (0/3) |
| | SARS-CoV-2 | 100% | 0% | 100% | 0% |

| | | | | | |
|--------------------------------------------|---------------------------------------|---------------|---------------|---------------|---------------|
| 3x LoD | 2000x LoD (1E6 cps/mL) | (3/3) | (0/3) | (3/3) | (0/3) |
| | RSV 1000x LoD (1E6 cps/mL) | 100% (3/3) | 0% (0/3) | 0% (0/3) | 100% (3/3) |
| Influenza B/Austria/1359417/2021 3x LoD | Influenza A 2000x LoD (1E6 cps/mL) | 100% (3/3) | 100% (3/3) | 0% (0/3) | 0% (0/3) |
| | SARS-CoV-2 2000x LoD (1E6 cps/mL) | 0% (0/3) | 100% (3/3) | 100% (3/3) | 0% (0/3) |
| | RSV B 1000x LoD (1E6 cps/mL) | 0% (0/3) | 100% (3/3) | 0% (0/3) | 100% (3/3) |
| | Influenza A 2000x LoD (1E6 cps/mL) | 100% (3/3) | 0% (0/3) | 100% (3/3) | 0% (0/3) |
| SARS-CoV-2, Lineage BA.2.3 3x LoD | Influenza B 2000x LoD (1E6 cps/mL) | 0% (0/3) | 100% (3/3) | 100% (3/3) | 0% (0/3) |
| | RSV B 1000x LoD (1E6 cps/mL) | 0% (0/3) | 0% (0/3) | 100% (3/3) | 100% (3/3) |
| | Influenza A 2000x LoD (1E6 cps/mL) | 100% (3/3) | 0% (0/3) | 0% (0/3) | 100% (3/3) |
| RSV, B CH93(18)-18 3x LoD | Influenza B 2000x LoD (1E6 cps/mL) | 0% (0/3) | 100% (3/3) | 0% (0/3) | 100% (3/3) |
| | SARS-CoV-2 2000x LoD (1E6 cps/mL) | 0% (0/3) | 0% (0/3) | 100% (3/3) | 100% (3/3) |
| | Influenza A 2000x LoD (1E6 cps/mL) | 100% (3/3) | 0% (0/3) | 0% (0/3) | 100% (3/3) |

4. Assay Reportable Range:

Not applicable; this is a qualitative assay.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a) Controls

The assay contains an Internal Control (RNA-IC) added to each reaction and external positive and negative controls. For more information, see section **IV.C.5.Quality Control**, above.

b) Sample Stability

Stability studies have been performed to support the following sample stability claims:

Primary Specimen (NPS and NS specimens collected in Universal Transport Medium (UTM), BD Universal Viral Transport (UVT) or M4RT)

- Stored at 2–8°C for up to 7 days
- ≤–70°C for up to 3 months.
- up to three freeze/thaw cycles at ≤ –70°C.

c) Kit Stability

The Simplexa COVID-19/FLUA/B & RSV Direct assay Reaction Mix and the Simplexa COVID-19/FLUA/B & RSV Direct assay Positive Control

- 30 minutes post-thaw at room temperature.
- shelf life of 6 months when stored at $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$.

d) Shipping Stability

The Simplexa COVID-19/FLUA/B & RSV Direct assay Reaction Mix and the Simplexa COVID-19/FLUA/B & RSV Direct assay Positive Control

The shipping validation study for the Simplexa COVID-19/Flu A/B & RSV Direct Kit and its associated Positive Control Pack was conducted to demonstrate that the product maintains its integrity and performance following international shipping conditions. A total of twelve reaction kits and ten positive control packs were shipped from DiaSorin Molecular's manufacturing facility in Cypress, CA to the FedEx Packaging Lab in Collierville, TN, where they were subjected to the 72-hour HEAT profile simulating worst-case summer shipping conditions. The kits were then returned to the manufacturing site for inspection and testing. Temperature monitoring confirmed that internal temperatures remained below -10°C throughout the 122-hour shipping duration, with no significant excursions. Upon receipt, all kits were visually inspected and confirmed to be free of physical damage, with all liquid components remaining frozen and intact.

