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

I Background Information:

A 510(k) Number
K233410

B Applicant
Luminex Corporation

C Proprietary and Established Names
LIAISON PLEX Respiratory *Flex* Assay

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QOF[1]	Class II	21 CFR 866.3981 - Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The SARS-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test	MI - Microbiology
OEM	Class II	21 CFR 866.3980 – Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
OOU	Class II	21 CFR 866.3980 – Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
OTG	Class II	21 CFR 866.3980 – Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
OZE	Class II	21 CFR 866.3980 – Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
OZX	Class II	21 CFR 866.3980 – Respiratory viral panel	MI - Microbiology

		multiplex nucleic acid assay	
OZY	Class II	21 CFR 866.3980 – Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
OZZ	Class II	21 CFR 866.3980 – Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
OCC	Class II	21 CFR 866.3980 – Respiratory viral panel multiplex nucleic acid assay	MI - Microbiology
NSU	Class II	21 CFR 862.2570 – Instrumentation for clinical multiplex test systems	CH - Clinical Chemistry

[1]Primary Product Code

II Submission/Device Overview:

A Purpose for Submission:

The purpose of this submission is to show that the LIASION PLEX Respiratory *Flex* Assay is substantially equivalent to the BioFire Respiratory Panel 2.1 (RP2.1) (DEN200031) and to obtain clearance for the LIASION PLEX Respiratory *Flex* Assay.

B Measurand:

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

C Type of Test:

A multiplexed nucleic acid test intended for use with the automated LIAISON PLEX instrument for the qualitative *in vitro* detection and identification of multiple respiratory pathogen nucleic acids in nasopharyngeal swabs (NPS) collected in BD Universal Transport Media (UVT) or Copan Universal Transport Media (UTM) and obtained from individuals with signs and symptoms of respiratory tract infections.

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

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.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only
For *in vitro* diagnostic use only

D Special Instrument Requirements:
For use with the LIAISON PLEX System, only.

IV Device/System Characteristics:

A Device Description:

The LIAISON PLEX Respiratory *Flex* Assay is performed on the LIAISON PLEX System. 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. The LIAISON PLEX Respiratory *Flex* Assay components required to perform the test include the following single-use, disposables:

- LIAISON PLEX Respiratory *Flex* Assay Test Cartridge
- LIAISON PLEX Respiratory *Flex* Assay Transfer Pipettes (or equivalent)

Prior to initiating a test on the LIAISON PLEX System, a 300 µL aliquot of NPS in Viral Transport Media (VTM) is pipetted by the user into the Sample Port within the Sample Prep Tray (recommended, but not required), followed by closing the Sample Port Closure. Next, the Sample ID barcode on the sample tube is scanned with the hand-held barcode reader, or the Sample ID is manually entered using the touchscreen keyboard. The user then scans the assay cartridge ID barcode with the hand-held barcode scanner. Lastly, the Respiratory *Flex* Assay Cartridge is inserted into the module by the user. 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. Once the test is finished running (~ 2 hours), the user ejects the assay cartridge by selecting the green check mark or the eject icon on the touchscreen.

The Respiratory *Flex* Assay has 19 different reportable targets (organisms and influenza A subtypes H1 and H3). Reporting of these targets is based on detection of one or more of the nucleic acid targets. For each intended Respiratory *Flex* Assay target, four sequence components, referred to as oligonucleotides (Oligos), consisting of one or more Capture probe(s), Mediator probe(s), forward primer(s), and reverse primer(s), are required.

The LIAISON PLEX Respiratory *Flex* Assay can be run in Fixed mode, which will return the results for all target analytes. To allow testing flexibility, the LIAISON PLEX Respiratory *Flex* Assay can be run in *Flex* mode. This feature allows laboratories to generate custom panels, which include only a subset of analytes selected by the laboratory. No pre-set *Flex*-panels are defined, and laboratories can choose to create custom panels of specific targets for certain populations and/or seasons, if they desire to incorporate the *Flex* reporting feature. Target assays that are initially masked can later be unmasked using *Flex* credits when ordered by a physician. When *Flex* mode is implemented, all target assays are performed, however the raw data is not analyzed for masked targets until they are unmasked.

Interpretation of Results

The Respiratory *Flex* Assay provides a qualitative result for the presence (Detected) or absence (Not Detected) of the Respiratory *Flex* target nucleic acid gene sequences. The image analysis of the Substrate provides light signal intensities from the target-specific capture spots as well as the internal processing controls, negative control, background, and imaging control spots. The mean signal intensity of a target, after background subtraction, is compared to the assay's signal detection threshold to make a determination. **Table 2** below lists the possible test results generated by the Respiratory *Flex* Assay representing identification of viral and bacterial nucleic acid sequences/targets.

Table 2. Respiratory *Flex* Assay Calls for Valid Tests

Test Result Reported as "Detected"	Reported Target
Viral Targets	
Adenovirus (inclusive to A, B, C, D, E, and F)	Adenovirus
Human Metapneumovirus	Human Metapneumovirus
Human Parainfluenza Virus 1	Human Parainfluenza Virus 1
Human Parainfluenza Virus 2	Human Parainfluenza Virus 2
Human Parainfluenza Virus 3	Human Parainfluenza Virus 3
Human Parainfluenza Virus 4	Human Parainfluenza Virus 4
Human Coronavirus (inclusive to HKU1, NL63, OC43, and 229E)	Human Coronavirus
Influenza A*	Influenza A
Influenza A subtype H1**	Influenza A subtype H1
Influenza A subtype H3**	Influenza A subtype H3
Influenza B	Influenza B
Enterovirus	Enterovirus/Rhinovirus
Rhinovirus	
Respiratory Syncytial Virus (inclusive to RSV A and RSV B)	Respiratory Syncytial Virus
SARS-CoV-2	SARS-CoV-2
Bacterial Targets	
Bordetella holmesii	*Bordetella holmesii*
Bordetella pertussis (Toxin Promoter Region)	*Bordetella pertussis*
Bordetella parapertussis (IS*1001*)	*Bordetella parapertussis*
Chlamydia pneumoniae	*Chlamydia pneumoniae*
Mycoplasma pneumoniae	*Mycoplasma pneumoniae*
Test Result Reported as "Not Detected"	
All Analytes Not Detected	

*Detection of Influenza A without an Influenza A/H1 or Influenza A/H3 subtype may occur at low titer of the virus in the specimen or may indicate a false positive due to contamination. The result could also indicate a novel Influenza A strain. In these cases, the sample should be retested. If an Influenza A detected result is obtained without detection of an Influenza A/H1 or A/H3 subtype upon retesting, contact local or state public health authorities for confirmatory testing.

**Detection of Influenza A/H1 or Influenza A/H3 subtypes without an Influenza A "Detected" result may occur at low titer of the virus in the specimen or may indicate a false positive due to contamination. The result could also indicate potential genetic mutations in the Matrix protein gene among circulating seasonal Influenza A viruses. In these cases, the sample should be retested. If an Influenza A/H1 or A/H3 subtype detected result is obtained again without detection of Influenza A upon repeat testing, further investigations may be warranted.

Reasons for invalid (no call) results, together with the appropriate recourse which should be taken by the user, are described in **Table 3**.

Table 3. LIAISON PLEX Respiratory *Flex* Assay Invalid Calls and Recourse

Call	Reason	Recourse
No Call	The hybridization internal control (IC) is not detected[1]	Retest from the primary sample beginning with the assay package insert
	The amplification IC or extraction IC are not detected AND no DNA or RNA target pathogen is detected, respectively[1]	

	Signal in regions of the microarray which do not contain capture oligos is too high	section *Procedure*, using a new cartridge
	Signal in regions of the microarray containing oligomer spots to ensure proper stringency	
	The coefficient of variation of intensities for spots within at least one spot group is high	
	The overall signal across all spot groups, excluding Negative Control and Background, is too high	

[1]Additional information on the ICs (hybridization, amplification, and extraction) is provided in **Section IV.C.Instrument Descriptive Information.5.Quality Control.Internal Controls**, below.

