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

I Background Information:

A 510(k) Number

K251978

B Applicant

Diasorin Molecular LLC

C Proprietary and Established Names

LIAISON NES FLU A/B, RSV & COVID-19

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 demonstrate that the LIAISON NES FLU A/B, RSV & COVID-19 assay is substantially equivalent to the BIOFIRE SPOTFIRE Respiratory Panel Mini (SPOTFIRE R Panel Mini, K230719).

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 LIAISON NES FLU A/B, RSV & COVID-19 assay is a real-time RT-PCR assay intended for use on the LIAISON NES 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 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 LIAISON NES FLU A/B, RSV & COVID-19 assay is intended for use as an aid in the differential diagnosis of SARS-CoV-2, influenza A, influenza B, and RSV infections when 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 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 LIAISON NES FLU A/B, RSV & COVID-19 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 NES Instrument

IV Device/System Characteristics:

A Device Description:
The LIAISON NES Instrument is capable of analysis of a single cartridge containing a single specimen. A set of parameters specific to the assay is included in the instrument software to name target molecules, assign dyes to probes, specify cycling conditions, and to analyze data from runs. Fluorescence intensity is monitored at each PCR

cycle by detection modules within the instrument. The instrument software controls the thermocycling and, upon completion of the run, automatically interprets and displays results for the specimen.

The LIAISON NES instrument is designed to accept a cartridge, containing either a quality control (QC) or patient sample, to process and detect for the target nucleic acid.

The LIAISON NES FLU A/B, RSV & COVID-19 assay used on the LIAISON NES instrument is a real-time RT-PCR system that enables the direct amplification, detection, and differentiation of influenza A RNA, influenza B RNA, RSV RNA, and SARS-CoV- 2 RNA directly from anterior nasal swabs.

B Principle of Operation:

The anterior nasal swab specimen is collected by a healthcare professional or the patient under the supervision of the healthcare professional as indicated in section **IV. C 3** Specimen Sampling and Handling.

The LIAISON NES instrument automates the LIAISON NES FLU A/B, RSV & COVID-19 assay sample analysis steps within a single cartridge. The sample analysis steps that occur within the cartridge are described below.

Sample Preparation: The specimen is processed inside the instrument with heating and pipetting to release the nucleic acids.

PCR Reagents Preparation: Nucleic acids released from the specimen are mixed with lyophilized PCR reagents (primers, nucleotides and enzymes needed for the amplification process) contained within the cartridge.

Target Amplification: Multiplex RT-PCR amplification of the target specific amplicons is performed using thermal cycling. Primers and fluorescent probes are used to specifically amplify conserved regions of influenza A RNA, influenza B RNA, RSV RNA, and SARS-CoV-2 RNA, while the internal control (IC) RNA is used to detect any PCR failures and/or inhibition. The genes targeted by the primers and probes include the Matrix gene for Influenza A, Influenza B, and RSV, and the Spike and ORF7A genes for SARS-CoV-2.

Analysis: The instrument's optical modules continuously monitor the fluorescence emitted during each PCR cycle. This real-time detection enables the system to determine the presence of specific pathogens. The instrument calculates the threshold cycle (Ct) value, which is the cycle number at which the fluorescence signal exceeds a predefined threshold.

Final results are displayed on the instrument's screen, indicating whether the sample is "Positive" or "Negative" for the targeted pathogens (e.g., influenza A, influenza B, RSV, SARS-CoV-2). These results are linked to the test and patient information entered at the beginning of each test to provide a results report.

C Instrument Description Information:

1. Instrument Name:

LIAISON NES instrument

2. Specimen Identification:
   The instrument incorporates a barcode reader to scan in specimen identification. Specimen identification may also be entered manually using an on-screen keyboard.

3. Specimen Sampling and Handling:
   The NES Swab is utilized to collect the specimen from the patient by inserting the swab approximately 1 inch into one nostril, gently rotating the swab 5 times against the nasal wall for approximately 15 seconds, then repeating these steps in the other nostril. Specimen collection can be completed by the healthcare provider or by the patient under supervision of a healthcare provider.

   After collection of the specimen from the patient, the operator inserts the nasal swab into the NES Sample Vial and squeezes the swab head while rotating the swab 10 times. Following rotation of the swab in the sample vial, the operator snaps the swab shaft off at a pre-defined mark on the swab shaft, leaves the swab head in the sample vial, and places the cap back on the sample vial. The operator then twists off the cap at the end of the sample vial and squeezes the body of the sample vial to deliver all of the liquid into the cartridge.

4. Calibration:
   The instrument is factory calibrated. No user calibration is performed.

5. Quality Control:

   Internal Controls

   Each LIAISON NES FLU A/B, RSV & COVID-19 cartridge includes an internal control to ensure performance of sample preparation, amplification, and detection. The internal control must generate a signal above threshold in the Q705 channel for the system to report a valid test result when target RNA is not detected. Detection of the internal control is not required for a valid result when target RNA is detected.

   External Controls

   The LIAISON NES FLU A/B, RSV & COVID-19 Control Swab Kit contains positive and negative control swabs as external controls. Positive and negative external controls should be tested with each new lot or shipment of reagents, or monthly, (whichever occurs first), or in accordance with updated local, regional, state, and/or federal guidelines, per the instructions for use and facility procedures.

   The NES FLU A/B, RSV & COVID-19 Positive Control Swab contains influenza A, influenza B, RSV A, and SARS-CoV-2 viral particles that have been inactivated using irradiation and chemical treatments. The viral particle solution is lyophilized into the swab. Viral particles included on the swab are influenza A (California 07/2009), Influenza B (Colorado 06/2017), RSV (A2) and SARS-CoV-2 (Washington 1/2020).

V Substantial Equivalence Information:

A Predicate Device Name(s):
BIOFIRE SPOTFIRE Respiratory (R) Panel Mini

B Predicate 510(k) Number(s):
K230719

C Comparison with Predicate(s):

Device & Predicate Device(s):	K251978	K230719
Device Trade Name	LIAISON NES FLU A/B, RSV & COVID-19	BIOFIRE SPOTFIRE Respiratory Panel Mini (SPOTFIRE R Panel Mini)
General Device Characteristic Similarities		
Intended Use/Indications For Use	The LIAISON NES FLU A/B, RSV & COVID-19 assay is a real-time RT-PCR assay intended for use on the LIAISON NES 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 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 LIAISON NES FLU A/B, RSV & COVID-19	The BIOFIRE SPOTFIRE Respiratory Panel Mini (SPOTFIRE R Panel Mini) is a multiplexed polymerase chain reaction (PCR) test intended for use with the BIOFIRE SPOTFIRE System for the simultaneous, qualitative detection and identification of multiple respiratory viral nucleic acids in nasopharyngeal swab (NPS) specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19. The following organism types and subtypes are identified and differentiated using the SPOTFIRE R Panel Mini: • Coronavirus SARS-CoV-2 • Human rhinovirus • Influenza A virus • Influenza B virus • Respiratory syncytial

	assay is intended for use as an aid in the differential diagnosis of SARS-CoV-2, influenza A, influenza B, and RSV infections when 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 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 LIAISON NES FLU A/B, RSV & COVID-19 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.	virus Nucleic acids from the viral organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection are indicative of the presence of the identified micro-organism 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. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the SPOTFIRE R Panel Mini may not be the definite cause of disease. Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible

		respiratory tract infection.
Measurand	Same	Nucleic acid from Organisms detected
Patient Population	Individuals with signs and symptoms of respiratory tract infection	Individuals with signs and symptoms of respiratory tract infection, including COVID-19.
Organisms detected	Same, except no assay for Human rhinovirus	• Coronavirus SARS-CoV-2 • Human rhinovirus • Influenza A virus • Influenza B virus • Respiratory syncytial virus
Automated System (Sample to Answer)	Same	Automated test interpretation and reporting. User cannot access raw data.
Reporting Features	Same	System software analyzes processed image data and provides test results for each panel target
Assay Software Functions	Same	Defines panel-specific parameters, instrument protocols and report requirements.
Time to Result	~18 minutes	~15 minutes
General Device Characteristic Differences		
Sample Type	Anterior Nasal Swab (ANS)	Nasopharyngeal Swab (NPS)
Instrumentation	LIAISON NES Instrument	BIOFIRE SPOTFIRE System
Technological Principle	Highly multiplexed real-time RT- PCR with fluorescence detection and analysis	Highly multiplexed PCR with DNA melting analysis

VI Standards/Guidance Documents Referenced:

- Special controls listed under 21 CFR 866.3981—Respiratory Viral Panel Multiplex Nucleic Acid Assay – Class II Special Controls Guidance for Industry and FDA Staff (October 9, 2009)
- 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: 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

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

Analytical studies were performed using pooled negative human nasal matrix.

1. Precision/Reproducibility:

a. Reproducibility

A reproducibility study was conducted assessing the total variability of the LIAISON NES FLU A/B, RSV & COVID-19 assay across operators, study sites, testing days and instruments.