Post-shipping analytical performance testing was conducted using the LIAISON MDX platform and included 30 replicates of the shipped reaction mix tested with both non-template controls (NTCs) and positive controls (PCs), as well as 30 replicates of the shipped positive control tested with reaction mix that were not exposed to the simulated shipping condition. Comparator kits from the same lot stored at $-20^{\circ}\text{C} \pm 10^{\circ}\text{C}$ served as controls. All NTC replicates were valid for the internal control, with no detection of Flu A, Flu B, SARS-CoV-2, or RSV in the respective channels, except for one Flu B detection, which triggered extended testing per protocol. The additional 17 NTC replicates yielded no further detections. All PC replicates demonstrated 100% detection across all targets. Result of the study These support the use of the validated shipping configuration for both domestic and international distribution and demonstrate that the shipping process preserves product integrity and performance.

6. Detection Limit:

The purpose of this study was to determine the limit of detection (LoD) of the Simplexa COVID-19/FLUA/B & RSV Direct assay for the detection of influenza A, influenza B, SARS-CoV-2 and RSV as single analytes in nasopharyngeal swab (NPS) and anterior nasal swab (ANS) specimens. The study assessed two active strains of influenza A (influenza A H1N1, Victoria/4897/2022 and influenza A H3N2, Darwin/9/21), two active strains of influenza B (influenza B Victoria, Austria/1359417/2021 and influenza B Yamagata, Phuket/3073/2013), two inactivated strains of SARS-CoV-2 (USA-WA1/2020 and Omicron

BA.2.3) and two active strains of RSV (RSV A 1/2015, RSV B CH93(18)-18). Each organism was individually spiked into pooled negative clinical NPS or NS matrix at the appropriate final concentration for testing per the instructions for use for the Simplexa COVID-19/FLUA/B & RSV Direct assay. Each virus was tested in a range finding study that included five concentrations, where each concentration was tested with six replicates. The lowest concentration with 100% detection was estimated to be the LoD. The estimated LoD for each strain was verified by testing 60 replicates at the estimated LoD concentration and demonstrating that at least 57/60 replicates were positive. If the criteria of at least 95% of positive replicates were not met, testing was repeated with a higher concentration until at least 95% of replicates gave a positive result.

The LoD of the Simplexa COVID-19/FLUA/B & RSV Direct test for influenza A, influenza B, SARS-CoV-2 and RSV in NPS and ANS specimen are summarized in **Table 14**.

Table 14: Confirmed LoD for the Simplex COVID-19/FLUA/B & RSV Direct Assay

| Target | Confirmed LoD (cps/mL) NPS | Confirmed LoD (cps/mL) ANS |
|----------------------------------------------------|----------------------------|----------------------------|
| Influenza A Victoria/4897/2022 (H1N1) | 500 | 750 |
| Influenza A Darwin/9/21 (H3N2) | 750 | 750 |
| Influenza B Austria/1359417/2021 (Victoria) | 500 | 500 |
| Influenza B Phuket/3073/2013 (Yamagata) | 500 | 500 |
| SARS-CoV-2 USA/WA 1/2020 | 500 | 500 |
| SARS-CoV-2 Lineage BA.2.3; Omicron Variant | 500 | 500 |
| Respiratory syncytial virus (RSV) A 1/2015 Isolate | 1000 | 2000 |
| Respiratory syncytial virus (RSV) B CH93(18)-18 | 1000 | 1000 |

7. Assay Cut-Off:

Data from several analytical studies were used to analyze the fluorescence signal distribution and set cutoffs for all organisms to maximize sensitivity and specificity.

8. Accuracy (Instrument):

Not applicable.

9. Carry-Over:

The carry-over and cross-contamination rate for the Simplexa COVID-19/FLUA/B & RSV Direct assay was determined by testing 28 samples of pooled negative NPS matrix and 28 high positive SARS-CoV-2 samples at a concentration of 1E6 cps/mL in an alternating sequence across seven LIAISON MDX instruments. All 28 replicates of the negative sample were negative, resulting in a cross-contamination rate of 0%.

An additional study was conducted to evaluate the risk for cross-contamination and to demonstrate the re-usability of the DAD consumable up to eight times. A total of 18 DADs were tested by loading fluorescent dyes, FAM and CFR610 alternatively into the eight wells

of each disc to detect potential cross-contamination. No carryover contamination events were observed across any of the 18 discs.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Not applicable.

2. Media Equivalency Study:

Equivalency between NPS stabilized in UTM and M4RT was evaluated. Pooled negative clinical NPS matrix was spiked with RSV, influenza A, SARS-CoV-2 or influenza B. For each target and media type, 30 samples were tested at 2x LoD, 10 at 5x LoD, and 10 negative samples. All replicates tested were positive for the respective viral target for both matrices with 100% hit rate.

