March 1, 2024

Luminex Corporation
Tara Viviani
Sr. Director Molecular Regulatory Affairs
4088 Commercial Avenue
Northbrook, Illinois 60062

Re: K233410
Trade/Device Name: LIAISON PLEX Respiratory *Flex* Assay
Regulation Number: 21 CFR 866.3981
Regulation Name: Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The SARS-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test
Regulatory Class: Class II
Product Code: QOF, OEM, OOU, OTG, OZE, OZX, OZY, OZZ, OCC, NSU
Dated: October 6, 2023
Received: October 6, 2023

Dear Tara Viviani:

We have reviewed your section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (the Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Additional information about changes that may require a new premarket notification are provided in the FDA guidance documents entitled "Deciding When to Submit a 510(k) for a Change to an Existing Device"

(https://www.fda.gov/media/99812/download) and "Deciding When to Submit a 510(k) for a Software Change to an Existing Device" (https://www.fda.gov/media/99785/download).

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

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR Part 803) for devices or postmarketing safety reporting (21 CFR Part 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR Part 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR Parts 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

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

Sincerely,

Joseph Briggs -S

Joseph Briggs, Ph.D.
Deputy Branch Chief
Division of Microbiology Devices
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 07/31/2026
See PRA Statement below.

510(k) Number *(if known)*
K233410

Device Name
LIAISON PLEX Respiratory Flex Assay

Indications for Use *(Describe)*
The LIAISON PLEX Respiratory Flex (RSP Flex) Assay is a multiplexed qualitative test for the simultaneous in vitro detection and identification of multiple bacterial and viral nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals with clinical signs and symptoms of respiratory tract infection, including SARS-CoV-2. The test is performed on the automated LIAISON PLEX System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific nucleic acid gene sequences of the following organism types and subtypes:

Viruses:
Adenovirus
Human Coronavirus (HKU1, NL63, OC43, and 229E not differentiated)
Human Enterovirus/Rhinovirus (not differentiated)
Human Metapneumovirus,
Influenza A
  Influenza A (subtype H1)
  Influenza A (subtype H3)
Influenza B
Parainfluenza 1
Parainfluenza 2
Parainfluenza 3
Parainfluenza 4
Respiratory Syncytial Virus
Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2)

Bacteria:
Bordetella holmesii
Bordetella parapertussis
Bordetella pertussis
Chlamydia pneumoniae
Mycoplasma pneumoniae

Nucleic acids from the bacterial and viral organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. Detecting and identifying specific bacterial and viral nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory infection, if used in conjunction with other clinical, epidemiological, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or patient management decisions.

Negative results in the presence of a respiratory illness may be due to infection with pathogens that are not detected by this test or due to lower respiratory tract infection that is not detected by an NPS specimen. Conversely, positive results do not rule out infection or co-infection with organisms not detected by the LIAISON PLEX Respiratory Flex (RSP Flex) Assay. The agent(s) detected may not be the definite cause of disease.

The use of additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography), may be necessary when evaluating a patient with possible respiratory tract infection.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) Summary

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

Preparation Date: 21-February-2024

A. 510(k) Number:

K233410

B. Purpose for Submission:

Traditional 510(k), New Device

C. Measurand:

Adenovirus, *Bordetella holmesii, Bordetella parapertussis, Bordetella pertussis, Chlamydia pneumoniae*, Human Coronavirus (HKU1, NL63, OC43, and 229E not differentiated), Human Enterovirus/Rhinovirus (not differentiated), Human Metapneumovirus, Influenza A, Influenza A (subtype H1), Influenza A (subtype H3), Influenza B, *Mycoplasma pneumoniae*, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Respiratory Syncytial Virus (RSV), and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) nucleic acid target sequences

D. Type of Test:

Qualitative Multiplexed Nucleic Acid Test that Utilizes Reverse Transcription, Real Time Polymerase Chain Reaction (PCR), and Array Hybridization.

E. Applicant:

Tara Viviani,
Luminex Corporation
4088 Commercial Avenue
Northbrook, IL 60062
(847) 400-9000

F. Proprietary and Established Names:

LIAISON PLEX® Respiratory *Flex* Assay

G. Regulatory Information:

Primary Product Code	Classification	Regulation Section	Panel
QOF	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

H. Intended Use:

Intended use(s):

The LIAISON PLEX Respiratory *Flex* (RSP *Flex*) Assay is a multiplexed qualitative test for the simultaneous *in vitro* detection and identification of multiple bacterial and viral nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals with clinical signs and symptoms of respiratory tract infection, including SARS-CoV-2. The test is performed on the automated LIAISON PLEX System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific nucleic acid gene sequences of the following organism types and subtypes:

Viruses:
Adenovirus
Human Coronavirus (HKU1, NL63, OC43, and 229E not differentiated)
Human Enterovirus/Rhinovirus (not differentiated)
Human Metapneumovirus,
Influenza A
- Influenza A (subtype H1)
- Influenza A (subtype H3)

Influenza B
Parainfluenza 1
Parainfluenza 2
Parainfluenza 3
Parainfluenza 4
Respiratory Syncytial Virus
Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2)

Bacteria:
Bordetella holmesii
Bordetella parapertussis
Bordetella pertussis
Chlamydia pneumoniae
Mycoplasma pneumoniae

Nucleic acids from the bacterial and viral organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. Detecting and identifying specific bacterial and viral nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory infection, if used in conjunction with other clinical, epidemiological, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or patient management decisions.

Negative results in the presence of a respiratory illness may be due to infection with pathogens that are not detected by this test or due to lower respiratory tract infection that is not detected by an NPS specimen. Conversely, positive results do not rule out infection or co-infection with organisms not detected by the LIAISON PLEX Respiratory *Flex* (RSP *Flex*) Assay. The agent(s) detected may not be the definite cause of disease.

The use of additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography), may be necessary when evaluating a patient with possible respiratory tract infection.

Indication(s) for use:

Same as intended use.

Special conditions for use statement(s):

For prescription use only.

For *in vitro* diagnostic use only

Special instrument requirements:

For use with LIAISON PLEX Systems only

I. Device Description:

The LIAISON PLEX® Respiratory *Flex* Assay is a multiplexed nucleic acid test system composed of the LIAISON PLEX Instrument, the LIAISON PLEX® System Software (preinstalled on the LIAISON PLEX Instrument), the LIAISON PLEX® Respiratory *Flex* Assay cartridge, and the LIAISON PLEX® Respiratory *Flex* Assay File. The LIAISON PLEX® Respiratory *Flex* Assay cartridge contains the reagents to perform nucleic acid extraction and purification, reverse transcription, PCR, and array hybridization. Specifically, the LIAISON PLEX® Respiratory *Flex* Assay detects bacteria and viruses from nasopharyngeal swab (NPS) specimens collected from individuals with signs and symptoms of respiratory infection.

The LIAISON PLEX System consists of a touchscreen user interface that includes the software for running and analyzing assay results, one to six processing/imaging LIAISON PLEX modules, and a handheld barcode reader. Each LIAISON PLEX module processes one sample at a time under the control of the LIAISON PLEX System software.

LIAISON PLEX® automates the sample processing through analysis within a single cartridge. Processing steps include 1.) Sample Preparation: Nucleic acid extraction from organisms by chemical and mechanical means and isolation of nucleic acid on magnetic beads 2.) Target Amplification: Multiplex PCR and RT-PCR based amplification of extracted nucleic acid to generate target specific amplicons 3.) Hybridization: Amplicons hybridize with their target specific DNA probe arranged in a microarray format and that are attached to mediator and gold nanoparticles 4.) Analysis: Gold nanoparticles specifically bound to target amplicons are silver enhanced and the light scatter from microarray spot is measured and analyzed to confirm presence (Detected) or absence (not Detected) of a target.

The LIAISON PLEX Respiratory *Flex* Assay has the option of creating and processing results for custom panels using *Flex*® Software. *Flex* Software allows users to randomly select and group targets in tiers for result processing. Up to 7 targets may be selected for the initial test tier. After the first tier, each additional tier requires a specific number of credits. *Flex*™ credits allow the end-user to create custom panels and pay for a smaller subset of results tailored to the individual patient's clinical presentation. Alternatively, a laboratory may choose the fixed price option where all target results are processed at the same time.

J. Substantial Equivalence Information:

Predicate device name(s):

BioFire Respiratory Panel 2.1 (RP2.1)

Predicate 510(k) number(s):

DEN200031

Comparison with predicate:

The following table compares Luminex's LIAISON PLEX® Respiratory *Flex* Assay to the BioFire Respiratory Panel 2.1 (RP2.1) (DEN200031).

Comparison to Predicate

Comparison to Predicate Device	Predicate Device: BioFire Respiratory Panel 2.1 (RP2.1) (DEN200031)	Candidate Device: LIAISON PLEX® Respiratory *Flex* Assay
Product Code	QOF	QOF
Regulation Number	21 CFR 866.3981	21 CFR 866.3981

Comparison to Predicate Device	Predicate Device: BioFire Respiratory Panel 2.1 (RP2.1) (DEN200031)	Candidate Device: LIAISON PLEX® Respiratory *Flex* Assay
Organisms Detected	Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2), Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, including subtypes H1, H1-2009, and H3, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus, *Bordetella parapertussis* (IS1001), *Bordetella pertussis* (ptxP), *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*	Adenovirus, *Bordetella holmesii, Bordetella parapertussis, Bordetella pertussis, Chlamydia pneumoniae*, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Enterovirus/Rhinovirus, Human Metapneumovirus, Influenza A, Influenza A (subtype H1), Influenza A (subtype H3), Influenza B, *Mycoplasma pneumoniae*, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, Respiratory Syncytial Virus, and SARS-CoV-2
Measurand	Nucleic acid from Organisms detected	Nucleic acid from Organisms detected
Intended Use	The BioFire Respiratory Panel 2.1 (RP2.1) is a PCR-based multiplexed nucleic acid test intended for use with the BioFire FilmArray 2.0 or BioFire FilmArray Torch systems for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections, including COVID-19. The following organism types and subtypes are identified using the BioFire RP2.1: Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2), Human Metapneumovirus, Human Rhinovirus/Enterovirus, Influenza A, including subtypes H1, H1-2009, and H3, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus, *Bordetella parapertussis* (IS1001),	The LIAISON PLEX Respiratory *Flex* (RSP *Flex*) Assay is a multiplexed qualitative test for the simultaneous in *vitro* detection and identification of multiple bacterial and viral nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals with clinical signs and symptoms of respiratory tract infection, including SARS-CoV-2. The test is performed on the automated LIAISON PLEX System utilizing reverse transcription (RT), polymerase chain reaction (PCR), and array hybridization to detect specific nucleic acid gene sequences of the following organism types and subtypes: Viruses: Adenovirus Human Coronavirus (HKU1, NL63, OC43, and 229E not differentiated) Human Enterovirus/Rhinovirus (not differentiated) Human Metapneumovirus, Influenza A Influenza A (subtype H1) Influenza A (subtype H3) Influenza B Parainfluenza 1 Parainfluenza 2 Parainfluenza 3 Parainfluenza 4 Respiratory Syncytial Virus Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2)

Comparison to Predicate Device	Predicate Device: BioFire Respiratory Panel 2.1 (RP2.1) (DEN200031)	Candidate Device: LIAISON PLEX® Respiratory *Flex* Assay
	Bordetella pertussis (ptxP), *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* Nucleic acids from the respiratory viral and bacterial organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or symptoms of respiratory infection is indicative of the presence of the identified microorganism and aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the BioFire RP2.1 may not be the definite cause of disease. Additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.	Bacteria: *Bordetella holmesii* *Bordetella parapertussis* *Bordetella pertussis* *Chlamydia pneumoniae* *Mycoplasma pneumoniae* Nucleic acids from the bacterial and viral organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. Detecting and identifying specific bacterial and viral nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory infection, if used in conjunction with other clinical, epidemiological, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or patient management decisions. Negative results in the presence of a respiratory illness may be due to infection with pathogens that are not detected by this test or due to lower respiratory tract infection that is not detected by an NPS specimen. Conversely, positive results do not rule out infection or co-infection with organisms not detected by the LIAISON PLEX Respiratory *Flex* (RSP *Flex*) Assay. The agent(s) detected may not be the definite cause of disease. The use of additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography), may be necessary when evaluating a patient with possible respiratory tract infection.
Automated System (Sample to Answer)	Automated	Same
Instrumentation	BioFire® FilmArray® 2.0 or BioFire® FilmArray® Torch Systems	LIAISON PLEX®
Sample Types	Nasopharyngeal Swab (NPS) Specimens	Same
Technological Principles	Highly multiplexed nested nucleic acid amplification with melt analysis.	Highly multiplexed nucleic acid PCR and RT-PCR test with microarray detection
Internal Controls	Two controls are included in each reagent pouch to control for sample processing	Multiple internal controls contained in the cartridge monitor sample processing and RT and PCR

Comparison to Predicate Device	Predicate Device: BioFire Respiratory Panel 2.1 (RP2.1) (DEN200031)	Candidate Device: LIAISON PLEX® Respiratory *Flex* Assay
	and both stages of PCR and melt analysis.	functions.
Bordetella Species Detected	*Bordetella parapertussis* *Bordetella pertussis*	• *Bordetella parapertussis* • *Bordetella pertussis* • *Bordetella holmesii*
Human Coronavirus Result Reporting	Each target human coronavirus species (i.e., HKU1, OC43, 229E, NL63) is reported independently.	The human coronavirus target species (i.e., HKU1, OC43, 229E, NL63) are not differentiated.
Influenza A Subtyping	Influenza A subtypes H1, H1-2009, and H3 detected/reported.	Influenza A subtypes H1 and H3 detected/reported.
Time to Result	~45 minutes	~2 hours

K. Standards/Guidance Documents Referenced:

Standards

- CLSI. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline - Second Edition. CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.
- CLSI. Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline. CLSI document EP25-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
- CLSI. Interference Testing in Clinical Chemistry. 3rd ed. CLSI guideline EP07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.
- ISO 14971:2019 Medical devices - Application of risk management to medical devices
- IEC 62366-1:2015 Medical devices - Part 1: Application of usability engineering to medical devices
- ISO 62304:2006 Medical device software - Software life-cycle processes
- ISO 15223-1:2016: Medical Devices - Symbols to be used with medical device labels, labeling and information to be supplied - Part 1: General requirements
- IEC 61010-1 Ed. 3.0 2010: Safety requirements for electrical equipment for measurement, control, and laboratory use - Part 1: General requirements
- EN 61010-2-101:2002/IEC 61010-2-101:2015: Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment.
- IEC 60601-1-2:2014 (Edition 4.0): Medical electrical equipment - Part 1-2: General requirements for basic safety and essential performance - Collateral Standard: Electromagnetic disturbances - Requirements and tests
- ISO 13485:2016/EN ISO 13485:2016; Medical devices - Quality Management System - Requirements for regulatory purposes
- ISO 20916:2019; In vitro diagnostic medical devices. Clinical performance studies using specimens from human subjects. Good study practice
- EN ISO 18113-1:2011; In vitro diagnostic medical devices - Information supplied by the manufacturer (labeling). Terms, definition and general requirements
- EN ISO 18113-2:2011; In vitro diagnostic medical devices - Information supplied by the manufacturer (labeling) – Part 2: In vitro diagnostic reagents for professional use

- EN ISO 18113-3:2011; In vitro diagnostic medical devices - Information supplied by the manufacturer (labeling) – Part 3: In vitro diagnostic instruments for professional use
- EN ISO 23640:2015; In vitro diagnostic medical devices - Evaluation of stability of in vitro
- IEC 61326-1:2012; Electrical equipment for measurement control and laboratory use - EMC requirements - Part 1: General requirements
- EN 61326-2-6:2006/IEC 61326-2-6:2012; Electrical equipment for measurement control and laboratory use - EMC requirements - Part 2-6: Particular requirements - In vitro diagnostic (IVD) medical equipment

Special Controls

- Class II Special Controls as per 21 CFR 866.3981

Guidance Documents

- Electronic Submission Template for Medical Device 510(k) Submissions - Guidance for Industry and Food and Drug Administration Staff (October 2, 2023).
- Respiratory Viral Panel Multiplex Nucleic Acid Assay - Class II Special Controls Guidance for Industry and FDA Staff (October 9, 2009).
- Content of Premarket Submissions for Device Software Functions - Guidance for Industry and Food and Drug Administration Staff (June 14, 2023).
- Cybersecurity in Medical Devices: Quality System Considerations and Content of Premarket Submissions - Guidance for Industry and Food and Drug Administration Staff (September 23, 2023).
- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests - Guidance for Industry and FDA Staff (March 13, 2007).