Samples were prepared by a designated staff member of the Diasorin Molecular R&D study team. Each contrived sample panel member was randomized and labeled with a sample ID that did not contain any information regarding the sample content to ensure a minimum level of bias. Eight positive panel members consisted of contrived samples of influenza A virus, influenza B virus, RSV, and SARS-CoV-2 individually spiked onto dry nasal swabs at low positive (~2x LoD) and moderately positive (~5x LoD) concentrations. In addition, No Template Controls (NTC) which consisted of pooled negative human nasal matrix, and external positive controls, PC – LIAISON NES FLU A/B, RSV & COVID-19 Positive Control Swabs (PC), were included. The panel members are shown in **Table 1**. The reproducibility study was performed at three geographically distinct external sites, all under CLIA-waived status. There were two operators at each site and each operator tested two replicates of each panel member each day for eight non-consecutive days.

Table 1. Reproducibility Panel Members

Panel Member		Strain	Concentration
1	Influenza A	Darwin/9/21	2x LoD
2	Influenza A		5x LoD
3	Influenza B	Phuket 3073/2013	2x LoD
4	Influenza B		5x LoD
5	RSV	RSV B 12/2014	2x LoD
6	RSV		5x LoD
7	SARS-CoV-2	USA/WA 1/2020	2x LoD
8	SARS-CoV-2		5x LoD
9	NTC – Pooled Negative Human Nasal Matrix	NA	NA
10	PC - LIAISON NES FLU A/B, RSV & COVID-19 Positive Control Swab (PC)	NA	5-10x LoD

LoD = Limit of Detection, NTC = No Template Control, PC = Positive Control

Reproducibility was evaluated with reference to CLSI, EP05-A3 *Evaluation of Precision of Quantitative Measurement Procedures*.

Table 2 shows the qualitative summary for influenza A virus, influenza B virus, RSV and SARS-CoV-2 during panel member testing. The LIAISON NES FLU A/B, RSV & COVID-19 assay demonstrated 100% agreement (96/96) for each target analyte with the moderate panel members (5x LoD). For low positive panel members (2x LoD), the assay yielded 100% agreement with the exception of influenza B, which yielded 99% (95/96) agreement. All negative (NTC) and positive control (PC) panel members also yielded 100% agreement with the expected results. This performance is acceptable and demonstrates acceptable assay reproducibility.

Table 2. Qualitative Summary of Results and Mean Ct Values +/- SD (%CV)

	Site 1		Site 2		Site 3[a]		All Sites		
Sample	**Agreement with expected results**	**Mean Ct ±SD (%CV)**	**Agreement with expected results**	**Mean Ct ± SD (%CV)**	**Agreement with expected results**	**Mean Ct ± SD (%CV)**	**Agreement with expected results**	**Mean Ct ± SD (%CV)**	**95% CI**
Influenza A 2x	100% (32/32)	36.6 ± 0.77 (2.1%)	100% (32/32)	36.8 ± 1.28 (3.5%)	100% (32/32)	36.9 ± 1.18 (3.2%)	100% (96/96)	36.8 ± 1.09 (3.0%)	96.2% - 100%
Influenza A 5x	100% (32/32)	34.6 ± 1.09 (3.2%)	100% (32/32)	34.7 ± 1.16 (3.4%)	100% (32/32)	35.7 ± 1.73 (4.8%)	100% (96/96)	35.0 ± 1.43 (4.1%)	96.2% - 100%
Influenza B 2x	100% (32/32)	39.0 ± 1.89 (4.8%)	100% (32/32)	38.2 ± 0.88 (2.3%)	96.9% (31/32[a]	38.2 ± 1.42 (3.7%)	99% (95/96)	38.5 ± 1.48 (3.9%)	94.3% - 99.8%
Influenza B 5x	100% (32/32)	37.1 ± 1.40 (3.8%)	100% (32/32)	36.1 ± 0.78 (2.2%)	100% (32/32)	36.2 ± 1.47 (4.1%)	100% (96/96)	36.4 ± 1.32 (3.6%)	96.2% - 100%

RSV 2x	100% (32/32)	36.7 ± 0.96 (2.6%)	100% (32/32)	37.1 ± 1.05 (2.8%)	100% (32/32)	37.1 ± 1.38 (3.7%)	100% (96/96)	37.0 ± 1.15 (3.1%)	96.2% - 100%
RSV 5x	100% (32/32)	35.2 ± 1.01 (2.9%)	100% (32/32)	35.1 ± 0.85 (2.4%)	100% (32/32)	35.3 ± 1.57 (4.4%)	100% (96/96)	35.2 ± 1.17 (3.3%)	96.2% - 100%
SARS-CoV-2 2x	100% (32/32)	37.5 ± 1.41 (3.8%)	100% (32/32)	36.6 ± 0.93 (2.6%)	100% (32/32)	37.2 ± 1.73 (4.7%)	100% (96/96)	37.1 ± 1.43 (3.9%)	96.2% - 100%
SARS-CoV-2 5x	100% (32/32)	35.2 ± 0.91 (2.6%)	100% (32/32)	35.0 ± 0.67 (1.9%)	100% (32/32)	35.5 ± 1.84 (5.2%)	100% (96/96)	35.2 ± 1.25 (3.5%)	96.2% - 100%
PC Influenza A	100% (32/32)	28.6 ± 0.43 (1.5%)	100% (32/32)	28.9 ± 0.43 (1.5%)	100% (32/32)	28.0 ± 1.15 (4.1%)	100% (96/96)	28.5 ± 0.83 (2.9%)	96.2% - 100%
PC Influenza B	100% (32/32)	29.4 ± 0.39 (1.3%)	100% (32/32)	29.4 ± 0.28 (0.9%)	100% (32/32)	28.6 ± 0.73 (2.6%)	100% (96/96)	29.1 ± 0.62 (2.1%)	96.2% - 100%
PC RSV	100% (32/32)	29.8 ± 0.55 (1.8%)	100% (32/32)	29.8 ± 0.31 (1.0%)	100% (32/32)	30.4 ± 0.83 (2.7%)	100% (96/96)	30.0 ± 0.66 (2.2%)	96.2% - 100%
PC SARS-CoV-2	100% (32/32)	30.9 ± 1.21 (3.9%)	100% (32/32)	30.8 ± 0.29 (0.9%)	100% (32/32)	30.3 ± 1.08 (3.6%)	100% (96/96)	30.6 ± 0.97 (3.2%)	96.2% - 100%
NTC	100% (32/32)	NA	100% (32/32)	NA	100% (32/32)	NA	100% (96/96)	NA	96.2% - 100%

n = number of replicates, Ct = Cycle threshold, SD= Standard Deviation, %CV = Percent Coefficient of Variation.
NTC = No Template Control, PC = Positive Control
[a] One NTC tested at site 3 failed to detect the IC and was repeated in accordance with product labeling.

The total Ct variability, as measured by the standard deviation, was less than or equal to 1.72 across all target viruses and concentrations. These results, shown in **Table 3**, indicate that the reproducibility of the LIAISON NES FLU A/B, RSV & COVID-19 assay is acceptable. Internal control reproducibility was also carried out and the result is acceptable.

Table 3. Quantitative Summary of Reproducibility

			Reproducibility									
			Between Sites		**Between Operator (Site)**		**Between Day (Operator Site)**		**Between Instruments (Days Operator Site)**		**Total Reproducibility**	
Sample	**N**	**Mean Ct**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
Influenza A 2x	96	36.8	0.14	0.4	0.58	1.6	0.80	2.2	1.07	2.9	1.20	3.3
Influenza A 5x	96	35.0	0.61	1.8	0.97	2.8	1.20	3.4	1.03	3.0	1.60	4.6
Influenza B 2x	95	38.5	0.44	1.1	0.86	2.2	1.20	3.1	1.48	3.9	1.50	4.0
Influenza B 5x	96	36.4	0.54	1.5	0.82	2.3	1.06	2.9	1.22	3.3	1.54	4.2
RSV 2x	96	37.0	0.21	0.6	0.66	1.8	0.88	2.4	1.04	2.8	1.33	3.6
RSV 5x	96	35.2	0.09	0.3	0.82	2.3	1.02	2.9	0.81	2.3	1.33	3.8
SARS-CoV-2 2x	96	37.1	0.45	1.2	0.83	2.2	1.08	2.9	1.30	3.5	1.72	4.6
SARS-CoV-2 5x	96	35.2	0.26	0.7	0.91	2.6	1.12	3.2	0.76	2.2	1.52	4.3
PC Influenza A	96	28.5	0.46	1.6	0.57	2.0	0.75	2.7	0.45	1.6	0.92	3.2
PC Influenza B	96	29.1	0.45	1.5	0.53	1.8	0.58	2.0	0.32	1.1	0.69	2.4
PC RSV	96	30.0	0.33	1.1	0.49	1.6	0.59	2.0	0.42	1.4	0.80	2.7
PC SARS-CoV-2	96	30.6	0.30	1.0	0.50	1.6	0.84	2.7	0.88	2.9	1.01	3.3

N = Number of replicates, Ct = Cycle threshold, SD= Standard Deviation, %CV = Percent Coefficient of Variation

b. Within-Laboratory Precision

Within-laboratory precision of the LIAISON NES FLU A/B, RSV & COVID-19 assay was evaluated with four different lots of LIAISON NES FLU A/B, RSV & COVID-19 Cartridges and four LIAISON NES instruments. The study was conducted by one operator over the course of twenty-four non-consecutive days. The qualitative study result is shown in **Table 4.** Target concentrations for Low Positive (LP) and Medium Positive (MP) were 2x LoD and 5x LoD, respectively.