C Clinical Studies:

1. Clinical Sensitivity:

The clinical performance of the Simplexa COVID-19/ Flu A/B & RSV Direct assay was evaluated versus a FDA 510(k) cleared comparator in NPS and NS specimens from individuals experiencing signs and symptoms of respiratory viral infection. Fresh specimens were collected at nine (9) collection sites during the 2024-2025 respiratory viral season and tested with the Simplexa COVID-19/ Flu A/B & RSV Direct assay at five (5) testing sites.

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 and, NPS samples are summarized in **Tables 15 and 16**. The performance of the Simplexa COVID-19/ Flu A/B & RSV Direct assay in unpaired NS and NPS samples is summarized in **Table 17**. Samples that produced a discordant call between the Simplexa COVID-19/ Flu A/B & RSV Direct assay and the comparator test were further tested with a second FDA-cleared molecular assay and/or PCR/bidirectional sequencing (BDS). Results from discordant sample testing are presented as footnotes below the table but were not used in performance calculations

Demographics of subjects that were included in the final performance analysis are summarized in **Table 18**.

Table 15: 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% |
| SARS-CoV-2 | 61/65 ^g | 93.8% | 85.2%-97.6% | 1281/1288 ^h | 99.5% | 98.9%-99.7% |

^aFive (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.

^bThree (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.

^cThe one (1) Flu B False Negative specimen was negative by PCR/BDS.

^dBoth 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.

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

^fFive (5) of the six (6) RSV False Positive specimens were positive by PCR/BDS.

^gTwo (2) of the four (4) SARS-CoV-2 False Negative specimens were negative by PCR/BDS.

^hFour (4) of the seven (7) COVID-19 False Positive specimens were positive by PCR/BDS.

Table 16: Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of Prospective Data Set (ANS)

| 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% |
| SARS-CoV-2 | 42/43 ^g | 97.7% | 87.9%-99.6% | 882/887 ^h | 99.4% | 98.7%-99.8% |

^aFour (4) of the five (5) Flu A False Negative specimens were negative by PCR/BDS. The other specimen was negative by Standard of Care.

^bAll thirteen (13) Flu A False Positive specimens were positive by PCR/BDS.

^cThe one (1) Flu B False Negative specimen was negative by Standard of Care.

^dThe two (2) Flu B False Positive specimens were negative by PCR/BDS.

^eTwo (2) of the seven (7) RSV False Negative specimens were negative by PCR/BDS.

^fOne (1) of the two (2) RSV False Positive specimens was positive by PCR/BDS.

^gThe SARS-CoV-2 False Negative specimen was negative by PCR/BDS.

^hTwo (2) of the five (5) SARS-CoV-2 False Positive specimens were positive by PCR/BDS.

Table 17: Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of Prospective Data Set (Unpaired)

| 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% |
| SARS-CoV-2 | 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% |

^aFive (5) of the eleven (11) Flu A False Negative specimens were negative by PCR/BDS.

^bNine (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.

^cThe one (1) Flu B False Negative specimen was negative by PCR/BDS.

^dFour (4) of the seven (7) RSV False Negative specimens were negative by PCR/BDS. One (1) additional specimen was negative by Standard of Care.

^eFive (5) of the six (6) RSV False Positive specimens were positive by PCR/BDS.

^fTwo (2) of the four (4) SARS-CoV-2 False Negative specimens were negative by PCR/BDS.

^gOne (1) of the four (4) SARS-CoV-2 False Positive specimens was positive by PCR/BDS.

Table 18: General Demographic Details of the Prospective Study Population

| | NPS (N = 1361) | NS (N=932) |
|-------------------------|-----------------------|-------------------|
| Sex | | |
| 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 - 14 | 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%) |
| (80.9%) | 84 (6.2%) | 0 (0.0%) |
| Outpatient | 584 (42.9%) | 0 (0.0%) |

| | | |
|----------------|-------------|------------|
| Unknown | 0 (0.0%) | 2 (0.2%) |
| Total | 1361 (100%) | 932 (100%) |

2. Clinical Specificity:

See section VII. Performance Characteristics. C. Clinical Studies 1. Clinical Sensitivity, above.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The Simplexa COVID-19/ Flu A/B & RSV Direct assay prospective clinical studies included a total of 1,401 prospectively collected NPS specimens and 978 NS specimens during the 2024-2025 respiratory viral season. Forty NPS specimens and forty-six NS specimens were not evaluable due to protocol deviations, sample handling issues and/or invalid reference testing result. The number and percentage of cases positive for SARS-CoV-2, influenza A, influenza B, and RSV, as determined by the Simplexa COVID-19/ Flu A/B & RSV Direct assay from NPS samples, are presented in **Tables 19 - 20**, stratified by collection site and age. **Tables 21 -22** below shows the expected values for NS specimens, as determined by the Simplexa COVID-19/ Flu A/B & RSV Direct assay, stratified by the collection site and patient's age.