B Principle of Operation:

The Respiratory *Flex* Assay is a multiplexed molecular assay with automated nucleic acid isolation, amplification, and detection of unique genomic sequences of target pathogens. The Respiratory *Flex* Assay is performed using the LIAISON PLEX System, which is a bench-top sample-to-result molecular diagnostics workstation consisting 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. The LIAISON PLEX System automates the Respiratory *Flex* Assay sample analysis steps, which occur within the cartridge, including: (1) Specimen Extraction – Chemical and mechanical RNA/DNA extraction from nasopharyngeal swab specimens obtained from symptomatic patients; (2) Target Amplification - Multiplex RT-PCR- and PCR-based amplification of the extracted nucleic acids to generate target-specific amplicons; (3) Hybridization - Amplicon hybridization to target specific capture DNA in a microarray format and mediator and gold-nanoparticle probe hybridization to captured amplicons. Silver enhancement of the gold nanoparticle probes bound at the capture sites results in gold-silver aggregates that are imaged optically with high efficiency by the LIAISON PLEX System. The user can monitor the status of the assay via the touch screen on the instrument, which displays the run time.

Contamination Control
The Respiratory *Flex* Assay includes an Uracil DNA Glycosylase (UDG) enzyme-based strategy to eliminate amplicon contamination. Briefly, the lyophilized amplification master mix formulation contains deoxyuridine triphosphate (dUTP) in place of the standard deoxythymidine triphosphate (dTTP), and during the multiplexed RT-PCR step dUTP is incorporated into the amplicons. Prior to the start of an amplification step, the UDG enzyme renders any dUTP-containing *previously generated* amplicons non-amplifiable by selectively hydrolyzing at the uracil base, while not impacting the integrity of dTTP containing target RNA. The assay also uses a thermolabile version of UDG enzyme which is inactivated by heat prior to the RT step and does not interfere with the newly generated cDNA and/or the amplicon from the test. While the UDG-based strategy mitigates false positive risk due to lab-based carryover and cross-contamination, incomplete hydrolysis of uracil-containing amplicons may lead to amplification and detection of a contaminant. Additionally, this strategy does not address genomic contamination during the preparation of the samples. Strict adherence to the prescribed handling/preparation of samples and laboratory/system cleaning protocols and careful disposal of the used consumables can reduce the likelihood of contamination from user-based sources.

End-Point Detection and Analysis
The target-specific amplicon is detected in an endpoint assay that utilizes a microarray format. For each of the bacterial or viral nucleic acid sequences/analytes detected by the Respiratory *Flex* Assay, two types of oligonucleotides are required for the endpoint gold nanoparticle probe-based detection: (1) Capture oligonucleotides (or captures) and (2) Mediator oligonucleotides (or mediators). The

Capture oligonucleotides are arrayed on the surface of a substrate (a microarray) within the test cartridge and are designed to specifically bind to one part of the analyte-specific target amplicon. The Mediator oligonucleotides bind to a different portion of the same amplicon and enable binding of gold nanoparticle probes. Notably, in a multiplexed detection system, numerous unique target-specific mediators can coexist and form unique hybridizations at the different captures on the microarray. Since all the mediators have a target specific region and a poly-A tail region, a single, universal gold nanoparticle poly-T probe is sufficient for target/mediator labeling. Silver enhancement of the bound gold nanoparticle probes at the capture sites results in gold-silver aggregates that scatter light with high efficiency. Light scatter from the capture spots is imaged by the LIAISON PLEX System and intensities from the microarray spots are processed by a decision algorithm to make calls regarding the presence (Detected) or absence (Not Detected) of a nucleic acid sequence/analyte.

C Instrument Description Information:

1. Instrument Name:
   LIAISON PLEX System, software version 1.0.0.144.

2. Specimen Identification:
   Specimen identification information is entered either manually or via barcode.

3. Specimen Sampling and Handling:
   Nasopharyngeal swab (NPS) specimens collected in BD UVT or Copan UTM.

4. Calibration:
   LIAISON PLEX modules are calibrated during the manufacturing process; calibration is not performed by the user.

5. Quality Control:

   Internal Controls
   The Assay contains three sets of internal controls to check to ensure performance of sample preparation amplification, and detection. They are described in more detail, below:

   1. **Extraction control**. The extraction control verifies the presence of an amplicon for Bacteriophage MS2, which is added to the sample prior to the nucleic acid extraction step. The extracted control product is amplified and subsequently detected by unique spots on the hybridization array, thereby confirming successful nucleic acid extraction, Reverse Transcription, PCR amplification of RNA targets, and detection.

   2. **Amplification Control**. The amplification control verifies the presence of an amplicon for a synthetic DNA oligonucleotide sequence in the lyophilized PCR master mix. The product is detected by a unique spot on the hybridization array, thereby confirming successful PCR amplification and detection of DNA targets.

   3. **Hybridization Control**. The hybridization control target and mediator oligonucleotide are contained within the Sample Buffer and added to the post-amplification product prior to hybridization. The hybridization control is detected by a unique spot on the hybridization array, thereby confirming successful processing of hybridization and signal enhancement steps.

Internal controls results are reported as Pass, Fail, or N/A (see **Table 4** 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.

Table 4. Interpretation of Internal Control Results for the LIAISON PLEX Respiratory *Flex* Assay

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. • An RNA pathogen target was detected, indicating successful extraction. If an 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 an 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 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.	Repeat test with a new cartridge

	• The amplfication control, or a DNA pathogen was detected, indicating successful amplifcation. • The extraction control, or a RNA pathogen, was not detected, indicating extraction was not successful.	

External Controls
External controls are not provided with the Respiratory *Flex* Assay. However, five external control mixes (see **Table 5** below) were provided to the clinical study sites for daily testing during the prospective clinical study. External controls were tested on each day of testing, utilizing one external negative control and one of four external positive controls (tested on a rotating basis) representing all Respiratory *Flex* targets.

Table 5. External Controls Utilized in the Clinical Studies

External Control	Expected Calls
Positive Run Control - Pool 1	Adenovirus, *Chlamydia pneumoniae*, human coronavirus, hMPV, Influenza B, Parainfluenza 1, Parainfluenza 2, RSV
Positive Run Control - Pool 2	*Bordetella holmesii*, *Bordetella pertussis*, Enterovirus/Rhinovirus, Influenza A, Influenza A (subtype H1), Influenza A (subtype H3), *Mycoplasma pneumoniae*, Parainfluenza 3, Parainfluenza 4
Positive Run Control - Pool 3	SARS-CoV-2
Positive Run Control - Pool 4	*Bordetella parapertussis*, *Bordetella pertussis*
Negative Run Control	NA

The sponsor is also including the following in the product package insert, "*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. 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*."