There was 100% agreement between the expected results and the observed qualitative results. Each panel member showed 100% expected result (24/24). The results of the study demonstrate acceptable assay variability.

Table 4. Summary of Qualitative Performance for Reaction Mix Inter-Lot / Inter-Instrument Reproducibility

Panel Member	Strain/ Concentration	Expected Qualitative Result	Observed Qualitative Result % Detection (# Detected/# Tested)
ILII Repro 1	Influenza A Low Positive (LP)	100% of the tested replicate results are Influenza A Positive	100% (24/24) Influenza A Positive
ILII Repro 2	Influenza A Medium Positive (MP)	100% of the tested replicate results are Influenza A Positive	100% (24/24) Influenza A Positive
ILII Repro 3	Influenza B Low Positive (LP)	100% of the tested replicate results are Influenza B Positive	100% (24/24) Influenza B Positive
ILII Repro 4	Influenza B Medium Positive (MP)	100% of the tested replicate results are Influenza B Positive	100% (24/24) Influenza B Positive
ILII Repro 5	RSV Low Positive (LP)	100% of the tested replicate results are RSV Positive	100% (24/24) RSV Positive
ILII Repro 6	RSV Medium Positive (MP)	100% of the tested replicate results are RSV Positive	100% (24/24) RSV Positive
ILII Repro 7	SARS-CoV-2 Low Positive (LP)	100% of the tested replicate results are SARS-CoV-2 Positive	100% (24/24) SARS-CoV-2 Positive
ILII Repro 8	SARS-CoV-2 Medium Positive (MP)	100% of the tested replicate results are SARS-CoV-2 Positive	100% (24/24) SARS-CoV-2 Positive
ILII Repro 9	Negative Sample (No Template Control)	100% of the tested replicate results are Negative	0% (0/24) Negative
Positive Control (PC)	Positive Control (PC)	100% of the tested replicate results are Positive for all targets	100% (24/24) Positive

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:
The inclusivity of the LIAISON NES FLU A/B, RSV & COVID-19 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. In silico

The inclusivity of the LIAISON NES FLU A/B, RSV & COVID-19 assay was evaluated using *in silico* analysis of the forward primers, reverse primers, and probes for the SARS-CoV-2 target. Analysis was performed against SARS-CoV-2 sequences available in the GISAID EpiCoV database as of May 5, 2025. The sequence set encompassed 5,641,034 SARS-CoV-2 sequences, including available sequences from variants of concern or variants of interest defined as of May 5, 2025.

In silico analysis of the dual target SARS- CoV-2 primer and probe binding regions indicate ~100% detection of the 5,641,034 SARS-CoV-2 sequences, including sequences of all defined variants of concern or variants of interest, available in the GISAID EpiCoV database as of May 5, 2025.

b. Wet-Testing

The inclusivity study evaluates the ability of the test to detect clinically relevant strains, serotypes, or subtypes of influenza A virus, influenza B virus, RSV, and SARS-CoV-2. The reactivity/inclusivity of the LIAISON NES FLU A/B, RSV & COVID-19 assay was evaluated with 36 influenza A (15 H1N1, 1 H1N2, 14 H3N2, 4 H5N1, 1 H7N7, and 1 H9N2) isolates, 14 influenza B (7 Victoria, 6 Yamagata, and 1 subtype unknown) isolates, 9 RSV (4 RSVA and 5 RSVB) isolates, and 19 SARS-CoV-2 isolates. The inclusivity panel was prepared by spiking various target microorganisms into pooled negative ANS matrix. The concentration which showed a 100% hit rate is shown in **Table 5** through **Table 8**.

Influenza A

The detected concentrations for the influenza A variants tested with the Diasorin LIAISON NES FLU A/B, RSV & COVID-19 assay are shown in **Table 5**.

All strains of influenza A (H1N1) have been tested at 1.8E4 cps/swab (3x LoD of influenza A Victoria/4897/2022 (H1N1)). All replicates were detected correctly at this concentration.

All strains of influenza A (H3N2) have been tested at 2.4E4 cps/swab (3x LoD of influenza A Darwin/9/21 (H3N2)). All replicates were detected correctly at this concentration with the exception of Flu A Hong Kong/8/68, which was detected at 5x LoD (4.0E4 cps/swab).

Other influenza A isolates were detected successfully as indicated.

Table 5 Influenza A Results

Strain	Concentration (cps/swab)	% Detection (#Detected/#Tested)	Mean Ct ± SD (%CV)
Influenza A Michigan/45/2015 (H1N1)	1.8E4	100% (3/3)	35.3 ± 1.1 (3.0%)
Influenza A Brisbane/59/07 (H1N1)	1.8E4	100% (3/3)	34.9 ± 0.1 (0.4%)
Influenza A NY/02/09 (H1N1)	1.8E4	100% (3/3)	34.0 ± 0.3 (1.0%)
Influenza A Mexico/4108/09 (H1N1)*	1.8E4	100% (3/3)	36.2 ± 0.8 (2.3%)
Influenza A New York/18/09 (H1N1)	1.8E4	100% (3/3)	37.0 ± 0.3 (0.9%)
Influenza A Taiwan/42/06 (H1N1)	1.8E4	100% (3/3)	37.1 ± 1.2 (3.1%)
Influenza A Brisbane/02/2018 (H1N1) (CDC panel)	1.8E4	100% (3/3)	35.8 ± 0.6 (3.1%)
Influenza A Wisconsin/588/2019 (H1N1)	1.8E4	100% (3/3)	37.0 ± 1.1 (3.0%)
Influenza A California/07/2009 (H1N1)	1.8E4	100% (3/3)	33.3 ± 0.5 (1.5%)
Influenza A New Caledonia/20/1999 (H1N1)	1.8E4	100% (3/3)	38.6 ± 2.5 (6.5%)
Influenza A Guangdong- Moanan/SWL1536/2019 (H1N1)	1.8E4	100% (3/3)	38.5 ± 0.4 (1.0%)
Influenza A Puerto Rico/8/34 (H1N1)	1.8E4	100% (3/3)	31.9 ± 0.3 (1.1%)
Influenza A Victoria/2570/2019 (H1N1)	1.8E4	100% (3/3)	37.9 ± 0.4 (1.0%)
Influenza A Nebraska/14/2018 (H1N1)	1.8E4	100% (3/3)	34.3 ± 0.3 (0.7%)
Influenza A Sydney/05/2021 (H1N1)	1.8E4	100% (3/3)	33.5 ± 1.4 (4.2%)
Influenza A swine/Ohio/09SW1484E/2009 (Extracted RNA) (H1N2)	1.8E4	100% (3/3)	30.7 ± 0.5 (1.7%)
Influenza A Switzerland/9715293/13 (H3N2)	2.4E4	100% (3/3)	33.7 ± 0.6 (1.8%)
Influenza A Hong Kong/4801/2014 (H3N2)	2.4E4	100% (3/3)	36.0 ± 0.5 (1.3%)
Influenza A Singapore/INFIMH-16-0019/2016 (H3N2)	2.4E4	100% (3/3)	33.0 ± 0.3 (0.8%)
Influenza A Perth/16/2009 (H3N2) (CDC *Panel*)	2.4E4	100% (3/3)	35.0 ± 0.5 (1.3%)
Influenza A Hong Kong/2671/2019 (H3N2) (CDC *Panel*)	2.4E4	100% (3/3)	36.4 ± 0.2 (0.7%)
Influenza A Hong Kong/8/68 (H3N2)	2.4E4 (3x LoD)	67% (2/3)	38.1 ± 0.1 (0.2%)
	3.2E4 (4x LoD)	67% (2/3)	38.9 ± 1.0 (2.6%)
	4E4 (5x LoD)	100% (3/3)	37.5 ± 0.3 (0.9%)
Influenza A Cambodia/E0826360/2020 (H3N2)	2.4E4	100% (3/3)	35.4 ± 0.4 (1.3%)
Influenza A Kansas/14/2017 (H3N2) (CDC Panel)	2.4E4	100% (3/3)	34.0 ± 0.6 (1.7%)
Influenza A Port Chalmers/1/73 (H3N2)	2.4E4	100% (3/3)	34.8 ± 0.6 (1.7%)
Influenza A Darwin/6/2021 (H3N2)	2.4E4	100% (3/3)	35.1 ± 0.4 (1.1%)
Influenza A Tasmania/503/2020 (H3N2)	2.4E4	100% (3/3)	35.1 ± 0.5 (1.4%)
Influenza A Thailand/08/2022 (H3N2)	2.4E4	100% (3/3)	33.4 ± 0.5 (1.6%)
Influenza A Texas/50/2012 (H3N2)	2.4E4	100% (3/3)	33.3 ± 0.1 (0.2%)
Influenza A Massachusetts/18/2022 (H3N2)	2.4E4	100% (3/3)	36.9 ± 0.5 (1.2%)
Influenza A/Anhui/01/2005 (H5N1)	1.8E4	100% (3/3)	30.2 ± 0.2 (0.7%)
Influenza A/Egypt/N03072/2010 (H5N1)	1.8E4	100% (3/3)	29.4 ± 0.1 (0.5%)
Influenza A/Hubei/1/2010 (H5N1)	1.8E4	100% (3/3)	30.2 ± 0.2 (0.7%)
Influenza A A/bovine/Ohio.B24OSU-439/2024 gRNA (H5N1)	1.8E4	100% (3/3)	33.0 ± 0.2 (0.5%)
Influenza A/Mallard/Netherlands/12/2000 (H7N7)	1.8E4	100% (3/3)	30.7 ± 0.3 (1.0%)
Influenza A/Hong Kong/33982/2009 (H9N2)	1.8E4	100% (3/3)	32.8 ± 0.5 (1.6%)

Ct = Cycle threshold, SD = Standard Deviation, %CV = Percent Coefficient of Variation
*One replicate was not completed due to instrumental error and was repeated as per protocol

Influenza B

All strains of influenza B (Victoria) were tested at 1.2E4 cps/swab (3x LoD of influenza B Austria/1359417/2021 (Victoria)). All replicates were detected correctly at this concentration.