L. Test Principle:

The LIAISON PLEX® Respiratory *Flex* Assay is performed on nasopharyngeal swab (NPS) specimens collected in Copan Universal Transport Medium™ or BD™ Universal Viral Transport Media. The system consists of an instrument and a single-use, disposable test cartridge and a transfer pipette. The user loads a portion of the sample into the sample port of the LIAISON PLEX Respiratory *Flex* Assay Cartridge. Next, the user sets up the sample order on the LIAISON PLEX System by first entering the sample information or scanning the barcode ID located on the sample tube, then scanning the barcode ID located on the test cartridge. Last, the user inserts the test cartridge into the processing module to initiate the test. The LIAISON PLEX System identifies the assay being run and automatically initiates the proper testing protocol to process the sample, analyze the data, and generate test results.

The LIAISON PLEX System automates the LIAISON PLEX Respiratory *Flex* Assay sample analysis through the following steps: a) Sample Preparation: Nucleic acid extraction via mechanical and chemical cell lysis and magnetic bead- based nucleic acid isolation of prepared specimens obtained from patients; b) Target Amplification: Multiplex PCR- and RT-PCR-based amplification of the extracted nucleic acids to generate target-specific amplicons; c) Hybridization: Amplicons hybridize to target-specific capture DNA on a microarray format, and target-specific mediator and gold nanoparticle probes hybridize to captured amplicons; d) Signal Analysis: Gold nanoparticle probes bound specifically to target-containing spots in the microarray are silver-enhanced, and light scatter from the spots is measured and further

analyzed to determine the presence (Detected) or absence (Not Detected) of a target.

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Within Laboratory Precision

Within laboratory precision of the LIAISON PLEX Respiratory *Flex* Assay was evaluated by testing three lots of LIAISON PLEX Respiratory *Flex* Assay cartridges at a single site over five non-consecutive days. Three target concentrations were prepared and tested. Targets consisted of a negative sample and a positive sample comprised of five targets (*B. pertussis*, Adenovirus, influenza B, human metapneumovirus, and SARS-CoV-2). All positive samples were diluted in a simulated NPS matrix to a low positive concentration (1.5X LoD) and a moderate positive concentration (5X LoD). Targets were randomized and blinded to the operators in an order that each operator tested each target (negative, 1.5X LoD, and 5X LoD) in triplicate on each of the testing days. Qualitative results of the within laboratory precision study are summarized in Table 1.

Table 1 - Within Laboratory Precision

Target	Panel Concentration	% Positive	% Agreement with Expected Results (95% CI)
Bordetella pertussis	Low Positive (1.5X LoD)	93.3% (42/45)	93.3% (82.1-97.7%)
	Moderate Positive (5X LoD)	100% (45/45)	100% (92.1-100%)
	Negative	0% (0/45)	100% (92.1-100%)
Adenovirus	Low Positive (1.5X LoD)	97.8% (44/45)	97.8% (88.4-99.6%)
	Moderate Positive (5X LoD)	97.8% (44/45)	97.8% (88.4-99.6%)
	Negative	0% (0/45)	100% (92.1-100%)
Influenza B	Low Positive (1.5X LoD)	100% (45/45)	100% (92.1-100%)
	Moderate Positive (5X LoD)	100% (45/45)	100% (92.1-100%)
	Negative	0% (0/45)	100% (92.1-100%)
hMPV	Low Positive (1.5X LoD)	100% (45/45)	100% (92.1-100%)
	Moderate Positive (5X LoD)	97.8% (44/45)	97.8% (88.4-99.6%)
	Negative	0% (0/45)	100% (92.1-100%)
SARS-CoV-2	Low Positive (1.5X LoD)	100% (45/45)	100% (92.1-100%)
	Moderate Positive (5X LoD)	100% (45/45)	100% (92.1-100%)
	Negative	0% (0/45)	100% (92.1-100%)

Note: Results are shown only for the intended targets. Panel members co-spiked with 5 different targets are tested in triplicate across 3 lots and tested over 5 non-consecutive days (45 total replicates).

Reproducibility

Reproducibility of the LIAISON PLEX Respiratory *Flex* Assay was evaluated by testing one lot of LIAISON PLEX Respiratory *Flex* Assay cartridges with two operators at each of three sites over five non-consecutive days. Three target concentrations were prepared and tested across all

sites and operators to evaluate site-to-site reproducibility. Targets consisted of a negative sample and a positive sample comprised of five assay targets (*B. pertussis*, Adenovirus, influenza B, human metapneumovirus, and SARS-CoV-2). All positive samples were diluted in a simulated NPS matrix to a low positive concentration (1.5X LoD) and a moderate positive concentration (5X LoD). Targets were randomized and blinded to the operators in an order that each operator tested each target (negative, 1.5X LoD, and 5X LoD) in triplicate on each of the testing days. Qualitative results of the reproducibility study are summarized in Table 2.

Table 2. Reproducibility Results

Organism	Target Concentration	% Agreement with Expected Results			
		Site 1	Site 2	Site 3	All Sites (95% Confidence)
Adenovirus	Low Positive (1.5X LoD)	96.7% (29/30)	100% (30/30)	96.7% (29/30)	97.8% (88/90) (92.3% - 99.4%)
	Moderate Positive (5X LoD)	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Bordetella pertussis	Low Positive (1.5X LoD)	93.3% (28/30)	100% (30/30)	96.7% (29/30)	96.7% (87/90) (90.7% - 98.9%)
	Moderate Positive (5X LoD)	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Influenza B	Low Positive (1.5X LoD)	93.3% (28/30)	100% (30/30)	96.7% (29/30)	96.7% (87/90) (90.7% - 98.9%)
	Moderate Positive (5X LoD)	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Human Metapneumovirus	Low Positive (1.5X LoD)	90.0% (27/30)	100% (30/30)	93.3% (28/30)	94.4% (85/90) (87.6% - 97.6%)
	Moderate Positive (5X LoD)	100% (30/30)	93.3% (28/30)	100% (30/30)	97.8% (88/90) (92.3% - 99.4%)
SARS-CoV-2	Low Positive (1.5X LoD)	96.7% (29/30)	100% (30/30)	100% (30/30)	98.9% (89/90) (93.9% - 99.8%)
	Moderate Positive (5X LoD)	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)
Negative NPS	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9% - 100%)

b. Linearity/assay reportable range:

Not applicable. The LIAISON PLEX® Respiratory *Flex* Assay is a qualitative assay.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Controls:

Each LIAISON PLEX Respiratory *Flex* Assay cartridge includes internal controls (extraction control, amplification control, and hybridization control) to ensure performance of sample preparation, amplification, and detection. **Extraction control** is automatically added to the sample prior to initiation of sample preparation and assesses extraction, nucleic acid recovery, amplification of RNA targets, and detection. Additionally, an **amplification control** present in the lyophilized PCR

master mix, serves as an independent amplification and detection control for DNA targets. Finally, a post-amplification **hybridization control** serves as an indicator of successful hybridization. Internal control results are reported as Pass, Fail, or N/A on the printed reports (see Table 3 for detailed explanations of each control result). Internal controls must either (1) generate a signal above threshold in each internal reaction for the system to report a valid test result, or (2) the amplification or extraction control result can be below the signal threshold if a DNA or RNA target pathogen is detected, respectively.

If the Test Result is "No Call" for reasons other than failure of internal controls, the Internal Control Result is reported as "N/A" and the user should repeat the test with a new cartridge. For additional assistance regarding assay failures unrelated to internal controls, please refer to Chapter 8 (Troubleshooting Unexpected Results/Failures) of the LIAISON PLEX® System User Manual.

Table 3. Interpretation of Controls on the LIAISON PLEX Respiratory *Flex* Assay Report

Internal Control Result	Explanation	Suggested Action
Pass	The hybridization control was detected, indicating successful hybridization. The amplification control was detected, indicating successful amplification. The extraction control was detected, indicating successful extraction.	Review and report results
N/A	The hybridization control was detected, indicating successful hybridization. A DNA pathogen target was detected, indicating successful amplification. If a DNA pathogen target is detected, the amplification control result is ignored. The extraction control was detected, indicating successful extraction.	Review and report results
N/A	The hybridization control was detected, indicating successful hybridization. The amplification control was detected, indicating successful amplification. A RNA pathogen target was detected, indicating successful extraction. If a RNA pathogen target is detected, the extraction control result is ignored.	Review and report results
Fail	The hybridization control was not detected indicating hybridization was not successful. The amplification control, or a DNA pathogen was detected, indicating successful amplification. The extraction control, or a RNA pathogen was detected, indicating successful extraction.	Repeat test with a new cartridge

Internal Control Result	Explanation	Suggested Action
Fail	The hybridization control was detected indicating successful hybridization. The amplification control, or a DNA pathogen was not detected, indicating amplification was not successful. The extraction control, or a RNA pathogen was detected, indicating successful extraction.	Repeat test with a new cartridge
Fail	The hybridization control was detected indicating successful hybridization. The amplification control, or a DNA pathogen was detected, indicating successful amplification. The extraction control, or a RNA pathogen, was not detected, indicating extraction was not successful.	Repeat test with a new cartridge

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. Positive and negative external controls are not provided with the LIAISON PLEX Respiratory *Flex* Assay. Verified negative nasopharyngeal swab (NPS) specimens can be used as the negative control. Previously characterized positive samples or verified negative NPS specimens spiked with well characterized organisms may be used as the external positive control. External controls should be used in accordance with laboratory protocols and in accordance with local, state, and federal accrediting organizations, as applicable.

Stability:

Specimen Stability

Contrived specimen stability at room temperature (15°C - 30°C), refrigerated (2°C - 8°C), and frozen (≤-70°C) storage was evaluated for use with the LIAISON PLEX® Respiratory *Flex* Assay. A representative panel of 5 Respiratory *Flex* target organisms (i.e., *Bordetella pertussis*, adenovirus, influenza B, hMPV, and SARS-CoV-2) was co-spiked into negative clinical NPS matrix at three concentrations - a low positive (2x LoD) sample, a moderate positive (5x LoD) sample, as well as the negative clinical matrix independently. Testing occurred at baseline and various time points up to 36 days for the frozen storage, up to 80 hours for refrigerated storage, and up to 9 hours for room temperature storage.

The results of this study demonstrated that specimens stored frozen (≤ -70°C) are stable for up to 30 days, specimens stored refrigerated (2°C - 8°C) are stable for up to 72 hours, and specimens stored at room temperature (15°C - 30°C) are stable for up to 8 hours.

Fresh vs. Frozen Specimen Stability

Performance of the LIAISON PLEX® Respiratory *Flex* Assay was assessed using contrived specimens tested fresh (i.e. unfrozen) and specimens tested frozen (stored at ≤ -70°C).

The effect of repeated freeze/thaw cycles was also assessed between freshly prepared contrived specimens and those that had undergone 1, 2, and 3 freeze/thaw cycles. Four contrived sample panels were prepared by co-spiking 5-6 targets into clinical negative NPS matrix at three concentrations - a low positive (2x LoD) sample, a moderate positive (5x LoD) sample, as well as the negative clinical matrix independently (see Table 4).

Table 4. Positive Sample Panels Evaluated in the Freeze/Thaw Study

Panel	Organism	Panel	Organism
A	*Bordetella pertussis*	C	*Bordetella parapertussis*
	Adenovirus		RSV A
	Influenza B		Parainfluenza 2
	hMPV (A2)		Influenza A (subtype H3)
	SARS-CoV-2		hMPV (B2)
B	*Bordetella holmesii*	D	RSV B
	Mycoplasma pneumoniae		*Chlamydia pneumoniae*
	Parainfluenza 4		Parainfluenza 1
	Influenza A (subtype H1)		Human coronavirus NL63
	Parainfluenza 3		Human coronavirus OC43
	Rhinovirus A		

Positive panels spiked at 2x were tested in replicates of 40 at T0 (fresh) and 20 replicates following 1, 2, and 3 freeze/thaw (F/T) cycles after storage at -70°C. Positive panels spiked at 5x LoD and the negative sample were tested in replicates of 10 at T0 (fresh) and following 1, 2, and 3 F/T cycles after storage at -70°C.

The results of the study support that NPS specimens in UVT/UTM can undergo up to two freeze/thaw cycles prior to testing with the LIAISON PLEX Respiratory *Flex* Assay.

d. Detection Limit:

A limit of detection study (LoD) was performed to evaluate the analytical sensitivity of the LIAISON PLEX RSP *Flex* Assay. Thirty-nine (39) strains and isolates that represent the 19 reportable targets of the LIAISON PLEX RSP *Flex* Assay were tested individually by serially diluting each target in NPS matrix. Testing was broken into two parts; LoD Determination and LoD Confirmation. The determined LoD concentrations were evaluated using a 3-fold dilution series and testing of at least six replicates per dilution. The determined LoD for each target was defined as the lowest concentration at which 100% of six replicates were positive for the intended reportable target. The confirmed LoD was evaluated using a dilution series around the determined LoD and testing of at least 20 replicates was performed at the confirmed LoD and the dilution below the confirmed LoD. The confirmed LoD for each organism was defined as the lowest concentration at which ≥ 95% of the 20 replicates were positive for the intended reportable target. The confirmed LoD for each target tested is listed in Table 5. The LoD for co-analyte spiked samples was also evaluated and shown to be equivalent to single spiked samples.

Table 5. LIAISON PLEX Respiratory Flex Assay Target Limit of Detection

Target Organism	Strain / Isolate		Concentration at LoD[1]	
Bacteria				
Bordetella holmesii	F061		7.29E+03 copies/mL	8.68E+01 CFU/mL
Bordetella pertussis	18323 NCTC 10739		3.80E+03 copies/mL	1.98E+03 CFU/mL
Bordetella parapertussis	C510		7.90E+02 copies/mL	2.06E+01 CFU/mL
Chlamydia pneumoniae	CM-1		5.68E+02 copies/mL	1.04E+02 IFU/mL
Mycoplasma pneumoniae	M129		1.30E+03 copies/mL	4.24E+01 CCU/mL
Viruses				
Adenovirus (A, B, C, D, E, F)	Type 31 (A)		1.76E+03 copies/mL	1.09E-02 $TCID_{50}$/mL
	Type 3 (B)		6.86E+02 copies/mL	1.69E-01 $TCID_{50}$/mL
	Type 1 (C)		1.12E+03 copies/mL	8.97E+01 $TCID_{50}$/mL
	Type 26 (D)		7.48E+02 copies/mL	1.10E-02 $TCID_{50}$/mL
	Type 4 (E)		3.53E+02 copies/mL	1.08E-02 $TCID_{50}$/mL
	Type 40 (F)		4.85E+02 copies/mL	2.29E-02 $TCID_{50}$/mL
Human Coronavirus (HKU1, 229E, NL63, OC43)	229E		4.00E+02 copies/mL	9.15E-02 $TCID_{50}$/mL
	HKU1		1.67E+03 copies/mL	N/A[2]
	NL63		7.64E+01 copies/mL	1.34E-02 $TCID_{50}$/mL
	OC43		9.48E+03 copies/mL	9.58E-01 $TCID_{50}$/mL
Human Metapneumovirus	(hMPV-9) A1		2.13E+03 copies/mL	2.09E-01 $TCID_{50}$/mL
	(hMPV-27) A2		2.04E+03 copies/mL	2.14E-01 $TCID_{50}$/mL
	(hMPV-3) B1		5.00E+03 copies/mL	4.31E-01 $TCID_{50}$/mL
	(hMPV-8) B2		1.50E+04 copies/mL	1.66E+00 $TCID_{50}$/mL
Influenza A | Influenza A (subtype H1)	Brisbane/02/18	Influenza A	1.35E+04 copies/mL	3.97E+00 $TCID_{50}$/mL
		H1 Subtype	1.50E+03 copies/mL	4.41E-01 $TCID_{50}$/mL
	Guangdong-Maonan/SWL/1536/19	Influenza A	1.37E+04 copies/mL	5.86E+00 $TCID_{50}$/mL
		H1 Subtype	1.37E+04 copies/mL	5.86E+00 $TCID_{50}$/mL
Influenza A | Influenza A (subtype H3)	HongKong/2671/19	Influenza A	1.59E+05 copies/mL	1.50E+01 $TCID_{50}$/mL
		H3 Subtype	5.30E+04 copies/mL	4.98E+00 $TCID_{50}$/mL
	A/Kansas/14/2017	Influenza A	1.96E+03 copies/mL	5.58E+00 $TCID_{50}$/mL
		H3 Subtype	1.96E+03 copies/mL	5.58E+00 $TCID_{50}$/mL
	Singapore/INFUMH-16-0019/16	Influenza A	4.55E+03 copies/mL	1.10E+01 $TCID_{50}$/mL
		H3 Subtype	4.55E+03 copies/mL	1.10E+01 $TCID_{50}$/mL
Influenza B	Alabama/2/17 (Victoria Lineage)		3.35E+02 copies/mL	7.30E-01 $TCID_{50}$/mL
	Washington/02/19 (Victoria Lineage)		3.02E+03 copies/mL	2.79E+01 $TCID_{50}$/mL
	Colorado/6/17 (Victoria Lineage)		3.02E+03 copies/mL	6.64E-01 $TCID_{50}$/mL
	Wisconsin/1/10 (Yamagata Lineage)		1.01E+03 copies/mL	3.23E-01 $TCID_{50}$/mL
Parainfluenza 1	N/A		7.61E+02 copies/mL	1.06E+01 $TCID_{50}$/mL
Parainfluenza 2	N/A		8.46E+03 copies/mL	1.55E+01 $TCID_{50}$/mL
Parainfluenza 3	N/A		1.93E+03 copies/mL	3.18E+00 $TCID_{50}$/mL
Parainfluenza 4	A		5.76E+03 copies/mL	6.65E+01 $TCID_{50}$/mL
Respiratory Syncytial Virus A	A (2006 Isolate)		3.83E+03 copies/mL	1.11E+00 $TCID_{50}$/mL
Respiratory Syncytial Virus B	B (3/2015 Isolate #1)		1.61E+04 copies/mL	7.48E-01 $TCID_{50}$/mL
Enterovirus / Rhinovirus	Human Rhinovirus 1A		8.19E+03 copies/mL	4.99E-01 $TCID_{50}$/mL