V Substantial Equivalence Information:

A Predicate Device Name(s):
BioFire Respiratory Panel 2.1 (RP2.1)

B Predicate 510(k) Number(s):
DEN200031

C Comparison with Predicate(s):

Device & Predicate Device(s):	K233410	DEN200031
Device Trade Name	LIAISON Plex Respiratory *Flex* Assay	BioFire Respiratory Panel 2.1 (RP2.1)
Regulation Number and Name	Same	21 CFR 866.3981; Devices to detect and identify nucleic acid targets in respiratory samples from

		microbial agents that cause the SARS-CoV-2 respiratory infection and other microbial agents when in a multi-analyte test.
Product Code	Same	QOF
Intended Use/Indications For Use	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: <u>*Viruses:*</u> 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) <u>*Bacteria:*</u> *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	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 2 (SARS-CoV-2), • Human Metapneumovirus, • Human Rhinovirus/Enterovirus, • Influenza A, including subtypes H1, H3 and H1-2009, • 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* 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

	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.	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 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 co-infection 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 possible respiratory tract infection.
Measurand	Same	Nucleic acids from target organisms
Sample Type	Same	Nasopharyngeal swab (NPS)
Instrumentation	LIAISON PLEX System	BioFire FilmArray 2.0 or BioFire FilmArray Torch Systems
Technological Principles	Highly multiplexed nucleic acid PCR and RT-PCR test with microarray detection.	Highly multiplexed nested nucleic acid amplification with melt analysis.
Internal Controls	Multiple internal controls contained in the cartridge monitor sample processing and RT and PCR functions.	Two controls are included in each reagent pouch to control for sample processing and both stages of PCR and melt analysis.
Automated Test Processes	Same	Nucleic Acid Extraction and Amplification, Detection and Results Interpretation
***Bordetella* Species Detected**	• *Bordetella parapertussis* • *Bordetella pertussis* • *Bordetella holmesii*	• *Bordetella parapertussis* • *Bordetella pertussis*
Human Coronavirus Result Reporting	The human coronavirus target species (i.e., HKU1, OC43, 229E, NL63) are not differentiated.	Each target human coronavirus species (i.e., HKU1, OC43, 229E, NL63) is reported independently.
Influenza A Subtyping	Influenza A subtypes H1 and H3 detected/reported.	Influenza A subtypes H1, H1-2009, and H3 detected/reported.

Time to Result	~ 2 hours	~45 minutes

VI Standards/Guidance Documents Referenced:

Standards

- ISTA 3A. Packaged-Products for Parcel Delivery System Shipment 70 kg (150 lb) or Less. (2018).
- ITSA 7D. Temperature Test for Transport Packaging.
- CLSI EP07. Interference Testing in Clinical Chemistry; Third Edition.
- CLSI EP37. Supplemental Tables for Interference Testing in Clinical Chemistry; First Edition.
- CLSI EP25-A. Evaluation of Stability of *In Vitro* Diagnostic Reagents; Approved Guideline.
- CLSI EP17-A2. Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition.
- CLSI EP05-A3. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition.
- CLSI EP12-A2. User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition.
- CLSI EP24-A2. Assessment of the Diagnostic Accuracy of Laboratory Testing Using Receiver Operating Characteristic Curves; Approved Guideline – Second Edition.
- ISO 14971. Medical Devices – Application of Risk Management to Medical Devices. Third Edition (2019-12).
- ISO 23640. *In Vitro* Diagnostic Medical Devices – Evaluation of Stability of *In Vitro* Diagnostic Reagents.
- ISO 15223-1. Medical Devices – Symbols to be Used with Information to be Supplied by the Manufacturer-Part 1: General Requirements. Fourth Edition (2021-07).
- ISO 3864-1. Graphic Symbols – Safety Colors and Safety Signs – Part 1. Design Principles for Safety Signs and Safety Markings (2011).
- IEC 61010-1 Edition 3.1, Consolidated Version. Safety Requirements for Electrical Equipment for Measurement Control and Laboratory Use – Part 1: General Requirements, Including Corrigendum 1. (2017-01).
- IEC 61326-2-6 Edition 3.0. Electrical Equipment for Measurement Control and Laboratory Use – EMC Requirements – Part 2-6: Particular Requirements – *In Vitro* Diagnostic (IVD) Medical Equipment (2010-10).
- IEC 60601-1-2 Edition 4.0. Medial Electrical Equipment – Part 1-2: General Requirements for Basic Safety and Essential Performance – Collateral Standard: Electromagnetic Disturbances-Requirements and Tests (2014-02).
- IEC 61000-3-2. Electromagnetic Compatibility (EMC) – Part 3-2: Limits for Harmonic Current Emissions, Input Current Up to & Including 16A Per Phase (2014).
- IEC 61000-3-3. Electromagnetic Compatibility (EMC) – Part 3-3: Limits – Limitation of Voltage Changes, Voltage Fluctuations and Flicker in Public Low – Voltage Supply Systems for Equipment (2013).

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).

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

a. Within-Laboratory Precision

Within-laboratory precision was evaluated at a single site using the Respiratory *Flex* Assay run on the LIAISON PLEX System. A total of three contrived panels containing known quantities of the target analytes were prepared in simulated NPS matrix, consisting of HeLa cells at a concentration of $2x10^3$ cells/mL in UTM. Data supporting the use of simulated matrix can be found in section **VII Performance Charactestics.B.2.Matrix Equivalency Study**, later in this document. The viral and bacterial materials used to generate the positive panel members are denoted in **Table 6**. The contrived positive panels consisted of five representative target organisms co-spiked at a low positive concentration (1.5x LoD) and moderate concentration (5x LoD). A negative panel was also included in the study (see **Table 7**). The study was conducted with one operator and three cartridge lots over the course of 5 non-consecutive days on two LIAISON PLEX Systems. Each panel member was tested in triplicate once per day on 5 different days generating a total of 45 replicates per panel member (1 Site x 1 Operator x 3 Lots x 5 Days X 1 Run per Day x 3 Replicates per Run).

Table 6. Viral and Bacterial Strains Used in the Within-Laboratory Precision Study

Description	Organism Type	Vendor	Catalog Number
Bordetella pertussis (9797)	Bacterium	ATCC	9797
Adenovirus (4E)	DNA virus	Zeptometrix	0810070CF
Influenza B (Colorado/06/2017)	RNA virus	Zeptometrix	0810573CF
hMPV (27A2)	RNA virus	Zeptometrix	0810164CF
SARS-CoV-2 (USA-WA1/2020)	RNA virus	Zeptometrix	0810587CFHI

Table 7. Precision Study Sample Panel

Panel ID	Description
1	*Bordetella pertussis*, Adenovirus, Influenza B, hMPV, SARS-CoV-2 (Low positive; all analytes at 1.5x LoD)
2	*Bordetella pertussis*, Adenovirus, Influenza B, hMPV, SARS-CoV-2 (Moderate positive; all analytes at 5x LoD)
3	Negative

The qualitative results (i.e., % agreement with expected results) from the study are illustrated in **Table 8**.

Table 8. Within-Laboratory Precision Study – Qualitative Results

Target	Panel ID	Panel Conc.	% Positive (pos n/ valid n)	% Agreement with Expected Results/ (95% CI)
Bordetella pertussis	1	Low positive	93.3% (42/45)	93.3% (82.1-97.7%)
	2	Mod. positive	100% (45/45)	100% (92.1-100%)
	3	Negative	0% (0/45)	100% (92.1-100%)
Adenovirus	1	Low positive	97.8% (44/45)	97.8% (88.4-99.6%)
	2	Mod. positive	97.8% (44/45)	97.8% (88.4-99.6%)
	3	Negative	0% (0/45)	100% (92.1-100%)
Influenza B	1	Low positive	100% (45/45)	100% (92.1-100%)
	2	Mod. positive	100% (45/45)	100% (92.1-100%)
	3	Negative	0% (0/45)	100% (92.1-100%)
hMPV	1	Low positive	100% (45/45)	100% (92.1-100%)
	2	Mod. positive	97.8% (44/45)	97.8% (88.4-99.6%)
	3	Negative	0% (0/45)	100% (92.1-100%)
SARS-CoV-2	1	Low positive	100% (45/45)	100% (92.1-100%)
	2	Mod. positive	100% (45/45)	100% (92.1-100%)
	3	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 presented 5 times.