All strains of influenza B (Yamagata) were tested at 1.5E4 cps/swab (3x LoD of influenza B Phuket/3073/2013 (Yamagata)). All replicates were detected correctly at this concentration.

Flu B Maryland/1/1959 (subtype unknown) was tested at 1.2E4 cps/swab (3x LoD of influenza B Austria/1359417/2021 (Victoria)). All three replicates were detected correctly at this concentration.

The results for influenza B strains/variants are presented in **Table 6**.

Table 6. Influenza B Results

Strain	Concentration (cps/swab)	% Detection (#Detected/#Tested)	Mean Ct ± SD (%CV)
Influenza B Brisbane/60/2008 (Victoria)	1.2E4	100% (3/3)	35.4 ± 0.2 (0.7%)
Influenza B Brisbane/33/2008 (Victoria)	1.2E4	100% (3/3)	35.4 ± 0.2 (0.6%)
Influenza B Washington/02/2019 (Victoria)	1.2E4	100% (3/3)	37.0 ± 0.9 (2.3%)
Influenza B Colorado/06/2017 (Victoria) (CDC *Panel*)	1.2E4	100% (3/3)	35.8 ± 0.3 (0.7%)
Influenza B Florida/02/06 (Victoria)	1.2E4	100% (3/3)	37.2 ± 0.7 (1.9%)
Influenza B Malaysia/2506/04 (Victoria)	1.2E4	100% (3/3)	35.8 ± 0.9 (2.5%)
Influenza B Texas/2/13 (Victoria) (CDC *Panel*)	1.2E4	100% (3/3)	35.0 ± 0.3 (1.0%)
Influenza B Maryland/1/1959 (subtype unknown)	1.2E4	100% (3/3)	43.3 ± 0.7 (1.5%)
Influenza B Massachusetts/02/2012 (Yamagata)	1.5E4	100% (3/3)	38.6 ± 0.2 (0.4%)
Influenza B Wisconsin/1/10 (Yamagata)	1.5E4	100% (3/3)	39.7 ± 0.5 (1.4%)
Influenza B Florida/07/04 (Yamagata)	1.5E4	100% (3/3)	38.9 ± 0.3 (0.7%)
Influenza B Florida/04/06 (Yamagata)	1.5E4	100% (3/3)	36.9 ± 0.6 (1.5%)
Influenza B Panama/45/90 (Yamagata)	1.5E4	100% (3/3)	36.5 ± 0.5 (1.4%)
Influenza B Utah/9/14 (Yamagata)	1.5E4	100% (3/3)	37.0 ± 0.2 (0.5%)

Ct = Cycle threshold, SD = Standard Deviation, %CV = Percent Coefficient of Variation

RSV

All strains of RSV were tested at 2.4E4 cps/swab (3x LoD of RSV A Isolate 2006 and RSV B Isolate 12/2014). All replicates were detected correctly at this concentration.

The results for RSV are presented in **Table 7**.

Table 7. RSV Results

Strain	Concentration (cps/swab)	% Detection (#Detected/#Tested)	Mean Ct ± SD (%CV)
RSV-A 1/2015 Isolate #1	2.4E4	100% (3/3)	39.3 ± 0.5 (1.4%)
RSV-A2	2.4E4	100% (3/3)	41.2 ± 0.1 (0.3%)
RSV-A 3/2015 Isolate #3	2.4E4	100% (3/3)	41.2 ± 1.2 (2.9%)
RSV-A 4/2015 Isolate 1	2.4E4	100% (3/3)	41.9 ± 0.7 (1.7%)
RSV-B 9320	2.4E4	100% (3/3)	40.7 ± 0.1 (0.2%)
RSV-B CH93(18)-18	2.4E4	100% (3/3)	37.8 ± 0.6 (1.5%)
RSV-B 3/2015 Isolate #1	2.4E4	100% (3/3)	41.3 ± 1.3 (3.0%)
RSV-B1	2.4E4	100% (3/3)	38.6 ± 0.6 (1.5%)
RSV-B 3/2015 Isolate #2	2.4E4	100% (3/3)	40.8 ± 1.3 (3.3%)

Ct = Cycle threshold, SD = Standard Deviation, %CV = Percent Coefficient of Variation

SARS-CoV-2

All strains of SARS-CoV-2 were tested at 3.0E3 cps/swab (3x LoD of SARS-CoV-2 USA/WA 1/2020 and SARS-CoV-2 USA/MDHP20874/2021 (B.1.1.529 Omicron)). All replicates were detected correctly at this concentration, except for SARS-CoV-2 Isolate

hCoV-19/USA/CA-Stanford -109_S21/2022 PANGO Lineage B.1.1.529, (XBB Omicron), which was detected at 4x LoD (4.0E3 cps/swab).

The results for SARS-CoV-2 are presented in **Table 8**.

Table 8. SARS-CoV-2 Results

Strain	Concentration (cps/swab)	% Detection (#Detected/#Tested)	Mean Ct ± SD (%CV)
SARS-CoV-2 Isolate hCoV-1 9/USA/PHC658/2021 B.1.617.2 (Delta)	3.0E3	100% (3/3)	38.1 ± 0.7 (1.8%)
SARS-CoV-2 Isolate Hong Kong/VM20001061/2020	3.0E3	100% (3/3)	36.1 ± 0.5 (1.5%)
SARS-CoV-2 Isolate hCoV- 19/Japan/TY7-503/2021 (Gamma Variant)	3.0E3	100% (3/3)	35.5 ± 0.3 (0.8%)
SARS-CoV-2 Isolate USA/MD- HP24556/2022 BA.2.3 (Omicron)	3.0E3	100% (3/3)	32.8 ± 0.3 (1.1%)
SARS-CoV-2 Isolate South Africa/KRISP-EC-K005325/2020 (B.1.351 South Africa variant)	3.0E3	100% (3/3)	34.4 ± 0.2 (0.5%)
SARS-CoV-2 Isolate England/204820464/2020 B.1.1.7 (UK variant)	3.0E3	100% (3/3)	35.7 ± 0.6 (1.6%)
SARS-CoV-2 USA/CA-Stanford- 15_S02/2021 B.1.617.1 (Kappa Variant)	3.0E3	100% (3/3)	37.1 ± 0.7 (2.0%)
SARS-CoV-2 USA/NY-Wadsworth- 21025952-01/2021 B.1.526_2021 (Iota Variant)	3.0E3	100% (3/3)	37.7 ± 1.0 (2.7%)
SARS-CoV-2 USA-WI 1/2020 (Lineage B)	3.0E3	100% (3/3)	36.5 ± 1.2 (3.4%)
SARS-CoV-2 NY-Wadsworth- 21006055-01/2021 Lineage P2_2021 (Zeta Variant)	3.0E3	100% (3/3)	38.3 ± 0.5 (1.4%)
SARS-Related Coronavirus 2 Isolate hCoV-1 9/USA/New York/PV96109/2023 (Omicron Variant JN.1)	3.0E3	100% (3/3)	34.5 ± 0.2 (0.6%)
SARS-CoV-2 Lineage JN.1.4 Omicron Variant USA/NY-Wadsworth-23068107-01/2023	3.0E3	100% (3/3)	36.2 ± 0.2 (0.5%)
SARS-CoV-2 Isolate USA-CA3/2020	3.0E3	100% (3/3)	38.4 ± 0.7 (1.9%)
SARS-CoV-2 Isolate Germany/BavPat1/2020 (Lineage B)	3.0E3	100% (3/3)	36.3 ± 0.7 (1.8%)
SARS-CoV-2 Isolate USA/CA/VRLC014/2021 PANGO Lineage B.1.429 (Epsilon)	3.0E3	100% (3/3)	35.5 ± 0.9 (2.6%)
SARS-CoV-2 Isolate hCoV-1 9/USA/VA-FBCH_675/2021 PANGO Lineage AY4.2	3.0E3	100% (3/3)	36.0 ± 1.0 (2.8%)
SARS-CoV-2 Isolate SARS-CoV-2 Peru/un-CDC-2-4069945/2021 PANGO Lineage C.37 (Lambda)	3.0E3	100% (3/3)	35.3 ± 0.9 (2.4%)
SARS-CoV-2 isolate hCoV-191 USA/MD-HP38861/2022 PANGO Lineage B.1.1.529, BQ.1.1 (Omicron)	3.0E3	100% (3/3)	34.9 ± 0.3 (0.9%)
SARS-CoV-2 Isolate hCoV- 19/USA/CA-Stanford -109_S21/2022 PANGO Lineage B.1.1.529, (XBB Omicron)	3.0E3 (3x LoD)	67% (2/3)	39.2 ± 0.6 (1.5%)
	4.0E3 (4x LoD)	100% (3/3)	37.6 ± 0.4 (1.1%)

Ct = Cycle threshold, SD = Standard Deviation, %CV = Percent Coefficient of Variation

This study demonstrates that the LIAISON NES FLU A/B, RSV & COVID-19 assay is able to detect multiple strains/variants for all four (4) viral targets.