Target Organism	Strain / Isolate	Concentration at LoD[1]	
	Human Rhinovirus B14	8.18E+03 copies/mL	1.10E+01 $TCID_{50}$/mL
	Human Rhinovirus C1	1.92E+04 copies/mL	N/A[2]
	Human Enterovirus Echovirus Type 6	2.25E+04 copies/mL	3.00E+01 $TCID_{50}$/mL
SARS-CoV-2	USA-WA1/2020	8.00E+03 copies/mL	4.04E+01 $TCID_{50}$/mL

[1]Concentrations in copies/mL were obtained by digital-droplet PCR.
[2]Testing for Coronavirus HKU1 and Rhinovirus 1C utilized a clinical specimen due to the lack of availability of a cultured isolate. Viral concentration was determined in RNA copies/mL by digital droplet PCR.

Limit of Detection Testing with the WHO International Standard for SARS-CoV-2 (NIBSC, 20/146)

An LoD study was performed to evaluate the analytical sensitivity of the Respiratory *Flex* Assay with the World Health Organization (WHO) Internal Standard for SARS-CoV-2 (Table 6). The WHO International SARS-CoV-2 standard was reconstituted then serially diluted in NPS matrix. As with the LoD study described above, testing was broken into two parts: preliminary and confirmatory LoD testing. For the preliminary LoD study, testing at multiple concentrations in triplicate was performed. The preliminary LoD was defined as the lowest concentration at which 100% of replicates were positive for SARS-CoV-2. The confirmed LoD was determined by testing a 3-fold dilution series of multiple concentrations around the preliminary LoD in replicates of 20. The confirmed LoD was defined as the lowest concentration at which ≥ 95% of the replicates were positive for the intended reportable target. To confirm the LoD, at least one dilution below the LoD was required to result in less than 95% positivity. The confirmed LoD for the Respiratory *Flex* Assay with the WHO International Standard was $7.7x10^5$ IU/mL.

Table 6. Limit of Detection Results for WHO International Standard for SARS-CoV-2 (NIBSC, 20/146) Target

	Concentration Tested (IU/mL)	SARS-CoV-2 Positivity
International WHO SARS-CoV-2 Standard	7.70E+05	95.0% (19/20)
	2.57E+05	65.0% (13/20)

e. Analytical Reactivity (Inclusivity)

Laboratory (Wet Testing)

The analytical reactivity (inclusivity) of the LIAISON PLEX Respiratory *Flex* Assay was evaluated by using a collection of 181 isolates and clinical samples (34 bacteria and 147 viruses), representing the genetic diversity of the analytes in the LIAISON PLEX Respiratory *Flex* Assay. The organisms were diluted to a final concentration of 3X the target LoD in simulated NPS matrix and each diluted organism was tested in triplicate. In cases where 100% positivity was not achieved at 3X LoD, samples were reprepared at the same concentration and retested in triplicate. If 100% positivity was obtained during retesting, no additional testing was performed. If less than 100% positivity was obtained during retesting, the organism was prepared at a higher concentration and tested until 100% positivity was obtained.

Of the 181 strains tested, a total of 176 strains were detected with 100% positivity at 3X LoD in the

laboratory. Four strains (influenza A Brisbane (02/18), coronavirus NL63 (NR-470), coronavirus 229E (VR-740) and SARS-CoV-2 (Stanford)) were detected at 9X LoD and one strain (*B. holmesii* (CIP 104396)) was detected at 27X LoD. Results of laboratory (wet testing) are shown in Table 7.

Three influenza A variant strains (H1N1v, H1N2v, and H3N2v) and two influenza A strains (H2N2 and H7N9) were included in the evaluation. The influenza A variant strains were tested in the laboratory using synthetic DNA. Based on this analysis, the three influenza A variant strains (H1N1v, H1N2v, and H3N2v) strains are expected to be detected as influenza A positive and subtype H1, H1, and H3 positive, respectively. Influenza H2N2 and H7N9 are predicted to be detected as influenza A, H1/H3 negative.

Table 7. Inclusivity of LIAISON PLEX Respiratory *Flex* Assay

Reportable Target & Subtype	Serovars/Group/Species	Concentration		Concentration	% Detected (# Detected/# Tested)
		Value	Units	copies/mL	
Bacteria					
Bordetella holmesii	F061	2.60E+02	CFU/mL	2.19E+04	100% (3/3)
	CDC F5101 [CDC 84-013939]	1.95E+02	CFU/mL	2.19E+04	83.3% (5/6)
	CIP 104396	N/A[1]		1.97E+05[6]	100% (3/3)
	CIP 104395 [G7702; 92A2997]	N/A[1]		2.19E+04	80.0% (4/5)
Bordetella parapertussis	NCTC 5952 [522]	1.05E+02	CFU/mL	2.37E+03	100% (3/3)
	508 & 344 [NCTC 10853]	9.15E+00	CFU/mL	2.37E+03	100% (3/3)
	517	3.12E+01	CFU/mL	2.37E+03	100% (3/3)
	12822	7.68E+01	CFU/mL	2.37E+03	100% (3/3)
	509 and 609	6.02E+01	CFU/mL	2.37E+03	100% (3/3)
	PT28G	3.32E+01	CFU/mL	2.37E+03	100% (3/3)
	PT 26/28G	2.73E+01	CFU/mL	2.37E+03	100% (3/3)
	C510	6.18E+01	CFU/mL	2.37E+03	100% (3/3)
Bordetella pertussis	18323 [NCTC 10739]	4.12E+03	CFU/mL	1.14E+04	100% (3/3)
	CNCTC Hp 12/63 [623]	4.87E+03	CFU/mL	1.14E+04	100% (3/3)
	10-536	2.47E+03	CFU/mL	1.14E+04	100% (3/3)
	5 [17921]	2.99E+03	CFU/mL	1.14E+04	100% (3/3)
	Tohama I	5.71E+03	CFU/mL	1.14E+04	100% (3/3)
	MN2531	N/A[1]		1.14E+04	100% (3/3)
	PT9/28G [W28]	1.99E+03	CFU/mL	1.14E+04	100% (3/3)
	589	1.95E+03	CFU/mL	1.14E+04	100% (3/3)
	F	N/A[1]		1.14E+04	100% (3/3)
Chlamydia pneumoniae	CWL-029	1.16E+02	IFU/mL	1.71E+03	100% (3/3)
	AR-39	1.93E+02	IFU/mL	1.71E+03	100% (3/3)
	J-21	9.48E-01	$TCID_{50}$/mL	1.71E+03	100% (3/3)
	2023	9.32E+01	IFU/mL	1.71E+03	100% (3/3)
Mycoplasma pneumoniae	M129	1.27E+02	CCU/mL	3.89E+03	100% (3/3)
	15531-TTR	1.23E+03	CFU/mL	3.89E+03	100% (3/3)
	Mac	1.45E+02	CCU/mL	3.89E+03	100% (3/3)
	PI 1428	1.56E+02	CCU/mL	3.89E+03	100% (3/3)
	Bru	N/A[1]		3.89E+03	100% (3/3)
	M52	1.83E+01	CFU/mL	3.89E+03	100% (3/3)
	UTMB-10P	7.83E-01	CCU/mL	3.89E+03	100% (3/3)
	Mutant 22	9.47E+01	CFU/mL	3.89E+03	100% (3/3)
	M129-B7	1.33E-01	CFU/mL	3.89E+03	100% (3/3)
Adenovirus	A 31	1.79E-02	$TCID_{50}$/mL	5.28E+03	100% (3/3)
	B 3	5.08E-01	$TCID_{50}$/mL	2.06E+03	100% (3/3)
	B 7A	7.05E+00	$TCID_{50}$/mL	2.06E+03	100% (3/3)
	B 21	3.60E-01	$TCID_{50}$/mL	2.06E+03	100% (3/3)
	B 11	1.43E+00	$TCID_{50}$/mL	2.06E+03	100% (3/3)

Reportable Target & Subtype	Serovars/Group/Species	Concentration		Concentration	% Detected (# Detected/# Tested)
		Value	Units	copies/mL	
	B 14	5.68E-01	$TCID_{50}$/mL	2.06E+03	100% (3/3)
	B 34	1.19E+02	$TCID_{50}$/mL	2.06E+03	83.3% (5/6)
	B 35	9.52E+00	$TCID_{50}$/mL	2.06E+03	100% (3/3)
	C 1	2.69E+02	$TCID_{50}$/mL	3.35E+03	100% (3/3)
	C 2	1.30E+02	$TCID_{50}$/mL	3.35E+03	100% (3/3)
	C 5	1.10E+02	$TCID_{50}$/mL	3.35E+03	100% (3/3)
	C 6	4.81E+01	$TCID_{50}$/mL	3.35E+03	100% (3/3)
	D 26	3.51E-01	$TCID_{50}$/mL	2.24E+03	100% (3/3)
	D 37	1.43E-01	$TCID_{50}$/mL	2.24E+03	100% (3/3)
	E 4	3.23E-02	$TCID_{50}$/mL	1.06E+03	83.3% (5/6)
	F 40-Dugan	7.60E-02	$TCID_{50}$/mL	1.45E+03	100% (3/3)
	F 41-Tak	8.97E-03	$TCID_{50}$/mL	1.45E+03	100% (3/3)
Human Coronavirus	**HKU1**	N/A[2]		5.00E+03	100% (3/3)
	HKU1			5.00E+03	100% (3/3)
	NL63 Source #: NR-470[5]	5.41E-03	$TCID_{50}$/mL	6.88E+02[6]	100% (3/3)
	NL63 Source #: 0810228CF[5]	4.02E-02	$TCID_{50}$/mL	2.29E+02	100% (3/3)
	OC43 Source #: 0810024CF[5]	2.87E+00	$TCID_{50}$/mL	2.84E+04	100% (3/3)
	OC43 Source #: VR-1558[5]	1.02E+00	$TCID_{50}$/mL	2.84E+04	100% (3/3)
	229E Source #: 0810229CF[5]	2.74E-01	$TCID_{50}$/mL	1.20E+03	100% (3/3)
	229E Source #: VR-740[5]	1.51E+00	$TCID_{50}$/mL	3.60E+03[6]	100% (3/3)
Enterovirus/ Rhinovirus	**Human Enterovirus Coxsackievirus A10**	3.53E+02	$TCID_{50}$/mL	6.75E+04	100% (3/3)
	Human Enterovirus Coxsackievirus 71 (2003)	1.18E+00	$TCID_{50}$/mL	6.75E+04	100% (3/3)
	Human Enterovirus Coxsackievirus A9	1.98E+02	$TCID_{50}$/mL	6.75E+04	100% (3/3)
	Human Enterovirus Coxsackievirus B3	1.79E+01	$TCID_{50}$/mL	6.75E+04	100% (3/3)
	Human Enterovirus Coxsackievirus B4	1.04E+02	$TCID_{50}$/mL	6.75E+04	100% (3/3)
	Human Enterovirus Echovirus 6	9.01E+01	$TCID_{50}$/mL	6.75E+04	100% (3/3)
	Human Enterovirus Echovirus 9	5.21E+00	$TCID_{50}$/mL	6.75E+04	100% (3/3)
	Human Enterovirus Echovirus 11	1.02E+03	$TCID_{50}$/mL	6.75E+04	100% (3/3)
	Human Enterovirus Echovirus 30	1.20E+02	$TCID_{50}$/mL	6.75E+04	100% (3/3)
	Human Enterovirus Coxsackievirus A21	2.02E+01	$TCID_{50}$/mL	6.75E+04	100% (3/3)
	Human Enterovirus Coxsackievirus A24	8.50E+00	$TCID_{50}$/mL	6.75E+04	100% (3/3)
	Human Enterovirus 68	1.05E+01	$TCID_{50}$/mL	6.75E+04	100% (3/3)
	Human Rhinovirus A16	1.45E+02	$TCID_{50}$/mL	2.46E+04	100% (3/3)
	Human Rhinovirus A2	4.89E-01	$TCID_{50}$/mL	2.46E+04	100% (3/3)
	Human Rhinovirus A34	1.69E+02	$TCID_{50}$/mL	2.46E+04	100% (3/3)
	Human Rhinovirus A57	1.04E+01	$TCID_{50}$/mL	2.46E+04	100% (3/3)

Reportable Target & Subtype		Serovars/Group/Species	Concentration		Concentration	% Detected (# Detected/# Tested)
			Value	Units	copies/mL	
		Human Rhinovirus A7	1.20E+01	$TCID_{50}$/mL	2.46E+04	100% (3/3)
		Human Rhinovirus A77	2.15E-01	$TCID_{50}$/mL	2.46E+04	100% (3/3)
		Human Rhinovirus A85	7.60E+02	$TCID_{50}$/mL	2.46E+04	100% (3/3)
		Human Rhinovirus B14	3.31E+01	$TCID_{50}$/mL	2.45E+04	100% (3/3)
		Human Rhinovirus B17	1.75E+02	$TCID_{50}$/mL	2.45E+04	100% (3/3)
		Human Rhinovirus B27	5.09E+00	$TCID_{50}$/mL	2.45E+04	100% (3/3)
		Human Rhinovirus B3	5.68E+01	PFU/mL	2.45E+04	100% (3/3)
		Human Rhinovirus B42	8.64E+00	$TCID_{50}$/mL	2.45E+04	100% (3/3)
		Human Rhinovirus B83	1.13E+01	$TCID_{50}$/mL	2.45E+04	100% (3/3)
		Human Rhinovirus C1	NA[2]		5.75E+04	100% (3/3)
		Human Rhinovirus C1	NA[2]		5.75E+04	100% (3/3)
		Human Rhinovirus C1	NA[2]		5.75E+04	100% (3/3)
		Human Rhinovirus C1	NA[2]		5.75E+04	100% (3/3)
Human Metapneumovirus		**(hMPV-9) A1**	6.26E-01	$TCID_{50}$/mL	6.40E+03	100% (3/3)
		(hMPV-16) A1	4.01E+00	$TCID_{50}$/mL	6.40E+03	100% (3/3)
		(hMPV-20) A2	NA[2]		6.12E+03	100% (3/3)
		(hMPV-27) A2	6.41E-01	$TCID_{50}$/mL	6.12E+03	100% (3/3)
		(hMPV-3) B1	1.29E+00	$TCID_{50}$/mL	1.50E+04	100% (3/3)
		(hMPV-5) B1	9.50E+00	$TCID_{50}$/mL	1.50E+04	100% (3/3)
		(hMPV-4) B2	1.37E+03	$TCID_{50}$/mL	4.50E+04	100% (3/3)
		(hMPV-8) B2	4.99E+00	$TCID_{50}$/mL	4.50E+04	100% (3/3)
		(hMPV-18) B2	2.78E+01	$TCID_{50}$/mL	4.50E+04	100% (3/3)
Influenza A	**H1N1**	**A/Wisconsin/588/2019**	4.06E+01	FFU/mL	4.50E+03	Matrix: 100% (3/3) H1: 100% (3/3)
		A/Hawaii/66/2019 X-345A	2.45E+03	$CEID_{50}$/mL	4.50E+03	Matrix: 100% (6/6) H1: 83.3% (5/6)
		A/Indiana/02/2020	2.06E+03	$CEID_{50}$/mL	4.50E+03	Matrix: 100% (3/3) H1: 100% (3/3)
		A/Michigan/272/2017	1.86E+01	$TCID_{50}$/mL	4.50E+03	Matrix: 100% (3/3) H1: 100% (3/3)
		A/Idaho/07/2018	8.73E-01	$TCID_{50}$/mL	4.50E+03	Matrix: 100% (3/3) H1: 100% (3/3)
		A/Wisconsin/505/2018	5.40E+00	$TCID_{50}$/mL	4.50E+03	Matrix: 100% (6/6) H1: 83.3% (5/6)
		Guangdong-Maonan/SWL 1536/19	1.93E+00	$TCID_{50}$/mL	4.50E+03	Matrix: 100% (6/6) H1: 83.3% (5/6)
		Brisbane/02/18	3.97E+00	$TCID_{50}$/mL	1.35E+04[6]	Matrix: 100% (3/3) H1: 100% (3/3
		A/St.Petersburg/61/2015	1.82E+03	$CEID_{50}$/mL	4.50E+03	Matrix: 100% (3/3) H1: 100% (3/3)
		A/Bangladesh/3002/2015	7.78E+02	$CEID_{50}$/mL	4.50E+03	Matrix: 100% (3/3) H1: 100% (3/3)
		A/Denver/1/57	6.84E+02	$CEID_{50}$/mL	4.50E+03	Matrix: 100% (3/3) H1: 100% (3/3)
		New Caledonia/20/99	8.71E+00	$TCID_{50}$/mL	4.50E+03	Matrix: 100% (3/3) H1: 100% (3/3)
		PR/8/34	1.58E+00	$TCID_{50}$/mL	4.50E+03	Matrix: 100% (3/3) H1: 100% (3/3)
		Singapore/63/04	6.23E-01	$TCID_{50}$/mL	4.50E+03	Matrix: 100% (3/3) H1: 100% (3/3)
		Solomon Islands/03/06	5.00E-01	$TCID_{50}$/mL	4.50E+03	Matrix: 100% (3/3) H1: 100% (3/3)