All low positive (1.5x LoD) panel members were positive >97.8%, except for *Bordetella pertussis*, which had a positivity of 93.3% (42/45). All moderate positive (5x LoD) panel members were 100% positive for the spiked target analytes, except for adenovirus and hMPV, which yielded 97.8% positivity (44/45). The negative panel member was 100% negative. There was no lot-to-lot variability observed in the study. The results of the study demonstrate acceptable assay variability.

b. Reproducibility

A reproducibility study was conducted at three testing sites using the Respiratory *Flex* Assay run on the LIAISON PLEX System. The study incorporated potential sources of variation introduced by site (three testing sites), day (5 different days), operator (two operators per site), and instrument (six LIAISON PLEX Systems). One lot of Respiratory *Flex* Assay cartridges was tested at three sites by two operators per site over five days.

The same three contrived panels used to evaluate precision (see **Table 7**) were included in the reproducibility study. Three replicates of each panel member were tested by each operator at each site on all 5 days of testing generating a total of 90 replicates per panel member. The qualitative results of the study are illustrated in **Table 9**.

Table 9. Reproducibility Study – Qualitative Results

Target	Panel ID	Panel Conc.	% Agreement with Expected Results			
			Site 1	Site 2	Site 3	Overall/ (95% CI)
Bordetella pertussis	1	Low positive	93.3% (28/30)	100% (30/30)	96.7% (29/30)	96.7% (87/90) (90.7-98.9%)

	2	Mod. positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9-100%)
	3	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9-100%)
Adenovirus	1	Low positive	96.7% (29/30)	100% (30/30)	96.7% (29/30)	97.8% (88/90) (92.3-99.4%)
	2	Mod. positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9-100%)
	3	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9-100%)
Influenza B	1	Low positive	93.3% (28/30)	100% (30/30)	96.7% (29/30)	96.7% (87/90) (90.7-98.9%)
	2	Mod. positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9-100%)
	3	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9-100%)
hMPV	1	Low positive	90.0% (27/30)	100% (30/30)	93.3% (28/30)	94.4% (85/90) (87.6-97.6%)
	2	Mod. positive	100% (30/30)	93.3% (28/30)	100% (30/30)	97.8% (88/90) (92.3-99.4%)
	3	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9-100%)
SARS-CoV-2	1	Low positive	96.7% (29/30)	100% (30/30)	100% (30/30)	98.9% (89/90) (94.0-99.8%)
	2	Mod. positive	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9-100%)
	3	Negative	100% (30/30)	100% (30/30)	100% (30/30)	100% (90/90) (95.9-100%)

Mod – moderate; *Note:* Results are shown only for the intended targets. Panel members co-spiked with 5 different targets are presented 5 times.

The negative panel was negative 100% of the time. The moderate positive (5x LoD) target gave expected results 100% of the time for adenovirus, *Bordetella pertussis*, influenza B, and SARS-CoV-2. The moderate positive for human metapneumovirus yielded 97.8% (88/90) positive results.

For the low positive (1.5x LoD) target, all targets except for human metapneumovirus yielded at least 96.7% (87/90) positivity. The overall positivity for the human metapneumovirus low positive sample was 94.4% (85/90).

2. Linearity:
Not applicable; this is a qualitative assay.

3. Analytical Specificity/Interference:

Analytical Reactivity (Inclusivity)
The inclusivity of the LIAISON PLEX Respiratory *Flex* Assay was evaluated using a combination of *in silico* analysis of publicly available sequence information and laboratory testing of contrived specimens containing viral and bacterial isolates that were selected to represent phylogenetic, geographic, and temporal diversity.

a. Wet-Testing
This study was performed to determine the analytical reactivity of the Respiratory *Flex* Assay with clinically relevant strains, serotypes, or subtypes of the target species. The inclusivity panel was prepared by spiking various target microorganism strains/serotypes/subtypes encompassing temporal and geographical diversity into simulated NPS matrix at a concentration of ~3x LoD and testing in triplicate. Data supporting the use of simulated matrix can be found in section **VII Performance Charactestics.B.2.Matrix Equivalency Study**, later in this document. Strains that did not yield 100% reactivity at 3x LoD were reprepared at the same concentration and retested in triplicate. If 100% reactivity was obtained during retesting, reactivity was reached. In this situation, the original and retesting results were pooled for performance calculations. If less than 100% reactivity was observed during retesting, the strain was prepared at a higher concentration and tested until 100% reactivity was achieved. The strains evaluated and the lowest concentration that met the reactivity criteria outlined above are shown in **Table 10** - **Table 23**, below.

Table 10. Inclusivity Testing – Adenovirus Results

Strain	Concentration		% Detected (# Detected/#Tested)
	Copies/mL	xLoD	
A 31	$5.28x10^3$	3x	100% (3/3)
B 3	$2.06x10^3$		100% (3/3)
B 7A	$2.06x10^3$		100% (3/3)
B 21	$2.06x10^3$		100% (3/3)
B 11	$2.06x10^3$		100% (3/3)
B 14	$2.06x10^3$		100% (3/3)
B 34	$2.06x10^3$		83.3% (5/6)[1]
B 35	$2.06x10^3$		100% (3/3)
C 1	$3.35x10^3$		100% (3/3)
C 2	$3.35x10^3$		100% (3/3)
C 5	$3.35x10^3$		100% (3/3)
C 6	$3.35x10^3$		100% (3/3)
D 26	$2.24x10^3$		100% (3/3)
D 37	$2.24x10^3$		100% (3/3)
E 4	$1.06x10^3$		83.3% (5/6)[1]
F 40-Dugan	$1.45x10^3$		100% (3/3)
F 41-Tak	$1.45x10^3$		100% (3/3)

[1]The original three replicates tested resulted in 66.7% (2/3) positivity for adenovirus. New test material was prepared and tested, resulting in 100% (3/3) positivity for adenovirus.

Table 11. Inclusivity Testing – *Bordetella holmesii* Results

Strain	Concentration		% Detected (# Detected/#Tested)
	Copies/mL	xLoD	
FA061	$2.19x10^4$	3x	100% (3/3)
CDC F5101 [CDC 84-013939]	$2.19x10^4$		83.3% (5/6)[1]
CIP 104395 [G7702; 92A2997]	$2.19x10^4$		80.0% (4/5)[2]
CIP 104396	$1.97x10^5$	27x[3]	100% (3/3)

[1]The original three replicates tested resulted in 66.7% (2/3) positivity for *B. holmesii*. New test material was prepared and tested, resulting in 100% (3/3) positivity for *B. holmesii.*
[2]The original three replicates tested resulted in 33% (1/3) invalid replicates and 50% (1/2) positivity for *B. holmesii*. New test material was prepared and tested, resulting in 100% (3/3) positivity for *B. holmesii.*
[3]Testing at lower concentrations (i.e., 3x and 9x LoD) failed to yield 100% detection. The lowest concentration that yielded 100% detection was 27x LoD.

Table 12. Inclusivity Testing – *Bordetella parapertussis* Results

Strain	Concentration		% Detected (# Detected/#Tested)
	Copies/mL	xLoD	
NCTC 5952 [522]	$2.37x10^3$	3x	100% (3/3)
508 and 344 [NCTC10853]			100% (3/3)
517			100% (3/3)
12822			100% (3/3)
509 and 609			100% (3/3)
PT28G			100% (3/3)[1]
PT 26/28G			100% (3/3)[1]
C510			100% (3/3)

[1]Testing results in positivity for *B. parapertussis* and *B. pertussis*. CoAs from the vendor confirm that these are genetically modified strains, engineered to contain DNA sequences for both *B. parapertussis* and *B. pertussis* toxins.