Cross-Reactivity / Microbial Interference

Cross-reactivity (analytical specificity) of the LIAISON NES FLU A/B, RSV & COVID-19 assay was evaluated. The purpose of this study was to determine the ability of the assay to not react with microorganisms that have a similar genome to influenza A virus, influenza B

virus, RSV and SARS-CoV-2 or normal and pathogenic flora that are reasonably likely to be present in the clinical sample.

For this study, forty-eight potential cross-reactants have been tested. Samples containing whole organisms or purified nucleic acid from bacteria, fungi, parasites and viruses quantified and titered in CFU/mL, TCID50/mL, cells/mL, cps/mL or other industry-accepted units were diluted in Pooled Negative Human Nasal Matrix and spiked on a dry swab. Each potential cross-reactant was tested in triplicate.

The results of the study are shown in **Table 9**. No cross-reactivity of LIAISON NES FLU A/B, RSV & COVID-19 was observed with any of the potential cross-reactants tested.

Table 9. Cross-Reactivity Results for Non-Target Microorganisms.

Organism	Concentration tested	Influenza A	Influenza B	RSV	SARS-CoV-2	IC
		% Detection (#Detected /#Tested)	% Detection (#Detected /#Tested)	% Detection (#Detected /#Tested)	% Detection (#Detected /#Tested)	% Detection (#Detected /#Tested)
Bordetella parapertussis	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Bordetella pertussis	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Candida albicans	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Corynebacterium diphtheriae	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Escherichia coli	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Fusobacterium necrophorum	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Haemophilus influenzae	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Lactobacillus casei	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Legionella pneumophila	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Moraxella catarrhalis	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Neisseria elongata	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Neisseria gonorrhoeae	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Neisseria meningitidis	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Pneumocystis jirovecii	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Pseudomonas aeruginosa	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Staphylococcus epidermidis BAA-3171	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Streptococcus pneumoniae	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Streptococcus pyogenes M1	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Streptococcus salivarius	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Staphylococcus aureus	1E6 CFU/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Aspergillus fumigatus	1E6 cps/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Chlamydia pneumoniae	1E6 cps/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Mycoplasma genitalium	1E6 cps/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)

Mycoplasma pneumonia	1E6 cps/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Mycobacterium tuberculosis (genomic DNA)	1E6 cps/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Adenovirus 7A	1E6 cps/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Adenovirus Type 31	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
CMV AD-169	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Coronavirus 229E	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Coronavirus NL63	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Coronavirus OC43	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Enterovirus Type 68	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
hMPV 9	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Influenza C SendaiITU2IO8	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Measles	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Mumps Virus	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Parainfluenza Virus Type 2	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Parainfluenza Virus Type 3	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Parainfluenza Virus Type 4A	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Parechovirus Type 1	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Rhinovirus Type 1A	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
EBV	1E5 cps/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
MERS Coronavirus (Florida/USA-2_Saudi Arabia_2014)	1E5 cps/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Parainfluenza Virus Type 1	1E5 TCID50/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Coronavirus HKU1 (synthetic RNA)	1E5 genome copies/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Leptospira (interrogans)	Quantification not provided[a]	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
SARS Coronavirus	Quantification not provided[a]	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Pooled human nasal wash	10%	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)

[a] Quantification not provided by the manufacturer. Maximum testable volume used.

Microbial interference of the LIAISON NES FLU A/B, RSV & COVID-19 assay was evaluated. The purpose of this study was to determine the ability of the assay to detect influenza A virus, influenza B virus, RSV and SARS-CoV-2 in nasal swab specimens that contain other bacteria, fungi, and viruses commonly found in the respiratory tract, using contrived dry nasal swab samples. Forty-seven microorganisms were tested for cross-reactivity and interference along with pooled human nasal wash.

The test samples consisted of contrived dry nasal swabs spiked with low concentrations (3x LoD) of influenza A virus, influenza B virus, RSV and SARS-CoV-2 respectively, prepared together with dilutions of bacteria, fungi, parasites and viruses from stocks that have been quantified and titered in CFU/mL, TCID50/mL, cells/mL or other industry-accepted units. Test samples were prepared in pooled negative human nasal matrix that was confirmed influenza A virus, influenza B virus, RSV and SARS-CoV-2-negative through screening.

Results of the microbial interference study are presented in **Table 10**.

Two instances of microbial interference with the detection of influenza B by the LIAISON NES FLU A/B, RSV & COVID-19 assay were observed. At 1E6 CFU/mL, *Staphylococcus aureus* completely inhibited the detection of influenza B (0/3). The assay was repeated with *Staphylococcus aureus* at 3.13E5 CFU/mL. Inhibition of Influenza B detection was no longer observed with 100% of the influenza B samples detected.

At 1E5 $TCID_{50}$/mL, Parainfluenza Virus Type 1 resulted in detection of 33% (1/3) of influenza B samples. Repeating the assay after reducing the concentration of Parainfluenza to 3.31E4 $TCID_{50}$/mL resulted in the detection of 100% (3/3) of influenza B samples.

No microbial inhibition was observed with any of the other microorganisms tested.

Table 10. Microbial Interference Results for Non-Target Microorganisms.

Organism	Concentration tested	Influenza A	Influenza B	RSV	SARS-CoV-2	IC
		% Detection (#Detected /#Tested)	% Detection (#Detected /#Tested)	% Detection (#Detected /#Tested)	% Detection (#Detected /#Tested)	% Detection (#Detected /#Tested)
Bordetella parapertussis	1E6 CFU/mL	100% (3/3)	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)	100% (3/3)
Candida albicans	1E6 CFU/mL	100% (3/3)	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)	100% (3/3)
Escherichia coli	1E6 CFU/mL	100% (3/3)	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)	100% (3/3)
Haemophilus influenzae	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Lactobacillus casei	1E6 CFU/mL	100% (3/3)	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)	100% (3/3)
Moraxella catarrhalis	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Neisseria elongata	1E6 CFU/mL	100% (3/3)	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)	100% (3/3)
Neisseria meningitidis	1E6 CFU/mL	100% (3/3)	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)	100% (3/3)
Pseudomonas aeruginosa	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Staphylococcus epidermidis BAA-3171	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Streptococcus pneumoniae	1E6 CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Streptococcus pyogenes M1	1E6 CFU/mL	100% (3/3)	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)	100% (3/3)
*Staphylococcus aureus**	1E6 CFU/mL	100% (3/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)
	3.13E5 CFU/mL	N/A	100% (3/3)	N/A	N/A	N/A

Aspergillus fumigatus	1E6 cps/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Chlamydia pneumoniae	1E6 cps/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Mycoplasma genitalium	1E6 cps/mL	100% (3/3)	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)	100% (3/3)
Mycobacterium tuberculosis (genomic DNA)	1E6 cps/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Adenovirus 7A	1E6 cps/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Adenovirus Type 31	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
CMV AD-169	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Coronavirus 229E	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Coronavirus NL63	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Coronavirus OC43	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Enterovirus Type 68	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
hMPV 9	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Influenza C SendaiITU2IO8	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Measles	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Mumps Virus	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parainfluenza Virus Type 2	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parainfluenza Virus Type 3	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parainfluenza Virus Type 4A	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parechovirus Type 1	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Rhinovirus Type 1A	1E5 TCID50/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
EBV	1E5 cps/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
MERS Coronavirus (Florida/USA-2 Saudi Arabia_2014)	1E5 cps/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Parainfluenza Virus Type 1**	1E5 TCID50/mL	100% (3/3)	33% (1/3)	100% (3/3)	100% (3/3)	100% (3/3)
	3.31E4 TCID50/mL	N/A	100% (3/3)	N/A	N/A	N/A
Coronavirus HKU1 (synthetic RNA)	1E5 genome copies/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Leptospira (interrogans)	Quantification not provided[a]	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
SARS Coronavirus	Quantification not provided[a]	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Pooled human nasal wash	10%	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

* Inhibition of Influenza B detection was observed with *Staphylococcus aureus* tested at 1E6 CFU/mL. No inhibition was observed when a lower concentration of 3.13E5 CFU/mL was tested.
** Inhibition of Influenza B detection was observed with Parainfluenza Virus Type 1 tested at 1E5 TCID50/mL. No inhibition was observed when a lower concentration of 3.31E4 TCID50/mL was tested.
[a] Quantification not provided by the manufacturer. Maximum testable volume used.