Table 19. Simplexa COVID-19/FLU A/B & RSV Direct Expected Values for Prospective NPS Specimens by Site (N=1353)¹

| | Site 01 (N = 189) | | Site 02 (N = 127) | | Site 03 (N = 27) | | Site 04 (N = 9) | | Site 06 (N = 141) | | Site 09 (N = 308) | | Site 10 (N = 144) | | Site 11 (N = 398) | | Site 13 (N = 10) | | Total (N = 1353) | |
|------------|----------------------|-------------------|----------------------|-------------------|---------------------|------------------|--------------------|----------------|----------------------|-------------------|----------------------|-------------------|----------------------|-------------------|----------------------|--------------------|---------------------|-----------------|---------------------|---------------------|
| Target | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) |
| Flu A | 58 | 30.7% (58/189) | 24 | 18.9% (24/127) | 18 | 66.7% (18/27) | 2 | 22.2% (2/9) | 45 | 31.9% (45/141) | 79 | 25.6% (79/308) | 14 | 9.7% (14/144) | 128 | 32.2% (128/398) | 1 | 10.0% (1/10) | 369 | 27.3% (369/1353) |
| Flu B | 7 | 3.7% (7/189) | 11 | 8.7% (11/127) | 0 | 0.0% (0/27) | 0 | 0.0% (0/9) | 3 | 2.1% (3/141) | 0 | 0.0% (0/308) | 17 | 11.8% (17/144) | 9 | 2.3% (9/398) | 0 | 0.0% (0/10) | 47 | 3.5% (47/1353) |
| RSV | 2 | 1.1% (2/184) | 7 | 5.5% (7/127) | 1 | 3.7% (1/27) | 0 | 0.0% (0/9) | 13 | 9.2% (13/141) | 12 | 3.9% (12/307) | 20 | 13.9% (20/144) | 46 | 11.6% (46/397) | 0 | 0.0% (0/10) | 101 | 7.5% (101/1346) |
| SARS-CoV-2 | 5 | 2.6% (5/189) | 5 | 3.9% (5/127) | 1 | 3.7% (1/27) | 0 | 0.0% (0/9) | 8 | 5.7% (8/141) | 21 | 6.8% (21/308) | 2 | 1.4% (2/144) | 25 | 6.3% (25/398) | 1 | 10.0% (1/10) | 68 | 5.0% (68/1353) |

¹Eight (8) NPS specimens returned invalid results on the Simplexa COVID-19 / FLU A/B & RSV Direct assay and are excluded from this analysis.

Table 20. Simplexa COVID-19/FLU A/B & RSV Direct Expected Values for Prospective NPS Specimens by Age (N=1353)¹

| | <=5 Years (N=506) | 6-18 Years (N=483) | 19-40 Years (N=153) | 41-60 Years (N=100) | 61+ Year (N=105) | Unknown (N=6) | Overall (N=1353) |
|--|----------------------|-----------------------|------------------------|------------------------|---------------------|------------------|---------------------|
|--|----------------------|-----------------------|------------------------|------------------------|---------------------|------------------|---------------------|

| Target | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) |
|------------|------|--------------------|------|--------------------|------|-------------------|------|-------------------|------|-------------------|------|----------------|------|---------------------|
| Flu A | 135 | 26.7% (135/506) | 153 | 31.7% (153/483) | 35 | 22.9% (35/153) | 26 | 26.0% (26/100) | 19 | 18.1% (19/105) | 1 | 16.7% (1/6) | 369 | 27.3% (369/1353) |
| Flu B | 12 | 2.4% (12/506) | 33 | 6.8% (33/483) | 2 | 1.3% (2/153) | 0 | 0.0% (0/100) | 0 | 0.0% (0/105) | 0 | 0.0% (0/6) | 47 | 3.5% (47/1353) |
| RSV | 73 | 14.5% (73/505) | 17 | 3.5% (17/479) | 3 | 2.0% (3/152) | 2 | 2.0% (2/100) | 5 | 4.8% (5/104) | 1 | 16.7% (1/6) | 101 | 7.5% (101/1346) |
| SARS-CoV-2 | 27 | 5.3% (27/506) | 16 | 3.3% (16/483) | 6 | 3.9% (6/153) | 6 | 6.0% (6/100) | 12 | 11.4% (12/105) | 1 | 16.7% (1/6) | 68 | 5.0% (68/1353) |

¹Eight (8) NPS specimens returned invalid results on the Simplexa COVID-19 / FLU A/B & RSV Direct assay and are excluded from this analysis.