Table 13. Inclusivity Testing – *Bordetella pertussis* Results

Strain	Concentration		% Detected (# Detected/#Tested)
	Copies/mL	xLoD	
18323 [NCTC 10739]	$1.14x10^4$	3x	100% (3/3)
CNCTC Hp 12/63 [623]			100% (3/3)
10-536			100% (3/3)
5 [17921]			100% (3/3)
Tohama I			100% (3/3)
MN2531			100% (3/3)
PT9/28G [W28]			100% (3/3)
589			100% (3/3)
F			100% (3/3)

Table 14. Inclusivity Testing – *Chlamydia pneumoniae* Results

Strain	Concentration		% Detected (# Detected/#Tested)
	Copies/mL	xLoD	
CWL-029	$1.71x10^3$	3x	100% (3/3)
AR-39			100% (3/3)
J-21			100% (3/3)
2023			100% (3/3)

Table 15. Inclusivity Testing – Human Coronavirus Results

Species/Strain		Concentration		% Detected
		Copies/mL	xLoD	(# Detected/#Tested)
HKU1	CS-Lum2020-Resp-1146	$5.0x10^3$	3x	100% (3/3)
	CS-83254			100% (3/3)
NL63	Source #: 0810228CF	$2.29x10^2$	3x	100% (3/3)
	Source #: NR-470	$6.88x10^2$	9x[1]	100% (3/3)
OC43	Source #: 0810024CF	$2.84x10^4$	3x	100% (3/3)
	Source #: VR-1558			100% (3/3)
229E	Source #: 0810229CF	$1.20x10^3$	3x	100% (3/3)
	Source #: VR-740	$3.60x10^3$	9x[1]	100% (3/3)

CS-Clinical sample

[1]Testing at a lower concentration (i.e., 3x LoD) failed to yield 100% detection. The lowest concentration that yielded 100% detection was 9x LoD.

Table 16. Inclusivity Testing – Enterovirus/Rhinovirus Results

Species/Strain		Concentration		% Detected
		Copies/mL	xLoD	(# Detected/#Tested)
Enterovirus A	Coxsackievirus A10	$6.75x10^4$	3x	100% (3/3)
	Coxsackievirus 71			100% (3/3)
Enterovirus B	Coxsackievirus A9			100% (3/3)
	Coxsackievirus B3			100% (3/3)
	Coxsackievirus B4			100% (3/3)
	Echovirus 6			100% (3/3)
	Echovirus 9			100% (3/3)
	Echovirus 11			100% (3/3)
	Echovirus 30			100% (3/3)
Enterovirus C	Coxsackievirus A21			100% (3/3)
	Coxsackievirus A24			100% (3/3)
Enterovirus D	68			100% (3/3)
Rhinovirus A	16	$2.46x10^4$	3x	100% (3/3)
	2			100% (3/3)
	34			100% (3/3)
	57			100% (3/3)
	7			100% (3/3)
	77			100% (3/3)
	85			100% (3/3)
Rhinovirus B	14	$2.45x10^4$	3x	100% (3/3)
	17			100% (3/3)
	27			100% (3/3)
	3			100% (3/3)
	42			100% (3/3)
	83			100% (3/3)
Rhinovirus C	CS-75029 (H7-3)	$5.75x10^4$	3x	100% (3/3)
	CS-75466 (J3-3)			100% (3/3)
	CS-NPS/UTU NEG 178			100% (3/3)
	CS-NEG004 (SAR-4)			100% (3/3)

CS-Clinical sample

Table 17. Inclusivity Testing –Human Metapneumovirus Results

Strain	Concentration		% Detected (# Detected/#Tested)
	Copies/mL	xLoD	
hMPV-9 (Type A1)	$6.40x10^3$	3x	100% (3/3)
hMPV-16 (Type A1)			100% (3/3)
hMPV-20 (Type A2)	$6.12x10^3$	3x	100% (3/3)
hMPV-27 (Type A2)			100% (3/3)
hMPV-3 (Type B1)	$1.50x10^4$	3x	100% (3/3)
hMPV-5 (Type B1)			100% (3/3)
hMPV-4 (Type B2)	$4.50x10^4$	3x	100% (3/3)
hMPV-8 (Type B2)			100% (3/3)
hMPV-18 (Type B2)			100% (3/3)

Table 18. Inclusivity Testing – Influenza A Results

Species/Strain		Concentration		% Detected (# Detected/#Tested)
		Copies/ mL	xLoD	
H1N1	A/Wisconsin/588/2019	$4.5x10^3$	3x	Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
	A/Hawaii/66/2019 X-345A			Matrix: 100% (6/6)
				Subtype H1: 83.3% (5/6)[1]
	A/Indiana/02/2020			Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
	A/Michigan/272/2017			Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
	A/Idaho/07/2018			Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
	A/Wisconsin/505/2018			Matrix: 100% (6/6)
				Subtype H1: 83.3% (5/6)[1]
	Guangdong-Maonan /SWL 1536/19			Matrix: 100% (6/6)
				Subtype H1: 83.3% (5/6)[1]
	Brisbane/02/18	$1.35x10^4$	$9x^3$	Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
	A/St.Petersburg/61/2015	$4.5x10^3$	3x	Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
	A/Bangladesh/3002/2015			Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
	A/Denver/1/57			Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
	New Caledonia/20/99			Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
	PR/8/34			Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
	Singapore/63/04			Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
	Solomon Islands/03/06			Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
	Taiwan/42/06			Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
H1N1v	A/Ohio/09/2015 (Subtype Synthetic DNA)	$4.5x10^3$		Matrix: 0% (0/3)[2]
				Subtype H1: 100% (3/3)
	A/Ohio/09/2015			Matrix: 100% (3/3)

Species/Strain		Concentration		% Detected (# Detected/#Tested)
		Copies/ mL	xLoD	
	(Matrix Synthetic DNA)		3x	Subtype H1: 0% (0/3)[2]
H1N2	A/swine/Ohio/09SW1484E/2009	$4.5x10^3$		Matrix: 100% (3/3)
				Subtype H1: 100% (3/3)
H1N2v	A/Minnesota/19/2011 (Subtype Synthetic DNA)	$4.5x10^3$		Matrix: 0% (0/3)[2]
				Subtype H1: 100% (3/3)
	A/Minnesota/19/2011 (Matrix Synthetic DNA)			Matrix: 100% (3/3)
				Subtype H1: 0% (0/3)[2]
H3N2	A/Kansas/14/2017 NYMC X-327	$5.89x10^3$		Matrix: 100% (3/3)
				Subtype H3: 100% (3/3)
	A/Texas/71/2017			Matrix: 100% (3/3)
				Subtype H3: 100% (3/3)
	A/Wisconsin/04/2018			Matrix: 100% (3/3)
				Subtype H3: 100% (3/3)
	A/Arizona/45/2018			Matrix: 100% (3/3)
				Subtype H3: 100% (3/3)
	A/Hong Kong/45/2019			Matrix: 100% (3/3)
				Subtype H3: 100% (3/3)
	A/Tasmania/503/2020			Matrix: 100% (3/3)
				Subtype H3: 100% (3/3)
	A/Delaware/01/2021			Matrix: 100% (3/3)
				Subtype H3: 100% (3/3)
	A/Singapore/INFIMH-16-0019/2016			Matrix: 100% (3/3)
				Subtype H3: 100% (3/3)
	/California/55/2020			Matrix: 100% (3/3)
				Subtype H3: 100% (3/3)
	A/Alaska/232/2015			Matrix: 100% (3/3)
				Subtype H3: 100% (3/3)
H3N2v	A/Hawaii/28/2020 (Subtype Synthetic DNA)	$5.89x10^3$		Matrix: 0% (0/3)[2]
				Subtype H3: 100% (3/3)
	A/Hawaii/28/2020 (Matrix Synthetic DNA)			Matrix: 100% (3/3)
				Subtype H3: 0% (0/3)[2]
H5N1	A/Egypt/N03072/2010	$5.89x10^3$		Matrix: 100% (3/3)
				Subtype: 0% (0/3)[2]
	A/Hubei/1/2010			Matrix: 100% (3/3)
				Subtype: 0% (0/3)[2]
	A/Anhui/01/2005			Matrix: 100% (3/3)
				Subtype: 0% (0/3)[2]
H7N2	A/turkey/Virginia/4529/2002	$5.89x10^3$		Matrix: 100% (3/3)
				Subtype: 0% (0/3)[2]
H7N7	A/mallard/Netherlands/12/2000	$5.89x10^3$		Matrix: 100% (3/3)
				Subtype: 0% (0/3)[2]
H9N2	A/Hong Kong/33982/2009	$5.89x10^3$		Matrix: 100% (3/3)
				Subtype: 0% (0/3)[2]