Reportable Target & Subtype		Serovars/Group/Species	Concentration		Concentration	% Detected (# Detected/# Tested)
			Value	Units	copies/mL	
		Taiwan/42/06	3.58E-01	$TCID_{50}$/mL	4.50E+03	Matrix: 100% (3/3) H1: 100% (3/3)
	H1N1v	A/Ohio/09/2015 (Subtype Synthetic DNA)	N/A[3]		4.50E+03	Matrix: 0% (0/3)[7] H1: 100% (3/3)[7]
		A/Ohio/09/2015 (Influenza A Synthetic DNA)	N/A[3]		4.50E+03	Matrix: 100% (3/3)[7] H1: 0% (0/3)[7]
	H1N2	A/swine/Ohio/09SW1484E/2009	4.15E+03	$TCID_{50}$/mL	4.50E+03	Matrix: 100% (3/3) H1: 100% (3/3)
	H1N2v	A/Minnesota/19/2011 (Subtype Synthetic DNA)	N/A[3]		4.50E+03	Matrix: 0% (0/3)[7] H1: 100% (3/3)[7]
		A/Minnesota/19/2011 (Influenza A Synthetic DNA)	N/A[3]		4.50E+03	Matrix: 100% (3/3)[7] H1: 0% (0/3)[7]
	H3N2	A/Kansas/14/2017 NYMC X-327	1.87E+03	$CEID_{50}$/mL	5.89E+03	Matrix: 100% (3/3) H3: 100% (3/3)
		A/Texas/71/2017	4.35E+01	FFU/mL	5.89E+03	Matrix: 100% (3/3) H3: 100% (3/3)
		A/Wisconsin/04/2018	2.38E+01	FFU/mL	5.89E+03	Matrix: 100% (3/3) H3: 100% (3/3)
		A/Arizona/45/2018	8.27E+01	FFU/mL	5.89E+03	Matrix: 100% (3/3) H3: 100% (3/3)
		A/Hong Kong/45/2019	6.09E+01	FFU/mL	5.89E+03	Matrix: 100% (3/3) H3: 100% (3/3)
		A/Tasmania/503/2020	1.99E+01	FFU/mL	5.89E+03	Matrix: 100% (3/3) H3: 100% (3/3)
		A/Delaware/01/2021	7.01E+01	FFU/mL	5.89E+03	Matrix: 100% (3/3) H3: 100% (3/3)
		A/Singapore/INFIMH-16-0019/2016	8.93E+02	$CEID_{50}$/mL	5.89E+03	Matrix: 100% (3/3) H3: 100% (3/3)
		A/California/55/2020	8.55E+01	FFU/mL	5.89E+03	Matrix: 100% (3/3) H3: 100% (3/3)
		A/Alaska/232/2015	1.14E+03	$CEID_{50}$/mL	5.89E+03	Matrix: 100% (3/3) H3: 100% (3/3)
	H3N2v	A/Hawaii/28/2020 (Subtype Synthetic DNA)	N/A[3]		5.89E+03	Matrix: 0% (0/3)[7] H3: 100% (3/3)[7]
		A/Hawaii/28/2020 (Influenza A Synthetic DNA)	N/A[3]		5.89E+03	Matrix: 100% (3/3)[7] H3: 0% (0/3)[7]
	H5N1	A/Egypt/N03072/2010	4.70E-03	HA[4]	5.89E+03	Matrix: 100% (3/3)[8] Subtype: 0% (0/3)[8]
		A/Hubei/1/2010	3.97E-03	HA[4]	5.89E+03	Matrix: 100% (3/3)[8] Subtype: 0% (0/3)[8]
		A/Anhui/01/2005	1.54E-02	HA[4]	5.89E+03	Matrix: 100% (3/3)[8] Subtype: 0% (0/3)[8]
	H7N2	A/turkey/Virginia/4529/2002	4.40E-02	HA[4]	5.89E+03	Matrix: 100% (3/3)[8]

Reportable Target & Subtype		Serovars/Group/Species	Concentration		Concentration	% Detected (# Detected/# Tested)
			Value	Units	copies/mL	
						Subtype: 0% (0/3)[8]
	H7N7	A/mallard/Netherlands/12/2000	8.03E-03	HA[4]	5.89E+03	Matrix: 100% (3/3)[8]
						Subtype: 0% (0/3)[8]
	H9N2	A/Hong Kong/33982/2009	5.06E+03	$CEID_{50}$/mL	5.89E+03	Matrix: 100% (3/3)[8]
						Subtype: 0% (0/3)[8]
Influenza B		B/Washington/02/2019 (Victoria Lineage)	1.97E+02	$CEID_{50}$/mL	1.01E+03	100% (3/3)
		B/New Hampshire/01/2021 (Victoria Lineage)	9.28E-01	$TCID_{50}$/mL	1.01E+03	100% (3/3)
		B/Missouri/12/2018 (NA D197E) (Victoria Lineage)	1.35E+02	$TCID_{50}$/mL	1.01E+03	100% (3/3)
		B/Wisconsin/10/2016 (NA I221V) (Yamagata Lineage)	1.81E+03	$TCID_{50}$/mL	1.01E+03	100% (3/3)
		B/Indiana/17/2017 (NA I221T) (Yamagata Lineage)	1.79E+03	$TCID_{50}$/mL	1.01E+03	100% (3/3)
		B/Hawaii/01/2018 (NA D197N (Victoria Lineage)	4.56E+02	$TCID_{50}$/mL	1.01E+03	100% (3/3)
		B/Oklahoma/10/2018 (NA D197N) (Yamagata Lineage)	1.85E+03	$TCID_{50}$/mL	1.01E+03	100% (3/3)
		B/Michigan/01/2021 (Victoria Lineage)	7.91E+00	$TCID_{50}$/mL	1.01E+03	100% (3/3)
		B/Hong Kong/286/2017 (Victoria Lineage)	2.80E+00	$TCID_{50}$/mL	1.01E+03	100% (3/3)
		B/Colorado/6/2017 (Victoria Lineage)	2.33E+00	$TCID_{50}$/mL	1.01E+03	100% (3/3)
		B/Texas/43/2019 (Victoria Lineage)	1.55E+00	$TCID_{50}$/mL	1.01E+03	100% (3/3)
		B/Wisconsin/1/10 (Yamagata Lineage)	3.23E-01	$TCID_{50}$/mL	1.01E+03	100% (3/3)
		B/Florida/02/06 (Yamagata Lineage)	1.60E-01	$TCID_{50}$/mL	1.01E+03	100% (3/3)
		B/Florida/07/04 (Yamagata Lineage)	3.71E+01	$TCID_{50}$/mL	1.01E+03	100% (3/3)
		B/Phuket/3073/13 (Yamagata Lineage)	6.53E-01	$TCID_{50}$/mL	1.01E+03	100% (3/3)
Parainfluenza 1		N/A	3.19E+01	$TCID_{50}$/mL	2.28E+03	83.3% (5/6)
		C35	2.62E+00	$TCID_{50}$/mL	2.28E+03	100% (3/3)
Parainfluenza 2		N/A	4.65E+00	$TCID_{50}$/mL	2.54E+04	100% (3/3)
		Greer	4.81E+00	$TCID_{50}$/mL	2.54E+04	100% (3/3)
Parainfluenza 3		N/A	9.54E+00	$TCID_{50}$/mL	5.79E+03	100% (3/3)
		ATCC-2011-5	1.78E+02	$TCID_{50}$/mL	5.79E+03	83.3% (5/6)
		C243	8.83E+02	$TCID_{50}$/mL	5.79E+03	100% (3/3)
		NIH 47885	3.57E+01	$TCID_{50}$/mL	5.79E+03	100% (3/3)
Parainfluenza 4		4A	6.63E+00	$TCID_{50}$/mL	1.73E+04	100% (3/3)
		4A M-25	5.94E+00	$TCID_{50}$/mL	1.73E+04	100% (3/3)
		4B	5.40E+00	$TCID_{50}$/mL	1.73E+04	100% (3/3)
		4B CH 19503	8.79E+01	$TCID_{50}$/mL	1.73E+04	100% (3/3)
Respiratory Syncytial Virus		A 2006 Isolate	3.34E+00	$TCID_{50}$/mL	1.15E+04	100% (3/3)
		A2	1.10E+03	PFU/mL	1.15E+04	100% (3/3)
		A Long	1.11E+03	PFU/mL	1.15E+04	100% (3/3)
		B CH93(18)-18	1.46E+01	$TCID_{50}$/mL	4.82E+04	100% (3/3)
		B WV/14617/85	2.12E+00	$TCID_{50}$/mL	4.82E+04	100% (3/3)
		B 18537	3.16E+00	PFU/mL	4.82E+04	100% (3/3)
		B1	1.30E+03	$TCID_{50}$/mL	4.82E+04	100% (3/3)

Reportable Target & Subtype	Serovars/Group/Species	Concentration		Concentration	% Detected (# Detected/# Tested)
		Value	Units	copies/mL	
SARS-CoV-2	**USA-WA1/2020**	1.37E+01	$TCID_{50}$/mL	2.40E+04	100% (3/3)
	B.1.1.529 BA.1: USA/MD-HP20874/2021 (Omicron)	9.59E-01	$TCID_{50}$/mL	2.40E+04	100% (3/3)
	Italy-INMI1	1.44E+02	$TCID_{50}$/mL	2.40E+04	100% (3/3)
	Hong Kong/VM20001061/2020	2.37E+00	$TCID_{50}$/mL	2.40E+04	100% (3/3)
	B.1_2020: USA/NY-Wadsworth-103677-01/2020	9.74E-01	$TCID_{50}$/mL	2.40E+04	100% (3/3)
	B.1.1.7: England/204820464/2020 (Alpha)	1.53E+01	$TCID_{50}$/mL	2.40E+04	100% (3/3)
	B.1.1.7: USA/CA_CDC_5574/2020 (Alpha)	6.61E+00	$TCID_{50}$/mL	2.40E+04	100% (3/3)
	B.1.351: South Africa/KRISP-K005325/2020 (Beta)	5.84E+00	$TCID_{50}$/mL	2.40E+04	100% (3/3)
	P1: Japan/TY7-503/2021 (Gamma)	5.65E+00	$TCID_{50}$/mL	2.40E+04	100% (3/3)
	P2_2021: NY-Wadsworth-21006055-01/2021 (Zeta)	8.82E+00	$TCID_{50}$/mL	2.40E+04	100% (3/3)
	B.1.526_2021: USA/NY-Wadsworth-21025952-01/2021 Isolate 1 (Lota)	1.39E+01	$TCID_{50}$/mL	2.40E+04	100% (3/3)
	B.1.617.1: USA/CA-Stanford-15_S02/2021 (Kappa)	1.22E+01	$TCID_{50}$/mL	7.20E+04[6]	100% (3/3)
	B.1.617.2: USA/PHC658/2021 (Delta)	9.42E+00	$TCID_{50}$/mL	2.40E+04	100% (3/3)

[1]No supplier concentration listed.
[2]Clinical samples quantified in copies/mL based on digital droplet PCR (ddPCR) analysis.
[3]Synthetic DNA quantified in copies/mL based on spectrophotometric analysis and optical density measurements.
[4]Titer determined through hemagglutination assay using 0.5% turkey red blood cells.
[5]No additional strain information was provided by the supplier so different samples tested within the same species were distinguished using the vendor catalog number.
[6]Four strains (influenza A Brisbane (02/18), coronavirus NL63 (NR-470), coronavirus 229E (VR-740) and SARS-CoV-2 (Stanford)) were detected at 9X LoD and one strain (*B. holmesii* (CIP 104396)) was detected at 27X LoD.
[7]Subtype synthetic DNA and influenza A synthetic DNA were expected to yield positive results for the subtype and influenza A targets, respectively.
[8]Matrix positive, subtype negative results for influenza A strains H5N1, H7N2, H7N7, and H9N2 are expected results based on the assay design.

***In Silico* Inclusivity for SARS-CoV-2 and Influenza**

In addition to wet testing*, in silico* analyses for SARS-CoV-2 and Influenza were performed.

SARS-CoV-2:

For SARS-CoV-2, 5,622,325 sequences in GISAID (as of July 31, 2023) were included in the analysis. The LIAISON PLEX Respiratory *Flex* Assay targets three SARS-CoV-2 gene regions (E, ORF1ab, and ORF3a). The Respiratory *Flex* Assay result logic states that if at least 1 of these targets is detected, SARS-CoV-2 is positive. This same result logic was implemented for the in silico inclusivity assessment. Of the sequences included in this evaluation, 99.94% (5,619,069/5,622,325) have no mismatch in at least one gene oligo set and thus are predicted to be detected by the Respiratory *Flex* Assay. Of the 0.06% (3,256/5,622,325) of sequences with mismatches in at least one oligo binding region in all 3 SARS-CoV-2 target genes, a Tm analysis revealed that amplification/hybridization were expected to occur. Thus, it is expected that 100% of SARS-CoV-2 sequences evaluated in this study will be detected by the assay.

Influenza:

For influenza A, influenza A H1, influenza A H3, and influenza B, sequences uploaded to GISAID between September 1, 2015 and July 5, 2023 were included in the analysis. The following number of sequences were included in the evaluation of influenza A, A H1, A H3, and influenza B: 112,056, 54,364, 104,428, 26,470. Of the 26,470 influenza B sequences, 66.1% (17,509/26,470) were Victoria lineage, 30.9% (8,167/26,470) were Yamagata lineage, and 3.0% (794/26,470) were of unknown/unclassified lineage. The influenza in silico inclusivity analysis results are shown in Table 8. Based on the reactivity criteria (>90% homology), 99.9% (112,034/112,056) of influenza A sequences are expected to be detected, 98.9% (53,778/54,364) of influenza A H1 sequences are expected to be detected, 99.9% (104,315/104,428) of influenza A H3 sequences are expected to be detected, and 99.9% (26,433/26,470) of influenza B sequences are expected to be detected.

Table 8. Influenza *In Silico* Inclusivity Results

Reportable Target	Target Gene	of Sequences in Alignment	# of Sequences with Percent Oligo Identify >90%
Influenza A	Matrix protein (MP)	112,056	112,034[1]
Influenza A H1	HA	54,364	53,778
Influenza A H3	HA	104,428	104,315
Influenza B	Non-structural protein (NS)	26,470	26,433[2]

[1]Analysis included influenza A subtype H0, H1, H3, H5, H7, H9, and H10 strains.