Interfering Substances

An analytical study was performed to assess the potential inhibitory effects of endogenous and exogenous substances that may be commonly found in ANS specimens. Low positive samples were prepared by spiking low doses (3x LoD) of influenza A virus, influenza B virus, RSV and SARS-CoV-2 in nasal swab specimens. For negative samples, pooled negative human nasal matrix was used. Each interfering substance was tested in triplicate, at the indicated concentration, for each target. Results for the negative sample study are presented in **Table 11**.

Table 11: LIAISON NES FLU A/B, RSV & COVID-19 Assay Interference for Negative Sample

Potentially Interfering Substances	Active Ingredient	Interferent Concentration	Influenza A	Influenza B	RSV	SARS-CoV-2	IC
			% 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	0.004 mg/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)
Blood	N/A	2% (v/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Purified Mucin Protein	Bovine submaxillary gland mucin, type I-S	5 mg/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Nasal corticosteroid (Beconase AQ)	Beclomethasone	5% (w/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
	Dexamethasone	5% (w/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
	Mometasone	5% (w/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Nasal corticosteroid (Fluticasone)	Fluticasone	5% (v/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Tamiflu Antiviral drug	Oseltamivir	0.001 mM	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 % (v/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Budesonide	Budesonide	5% (v/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Flunisolide	Flunisolide	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)
Throat lozenges	*Avena sativa,* Zinc gluconate, *Sambucus nigra*, Echinacea, Rose hips, Licorice root	1.25% (w/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)

Remdesivir	Remdesivir	0.1 mg/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
		0.05 mg/mL	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)
Neo-Synephrine nasal spray	Phenylephrine hydrochloride	15% (v/v)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	100% (3/3)

mg/mL= milligram/milliliter, v/v= volume *per* volume, w/v= weight *per* volume, mM= millimolar

The results for the positive sample study are presented in **Table 12**. One replicate of the RSV target was not detected when tested with Remdesivir at a concentration of 0.1 mg/mL. The test was repeated in triplicate at a reduced concentration (Remdesivir, 0.05 mg/mL). No interference in the detection of the RSV target was observed at the lower concentration.

Table 12. LIAISON NES FLU A/B, RSV & COVID-19 assay Interference for Positive Sample

Potentially Interfering Substances	**Active Ingredient**	**Interferent Concentration**	**Influenza A**	**Influenza B**	**RSV**	**SARS-CoV-2**
			% 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)
Antibacterial, systemic	Tobramycin	0.004 mg/mL	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)
Blood	N/A	2% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Purified Mucin Protein	Bovine submaxillary gland mucin, type I-S	5 mg/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Nasal corticosteroid (Beconase AQ)	Beclomethasone	5% (w/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
	Dexamethasone	5% (w/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
	Mometasone	5% (w/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Nasal corticosteroid (Fluticasone)	Fluticasone	5% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Tamiflu Antiviral drug	Oseltamivir	0.001 mM	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Zicam Nasal Gel	*Luffa operculata*, *Galphimia glauca*, histaminum hydrochloricum	5 % (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Budesonide	Budesonide	5% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Flunisolide	Flunisolide	5% (v/v)	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)
Throat lozenges	*Avena sativa,* Zinc gluconate, *Sambucus nigra*, Echinacea, Rose hips, Licorice root	1.25% (w/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Remdesivir	Remdesivir	0.1 mg/mL	100% (3/3)	100% (3/3)	67% (2/3)[a]	100% (3/3)
		0.05 mg/mL	N/A	N/A	100% (3/3)	N/A
Neo-Synephrine nasal spray	Phenylephrine hydrochloride	15% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

mg/mL= milligram/milliliter, v/v= volume *per* volume, w/v= weight *per* volume, mM= millimolar

[a]0.1 mg/mL Remdesivir resulted in one out of three replicates 'Negative' for RSV. The concentration was reduced to 0.05 mg/mL and retested and no interference was observed.

Competitive Inhibition

A competitive interference study was performed to assess the ability of the LIAISON NES FLU A/B, RSV & COVID-19 assay to detect low concentration (3x LoD) of one target analyte in the presence of high concentration (1000x LoD) of another. Twelve target combinations were generated and each was tested in triplicate. A total of 38 tests (36 tests plus 2 repeats) were carried out by one operator in one day.

The four strains/isolates tested were:

- Influenza A Victoria/4897/2022
- Influenza B Austria/1359417/2021
- Respiratory Syncytial Virus A 2006 Isolate
- SARS-CoV-2 USA/MDHP20874/2021

Results of the competitive inhibition assay are presented in **Table 13**. No evidence of competitive inhibition was observed.

Table 13. Results for Competitive Inhibition Target Combinations

Target combinations	Influenza A		Influenza B		RSV		SARS-CoV-2		Internal Control	
	% Detection (#Detected/ #Tested)	Mean Ct ± SD (%CV)	% Detection (#Detected/ #Tested)	Mean Ct ± SD (%CV)	% Detection (#Detected/ #Tested)	Mean Ct ± SD (%CV)	% Detection (#Detected/ #Tested)	Mean Ct ± SD (%CV)	% Detection (#Detected/ #Tested)	Mean Ct ± SD (%CV)
Influenza B HP/ Influenza A LP	100% (3/3)	32.2 ± 0.3 (1.1%)	100% (3/3)	25.4 ± 0.1 (0.6%)	0% (0/3)	N/A	0% (0/3)	N/A	100% (3/3)	24.6 ± 0.1 (0.2%)
RSVA HP/ Influenza A LP	100% (3/3)	33.0 ± 0.6 (1.7%)	0% (0/3)	N/A	100% (3/3)	23.6 ± 0.2 (0.9%)	0% (0/3)	N/A	100% (3/3)	24.5 ± 0.3 (1.2%)
SARS-CoV-2 HP/ Influenza A LP	100% (3/3)	32.3 ± 0.8 (2.5%)	0% (0/3)	N/A	0% (0/3)	N/A	100% (3/3)	25.2 ± 0.5 (1.8%)	100% (3/3)	24.8 ± 0.2 (0.9%)
Influenza A HP/ Influenza B LP	100% (3/3)	23.2 ± 0.9 (4.0%)	100% (3/3)	35.4 ± 0.6 (1.6%)	0% (0/3)	N/A	0% (0/3)	N/A	100% (3/3)	24.5 ± 0.2 (1.0%)
RSVA HP/ Influenza B LP*	0% (0/3)	N/A	100% (3/3)	36.9 ± 1.8 (5.0%)	100% (3/3)	24.4 ± 0.1 (0.6%)	0% (0/3)	N/A	100% (3/3)	24.4 ± 0.2 (0.9%)
SARS-CoV-2 HP/ Influenza B LP	0% (0/3)	N/A	100% (3/3)	36.8 ± 1.9 (5.1%)	0% (0/3)	N/A	100% (3/3)	25.4 ± 0.1 (0.2%)	100% (3/3)	25.0 ± 0.3 (1.2%)
Influenza A HP/ RSVA LP	100% (3/3)	23.7 ± 0.7 (2.9%)	0% (0/3)	N/A	100% (3/3)	36.6 ± 0.6 (1.8%)	0% (0/3)	N/A	100% (3/3)	25.3 ± 0.2 (1.0%)
Influenza B HP/ RSVA LP	0% (0/3)	N/A	100% (3/3)	25.3 ± 0.3 (1.0%)	100% (3/3)	35.7 ± 0.5 (1.3%)	0% (0/3)	N/A	100% (3/3)	24.9 ± 0.3 (1.2%)
SARS-CoV-2 HP/ RSVA LP*	0% (0/3)	N/A	0% (0/3)	N/A	100% (3/3)	36.9 ± 1.1 (3.0%)	100% (3/3)	25.7 ± 0.4 (1.5%)	100% (3/3)	25.3 ± 0.1 (0.6%)
Influenza A HP/ SARS-CoV-2 LP	100% (3/3)	23.2 ± 0.1 (0.5%)	0% (0/3)	N/A	0% (0/3)	N/A	100% (3/3)	35.7 ± 0.4 (1.2%)	100% (3/3)	25.0 ± 0.1 (0.6%)

Influenza B HP/ SARS-CoV-2 LP	0% (0/3)	N/A	100% (3/3)	25.3 ± 0.2 (0.7%)	0% (0/3)	N/A	100% (3/3)	37.3 ± 0.4 (1.0%)	100% (3/3)	25.0 ± 0.2 (0.7%)
RSVA HP/ SARS-CoV-2 LP	0% (0/3)	N/A	0% (0/3)	N/A	100% (3/3)	25.2 ± 0.3 (1.4%)	100% (3/3)	37.3 ± 1.4 (3.7%)	100% (3/3)	25.0 ± 0.1 (0.4%)

Ct = Cycle threshold, SD = Standard Deviation, %CV = Percent Coefficient of Variation, N/A = Not Applicable
* One (1) run was repeated due to an instrumental error (a total of 4 runs were carried out)

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 (IC) added to each test specimen and external positive and negative controls. For more information, see Section **IV.C.5. Quality Control**, above.

   b. Sample Stability

   Sample storage and handling were evaluated for LIAISON NES FLU A/V, RSV & COVID-19 to support the following:

   - After sample collection, the anterior nasal swab should be stored in its tube and processed within 120 minutes at 15-30ºC ambient temperature if immediate testing is not possible.

   c. Device Stability (Open pouch)

   - The LIAISON NES FLU A/B, RSV & COVID-19 Cartridge is stable for up to 2 hours after pouch opening at 15-30ºC and 80% RH.