[1]The original three replicates tested resulted in 66.7% (2/3) positivity for the influenza A H1 subtype. New test material was prepared and tested, resulting in 100% (3/3) positivity for the influenza A H1 subtype.
[2]No positivity was expected based on the strain and/or type of material being tested.
[3]Testing at a lower concentration (i.e., 3x LoD) failed to yield 100% detection. The lowest concentration that yielded 100% detection was 9x LoD.

Table 19. Inclusivity Testing – Influenza B Results

Lineage/Strain		Concentration		% Detected (# Detected/#Tested)
		Copies/mL	xLoD	
Victoria Lineage	B/Washington/02/2019	$1.01x10^3$	3x	100% (3/3)
	B/New Hampshire/01/2021			100% (3/3)
	B/Missouri/12/2018 (NA D197E)			100% (3/3)
	B/Hawaii/01/2018 (NA D197N)			100% (3/3)
	B/Michigan/01/2021			100% (3/3)
	B/Hong Kong/286/2017			100% (3/3)
	B/Colorado/6/2017			100% (3/3)
	B/Texas/43/2019			100% (3/3)
Yamagata Lineage	B/Wisconsin/1/10			100% (3/3)
	B/Florida/02/06			100% (3/3)
	B/Florida/07/04			100% (3/3)
	B/Phuket/3073/13			100% (3/3)
	B/Wisconsin/10/2016 (NA I221V)			100% (3/3)
	B/Indiana/17/2017 (NA I221T)			100% (3/3)
	B/Oklahoma/10/2018 (NA D197N)			100% (3/3)

Table 20. Inclusivity Testing – *Mycoplasma pneumoniae* Results

Strain	Concentration		% Detected (# Detected/#Tested)
	Copies/mL	xLoD	
M129	$3.89x10^3$	3x	100% (3/3)
15531-TTR			100% (3/3)
Mac			100% (3/3)
PI 1428			100% (3/3)
Bru			100% (3/3)
M52			100% (3/3)
UTMB-10P			100% (3/3)
Mutant 22			100% (3/3)
M129-B7			100% (3/3)

Table 21. Inclusivity Testing – Parainfluenza Results

Species/Strain		Concentration		% Detected (# Detected/#Tested)
		Copies/mL	xLoD	
PIV-1	N/A	$2.28x10^3$	3x	83.3% (5/6)[1]
	C35			100% (3/3)
PIV-2	N/A	$2.54x10^4$		100% (3/3)
	Greer			100% (3/3)
PIV-3	N/A	$5.79x10^3$		100% (3/3)
	ATCC-2011-5			83.3% (5/6)[2]
	C243			100% (3/3)
	NIH 47885			100% (3/3)
PIV-4a	N/A	$1.73x10^4$		100% (3/3)
	M-25			100% (3/3)

Species/Strain		Concentration		% Detected (# Detected/#Tested)
		Copies/mL	xLoD	
PIV-4b	N/A			100% (3/3)
	CH 19503			100% (3/3)

[1]The original three replicates tested resulted in 66.7% (2/3) positivity for parainfluenza 1. New test material was prepared and tested, resulting in 100% (3/3) positivity for parainfluenza 1.
[2]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% (3/3) positivity for parainfluenza 3.

Table 22. Inclusivity Testing – RSV Results

Species/Strain		Concentration		% Detected (# Detected/#Tested)
		Copies/mL	xLoD	
RSV-A	2006 Isolate	$1.15x10^4$	3x	100% (3/3)
	A2			100% (3/3)
	Long			100% (3/3)
RSV-B	CH93(18)-18	$4.82x10^4$		100% (3/3)
	B WV/14617/85			100% (3/3)
	18537			100% (3/3)
	B1			100% (3/3)

Table 23. Inclusivity Testing – SARS-CoV-2 Results

Strain	Concentration		% Detected (# Detected/#Tested)
	Copies/ mL	xLoD	
B.1.617.1: Isolate: USA/CA-Stanford-15_S02/2021 (Kappa)	$7.20x10^4$	9x[1]	100% (3/3)
B.1.1.529 BA.1: Isolate: USA/MD-HP20874/2021 (Omicron)	$2.4x10^4$	3x	100% (3/3)
Isolate: Italy-INMI1			100% (3/3)
Isolate: Hong Kong/VM20001061/2020			100% (3/3)
B.1_2020: Isolate: USA/NY-Wadsworth-103677-01/2020			100% (3/3)
B.1.1.7: Isolate: England/204820464/2020 (Alpha)			100% (3/3)
B.1.1.7: Isolate: USA/CA_CDC_5574/2020 (Alpha)			100% (3/3)
B.1.351: Isolate: South Africa/KRISP-K005325/2020 (Beta)			100% (3/3)
P1: Isolate: Japan/TY7-503/2021 (Gamma)			100% (3/3)
P2_2021: Isolate: NY-Wadsworth-21006055-01/2021 (Zeta)			100% (3/3)
B.1.526_2021: Isolate: USA/NY-Wadsworth-21025952-01/2021 Isolate 1 (Lota)			100% (3/3)
B.1.617.2: Isolate: USA/PHC658/2021 (Delta)			100% (3/3)
USA-WA1/2020			100% (3/3)

[1]Testing at a lower concentration (i.e., 3x LoD) failed to yield 100% detection. The lowest concentration that yielded 100% detection was 9x LoD.

The results from this study demonstrate that the Respiratory *Flex* Assay is capable of detecting multiple clinically relevant strains of each target analyte.

In silico

The inclusivity of the LIAISON PLEX Respiratory *Flex* Assay was evaluated using *in silico* analysis of the oligonucleotides (i.e., forward primer(s), reverse primer(s), capture and mediator probe(s)) for all assay targets in relation to sequences available in GISAID (for SARS-CoV-2, influenza A, influenza A/H1, influenza A/H3, and influenza B) and sequences in NCBI GenBank for all other target organisms.

Sequence alignments were generated using a publicly available sequence alignment program. Sequences with full coverage of all four oligo-binding regions (forward primer, reverse primer, mediator probe and capture probe) were included in the analyses. Partial target sequences and sequences with ambiguous or degenerate bases in an oligo binding region were excluded. A match (i.e., predicted reactivity) was based on the following criteria for all organisms other than SARS-CoV-2: ≥90% homology between the oligo and reference sequences. If <90% homology was observed, a melting temperature (Tm) analysis was performed using a publicly available Tm calculator and applying assay specific conditions. The Tm analysis involved comparing the calculated mismatch Tm values against the assay's PCR annealing temperature for the primers, against the RT temperature for the RT primers, and against the target hybridization temperature for the capture and mediator probes. If the Tm of the mismatch oligo:sequence pair was above the assay's temperature for the pertinent RT-PCR step (i.e., annealing, RT, or target hybridization), then reactivity was predicted. The ≥90% homology threshold was chosen because the Respiratory *Flex* Assay's oligos are designed to have a much higher Tm than the annealing/RT temperature for primers and the hybridization temperature for the capture and mediator probes. As such, the longest oligos in the Respiratory Flex Assay are more tolerant to mismatches.