[2]Analysis included 17,509 Victoria lineage strains, 8,167 Yamagata lineage strains, and 794 strains of unknown lineage.

f. Analytical specificity

Cross-Reactivity and Microbial Interference:

Off-Panel Cross Reactivity

Laboratory (Wet Testing)

Cross-reactivity was assessed in the laboratory by wet testing 60 off-panel viral, fungal, and bacterial organisms that may be found in a respiratory tract clinical specimen. The potential cross-reacting organisms were spiked into simulated NPS matrix that was negative for all targets on the assay. Bacteria were tested at concentrations ≥ $1x10^6$ CFU/mL (or equivalent) and viruses were tested at ≥ $1x10^5$ $TCID_{50}$/mL (or equivalent), or the highest available concentration. For *Mycobacterium tuberculosis*, genomic DNA (quantified in ng/uL) was evaluated to minimize pathogenic exposure to the test operator.

Of the 60 potential cross-reacting off-panel organisms tested in the laboratory (Table 9), 59 organisms yielded negative results for all targets and are considered non-reactive with the LIAISON PLEX Respiratory *Flex* Assay. *Mycoplasma genitalium* cross-reacted with the *Mycoplasma pneumoniae* assay at a concentration of $4x10^6$ CCU/mL. No cross-reactivity was observed when *Mycoplasma genitalium* was tested at a lower concentration of $1x10^6$ CCU/mL.

Table 9. Organisms Tested for Potential Cross-Reactivity (Off-Panel)

Organism	Conc./Unit	Organism	Conc./Unit
Acinetobacter baumannii	$1x10^6$ CFU/mL	*Legionella pneumophila*	$4x10^5$ CFU/mL[3]
Aspergillus flavus	$1x10^6$ CFU/mL	*Listeria innocua*	$1x10^6$ CFU/mL
Aspergillus fumigatus	$4x10^5$ CFU/mL[3]	*Listeria monocytogenes*	$1x10^6$ CFU/mL
Bordetella avium	$1x10^6$ CFU/mL	Measles	$1x10^5$ $TCID_{50}$/mL
Bordetella bronchiseptica	$1x10^6$ CFU/mL	MERS-CoV	NA[2]
Bordetella hinzii	$1x10^6$ CFU/mL	*Moraxella catarrhalis*	$1x10^6$ CFU/mL
Bordetella petrii	$1x10^6$ CFU/mL	Mumps Virus	$1x10^5$ $TCID_{50}$/mL
Bordetella trematum	$1x10^6$ CFU/mL	*Mycobacterium tuberculosis* (H37Rv gDNA)	2.88 ng/uL
Bordetella parapertussis Bpp5 (synthetic DNA)[1]	$1x10^6$ copies/mL	*Mycoplasma genitalium*[3]	$4x10^6$ CCU/mL
			$1x10^6$ CCU/mL
			$4x10^5$ CCU/mL
Candida albicans	$1x10^6$ CFU/mL	*Mycoplasma hominis*	$1x10^6$ CFU/mL
Candida glabrata	$1x10^6$ CFU/mL	Nasal Wash (pooled)	NA[2]
Chlamydia trachomatis Serovar D	$1x10^6$ IFU/mL	*Neisseria elongata*	$1x10^6$ CFU/mL
Coronavirus-SARS	NA[2]	*Neisseria gonorrhoeae*	$1x10^6$ CFU/mL
Corynebacterium diphtheriae	$1x10^6$ CFU/mL	*Neisseria lactamica*	$1x10^6$ CFU/mL
Corynebacterium pseudodiphtheriticum	$1x10^6$ CFU/mL	*Neisseria meningitidis*	$1x10^6$ CFU/mL
Corynebacterium striatum	$1x10^6$ CFU/mL	*Neisseria mucosa*	$1x10^6$ CFU/mL
Cytomegalovirus	$1x10^5$ $TCID_{50}$/mL	*Neisseria sicca*	$1x10^6$ CFU/mL
Epstein Barr Virus	$1x10^5$ copies/mL	*Pneumocystis jiroveci*	$1x10^6$ CFU/mL

Organism	Conc./Unit	Organism	Conc./Unit
Escherichia coli	$1x10^6$ CFU/mL	*Proteus vulgaris*	$1x10^6$ CFU/mL
Fluoribacter bozemanae	$4x10^6$ CFU/mL	*Pseudomonas aeruginosa*	$1x10^6$ CFU/mL
Fusobacterium necrophorum	$1x10^6$ CFU/mL	*Serratia marcescens*	$1x10^6$ CFU/mL
Haemophilus influenzae	$1x10^6$ CFU/mL	*Staphylococcus aureus*	$1x10^6$ CFU/mL
Haemophilus parainfluenzae	$1x10^6$ CFU/mL	*Staphylococcus epidermidis*	$1x10^6$ CFU/mL
Herpes Simplex Virus Type 1	$1x10^5$ $TCID_{50}$/mL	*Staphylococcus haemolyticus*	$1x10^6$ CFU/mL
Klebsiella pneumoniae	$1x10^6$ CFU/mL	*Streptococcus agalactiae*	$1x10^6$ CFU/mL
Lactobacillus acidophilus	$1x10^6$ CFU/mL	*Streptococcus pneumoniae*	$1x10^6$ CFU/mL
Lactobacillus plantarum	$1x10^6$ CFU/mL	*Streptococcus pyogenes*	$1x10^6$ CFU/mL
Legionella anisa	$1x10^6$ CFU/mL	*Streptococcus salivarius*	$1x10^6$ CFU/mL
Legionella feeleii	$1x10^6$ CFU/mL	*Ureaplasma urealyticum*	$1x10^6$ CCU/mL
Legionella longbeachae	$1x10^6$ CFU/mL	Varicella-Zoster Virus	$2.34x10^4$ $TCID_{50}$/mL[3]

CFU = Colony Forming Units; CCU = Colony Changing Units; IFU = Inclusion Forming Units; TCID50 = Median Tissue Culture Infectious Dose.

[1]A portion of the *B. parapertussis* Bpp5 genome was identified by *in-silico* analysis as potentially cross-reactive with *B. pertussis*. Synthetic DNA was tested that matched the region of high homology in the assay. Testing was included in the off-panel testing since the targeted sequence was not expected to be detected as *B. parapertussis* by the assay.

[2]No concentration provided by the supplier.

[3]The highest possible concentration was tested.

In Silico Cross-Reactivity

In silico analysis of assay specificity/exclusivity was performed by conducting a BLAST comparison of the assay's oligos sequences to the GenBank nt sequence database, as of July 14, 2023. Sequences for 83 off-panel organisms (68 bacteria/fungi and 15 viruses) that can be found in a respiratory specimen were included. Additionally, sequences for all on-panel organisms were included to evaluate intra-panel cross-reactivity. A summary of the results from the analysis is provided in Table 10. The LIAISON PLEX Respiratory *Flex* assays were shown to be specific for their respective analytes with the following exceptions:

- Cross-reaction of the Adenovirus assays with closely related Adenovirus G (serotype 52) strains.
- Cross-reaction of the SARS-CoV-2 assays with closely related bat and pangolin coronavirus sequences;
- Cross-reaction of the *B. parapertussis* assay with strains of *B. bronchiseptica* that carry IS*1001*;
- Cross-reaction of the influenza A H1 subtyping assay with 3 swine H3N2 strains and 1 avian H6N1 strain;
- Cross-reaction of the influenza A H3 subtyping assay with 59 swine H1N1 and swine H1N2 strains, 1 duck H5N2 strain, 1 ostrich H7N1 strain, 1 avian H7N9 strain, 1 avian H8N4 strain, and 1 avian H11N9 strain.

Table 10. Organisms Predicted by *In Silico* Analyses to Cross-React with the Respiratory *Flex* Assay.

Reportable Target	Predicted Cross-Reaction
Adenovirus	Adenovirus G (serotype 52) - strains
SARS-CoV-2	Bat coronavirus and Bat SARS-like coronavirus (accessions MG772933, MG772934, and MN996532)[1]
Bordetella parapertussis	*Bordetella bronchiseptica* containing IS*1001* element (accessions JX013523 to JX013527 and CP022962)[2]
Influenza A H1	H5N1 (accession CY110922)[3]; swine H3N2 (accessions KM110061, KM110062, KM110063, and OM935891); avian H6N1 (accession OP888980)
Influenza A H3	swine H1N1 and swine H1N2 – 59 strains; duck H5N2 (accession OK103962); ostrich H7N1 (accession AF202244); avian H7N9 (accession KP413675); avian H8N4 (accession OK103964); avian H11N9 (accession OK103956)

[1]It is unlikely that Bat coronavirus and Bat SARS-like coronavirus would be present in human clinical NPS specimens; but if present, the cross-reactive product(s) produced by the LIAISON PLEX Respiratory *Flex* Assay will be reported as SARS-CoV-2.

[2]The LIAISON PLEX Respiratory *Flex* Assay contains primers designed to target the *B. parapertussis* IS*1001* insertion sequence. Some strains of *Bordetella bronchiseptica*, which is rarely isolated from humans, carry the same sequence that is targeted by LIAISON PLEX Respiratory *Flex* primers for *B. parapertussis.* If present, the LIAISON PLEX Respiratory *Flex* Assay will report these specimens as *B. parapertussis.*

[3]This H5N1 human strain sequence is a chimeric sequence containing H1N1 sequence fragments. Therefore, detection of this sequence by the H1 oligos is not considered a cross-reaction.

On-Panel Cross Reactivity

Potential intra-panel cross-reactivity was evaluated using 28 on-panel organisms. The potential cross-reacting on-panel organisms were spiked into simulated NPS matrix that was negative for all targets on the assay. Bacteria were tested at concentrations ≥ $1x10^6$ CFU/mL (or equivalent) and viruses were tested at ≥ $1x10^5$ $TCID_{50}$/mL (or equivalent), or the highest available concentration.

All 28 on-panel organisms tested (Table 11) yielded the expected on-panel result and did not cross-react with other target assays of the LIAISON PLEX Respiratory *Flex* Assay.

Table 11. Organisms Tested for Potential Cross-Reactivity (On-Panel)

Organism	Conc./Unit
Adenovirus	$1x10^5$ $TCID_{50}$/mL
Bordetella holmesii	$1x10^6$ CFU/mL
Bordetella parapertussis	$1x10^6$ CFU/mL
Bordetella pertussis	$1x10^6$ CFU/mL
Chlamydia pneumoniae	$1x10^6$ IFU/mL
Human Coronavirus 229E	$1x10^5$ $TCID_{50}$/mL
Human Coronavirus HKU1	$6.62x10^4$ copies/mL
Human Coronavirus NL63	$1x10^5$ $TCID_{50}$/mL
Human Coronavirus OC43	$1x10^5$ $TCID_{50}$/mL

Organism	Conc./Unit
Echovirus (Enterovirus/Rhinovirus)	$1x10^5$ $TCID_{50}$/mL
Human Metapneumovirus	$1x10^5$ $TCID_{50}$/mL
Influenza B (Washington/02/2019/Victoria Lineage)	$1x10^5$ $TCID_{50}$/mL
Influenza B (Phuket/3073/13/Yamagata Lineage)	$1x10^5$ $TCID_{50}$/mL
Mycoplasma pneumoniae	$1x10^6$ CCU/mL
Parainfluenza 1	$1x10^5$ $TCID_{50}$/mL
Parainfluenza 2	$1x10^5$ $TCID_{50}$/mL
Parainfluenza 3	$1x10^5$ $TCID_{50}$/mL
Parainfluenza 4	$1x10^5$ $TCID_{50}$/mL
RSV A	$1x10^5$ PFU/mL
RSV B	$1x10^5$ $TCID_{50}$/mL
Influenza A H1N1	$1x10^5$ $TCID_{50}$/mL
Influenza A H3N2	$1x10^5$ $TCID_{50}$/mL
Influenza A H5N1[1]	$2x10^7$ copies/mL
Influenza A H7N2[1]	$6x10^6$ copies/mL
Influenza A H7N7[1]	$2x10^7$ copies/mL
Influenza A H9N2[1]	$4x10^8$ copies/mL
Influenza A H1N2[2]	$3x10^7$ copies/mL
SARS-CoV-2	$1x10^5$ $TCID_{50}$/mL

CFU = Colony Forming Units; IFU = Inclusion Forming Units; TCID50 = Median Tissue Culture Infectious Dose; PFU = Plaque Forming Units

[1]These influenza A non-subtype H1/H3 strains are expected to be inclusive to the influenza A matrix target, only (i.e., are expected to be reported as influenza A positive, subtype H1/subtype H3 negative). All were negative for both subtype H1 and subtype H3, as anticipated.

[2]This influenza A H1N2 strain is expected to be inclusive to the influenza A matrix target and influenza A subtype H1 target. This strain was positive for both the influenza A matrix target and influenza A subtype H1 target, as expected.

Microbial Interference

The impact of 16 potentially interfering non-panel microbial organisms commonly found in nasopharyngeal swab samples were tested (Table 12) in the presence of representative assay panel targets. Each potentially interfering non-panel organism was tested at a high concentration of ≥ $1x10^6$ CFU/mL (or equivalent) for bacteria and $1x10^5$ $TCID_{50}$/mL (or equivalent) for viruses, or the highest concentration available, in the presence of a multi-analyte panel consisting of five assay targets (B. pertussis, Adenovirus, influenza B, human metapneumovirus, and SARS-CoV-2) at a low concentration (3X LoD).

None of the potentially interfering organisms tested at high concentration interfered with detection for panel targets at a low concentration, except for *Streptococcus pyogenes* at $1x10^6$ CFU/mL and Legionella pneumophilia at $4x10^5$ CFU/mL, which both interfered with detection of Adenovirus in 1 of 6 replicates (83.3% positivity).

Table 12. Organisms Tested for Potential Microbial Interference

Candida albicans	***Pseudomonas aeruginosa***
Corynebacterium diphtheriae	***Streptococcus pneumoniae***
Cytomegalovirus	***Streptococcus pyogenes***
Haemophilus influenzae	**SARS[1]**
Herpes Simplex Virus 1	***Legionella pneumophilia[2]***
MERS[1]	**Measles Virus**
Neisseria meningitidis	***Moraxella catarrhalis***
Staphylococcus aureus	**Mumps**

[1]No concentration was provided for material. Material was tested at the highest available concentration by diluting stock material directly into representative multi-analyte panel preparation.
[2]Tested at the highest available concentration of $4x10^5$ CFU/mL

Interfering Substances:

The potential inhibitory effect of non-microbial substances (endogenous and exogenous) expected to be found in nasopharyngeal swab (NPS) specimens or introduced during sample handling, were evaluated for the LIAISON PLEX Respiratory *Flex* Assay. Potential interference from 36 interfering substances were evaluated in the presence and absence of a multi-analyte panel of targets (Table 13). Each interfering substance tested was diluted to a clinically relevant concentration and tested in the presence of a positive and negative target in triplicate. The positive target was a multi-analyte panel consisting of five assay targets (*B. pertussis*, Adenovirus, influenza B, human metapneumovirus, and SARS-CoV-2) at a low concentration (3X LoD), while the negative target was a simulated NPS matrix. Interference was observed for the substances/concentrations shown in Table 14.