   - The LIAISON NES FLU A/B, RSV & COVID-19 Positive and negative control swabs must be used immediately after opening the sleeved pouch.

6. Detection Limit:

   Limit of detection (LoD) studies were conducted to determine the lowest detectable concentration of influenza A virus, influenza B virus, RSV and SARS-CoV-2 at which ≥95% of all replicates test positive.

   The LoD was established with serial dilutions of two quantified viral stocks for each target (influenza A virus, influenza B virus, RSV and SARS-CoV-2) in pooled negative human nasal matrix. Subsequently, 10 µL of the dilution was spiked onto dry swabs for testing.

   Confirmation of the LoD was performed using a minimum of twenty replicates for each of three cartridge lots. The strains evaluated, as well as their corresponding LoD values are shown in **Table 14**.

Table 14. LoD Determination for Influenza A, Influenza B, RSV and SARS-CoV-2

Target	Strain/Isolate	LoD Concentration (Cps/swab)	Positivity
Influenza A	Victoria/4897/2022 (H1N1)	6E3	98% (59/60)
	Darwin/9/21 (H3N2)	8E3	98% (59/60)
Influenza B	Austria/1359417/2021 (Victoria)	4E3	98% (59/60)
	Phuket/3073/2013	5E3	98% (59/60)
Respiratory Syncytial Virus A	Isolate 2006	8E3	98% (59/60)
Respiratory Syncytial Virus B	Isolate 12/2014	8E3	98% (59/60)
SARS-CoV-2	USA/WA 1/2020	1E3	98% (59/60)
	USA/MDHP20874/2021 (B.1.1.529 Omicron)	1E3	98% (59/60)

7. Assay Cut-Off:

   For each channel, the respective target is detected if (1) the fluorescence signal crosses the fluorescence threshold, and (2) the cycle at which it crosses is at/before the last cycle (the Ct threshold, 45 cycles).

8. Accuracy (Instrument):
   Not applicable.

9. Carry-Over/Cross-Contamination:

   The carry-over and cross-contamination rate for the LIAISON NES FLU A/B, RSV & COVID-19 assay was assessed through testing of alternating high positive samples and negative samples between successive runs. The high positive sample was prepared by spiking SARS-CoV-2 USA/MDHP20874/2021 into pooled negative human nasal matrix at a final concentration of 1E8 copies/mL (1000x LoD). The negative sample consisted of pooled negative human nasal matrix spiked onto the swab. A total of 107 experimental runs were performed by one operator over the course of four non-consecutive days.

   Results of the study adequately demonstrate that alternating between highly positive and negative samples does not result in cross-contamination.

B Comparison Studies:

1. Method Comparison with Predicate Device:
   Not Applicable

2. Matrix Comparison:
   Not Applicable

C Clinical Studies:

1. Clinical Sensitivity:

Prospective Clinical Study

The clinical performance of the LIAISON NES FLU A/B, RSV & COVID-19 assay was evaluated using clinical specimens prospectively collected between October 2024 and March 2025 from seven geographically diverse clinical sites located within the United States. The clinical study utilized prospective specimens collected from pediatric and adult patients exhibiting clinical signs and symptoms of respiratory tract infection. Anterior nasal swabs (ANS) were professionally collected by a Healthcare Provider or self-collected under the Healthcare Provider's supervision. All collection and assay testing were performed at CLIA-waived facilities, representative of the intended use, point-of-care environment.

Subject/Specimen Enrollment

A total of 1692 unique prospectively collected specimens that met the pre-determined inclusion criteria, were enrolled in the study. 101 specimens were excluded from performance evaluation. Of these 101 (6.0%) specimens,

- 55 specimens did not have a reference method result due to fresh specimen testing time window violations,
- 19 specimens were excluded due to investigational device testing occurring outside of the allowed time window,
- 19 specimens were excluded due to improper sample collection,
- 5 specimens were excluded for sample handling issues,
- 1 specimen was excluded due to operator error,
- 1 specimen was excluded due to patient withdrawing consent, and
- 1 patient for whom the reference method testing swab could not be collected.

This resulted in 1591 specimens that were eligible for performance evaluation. The demographic data for the study population are shown in **Table 15**. Clinical sample testing using the LIAISON NES FLU A/B, RSV & COVID-19 assay was performed using the LIAISON NES device by untrained operators at each of the seven collection sites.

Table 15: Demographic Data for the Prospective Study Population (N=1591)

	Site 01	**Site 02**	**Site 03**	**Site 04**	**Site 05**	**Site 06**	**Site 13**	**Total**
Sex								
Male	191 (53.1%)	129 (42.7%)	104 (53.3%)	31 (40.3%)	99 (46.3%)	107 (51.4%)	100 (42.6%)	761 (47.8%)
Female	169 (46.9%)	173 (57.3%)	91 (46.7%)	46 (59.7%)	115 (53.7%)	101 (48.6%)	135 (57.4%)	830 (52.2%)
Total	360 (100.0%)	302 (100.0%)	195 (100.0%)	77 (100.0%)	214 (100.0%)	208 (100.0%)	235 (100.0%)	1591 (100.0%)
Age (years)								
0-1	47 (13.1%)	33 (10.9%)	42 (21.5%)	0 (0.0%)	9 (4.2%)	10 (4.8%)	5 (2.1%)	146 (9.2%)

>1-5	87 (24.2%)	47 (15.6%)	60 (30.8%)	0 (0.0%)	5 (2.3%)	32 (15.4%)	23 (9.8%)	254 (16.0%)
>5-21	208 (57.8%)	169 (56.0%)	90 (46.2%)	0 (0.0%)	22 (10.3%)	138 (66.3%)	59 (25.1%)	686 (43.1%)
>21-65	18 (5.0%)	52 (17.2%)	3 (1.5%)	68 (88.3%)	142 (66.4%)	28 (13.5%)	132 (56.2%)	443 (27.8%)
>65	0 (0.0%)	1 (0.3%)	0 (0.0%)	9 (11.7%)	36 (16.8%)	0 (0.0%)	16 (6.8%)	62 (3.9%)
Total	360 (100.0%)	302 (100.0%)	195 (100.0%)	77 (100.0%)	214 (100.0%)	208 (100.0%)	235 (100.0%)	1591 (100.0%)
Subject Status								
Emergency Department	0 (0.0%)	0 (0.0%)	184 (94.4%)	41 (53.2%)	214 (100.0%)	0 (0.0%)	0 (0.0%)	439 (27.6%)
Outpatient	360 (100.0%)	302 (100.0%)	6 (3.1%)	35 (45.5%)	0 (0.0%)	208 (100.0%)	235 (100.0%)	1146 (72.0%)
Other	0 (0.0%)	0 (0.0%)	5 (2.6%)	1 (1.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (0.4%)
Total	360 (100.0%)	302 (100.0%)	195 (100.0%)	77 (100.0%)	214 (100.0%)	208 (100.0%)	235 (100.0%)	1591 (100.0%)
Collection Method								
Healthcare Professional	285 (79.2%)	258 (85.4%)	186 (95.4%)	37 (48.1%)	153 (71.5%)	183 (88.0%)	155 (66.0%)	1257 (79.0%)
Self-collected	75 (20.8%)	44 (14.6%)	9 (4.6%)	40 (51.9%)	61 (28.5%)	25 (12.0%)	80 (34.0%)	334 (21.0%)
Total	360 (100.0%)	302 (100.0%)	195 (100.0%)	77 (100.0%)	214 (100.0%)	208 (100.0%)	235 (100.0%)	1591 (100.0%)

After a single test of each specimen, 1571 specimens generated valid LIAISON NES results for a success rate of 98.7% (1571/1591). There were 20 specimens (1.3%) with invalid results. Due to sample type limitations, repeat testing of specimens with invalid results was not possible. **Table 16** presents the number of positives identified stratified by clinical site and operator.