Table 21. Simplexa COVID-19/FLU A/B & RSV Direct Expected Values for Prospective NS Specimens by Site (N=930)¹

| | Site 01 (N = 190) | | Site 02 (N = 161) | | Site 03 (N = 139) | | Site 04 (N = 68) | | Site 06 (N = 149) | | Site 09 (N = 3) | | Site 13 (N = 220) | | Total (N = 930) | |
|------------|----------------------|-------------------|----------------------|-------------------|----------------------|-------------------|---------------------|------------------|----------------------|-------------------|--------------------|---------------|----------------------|-------------------|--------------------|--------------------|
| Target | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) |
| Flu A | 58 | 30.5% (58/190) | 36 | 22.4% (36/161) | 55 | 39.6% (55/139) | 11 | 16.2% (11/68) | 52 | 34.9% (52/149) | 0 | 0.0% (0/3) | 51 | 23.2% (51/220) | 263 | 28.3% (263/930) |
| Flu B | 8 | 4.2% (8/190) | 15 | 9.3% (15/161) | 1 | 0.7% (1/139) | 0 | 0.0% (0/68) | 3 | 2.0% (3/149) | 0 | 0.0% (0/3) | 3 | 1.4% (3/220) | 30 | 3.2% (30/930) |
| RSV | 3 | 1.6% (3/190) | 10 | 6.3% (10/160) | 7 | 5.0% (7/139) | 1 | 1.5% (1/68) | 15 | 10.3% (15/145) | 0 | 0.0% (0/3) | 18 | 8.2% (18/219) | 54 | 5.8% (54/924) |
| SARS-CoV-2 | 4 | 2.1% (4/190) | 5 | 3.1% (5/161) | 8 | 5.8% (8/139) | 8 | 11.8% (8/68) | 10 | 6.7% (10/149) | 0 | 0.0% (0/3) | 12 | 5.5% (12/220) | 47 | 5.1% (47/930) |

¹Two (2) NS specimens returned invalid results on the Simplexa COVID-19 / FLU A/B & RSV Direct assay and are excluded from this analysis.

Table 22. Simplexa COVID-19/FLU A/B & RSV Direct Expected Values for Prospective NS Specimens by Age (N=930)¹

| | <=5 Years (N=241) | | 6-18 Years (N=416) | | 19-40 Years (N=137) | | 41-60 Years (N=94) | | 61+ Year (N=42) | | Overall (N=930) | |
|------------|----------------------|-------------------|-----------------------|--------------------|------------------------|-------------------|-----------------------|------------------|--------------------|-----------------|--------------------|--------------------|
| Target | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) | #Pos | (%) |
| Flu A | 83 | 34.4% (83/241) | 133 | 32.0% (133/416) | 28 | 20.4% (28/137) | 12 | 12.8% (12/94) | 7 | 16.7% (7/42) | 263 | 28.3% (263/930) |
| Flu B | 2 | 0.8% (2/241) | 24 | 5.8% (24/416) | 2 | 1.5% (2/137) | 2 | 2.1% (2/94) | 0 | 0.0% (0/42) | 30 | 3.2% (30/930) |
| RSV | 28 | 11.7% (28/240) | 18 | 4.4% (18/413) | 2 | 1.5% (2/135) | 1 | 1.1% (1/94) | 5 | 11.9% (5/42) | 54 | 5.8% (54/924) |
| SARS-CoV-2 | 9 | 3.7% (9/241) | 16 | 3.8% (16/416) | 9 | 6.6% (9/137) | 8 | 8.5% (8/94) | 5 | 11.9% (5/42) | 47 | 5.1% (47/930) |

¹Two (2) NS specimens returned invalid results on the Simplexa COVID-19 / FLU A/B & RSV Direct assay and are excluded from this analysis.

F Other Supportive Instrument Performance Characteristics Data:

Not applicable.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.