For SARS-CoV-2, the criteria were stricter due to the propensity of this organism to acquire point mutations. Specifically, 100% homology was expected between the oligo and reference sequences for at least one gene oligo set. If <100% homology was observed for all gene oligo sets, the same melting temperature analysis and reactivity criteria described above were employed.

For SARS-CoV-2, 5,622,325 sequences in GISAID (as of July 31, 2023) were included in the analysis. These sequences included all variants of concern or variants of interest defined as of July 31, 2023. The LIAISON PLEX Respiratory *Flex* Assay targets three SARS-CoV-2 gene regions. 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 0.04% (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.

For influenza A, influenza A H1, influenza A H3, and influenza B, sequences uploaded to GISAID between September 1, 2015, and July 7, 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 24**. Based on the reactivity criteria (>90% homology), 99.9% (112,034/112,056) of influenza A (matrix gene) 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 24. 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.

For all other target organisms, *in silico* inclusivity analyses was performed using sequences available from the GenBank nt database as of July 7, 2023. The majority of sequences evaluated for each target organism are expected to be detected by the Respiratory *Flex* Assay.

Cross-Reactivity

a. Cross-Reactivity Wet-Testing

i. Off-Panel Cross-Reactivity

This study evaluated the analytical specificity (cross-reactivity) of the Respiratory *Flex* Assay in the presence of non-targeted microorganisms that may be found in a respiratory tract clinical specimen. Sixty (60) non-target microorganisms (**Table 25**) were evaluated in the study. Panel members were composed of one individual non-target microorganism spiked into simulated NPS matrix at $\geq 10^5$ $TCID_{50}$/mL (or equivalent) for viruses, $\geq 10^6$ CFU/mL (or equivalent) for bacteria/fungi, or the highest concentration available. Data supporting the use of simulated matrix can be found in section **VII Performance Charactestics.B.2.Matrix Equivalency Study**, later in this document. For *Mycobacterium tuberculosis*, genomic DNA was evaluated to minimize pathogenic exposure to the test operator. Additionally, DNA was evaluated for *Bordetella parapertussis* Bpp5, which was identified by *in silico* analysis to contain a sequence that was between 80.0% - 95.8% identical with the primers, mediators, or capture oligos of the Respiratory *Flex Bordetella pertussis* assay. Wet testing was performed with synthetic DNA consisting of the sequence for the matching region of the *B. parapertussis* genome to determine if cross-reactivity with the *Bordetella pertussis* assay occurred.

To evaluate cross-reactivity, each panel was evaluated in triplicate in the absence of the target organisms. No cross-reactivity was observed at the concentrations tested except for *Mycoplasma genitalium* at a concentration of $4x10^6$ CCU/mL, which cross-reacted with the *Mycoplasma pneumoniae* assay in 50% of replicates (3/6). The concentration of

Mycoplasma genitalium was lowered to $1x10^6$ and $4x10^5$ CCU/mL and tested; cross-reactivity no longer occurred at either of these lower concentrations.

Table 25. Off-Panel Organisms Evaluated for Cross-Reactivity

Organism	Conc./Unit	Organism	Conc./Unit
Acinetobacter baumannii	$1x10^6$ CFU/mL	*Legionella pneumophila*	$4x10^5$ CFU/mL
Aspergillus flavus	$1x10^6$ CFU/mL	*Listeria innocua*	$1x10^6$ CFU/mL
Aspergillus fumigatus	$4x10^5$ CFU/mL	*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*	$1x10^5$ $TCID_{50}$/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
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

CFU = Colony Forming Units; CCU = Colony Changing Units; IFU = Inclusion Forming Units; $TCID_{50}$ = 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 cross-reactivity study since the targeted sequence was not expected to be detected as *B. parapertussis* by the assay.

[2]No concentration provided by the supplier.

[3]At 4x10[6] CCU/mL, *Mycoplasma genitalium* was cross-reactive with the *Mycoplasma pneumoniae* assay in 50.0% (3/6) of replicates. The *Mycoplasma genitalium* concentration was lowered to 1x10[6] CCU/mL and 4x10[5] CCU/mL and had 0% (0/3) positivity for both concentrations.

ii. On-Panel Cross-Reactivity

Potential intra-panel cross-reactivity was evaluated with twenty-eight (28) on-panel microorganisms (**Table 26**). The on-panel organisms were evaluated by spiking each independently into simulated NPS matrix at $\geq 10^5$ $TCID_{50}$/mL (or equivalent) for viruses and $\geq 10^6$ CFU/mL (or equivalent) for bacteria, or the highest concentration available. Data supporting the use of simulated matrix can be found in section **VII Performance Charactestics.B.2.Matrix Equivalency Study**, later in this document. To evaluate potential intra-panel cross-reactivity, each panel was evaluated in triplicate. The results of testing are shown in **Table 26**.

Table 26. On-Panel Organisms Evaluated for Cross-Reactivity

Organism	Conc./Unit	Expected Target Positivity	Unexpected Target Positivity
Adenovirus	1x10[5] $TCID_{50}$/mL	100% (3/3)	0% (0/3)
Bordetella holmesii	1x10[6] CFU/mL	100% (3/3)	0% (0/3)
Bordetella parapertussis	1x10[6] CFU/mL	100% (3/3)	0% (0/3)
Bordetella pertussis	1x10[6] CFU/mL	100% (3/3)	0% (0/3)
Chlamydia pneumoniae	1x10[6] IFU/mL	100% (3/3)	0% (0/3)
Human Coronavirus 229E	1x10[5] $TCID_{50}$/mL	100% (3/3)	0% (0/3)
Human Coronavirus HKU1 (Lum2020-Resp-1528)	6.62x10[4] copies/mL	100% (3/3)	0% (0/3)
Human Coronavirus NL63	1x10[5] $TCID_{50}$/mL	100% (3/3)	0% (0/3)
Human Coronavirus OC43	1x10[5] $TCID_{50}$/mL	100% (3/3)	0% (0/3)
Echovirus (Enterovirus/Rhinovirus)	1x10[5] $TCID_{50}$/mL	100% (3/3)	0% (0/3)
Human Metapneumovirus	1x10[5] $TCID_{50}$/mL	100% (3/3)	0% (0/3)
Influenza B (Washington/02/2019/Victoria Lineage)	1x10[5] $TCID_{50}$/mL	100% (3/3)	0% (0/3)
Influenza B (Phuket/3073/13/Yamagata Lineage)	1x10[5] $TCID_{50}$/mL	100% (3/3)	0% (0/3)
Mycoplasma pneumoniae	1x10[6] CCU/mL	100% (3/3)	0% (0/3)
Parainfluenza 1	1x10[5] $TCID_{50}$/mL	100% (3/3)	0% (0/3)
Parainfluenza 2	1x10[5] $TCID_{50}$/mL	100% (3/3)	0% (0/3)
Parainfluenza 3	1x10[5] $TCID_{50}$/mL	100% (3/3)	0% (0/3)
Parainfluenza 4	1x10[5] $TCID_{50}$/mL	100% (3/3)	0% (0/3)

RSV A	$1x10^5$ PFU/mL	100% (3/3)	0% (0/3)
RSV B	$1x10^5$ $TCID_{50}$/mL	100% (3/3)	0% (0/3)
Influenza A H1N1	$1x10^5$ $TCID_{50}$/mL	Matrix: 100% (3/3) Subtype H1: 100% (3/3)	0% (0/3)
Influenza A H3N2	$1x10^5$ $TCID_{50}$/mL	Matrix: 100% (3/3) Subtype H3: 100% (3/3)	0% (0/3)
Influenza A H5N1[1]	$2x10^7$ copies/mL	Matrix: 100% (3/3)	0% (0/3)
Influenza A H7N2[1]	$6x10^6$ copies/mL	Matrix: 100% (3/3)	0% (0/3)
Influenza A H7N7[1]	$2x10^7$ copies/mL	Matrix: 100% (3/3)	0% (0/3)
Influenza A H9N2[1]	$4x10^8$ copies/mL	Matrix: 100% (3/3)	0% (0/3)
Influenza A H1N2[2]	$3x10^7$ copies/mL	Matrix: 100% (3/3) Subtype H1: 100% (3/3)	0% (0/3)
SARS-CoV-2	$1x10^5$ $TCID_{50}$/mL	100% (3/3)	0% (0/3)

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.

b. *In silico*

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 27**. The LIAISON PLEX Respiratory *Flex* assays were shown to be specific for their respective analytes with the following exceptions, which are noted in the device labeling:

- 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 IS1001;
- 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 27. Organisms Predicted by *In Silico* Analyses to Cross-React with the Respiratory Flex Assay.