Table 16. Operator Testing Summary

Clinical Site #	Operator #	Samples Tested	# of Positives	% Positive	# of Invalids	% Invalid
1	2[1]	358	122	34.1%	3	0.8%
	Unknown[2]	2	0	0.0%	2	100%
	Subtotal	**360**	**122**	**33.9%**	**5**	**1.4%**
2	1	244	80	32.8%	1	0.4%
	2	58	22	37.9%	0	0.0%
	Subtotal	**302**	**102**	**33.8%**	**1**	**0.3%**
3	1	21	9	42.9 %	2	9.5%
	2	30	15	50.0 %	0	0.0%
	3	16	10	62.5 %	1	6.3%
	4	73	38	52.1 %	0	0.0%
	5	24	17	70.8 %	0	0.0%
	6	29	15	51.7 %	1	3.4%
	Unknown[3]	2	0	0.0%	2	100%
	Subtotal	**195**	**104**	**53.3%**	**6**	**3.1%**

4	1	69	18	26.1%	2	2.9%
	2	1	0	0.0%	0	0.0%
	3	7	3	42.9%	0	0.0%
	Subtotal	**77**	**21**	**27.3%**	**2**	**2.6%**
5	1	40	13	32.5%	0	0.0%
	2	29	11	37.9%	1	3.4%
	3	7	1	14.3%	0	0.0%
	4	31	17	54.8%	0	0.0%
	5	23	14	60.9%	0	0.0%
	6	36	15	41.7%	0	0.0%
	7	30	15	50.0%	0	0.0%
	8	17	8	47.1%	0	0.0%
	9	1	0	0.0%	0	0.0%
	Subtotal	**214**	**94**	**43.9%**	**1**	**0.5%**
6	1	165	68	41.2%	2	1.2%
	2	43	22	51.2%	1	2.3%
	Subtotal	**208**	**90**	**43.3%**	**3**	**1.4%**
13	1	38	18	47.4%	0	0.0%
	2	6	3	50.0%	0	0.0%
	3	59	31	52.5%	0	0.0%
	4	26	7	26.9%	0	0.0%
	5	106	31	29.2%	2	1.9%
	Subtotal	**235**	**90**	**38.3%**	**2**	**0.9%**
Total		**1591**	**623**	**39.2%**	**20**	**1.3%**

[1]Two (2) operators at Site 1 used a single instrument login/user ID and therefore the testing could not be stratified by operator.
[2]Two (2) invalid runs at Site 1 were not assigned to an operator as no source data is available for these runs.
[3]Two (2) invalid runs at Site 3 were not assigned to an operator as no source data is available for these runs.

For each target in the assay panel, the diagnostic performance of the LIAISON NES FLU A/B, RSV & COVID-19 assay was compared to the reference method to determine the Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA), along with the associated 95% confidence intervals. PPA was calculated as 100% × (TP / (TP + FN)). True positive (TP) indicates that both the assay and the comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the assay was negative while the comparator result was positive. NPA was calculated as 100% × (TN / (TN + FP)). True negative (TN) indicates that both the assay and the comparator method produced negative results, and false positive (FP) indicates that the assay was positive while the comparator result was negative. The results for the sample analysis are summarized in **Table 17**.

The Clinical Study results are acceptable.

Table 17. Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) (N=1571)

Pathogen Target	Positive Percent Agreement			Negative Percent Agreement		
	TP / (TP+FN)	PPA (%)	95% CI	TN / (TN+FP)	NPA (%)	95% CI
Influenza A	384/398[a]	96.5%	94.2%-97.9%	1140/1173[b]	97.2%	96.1%-98.0%
Influenza B	34/35[c]	97.1%	85.5%-99.5%	1531/1536[d]	99.7%	99.2%-99.9%
Respiratory Syncytial Virus	102/113[e]	90.3%	83.4%-94.5%	1455/1458[f]	99.8%	99.4%-99.9%
SARS-CoV-2	63/64[g]	98.4%	91.7%-99.7%	1498/1507[h]	99.4%	98.9%-99.7%

[a] Eight (8) of the 14 Influenza A False Negative specimens were negative by Standard of Care.
[b] Twelve (12) of the 33 Influenza A False Positive specimens were positive by bi-directional sequencing (BDS).
[c] The one (1) Influenza B False Negative specimen was negative by BDS.
[d] Two (2) of the five (5) Influenza B False Positive specimens were positive by Standard of Care.
[e] One (1) of the 11 RSV False Negative specimens was negative by BDS, and another two (2) specimens were negative by Standard of Care.
[f] Two (2) of the three (3) RSV False Positive specimens were positive by another FDA-cleared molecular assay.
[g] The one (1) SARS-CoV-2 False Negative specimen was negative by Standard of Care.
[h] Five (5) of the nine (9) SARS-CoV-2 False Positive specimens were positive by Standard of Care.

Observed co-infection combinations are shown in **Table 18**. Of the four specimens in which both influenza A and COVID-19 were detected by the LIAISON NES FLU A/B, RSV & COVID-19 assay, influenza A was detected in 4/4 specimens by the comparator and SARS-CoV-2 was detected in 1/4 samples by the comparator. Of the 6 specimens in which both influenza A and RSV were detected by the assay, influenza A was detected in 5/6 specimens by the comparator and RSV was detected in 5/6 specimens by the comparator.

Table 18. Observed Co-infection Combinations by the LIAISON NES FLU A/B, RSV & COVID-19 Assay Stratified by Age (N=1571)

Age (years)	0-1 Years (N=145)		2-5 Years (N=246)		6-21 Years (N=679)		22-65 Years (N=439)		>65 Year (N=62)		Overall (N=1571)	
Co-infection	**#POS**	**%**	**#POS**	**%**	**#POS**	**%**	**#POS**	**%**	**#POS**	**%**	**#POS**	**%**
Influenza A | SARS-CoV-2	0	0.00% (0/145)	0	0.00% (0/246)	3	0.44% (3/679)	1	0.23% (1/439)	0	0.00% (0/62)	4	0.25% (4/1571)
Influenza A | RSV	1	0.68% (1/145)	2	0.79% (2/246)	1	0.15% (1/679)	1	0.23% (1/439)	1	1.61% (1/62)	6	0.38% (6/1571)

2. Clinical Specificity:
See **Section "Clinical Sensitivity"** above.

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

D Clinical Cut-Off:
Not applicable.

E Expected Values/Reference Range:

The LIAISON NES FLU A/B, RSV & COVID-19 assay prospective clinical study included a total of 1,591 prospectively collected ANS specimens, of which 1,571 specimens were evaluable. The number and percentage of cases positive for influenza A virus, influenza B virus, RSV, and SARS-CoV-2, as determined by the test, are presented in **Table 19**, stratified by collection site, and by age in **Table 20**.

Table 19. Expected Values for Prospective Specimens by Site (N=1571)[1]

	Site 1 (N=355)	Site 2 (N=301)	Site 3 (N=189)	Site 4 (N=75)	Site 5 (N=213)	Site 6 (N=205)	Site 13 (N=233)	Total (N=1571)

Target	#Pos	(%)	#Pos	(%)	#Pos	(%)	#Pos	(%)	#Pos	(%)	#Pos	(%)	#Pos	(%)	#Pos	(%)
Influenza A	78	22.0% (78/355)	58	19.3% (58/301)	81	42.9% (81/189)	10	13.3% (10/75)	76	35.7% (76/213)	57	27.8% (57/205)	57	24.5% (57/233)	417	26.5% (417/1571)
Influenza B	7	2.0% (7/355)	18	6.0% (18/301)	2	1.1% (2/189)	0	0.0% (0/75)	3	1.4% (3/213)	5	2.4% (5/205)	4	1.7% (4/233)	39	2.5% (39/1571)
RSV	35	9.9% (35/355)	14	4.7% (14/301)	12	6.3% (12/189)	2	2.7% (2/75)	6	2.8% (6/213)	16	7.8% (16/205)	20	8.6% (20/233)	105	6.7% (105/1571)
SARS-CoV-2	4	1.1% (4/355)	14	4.7% (14/301)	10	5.3% (10/189)	9	12.0% (9/75)	9	4.2% (9/213)	15	7.3% (15/205)	11	4.7% (11/233)	72	4.6% (72/1571)

[1]Twenty (20) specimens out of 1591 returned invalid results on the LIAISON NES FLU A/B, RSV & COVID-19 Assay and were excluded from this analysis.

Table 20. Expected Values for Prospective Specimens by Age (N=1571)[1]

	0-1 Years (N=145)		2-5 Years (N=246)		6-21 Years (N=679)		22-65 Years (N=439)		>65 Year (N=62)		Overall (N=1571)	
Target	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**
Influenza A	31	21.4% (31/145)	81	32.9% (81/246)	184	27.1% (184/679)	106	24.1% (106/439)	15	24.2% (15/62)	417	26.5% (417/1571)
Influenza B	0	0.0% (0/145)	4	1.6% (4/246)	29	4.3% (29/679)	6	1.4% (6/439)	0	0.0% (0/62)	39	2.5% (39/1571)
RSV	23	15.9% (23/145)	41	16.7% (41/246)	29	4.3% (29/679)	6	1.4% (6/439)	6	9.7% (6/62)	105	6.7% (105/1571)
SARS-CoV-2	8	5.5% (8/145)	4	1.6% (4/246)	23	3.4% (23/679)	30	6.8% (30/439)	7	11.3% (7/62)	72	4.6% (72/1571)

[1]Twenty (20) specimens out of 1591 returned invalid results on the LIAISON NES FLU A/B, RSV & COVID-19 Assay and were excluded from this analysis.

F Other Supportive Instrument Performance Characteristics Data:

Not applicable

VIII Proposed Labeling:

The labeling supports or 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.

The submitted information in this CLIA waiver application supports a CLIA waiver approval decision.