Table 13: Interfering Substances Tested

Substance/Class	Description/Active Ingredient	Concentration Tested
Nasal Corticosteroid	Beclomethasone dipropionate	25 µg/mL
Anesthetic	Benzocaine	10% w/v
Nasal Corticosteroid	Budesonide	$3.4x10^{-2}$ µmol/L
Nasal Corticosteroid	Dexamethasone	30.6 µmol/L
Nasal Corticosteroid	Flunisolide	25 µg/mL
FLONASE Sensimist Allergy Relief	Fluticasone furoate	$2.84x10^{-3}$ µmol/L
Fluticasone Propionate Nasal Spray	Fluticasone propionate	$2.84x10^{-3}$ µmol/L
DNA	Human DNA	20 ng/µL
Nasal Wash	Human Nasal Wash	9.1%
Sputum/Mucus	Human Sputum/Mucus	1 swab/1mL sample[1]
		1 swab/2mL sample[2]
Human Blood	Human Whole Blood	5.0% v/v
		4.5% v/v
		4.0% v/v
Human Cells	Leukocytes	1000 cells/µL
		666.7 cells/µL
		333.3 cells/µL
Oral Anesthetic and Analgesic	Menthol	1% w/v
Nasal Corticosteroid	Mometasone furoate	$8.63x10^{-4}$ µmol/L
Mucin	Mucin, bovine submaxillary Type I-S	100 µg/mL
Mucin	Mucin, porcine stomach Type II	100 µg/mL
Mucin	Mucin, porcine stomach Type III	100 µg/mL

Substance/Class	Description/Active Ingredient	Concentration Tested
Antibiotic, Nasal Ointment	Mupirocin	3.0 µmol/L
Anti-viral	Oseltamivir Phosphate	1.28 µmol/L
Afrin Nasal Spray	Oxymetazoline	1% v/v
Nasal Decongestant	Phenylephrine	$1.79x10^{-1}$ µmol/L
Saline Nasal Spray	Sodium Chloride	1% v/v
Nasal Corticosteroid	Triamcinolone acetonide	25 µg/mL
Antibiotic	Tobramycin	76.0 µmol/L
Anti-viral	Zanamivir	100 µg/mL
Anti-viral	Zinc	5% v/v
ZICAM Nasal Spray	Galphimia Glauca	1% v/v
	Histaminum Hydrochloricum	
	Luffa operculata	
	Sulfur	
NPS Swab	Nylon swab (Copan)	NA
Transport Media	Universal Transport Medium (Copan)	100%

[1]A nylon nasopharyngeal swab was fully coated with human sputum/mucus and then eluted into 1 mL of simulated NPS matrix, containing 5 representative target organisms at 3x LoD. The eluent was subsequently tested with the Respiratory *Flex* Assay.
[2]A nylon nasopharyngeal swab was fully coated with human sputum/mucus and then eluted into 2 mL of simulated NPS matrix, containing 5 representative target organisms at 3x LoD. The eluent was subsequently tested with the Respiratory *Flex* Assay.

Table 14 Substances that Interfered with Detection of at Least One Target Organism

Active Ingredient	Test Conc.	% Positivity (# Detected/# Tested)				
		Adenovirus	*B. pertussis*	Human Metapneumovirus	Influenza B	SARS-CoV-2
Human Sputum/ Mucus	1 swab/1mL sample	100% (6/6)	100% (6/6)	**33.3% (2/6)**[1]	**66.7% (4/6)**[1]	**83.3% (5/6)**[1]
	1 swab/2mL sample	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Human Whole Blood	5.0% v/v	100% (6/6)	**83.3% (5/6)**[1]	**66.7% (4/6)**[1]	**83.3% (5/6)**[1]	100% (6/6)
	4.5% v/v	100% (3/3)	100% (3/3)	**66.7% (2/3)**[1]	100% (3/3)	100% (3/3)
	4.0% v/v	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Leukocytes	1000 cells/µL	100% (3/3)	100% (3/3)	**33.3% (1/3)**[2]	100% (3/3)	**66.7% (2/3)**[2]
	666.7 cells/µL	100% (3/3)	100% (3/3)	**33.3% (1/3)**	**33.3% (1/3)**	**33.3% (1/3)**
	333.3 cells/µL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Mupirocin	3.0 µmol/L	100% (6/6)	100% (6/6)	100% (6/6)	**83.3% (5/6)**[3]	100% (6/6)
Tobramycin	76.0 µmol/L	100% (5/5)	100% (5/5)	**80% (4/5)**[4]	100% (5/5)	**80% (4/5)**[4]

[1]Unexpected negative results were obtained during original and repeat testing, therefore testing was performed at more dilute concentrations until 100% detection occurred.
[2]The original three replicates tested resulted in 33.3% (1/3) replicates being invalid and 50% (1/2) positivity for hMPV and 100% positivity (2/2) for Adenovirus, B. pertussis, Flu B, and SARS-CoV-2. New test material was prepared and tested, resulting in 66.6% (2/3) invalid results, and 0% (0/1) positivity for hMPV and SARS-CoV-2. Therefore, testing was performed at more dilute concentrations until 100% detection occurred.
[3]The original three replicates tested resulted in 66.7% (2/3) positivity for influenza B. New test material was prepared and tested, resulting in 100% (3/3) positivity for influenza B.
[4]The original three replicates tested resulted in 33.3% (1/3) replicates being invalid and 50% (1/2) positivity for hMPV and SARS-CoV-2. New

test material was prepared and tested, resulting in 100% (3/3) positivity for hMPV and SARS-CoV-2.

Competitive Inhibition/Co-infection:

Competitive inhibition of the LIAISON PLEX Respiratory *Flex* Assay was assessed by testing 27 pairings of clinically prevalent co-infections, as listed in Table 12. A single pairing consisted of one target at a high concentration with another target at a low concentration (Table 15). All low concentration targets were tested at 3X LoD. All high concentration targets were tested at $1x10^5$ $TCID_{50}$/mL (or equivalent), or the highest available concentration. Testing of each combination was performed in triplicate, at a minimum.

Of the 54 combinations tested, 48 combinations did not show evidence of inhibition and generated the expected results for both targets tested. Interference was observed for the following co-infections:

- Parainfluenza 3 (low concentration) in the presence of human coronavirus OC43 (high concentration of $1x10^5$ $TCID_{50}$/mL). Competitive interference was no longer observed when the human coronavirus OC43 concentration was decreased to $5x10^4$ $TCID_{50}$/mL.
- Parainfluenza 3 (low concentration) in the presence of adenovirus 37D (high concentration of $1x10^5$ $TCID_{50}$/mL).
- RSV A (low concentration) in the presence of adenovirus 37D (high concentration of $1x10^5$ $TCID_{50}$/mL). Competitive interference was no longer observed when the adenovirus 37D concentration was decreased to $5x10^4$ $TCID_{50}$/mL.
- Flu A H3N2 (low concentration) in the presence of adenovirus 37D (high concentration of $1x10^5$ $TCID_{50}$/mL). Specifically, detection of influenza A (matrix) was decreased in the presence of adenovirus 37D at a high concentration of $1x10^5$ $TCID_{50}$/mL. Competitive interference was no longer observed when the adenovirus 37D concentration was decreased to $5x10^4$ $TCID_{50}$/mL.
- Human coronavirus 229E (low concentration) in the presence of SARS-CoV-2 (high concentration of $1x10^5$ $TCID_{50}$/mL).
- SARS-CoV-2 (low concentration) in the presence of human coronavirus OC43 (high concentration of $1x10^5$ $TCID_{50}$/mL).

Table 15. Summary of Competitive Inhibition Results

Target 1 (High Conc.)		Target 2 (Low Conc.)[1]	% Detected (# Detected/ # Tested)	
Organism	**Conc. ($TCID_{50}$/mL)[2]**	**Organism**	**Target 1**	**Target 2**
Adenovirus 37D	$1x10^5$	Rhinovirus	100% (3/3)	100% (3/3)
Rhinovirus	$1x10^5$	hMPV	100% (3/3)	100% (3/3)
Adenovirus 37D	$1x10^5$	Coronavirus NL63	100% (3/3)	100% (3/3)
Rhinovirus	$1x10^5$	RSV A	100% (3/3)	100% (3/3)
Coronavirus OC43	$1x10^5$	PIV-3	100% (6/6)	**66.7% (4/6)**[3]
Coronavirus OC43	$5x10^4$	PIV-3	100% (3/3)	100% (3/3)
Rhinovirus	$1x10^5$	Coronavirus NL63	100% (3/3)	100% (3/3)
Adenovirus 37D	$1x10^5$	hMPV	100% (3/3)	100% (3/3)
Rhinovirus	$1x10^5$	Flu A H3N2	100% (3/3)	Matrix: 100% (3/3) Subtype H3: 100% (3/3)
Adenovirus 37D	$1x10^5$	PIV-3	100% (7/7)	**85.7% (6/7)**[4]
Coronavirus NL63	$1x10^5$	hMPV	100% (3/3)	100% (3/3)

Target 1 (High Conc.)		Target 2 (Low Conc.)[1]	% Detected (# Detected/ # Tested)	
Organism	**Conc. ($TCID_{50}$/mL)[2]**	**Organism**	**Target 1**	**Target 2**
Rhinovirus	$1x10^5$	SARS-CoV-2	100% (3/3)	100% (3/3)
Rhinovirus	$1x10^5$	PIV-3	100% (3/3)	100% (3/3)
Adenovirus 37D	$1x10^5$	RSV A	100% (6/6)	**66.7% (4/6)**[5]
Adenovirus 37D	$5x10^4$	RSV A	100% (3/3)	100% (3/3)
Rhinovirus	$1x10^5$	PIV-4	100% (3/3)	100% (3/3)
Rhinovirus	$1x10^5$	PIV-1	100% (3/3)	100% (3/3)
Adenovirus 37D	$1x10^5$	Flu A H3N2	100% (6/6)	**Matrix: 66.7% (4/6)**[6] **Subtype H3: 100% (6/6)**
Adenovirus 37D	$5x10^4$	Flu A H3N2	100% (3/3)	Matrix: 100% (3/3) Subtype H3: 100% (3/3)
Rhinovirus	$1x10^5$	Flu A H1N1	100% (3/3)	Matrix: 100% (3/3) Subtype H1: 100% (3/3)
SARS-CoV-2	$1x10^5$	Flu A H3N2	100% (6/6)	Matrix: 100% (3/3) Subtype H3: 100% (3/3)
SARS-CoV-2	$1x10^5$	Flu B	100% (3/3)	100% (3/3)
SARS-CoV-2	$1x10^5$	Coronavirus 229E	100% (6/6)	**83.3% (5/6)**[7]
SARS-CoV-2	$1x10^5$	Coronavirus NL63	100% (3/3)	100% (3/3)
SARS-CoV-2	$1x10^5$	Coronavirus OC43	100% (3/3)	100% (3/3)
SARS-CoV-2	$1x10^5$	Coronavirus HKU1	100% (3/3)	100% (3/3)
SARS-CoV-2	$1x10^5$	RSV A	100% (3/3)	100% (3/3)
SARS-CoV-2	$1x10^5$	Adenovirus 3B	100% (3/3)	100% (3/3)
SARS-CoV-2	$1x10^5$	Adenovirus 4E	100% (3/3)	100% (3/3)
SARS-CoV-2	$1x10^5$	Adenovirus 7A	100% (3/3)	100% (3/3)
Rhinovirus	$1x10^5$	Adenovirus 37D	100% (3/3)	100% (3/3)
hMPV	$1x10^5$	Rhinovirus	100% (3/3)	100% (3/3)
Coronavirus NL63	$1x10^5$	Adenovirus 37D	100% (3/3)	100% (3/3)
RSV A	$1x10^5$ (PFU/mL)	Rhinovirus	100% (3/3)	100% (3/3)
PIV-3	$1x10^5$	Coronavirus OC43	100% (3/3)	100% (3/3)
Coronavirus NL63	$1x10^5$	Rhinovirus	100% (3/3)	100% (3/3)
hMPV	$1x10^5$	Adenovirus 37D	100% (3/3)	100% (3/3)
Flu A H3N2	$1x10^5$ ($CEID_{50}$/mL)	Rhinovirus	100% (3/3)	100% (3/3)
PIV-3	$1x10^5$	Adenovirus 37D	100% (3/3)	100% (3/3)
hMPV	$1x10^5$	Coronavirus NL63	100% (3/3)	100% (3/3)
SARS-CoV-2	$1x10^5$	Rhinovirus	100% (3/3)	100% (3/3)
PIV-3	$1x10^5$	Rhinovirus	100% (3/3)	100% (3/3)

Target 1 (High Conc.)		Target 2 (Low Conc.)[1]	% Detected (# Detected/ # Tested)	
Organism	**Conc. ($TCID_{50}$/mL)[2]**	**Organism**	**Target 1**	**Target 2**
RSV A	$1x10^5$ (PFU/mL)	Adenovirus 37D	100% (3/3)	100% (3/3)
PIV-4	$1x10^5$	Rhinovirus	100% (3/3)	100% (3/3)
PIV-1	$1x10^5$	Rhinovirus	100% (3/3)	100% (3/3)
Flu A H3N2	$1x10^5$ ($CEID_{50}$/mL)	Adenovirus 37D	Matrix: 100% (3/3) Subtype H3: 100% (3/3)	100% (3/3)
Flu A H1N1	$1x10^5$ ($CEID_{50}$/mL)	Rhinovirus	Matrix: 100% (3/3) Subtype H1: 100% (3/3)	100% (3/3)
Flu A H3N2	$1x10^5$ ($CEID_{50}$/mL)	SARS-CoV-2	Matrix: 100% (3/3) Subtype H3: 100% (3/3)	100% (3/3)
Influenza B	$1x10^5$	SARS-CoV-2	100% (3/3)	100% (3/3)
Coronavirus 229E	$1x10^5$	SARS-CoV-2	100% (3/3)	100% (3/3)
Coronavirus NL63	$1x10^5$	SARS-CoV-2	100% (3/3)	100% (3/3)
Coronavirus OC43	$1x10^5$	SARS-CoV-2	100% (6/6)	**83.3% (5/6)**[8]
Coronavirus HKU1	$1.31x10^4$ (copies/mL)	SARS-CoV-2	100% (3/3)	100% (3/3)
RSV A	$1x10^5$ (PFU/mL)	SARS-CoV-2	100% (3/3)	100% (3/3)
Adenovirus 3B	$1x10^5$	SARS-CoV-2	100% (3/3)	100% (3/3)
Adenovirus 4E	$1x10^5$	SARS-CoV-2	100% (3/3)	100% (3/3)
Adenovirus 7A	$1x10^5$	SARS-CoV-2	100% (3/3)	100% (3/3)

[1]Low concentration target organisms were prepared at 3x LoD.
[2]Concentrations are in $TCID_{50}$/mL, unless otherwise noted.
[3]Unexpected negative results were obtained during original and repeat testing for parainfluenza 3, therefore testing was performed at more dilute coronavirus concentrations until 100% detection of parainfluenza 3 occurred.
[4]The original three replicates tested resulted in 66.7% (2/3) positivity for parainfluenza 3. New test material was prepared and tested, resulting in 100% (4/4) positivity for parainfluenza 3. Four replicates were performed during resting, rather than three because a single false positive SARS-CoV-2 result was obtained during retesting.
[5]Unexpected negative results were obtained during original and repeat testing for RSV A, therefore testing was performed at more dilute adenovirus concentrations until 100% detection of RSV A occurred.
[6]Unexpected negative results were obtained during original and repeat testing for influenza A (matrix), therefore testing was performed at more dilute adenovirus concentrations until 100% detection of influenza A (matrix) occurred.
[7]The original three replicates tested resulted in 66.7% (2/3) positivity for coronavirus. New test material was prepared and tested, resulting in 100% (3/3) positivity for coronavirus.
[8]The original three replicates tested resulted in 66.7% (2/3) positivity for SARS-CoV-2. New test material was prepared and tested, resulting in 100% (3/3) positivity for SARS-CoV-2.

Carry-Over and Cross-Contamination:

Carry-over and cross contamination for the LIAISON PLEX Respiratory *Flex* Assay was evaluated by testing positive and negative samples in an alternating series. A multi-analyte panel consisting of five assay targets (*B. pertussis*, Adenovirus, influenza B, human metapneumovirus, and SARS-CoV-2) at a high concentration (*B. pertussis* at $1x10^6$ CFU/mL and the viral targets at $1x10^5$ $TCID_{50}$/mL) was prepared in simulated NPS matrix and used for positive samples. Simulated negative NPS matrix was used for negative samples. Positive samples were tested in modules adjacent to negative samples in order to evaluate possible cross contamination. Immediately following the testing of a positive sample, a negative sample was run in the

same module to evaluate possible carry-over contamination. This alternating series of positive and negative samples was continued across five consecutive runs using two LIAISON PLEX Systems for a total of 30 positive and 30 negative tests. No carry-over or cross contamination was observed.

g. Assay cut-off

The specific assay parameters for the LIAISON PLEX® Respiratory *Flex* Assay are considered confidential and proprietary.

Comparison Studies:

h. Method comparison with predicate device:

Refer to Section 2 Clinical Performance.

i. Matrix Comparison:

Not applicable

2. *Clinical Performance:*

Prospective Clinical Evaluation

A multi-site prospective clinical study established the clinical performance of the LIAISON PLEX® Respiratory *Flex* Assay for the detection and identification of bacteria and viral targets from nasopharyngeal swab (NPS) specimens transported in Copan Universal Transport Medium™ or BD™ Universal Viral Transport Media, collected from patients exhibiting clinical signs and symptoms of respiratory tract infection (RTI). The clinical performance of the LIAISON PLEX Respiratory *Flex* Assay was evaluated using NPS clinical specimens prospectively collected between October 2022 to April 2023 from six geographically diverse clinical sites within the United States. The clinical study included remnant, de-identified specimens collected from pediatric and adult patients exhibiting clinical signs and symptoms of respiratory tract infections. Specimens were stored refrigerated at 2-8℃ for up to 72-hours before testing (i.e., Category I specimens) or if they could not be tested within 72-hours, after freezing at -70℃ (Category II specimens).

A total of 1911 unique prospective specimens that met the pre-determined inclusion criteria were enrolled in the study. Clinical runs and re-runs using LIAISON PLEX Respiratory *Flex* Assay were tested on the LIAISON PLEX System by trained operators at four clinical sites. Out of the 1911 specimens enrolled in the prospective study, 68 specimens were disqualified and removed from further analysis. Most of the specimen exclusions were due to non-compliance with the study protocol or due to not meeting the inclusion criteria after enrollment. This left 1843 clinical specimens for evaluation. Of these 1843 specimens, 66.3% (1221/1843) were tested fresh, while 33.7% (622/1843) were tested frozen. Patient demographic information for the 1843 prospectively collected NPS specimens is presented in Table 16.

Table 16. Prospective Study Demographic Details (N=1843)

	# Specimens (%)
Gender	
Male	839 (45.5%)
Female	1004 (54.5%)
Total	**1843 (100.0%)**
Age (years)	
0-1	350 (19.0%)
>1-5	274 (14.9%)
>5-21	447 (24.3%)
>21-65	535 (29.0%)
> 65	237 (12.9%)
Total	**1843 (100.0%)**
Subject Status	
Outpatient	590 (32.0%)
Hospitalized	317 (17.2%)
Emergency Room	913 (49.5%)
Unknown	23 (1.2%)
Total	**1843 (100.0%)**

The LIAISON PLEX Respiratory *Flex* Assay was evaluated for prospective clinical performance by comparing to an FDA-cleared molecular respiratory panel for all analytes, except the following: SARS-CoV-2, *B. holmesii*, *B. parapertussis*, and *B. pertussis*. Performance for SARS-CoV-2 was evaluated by comparing to an FDA-cleared molecular SARS-CoV-2 assay. Performance for the denoted *Bordetella* species was based on comparison to well-validated Fragment Analysis (FA) assays followed by PCR/Bi-Directional Sequencing (PCR/BDS) assays (see Table 17).

Table 17. Comparator Methods for the LIAISON PLEX Respiratory *Flex* Assay Clinical Study

LIAISON PLEX Respiratory *Flex* Target	Comparator Method
Adenovirus (inclusive to A, B, C , D, E, and F)	FDA-Cleared Molecular Respiratory Panel
Chlamydia pneumoniae	
Human Coronavirus (inclusive to HKU1, NL63, OC43, and 229E)	
Enterovirus/Rhinovirus	
Human Metapneumovirus	
Influenza A	
Influenza A subtype H1	
Influenza A subtype H3	
Influenza B	
Mycoplasma pneumoniae	
Parainfluenza 1	
Parainfluenza 2	
Parainfluenza 3	
Parainfluenza 4	
RSV (inclusive to RSV A and RSV B)	
SARS-CoV-2	FDA-Cleared Molecular SARS-CoV-2 Assay, Cleared Under 21 CFR 866.3981
Bordetella holmesii	Analytically Validated Fragment Analysis Assays Followed by PCR/Bi-Directional Sequencing
Bordetella parapertussis	
Bordetella pertussis	

Out of the 1843 prospective clinical specimens included in the prospective study analysis, 95.2% (1755/1843) generated valid Respiratory *Flex* Assay results (i.e., detected or not detected) on the first attempt, for an initial invalid rate of 4.8% (88/1843). Of the 88 specimens with initial invalid results, 77 produced valid results on repeat, 6 specimens remained invalid on repeat, and 5 specimens were not retested, resulting in a final invalid rate of 0.6% (11/1843). This left 1832 specimens with valid Respiratory *Flex* Assay results. The final number of evaluable results varied by target based on the number of valid comparator method results obtained.

Clinical Performance (Positive Percent Agreement, Negative Percent Agreement, and 95% confidence interval) of the LIAISON PLEX Respiratory *Flex* Assay vs the comparator method(s)is summarized in Table 18 for prospective specimens. Positive Percent Agreement (PPA) was calculated as 100% × (TP / (TP + FN)). True positive (TP) indicates that both the Respiratory *Flex* Assay and the comparator method had a positive result for the specific analyte, and false negative (FN) indicates that the Respiratory *Flex* Assay was negative while the comparator result was positive. Negative Percent Agreement (NPA) was calculated as 100% × (TN / (TN + FP)). True negative (TN) indicates that both the Respiratory *Flex* Assay and the comparator method had negative results, and false positive (FP) indicates that the Respiratory *Flex* Assay was positive while the comparator result was negative. Specimens that obtained discordant results underwent additional testing with either an FDA-cleared molecular respiratory panel or PCR/BDS for investigation.

Table 18. Prospective Clinical Performance of the LIAISON PLEX Respiratory *Flex* Assay with NPS Specimens

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI
Adenovirus	Fresh	75/75	100	95.1-100	1074/1129	95.1	93.7-96.2
	Frozen	19/19	100	83.2-100	578/597	96.8	95.1-98.0
	Overall	***94/94***	***100***	***96.1-100***	***1652/1726***[1]	***95.7***	***94.7-96.6***
Bordetella holmesii	Fresh	0/0	NE	NE	1127/1127	100	99.7-100
	Frozen	0/0	NE	NE	603/603	100	99.4-100
	Overall	***0/0***	***NE***	***NE***	***1730/1730***	***100***	***99.8-100***
Bordetella parapertussis	Fresh	4/4	100	51.0-100	1161/1163	99.8	99.4-100
	Frozen	0/1	0	0-79.3	604/605	99.8	99.1-100
	Overall	***4/5***	***80.0***	***37.6-96.4***	***1765/1768***[2]	***99.8***	***99.5-99.9***
Bordetella pertussis	Fresh	0/0	NE	NE	1146/1146	100	99.7-100
	Frozen	0/0	NE	NE	607/607	100	99.4-100
	Overall	***0/0***	***NE***	***NE***	***1753/1753***	***100***	**99.8-100**
Chlamydia pneumoniae	Fresh	0/0	NE	NE	1204/1204	100	99.7-100
	Frozen	0/0	NE	NE	616/616	100	99.4-100
	Overall	***0/0***	***NE***	***NE***	***1820/1820***	***100***	***99.8-100***
Human Coronavirus	Fresh	90/97	92.8	85.8-96.5	1100/1107	99.4	98.7-99.7
	Frozen	27/33	81.8	65.6-91.4	582/583	99.8	99.0-100
	Overall	***117/130***[3]	***90.0***	***83.6-94.1***	***1682/1690***[4]	***99.5***	***99.1-99.8***
Enterovirus/ Rhinovirus	Fresh	230/242	95.0	91.5-97.1	937/962	97.4	96.2-98.2
	Frozen	81/90	90.0	82.1-94.6	518/526	98.5	97.0-99.2
	Overall	***311/332***[5]	***93.7***	***90.5-95.8***	***1455/1488***[6]	***97.8***	***96.9-98.4***
hMPV	Fresh	113/118	95.8	90.5-98.2	1080/1086	99.4	98.8-99.7
	Frozen	12/13	92.3	66.7-98.6	603/603	100	99.4-100
	Overall	***125/131***[7]	***95.4***	***90.4-97.9***	***1683/1689***[8]	***99.6***	***99.2-99.8***
Influenza A	Fresh	18/18	100	82.4-100	1185/1186	99.9	99.5-100
	Frozen	111/111	100	96.7-100	490/505	97.0	95.2-98.2
	Overall	***129/129***	***100***	***97.1-100***	***1675/1691***[9]	***99.1***	***98.5-99.4***
Influenza A Subtype H1	Fresh	16/16	100	80.6-100	1187/1188	99.9	99.5-100
	Frozen	21/21	100	84.5-100	595/595	100	99.4-100
	Overall	***37/37***	***100***	***90.6-100***	***1782/1783***[10]	***99.9***	***99.7-100***
Influenza A Subtype H3	Fresh	2/3	66.7	20.8-93.9	1200/1201	99.9	99.5-100
	Frozen	102/104	98.1	93.3-99.5	509/512	99.4	98.3-99.8
	Overall	***104/107***[11]	***97.2***	***92.1-99.0***	***1709/1713***[12]	***99.8***	***99.4-99.9***
Influenza B	Fresh	4/4	100	51.0-100	1200/1200	100	99.7-100
	Frozen	4/4	100	51.0-100	612/612	100	99.4-100
	Overall	***8/8***	***100***	***67.6-100***	***1812/1812***	***100***	***99.8-100***
Mycoplasma pneumoniae	Fresh	0/0	NE	NE	1204/1204	100	99.7-100
	Frozen	0/0	NE	NE	616/616	100	99.4-100
	Overall	***0/0***	***NE***	***NE***	***1820/1820***	***100***	***99.8-100***

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI
Parainfluenza 1	Fresh	7/8	87.5	52.9-97.8	1196/1196	100	99.7-100
	Frozen	4/4	100	51.0-100	612/612	100	99.4-100
	Overall	***11/12***[13]	***91.7***	***64.6-98.5***	***1808/1808***	***100***	***99.8-100***
Parainfluenza 2	Fresh	9/10	90	59.6-98.2	1194/1194	100	99.7-100
	Frozen	3/3	100	43.9-100	613/613	100	99.4-100
	Overall	***12/13***	***92.3***	***66.7-98.6***	***1807/1807***	***100***	***99.8-100***
Parainfluenza 3	Fresh	37/39	94.9	83.1-98.6	1164/1165	99.9	99.5-100
	Frozen	4/5	80	37.6-96.4	611/611	100	99.4-100
	Overall	***41/44***[14]	***93.2***	***81.8-97.7***	***1775/1776***[15]	***99.9***	***99.7-100***
Parainfluenza 4	Fresh	4/4	100	51.0-100	1199/1200	99.9	99.5-100
	Frozen	4/5	80.0	37.6-96.4	611/611	100	99.4-100
	Overall	***8/9***[16]	***88.9***	***56.5-98.0***	***1810/1811***[17]	***99.9***	***99.7-100***
Respiratory Syncytial Virus	Fresh	37/38	97.4	86.5-99.5	1166/1166	100	99.7-100
	Frozen	81/85	95.3	88.5-98.2	531/531	100	99.3-100
	Overall	***118/123***[18]	***95.9***	***90.8-98.3***	***1697/1697***	***100***	***99.8-100***
SARS-CoV-2	Fresh	178/183	97.3	93.8-98.8	996/1000	99.6	99.0-99.8
	Frozen	68/72	94.4	86.6-97.8	521/525	99.2	98.1-99.7
	Overall	***246/255***[19]	***96.5***	***93.4-98.1***	***1517/1525***[20]	***99.5***	***99.0-99.7***

TP – true positive; FN – false negative; TN – true negative; FP – false positive; NE – not evaluable

[1]Of the 74 specimens with false positive adenovirus results by the Respiratory *Flex* Assay, 21 were positive by an FDA-cleared molecular respiratory panel, 21 were negative, and 32 were not tested.

[2]Of the 3 specimens with false positive *Bordetella parapertussis* results by the Respiratory *Flex* Assay, 1 was negative by an FDA-cleared molecular respiratory panel and 2 were not tested.

[3]Of the 13 specimens with false negative coronavirus results by the Respiratory *Flex* Assay, 3 were negative by PCR/BDS, 9 were positive, and 1 was not tested.

[4]Of the 8 specimens with false positive coronavirus results by the Respiratory *Flex* Assay, 5 were positive by PCR/BDS, 2 were negative, and 1 was not tested.

[5]Of the 21 specimens with false negative enterovirus/rhinovirus results by the Respiratory *Flex* Assay, 9 were positive by PCR/BDS, 8 were negative, and 4 were not tested.

[6]Of the 33 specimens with false positive enterovirus/rhinovirus results by the Respiratory *Flex* Assay, 4 were positive by PCR/BDS, 27 were negative, and 2 were not tested.

[7]Of the 6 specimens with false negative hPMV results by the Respiratory *Flex* Assay, 4 were positive by PCR/BDS and 2 were negative.

[8]Of the 6 specimens with false positive hPMV results by the Respiratory *Flex* Assay, 4 were positive by PCR/BDS and 2 were negative.

[9]Of the 16 specimens with false positive influenza A results by the Respiratory *Flex* Assay, 7 were positive by PCR/BDS and 9 were negative.

[10]The 1 specimen with a false positive influenza A subtype H1 result by the Respiratory *Flex* Assay was negative by PCR/BDS.

[11]The 3 specimens with false negative influenza A subtype H3 results by the Respiratory *Flex* Assay were all negative by PCR/BDS.

[12]The 4 specimens with false positive influenza A subtype H3 results by the Respiratory *Flex* Assay were all negative by PCR/BDS.

[13]The 1 specimen with a false negative parainfluenza 1 result by the Respiratory *Flex* Assay was positive by PCR/BDS.

[14]Of the 3 specimens with false negative parainfluenza 3 results by the Respiratory *Flex* Assay, 2 were negative by PCR/BDS and 1 was not tested.

[15]The 1 specimen with a false positive parainfluenza 3 result by the Respiratory *Flex* Assay was negative by PCR/BDS.

[16]The 1 specimen with a false negative parainfluenza 4 result by the Respiratory *Flex* Assay was negative by PCR/BDS.

[17]The 1 specimen with a false positive parainfluenza 4 result by the Respiratory *Flex* Assay was negative by PCR/BDS.

[18]Of the 5 specimens with false negative RSV results by the Respiratory *Flex* Assay, 1 was negative by PCR/BDS, and 3 were negative by an FDA-cleared molecular Flu/RSV assay.

[19]Of the 9 specimens with false negative SARS-CoV-2 results by the Respiratory *Flex* Assay, 5 were positive by PCR/BDS, 2 were negative, and 2 were not tested.

[20]Of the 8 specimens with false positive SARS-CoV-2 results by the Respiratory *Flex* Assay, 5 were positive by PCR/BDS, 2 were negative, and 1 was not tested.

The LIAISON PLEX Respiratory *Flex* Assay reported multiple organism detections in a total of 176 prospective specimens as shown in Table 19. Of these 176 specimens, comparator results were unavailable for at least 1 of the organisms identified in the co-infection for 3 specimens, which were excluded from further analysis. The remaining 173 co-infections represent 14.6% (173/1187) of positive prospective specimens and 9.4% (173/1840) of all prospective specimens. The majority of co-infections, 87.3% (151/173), contained two organisms, while 11.6% (20/173) of coinfections contained three organisms, and 1.2% (2/173) contained 4 organisms. Out of the 173 specimens with multiple

detections, 45.5% (77/173) contained one or more organisms that were not detected by the comparator method(s) (Table 19). Co-infections identified by the comparator methods which were not reported by the LIAISON PLEX Respiratory *Flex* Assay are illustrated in Table 20.

Table 19. Co-infections Reported by the LIAISON PLEX Respiratory *Flex* Assay in the Prospective Study

				Number of Specimens		
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Total**	**LIAISON PLEX Respiratory *Flex* Assay False Positives**	**LIAISON PLEX Respiratory *Flex* Assay False Positive Analyte(s)**
Adenovirus	*Bordetella parapertussis*	Enterovirus/ Rhinovirus		2	2	Adenovirus (2), *Bordetella parapertussis* (1)
Adenovirus	Human Coronavirus			10	5	Adenovirus
Adenovirus	Human Coronavirus	Enterovirus/ Rhinovirus		1	1	Adenovirus
Adenovirus	Human Coronavirus	hMPV		1	1	hMPV
Adenovirus	Human Coronavirus	Parainfluenza 3		1	0	NA
Adenovirus	Enterovirus/ Rhinovirus			35	18	Adenovirus (17), Enterovirus/ Rhinovirus (1)
Adenovirus	Enterovirus/ Rhinovirus	hMPV		3	1	Adenovirus (1), Enterovirus/ Rhinovirus (1)
Adenovirus	Enterovirus/ Rhinovirus	hMPV	SARS-CoV-2	1	1	Adenovirus (1), Enterovirus/ Rhinovirus (1)
Adenovirus	Enterovirus/ Rhinovirus	Influenza A & Influenza A (subtype H1)		1	1	Adenovirus
Adenovirus	Enterovirus/ Rhinovirus	Parainfluenza 1		1	1	Adenovirus
Adenovirus	Enterovirus/ Rhinovirus	Respiratory Syncytial Virus		1	1	Adenovirus, Enterovirus/ Rhinovirus
Adenovirus	Enterovirus/ Rhinovirus	SARS-CoV-2		1	0	NA
Adenovirus	hMPV			7	4	Adenovirus

				Number of Specimens		
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Total**	**LIAISON PLEX Respiratory *Flex* Assay False Positives**	**LIAISON PLEX Respiratory *Flex* Assay False Positive Analyte(s)**
Adenovirus	hMPV	Parainfluenza 2		1	0	NA
Adenovirus	hMPV	SARS-CoV-2		1	1	Adenovirus
Adenovirus	Influenza A & Influenza A (subtype H1)			2	2	Adenovirus
Adenovirus	Influenza A & Influenza A (subtype H3)			2	2	Adenovirus
Adenovirus	Influenza B			1	1	Adenovirus
Adenovirus	Parainfluenza 1	Respiratory Syncytial Virus		1	1	Adenovirus
Adenovirus	Parainfluenza 2			1	1	Adenovirus
Adenovirus	Parainfluenza 3			5	4	Adenovirus
Adenovirus	Respiratory Syncytial Virus			4	3	Adenovirus
Adenovirus	SARS-CoV-2			2	2	Adenovirus
Bordetella parapertussis	Human Coronavirus			1	1	*Bordetella parapertussis*
Bordetella parapertussis	Enterovirus/ Rhinovirus			2	1	*Bordetella parapertussis*
Bordetella parapertussis	hMPV			1	0	NA
Bordetella parapertussis	Parainfluenza 3			1	0	NA
Human Coronavirus	Enterovirus/ Rhinovirus			8	2	Human Coronavirus (1), Enterovirus/ Rhinovirus (1)
Human Coronavirus	Enterovirus/ Rhinovirus	hMPV		1	0	NA

				Number of Specimens		
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Total**	**LIAISON PLEX Respiratory *Flex* Assay False Positives**	**LIAISON PLEX Respiratory *Flex* Assay False Positive Analyte(s)**
Human Coronavirus	Enterovirus/ Rhinovirus	SARS-CoV-2		1	0	NA
Human Coronavirus	hMPV			6	2	Human Coronavirus
Human Coronavirus	Influenza A & Influenza A (subtype H1)			1	0	NA
Human Coronavirus	Parainfluenza 3			3	0	NA
Human Coronavirus	Respiratory Syncytial Virus			2	0	NA
Human Coronavirus	SARS-CoV-2			4	1	Human Coronavirus
Enterovirus/ Rhinovirus	hMPV			11	1	Enterovirus/ Rhinovirus
Enterovirus/ Rhinovirus	hMPV	Influenza A (subtype H3)	SARS-CoV-2	1	1	Influenza A H3, Influenza A, Enterovirus/ Rhinovirus, SARS-CoV-2
Enterovirus/ Rhinovirus	hMPV	SARS-CoV-2		1	1	Enterovirus/ Rhinovirus
Enterovirus/ Rhinovirus	Influenza A & Influenza A (subtype H1)			2	0	NA
Enterovirus/ Rhinovirus	Influenza A & Influenza A (subtype H3)			7	2	Enterovirus/ Rhinovirus
Enterovirus/ Rhinovirus	Influenza A & Influenza A (subtype H3)	Respiratory Syncytial Virus		2	2	Influenza A H3, Influenza A
Enterovirus/ Rhinovirus	Parainfluenza 1			3	1	Enterovirus/ Rhinovirus
Enterovirus/ Rhinovirus	Parainfluenza 3			5	2	Enterovirus/ Rhinovirus
Enterovirus/ Rhinovirus	Parainfluenza 4			4	1	Parainfluenza 4
Enterovirus/ Rhinovirus	Respiratory Syncytial Virus			9	0	NA

				Number of Specimens		
Analyte 1	**Analyte 2**	**Analyte 3**	**Analyte 4**	**Total**	**LIAISON PLEX Respiratory *Flex* Assay False Positives**	**LIAISON PLEX Respiratory *Flex* Assay False Positive Analyte(s)**
Enterovirus/ Rhinovirus	SARS-CoV-2			5	2	Enterovirus/ Rhinovirus
hMPV	Respiratory Syncytial Virus			1	1	hMPV
Influenza A & Influenza A (subtype H1)	Influenza A (subtype H3)			1	0	NA
Influenza A & Influenza A (subtype H1)	SARS-CoV-2			1	1	SARS-CoV-2
Influenza A & Influenza A (subtype H3)	Respiratory Syncytial Virus			1	1	Influenza A
Parainfluenza 2	SARS-CoV-2			1	0	NA
Parainfluenza 3	Respiratory Syncytial Virus			1	0	NA
Parainfluenza 3	SARS-CoV-2			1	1	SARS-CoV-2
			Total	**173**	**77**	
			Total Double Infections	**151**	**62**	
			Total Triple Infections	**20**	**13**	
			Total Quadruple Infections	**2**	**2**	

Table 20. Co-infections Identified by the Comparator Methods which were Not Reported by the LIAISON PLEX Respiratory Flex Assay in the Prospective Study

			Number of Specimens		
Analyte 1	**Analyte 2**	**Analyte 3**	**Total**	**LIAISON PLEX Respiratory *Flex* Assay False Negatives**	**LIAISON PLEX Respiratory *Flex* Assay False Negative Analyte(s)**
Adenovirus	Human Coronavirus		7	1	Human Coronavirus
Adenovirus	Enterovirus/		18	2	Enterovirus/

			Number of Specimens		
Analyte 1	**Analyte 2**	**Analyte 3**	**Total**	**LIAISON PLEX Respiratory *Flex* Assay False Negatives**	**LIAISON PLEX Respiratory *Flex* Assay False Negative Analyte(s)**
	Rhinovirus				Rhinovirus
Adenovirus	Enterovirus/ Rhinovirus	Parainfluenza 2	1	1	Parainfluenza 2
Adenovirus	hMPV		3	1	hMPV
Adenovirus	hMPV	SARS-CoV-2	1	1	SARS-CoV-2
Adenovirus	SARS-CoV-2		1	1	SARS-CoV-2
Human Coronavirus	Enterovirus/ Rhinovirus		8	1	Human Coronavirus
Human Coronavirus	Enterovirus/ Rhinovirus	SARS-CoV-2	2	1	Human Coronavirus
Human Coronavirus	hMPV		6	2	Human Coronavirus
Human Coronavirus	Influenza A & Influenza A (subtype H3)		1	1	Human Coronavirus
Human Coronavirus	Parainfluenza 3		5	2	Human Coronavirus (1), Parainfluenza 3 (1)
Human Coronavirus	Respiratory Syncytial Virus		3	1	Human Coronavirus
Influenza A & Influenza A (subtype H3)	Parainfluenza 4		1	1	Parainfluenza 4
Influenza A & Influenza A (subtype H3)	Respiratory Syncytial Virus		1	1	Respiratory Syncytial Virus
hMPV	SARS-CoV-2		4	1	hMPV
Respiratory Syncytial Virus	hMPV		1	1	hMPV
Respiratory Syncytial Virus	SARS-CoV-2		1	1	Respiratory Syncytial Virus
Enterovirus/ Rhinovirus	*Bordetella Parapertussis*		3	1	*Bordetella parapertussis*
Enterovirus/ Rhinovirus	hMPV		11	1	hMPV
Enterovirus/ Rhinovirus	Influenza A & Influenza A (subtype H1)		6	3	Enterovirus/Rhinovirus
Enterovirus/ Rhinovirus	Influenza A (subtype H3)		1	1	Influenza A (subtype H3)
Enterovirus/ Rhinovirus	Influenza A & Influenza A (subtype H3)	SARS-CoV-2	1	1	SARS-CoV-2
Enterovirus/ Rhinovirus	Respiratory Syncytial Virus		13	3	Enterovirus/Rhinovirus
Enterovirus/ Rhinovirus	SARS-CoV-2		5	3	Enterovirus/Rhinovirus (2), SARS-CoV-2 (1)

			Number of Specimens		
Analyte 1	**Analyte 2**	**Analyte 3**	**Total**	**LIAISON PLEX Respiratory *Flex* Assay False Negatives**	**LIAISON PLEX Respiratory *Flex* Assay False Negative Analyte(s)**
Parainfluenza 3	Enterovirus/ Rhinovirus	Respiratory Syncytial Virus	1	1	Parainfluenza 3 (1), Enterovirus/ Rhinovirus (1)
		Total	**105**	**34**	
		Total Double Infections	**99**	**29**	
		Total Triple Infections	**6**	**5**	

Testing of Preselected Archived Specimens

A number of analytes on the LIAISON PLEX Respiratory *Flex* Assay were of low prevalence during the prospective study and were not encountered in large enough numbers to adequately demonstrate system performance. To supplement the results of the prospective clinical study, an evaluation of preselected archived retrospective NPS specimens was performed.

A total of 256 pre-selected left-over frozen, de-identified specimens (Category III specimens) sourced from four sites/vendors in the United States were obtained and tested at three US sites. Pre-selected specimen collection dates ranged from November 2013 through June 2023. Pre-selected specimens were characterized by the same comparator methods as the prospective study (described above). The pre-selected specimens were tested in a randomized, blinded manner with negative specimens. A summary of the available demographic information of the tested specimens is provided in Table 21. Out of the 256 specimens included in the pre-selected study analysis, 241 (94.1%) generated valid Respiratory *Flex* Assay results (i.e., Detected or Not Detected) on the first attempt. There were 15 specimens (5.9%) with invalid results on the initial run that required retesting. Of the specimens with initial invalid results, all 15 specimens generated valid Respiratory *Flex* Assay results after retest for a final success rate of 100% (256/256). The results of the LIAISON PLEX Respiratory *Flex* Assay performance for these archived specimens are shown in Table 22.

Table 21. Archived Specimen Demographic Details (N=256)

	# Specimens (%)
Gender	
Male	117(45.7%)
Female	124(48.4%)
Unknown	15(5.9%)
Total	**256(100.0%)**

Age (years)	
0-1	44(17.2%)
>1-5	53(20.7%)
>5-21	69(27.0%)
>21-65	44(17.2%)
> 65	32(12.5%)
Unknown	14(5.5%)
Total	**256(100.0%)**
Subject Status	
Outpatient	0 (0.0%)
Hospitalized	0 (0.0%)
Emergency Room	0 (0.0%)
Unknown	256 (100.0%)
Total	**256 (100.0%)**

Table 22. LIAISON PLEX Respiratory *Flex* Assay Archived Performance Summary for NPS Specimens

Analyte	Positive Percent Agreement			Negative Percent Agreement		
	TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI
Adenovirus	6/6	100	61.0-100	241/250[1]	96.4	93.3-98.1
Bordetella holmesii	0/0	NE	NE	234/234	100	98.4-100
Bordetella parapertussis	8/8	100	67.6-100	233/236	98.7	96.3-99.6
Bordetella pertussis	23/23	100	85.7-100	214/217	98.6	96.0-99.5
Chlamydia pneumoniae	13/14	92.9	68.5-98.7	241/242	99.6	97.7-99.9
Human Coronavirus	4/4	100	51.0-100	249/252	98.8	96.6-99.6
Enterovirus/ Rhinovirus	24/27[2]	88.9	71.9-96.1	223/229[3]	97.4	94.4-98.8
hMPV	1/1	100	20.7-100	255/255	100	98.5-100
Influenza A	1/1	100	20.7-100	254/255[4]	99.6	97.8-99.9
Influenza A subtype H1	1/1	100	20.7-100	254/255[4]	99.6	97.8-99.9
Influenza A subtype H3	0/0	NE	NE	256/256	100	98.5-100
Influenza B	23/23	100	85.7-100	232/233	99.6	97.6-99.9
Mycoplasma pneumoniae	23/24	95.8	79.8-99.3	226/232	97.4	94.5-98.8
Parainfluenza 1	18/18	100	82.4-100	237/238	99.6	97.7-99.9
Parainfluenza 2	19/20	95.0	76.4-99.1	235/236	99.6	97.6-99.9
Parainfluenza 3	2/2	100	34.2-100	254/254	100	98.5-100
Parainfluenza 4	23/23	100	85.7-100	230/233[5]	98.7	96.3-99.6
Respiratory Syncytial Virus	9/9	100	70.1-100	246/247	99.6	97.7-99.9

TP – true positive; FN – false negative; TN – true negative; FP – false positive; NE – not evaluable

[1]Of the 9 specimens with false positive adenovirus results by the Respiratory *Flex* Assay, seven were negative by PCR/BDS and two were not tested.
[2]Of the 3 specimens with false negative enterovirus/rhinovirus results by the Respiratory *Flex* Assay, one was negative by PCR/BDS, one was positive by PCR/BDS, and one was not tested.
[3]Of the 6 specimens with false positive enterovirus/rhinovirus results by the Respiratory *Flex* Assay, four were negative by PCR/BDS and two were not tested.
[4]The 1 specimen with a false positive influenza A/influenza A H1 result by the Respiratory *Flex* Assay was negative for influenza A by PCR/BDS and not tested for influenza A H1.
[5]Of the 3 specimens with false positive parainfluenza 4 results, one was negative by PCR/BDS and two were not tested.

Contrived Specimen Testing

Contrived specimens were tested to supplement the positive clinical specimens in the prospective and pre-selected study cohorts for low prevalence targets, including *Bordetella holmesii, Bordetella parapertussis, Bordetella pertussis, Chlamydia pneumoniae,* and *Mycoplasma pneumoniae*. Positive contrived specimens for influenza A H1N1 pdm09 were prepared and tested prior to completion of the prospective clinical study, in anticipation of potentially low prevalence for influenza A H1. The prospective clinical study ended up yielding an adequate number of influenza A H1 positive specimens to demonstrate performance, however since the contrived data was already acquired, it's presented here . A total of 300 specimens were contrived, blinded, randomized and tested along with negative specimens at two testing sites during August 2023.
Out of the 300 specimens included in the contrived study analysis, 291 specimens (97.0%) generated valid RSP *Flex* Assay results (i.e., Detected or Not Detected) on the first attempt. There were 9 specimens (3.0%) with an invalid result on the initial run. Of the 9 specimens retested, all 9 generated a valid result after a single retest for a final success rate of 100% (300/300).

Results from contrived specimen testing with the LIAISON PLEX Respiratory *Flex* Assay are shown in Table 23.

Table 23. LIAISON PLEX Respiratory *Flex* Assay Performance with Contrived Specimens

Analyte	Target Conc. (xLoD)	Positive Percent Agreement			Negative Percent Agreement		
		TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI
Bordetella holmesii	2x	25/25	100	86.7-100	125/125	100	97.0-100
	10x	13/13	100	77.2-100	65/65	100	94.4-100
	100x	12/12	100	75.8-100	60/60	100	94.0-100
	Combined	***50/50***	***100***	***92.9-100***	***250/250***	***100***	***98.5-100***
Bordetella parapertussis	2x	25/25	100	86.7-100	125/125	100	97.0-100
	10x	12/13	92.3	66.7-98.6	65/65	100	94.4-100
	100x	12/12	100	75.8-100	60/60	100	94.0-100
	Combined	***49/50***	***98.0***	***89.5-99.6***	***250/250***	***100***	***98.5-100***
Bordetella pertussis	2x	25/25	100	86.7-100	125/125	100	97.0-100
	10x	13/13	100	77.2-100	65/65	100	94.4-100
	100x	12/12	100	75.8-100	60/60	100	94.0-100
	Combined	***50/50***	***100***	***92.9-100***	***250/250***	***100***	***98.5-100***
Chlamydia	2x	25/25	100	86.7-100	125/125	100	97.0-100

Analyte	Target Conc. (xLoD)	Positive Percent Agreement			Negative Percent Agreement		
		TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI
pneumoniae	10x	13/13	100	77.2-100	65/65	100	94.4-100
	100x	12/12	100	75.8-100	60/60	100	94.0-100
	Combined	***50/50***	***100***	***92.9-100***	***250/250***	***100***	***98.5-100***
Influenza A H1N1 pdm09	2x	24/25	96.0	80.5-99.3	125/125	100	97.0-100
	10x	13/13	100	77.2-100	65/65	100	94.4-100
	100x	12/12	100	75.8-100	60/60	100	94.0-100
	Combined	***49/50***	***98.0***	***89.5-99.6***	***250/250***	***100***	***98.5-100***
Mycoplasma pneumoniae	2x	24/25	96.0	80.5-99.3	125/125	100	97.0-100
	10x	13/13	100	77.2-100	64/65	98.5	91.8-99.7
	100x	12/12	100	75.8-100	60/60	100	94.0-100
	Combined	***49/50***	***98.0***	***89.5-99.6***	***249/250***	***99.6***	***97.8-99.9***

N. Proposed Labeling:

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

O. Conclusion:

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