Assay	Predicted Cross-Reaction
Adenovirus	Adenovirus G (serotype 52) - strains
SARS-CoV-2	Bat coronavirus and Bat SARS-like coronavirus (accessions MG772933, MG772934, and MN996532)
Bordetella parapertussis	*Bordetella bronchiseptica* containing IS1001 element (accessions JX013523 to JX013527 and CP022962)
Influenza A H1	H5N1 (accession CY110922)[1]; 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]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.

Microbial Interference

To determine if non-target organisms can interfere with detection of on-panel organisms in the same sample, a microbial interference study was conducted. The study evaluated 16 non-target organisms spiked into simulated NPS matrix at $\geq 10^5$ $TCID_{50}$/mL for viruses and $\geq 10^6$ CFU/mL for bacteria/fungi, or the highest concentration available. Data supporting the use of simulated matrix can be found in section **VII Performance Charactestics.B.2.Matrix Equivalency Study**, later in this document. In addition to each non-target organism, each sample was co-spiked with five representative on-panel targets (i.e., *B. pertussis*, adenovirus, influenza B, hMPV, and SARS-CoV-2) at 3x LoD. To evaluate microbial interference, testing was performed in triplicate. If on-panel organisms were detected at <100%, samples were reprepared at the same concentration and retested in triplicate. If 100% detection of the on-panel targets was obtained during retesting, no additional testing was performed. When this situation was encountered, the original and retesting results were pooled for performance calculations. Results of the study are shown in **Table 28**. No microbial interference was observed except for *Streptococcus pyogenes* at $1x10^6$ CFU/mL and *Legionella pneumophilia* at $4x10^5$ CFU/mL, which interfered with detection of adenovirus in 16.7% (1/6) of replicates.

Table 28. Microbial Interference Study Results

Off-Panel Organism	Conc./Unit	% Positivity (# Detected/# Tested)				
		Adenovirus	*B. pertussis*	Flu B	hMPV	SARS-CoV-2
Candida albicans	$1x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Corynebacterium diphtheriae	$1x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

Cytomegalovirus	$1x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Haemophilus influenzae	$1x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Herpes Simplex Virus 1	$1x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
MERS	NA	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Neisseria meningitidis	$1x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Staphylococcus aureus	$1x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Pseudomonas aeruginosa	$1x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Streptococcus pneumoniae	$1x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Streptococcus pyogenes	$1x10^6$ CFU/mL	83.3% (5/6)[1]	100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)
SARS	NA	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Legionella pneumophilia	$4x10^5$ CFU/mL	83.3% (5/6)[1]	100% (6/6)	100% (6/6)	100% (6/6)	100% (6/6)
Measles	$1x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Moraxella catarrhalis	$1x10^6$ CFU/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
Mumps	$1x10^5$ $TCID_{50}$/mL	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

[1]The original three replicates tested resulted in 66.7% (2/3) positivity for adenovirus. New test material was prepared and tested, resulting in 100% (3/3) positivity for adenovirus.

Interfering Substances

An analytical study was performed to assess the potential inhibitory effects of exogenous and endogenous substances that may be commonly found in NPS specimens. A representative panel of five Respiratory *Flex* target organisms - *Bordetella pertussis*, adenovirus, influenza B, hMPV, and SARS-CoV-2 were co-spiked at 3x LoD in simulated NPS matrix and challenged with the 34 substances illustrated in **Table 29**. Testing was performed in triplicate. Data supporting the use of simulated matrix can be found in section **VII Performance Charactestics.B.2.Matrix Equivalency Study**, later in this document.

Table 29. Substances Evaluated for Interference

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
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.

If an on-panel organism in the panel was detected at <100%, samples were reprepared at the same concentration (for both the on-panel organisms and the potential interfering substance) and retested in triplicate. If 100% detection of the on-panel targets was obtained during retesting, no additional testing was performed. When this situation was encountered, the original and retesting results were pooled for performance calculations. If less than 100% detection was observed during retesting, the sample was reprepared at a lower concentration of the interfering substance and tested until 100% detection was achieved.

No interference was observed except for the substances illustrated in **Table 30** and described in more detail below:

- Human sputum/mucus (prepared by coating a nylon NPS swab with human sputum/mucus, eluting the swab in 1 mL of simulated NPS matrix containing 5 representative target organisms at 3x LoD, and then testing the eluate) interfered with detection of hMPV, influenza B, and SARS-CoV-2. For hMPV and influenza B, interference was observed during both initial testing and retesting, so per the study protocol, testing was performed at a less challenging concentration until interference was no longer observed (i.e., eluting the

swab into 2 mL of simulated NPS matrix containing 5 representative target organisms at 3x LoD). Interference was no longer observed when human sputum/mucus was eluted into 2 mL of simulated NPS matrix containing 5 representative analytes.

- Human whole blood at 5.0% v/v interfered with detection of *Bordetella pertussis*, hMPV, and influenza B. For hMPV, interference was observed during both initial testing and retesting, so per the study protocol, testing was performed at a less challenging concentration until interference was no longer observed. Interference caused by human whole blood no longer occurred at a concentration of 4.0% v/v.
- Leukocytes at 1000 cells/µL interfered with detection of hMPV and SARS-CoV-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 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. Per the study protocol, testing was performed at more dilute concentrations until 100% detection occurred. Interference caused by leukocytes was no longer observed at a concentration of 333.3 cells/µL.
- Mupirocin at 3.0 µmol/L interfered with detection of influenza B in 33% (1/3) of replicates during initial testing. Upon repeat testing, no interference was observed.
- Tobramycin at 76.0 µmol/L interfered with detection of hMPV and SARS-CoV-2 in 50% (1/2) of replicates during initial testing. In addition, one replicate was invalid during original testing. Upon repeat testing, no interference was observed.

Table 30. Substances that Interfered with Detection of at Least One Target Organism

Active Ingredient	Test Conc.	% Positivity (# Detected/# Tested)				
		Adenovirus	*B. pertussis*	hMPV	Flu 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 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)	66.7% (4/6)[1]	83.3% (5/6)	100% (6/6)
	4.5% v/v	100% (3/3)	100% (3/3)	66.7% (2/3)	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 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 Interference (Co-Infection)

The impact of competitive interference, caused by co-infections with on-target analytes, was evaluated for the Respiratory *Flex* Assay by testing contrived samples consisting of an on-target analyte at a low concentration (3x LoD) in the presence of a different target organism at a high concentration ($\geq$1x10^5 $TCID_{50}$/mL, PFU/mL, or $CEID_{50}$/mL) in simulated NPS matrix. Data supporting the use of simulated matrix can be found in section **VII Performance Charactestics.B.2.Matrix Equivalency Study**, later in this document. For high concentration Coronavirus HKU1 testing, the highest concentration available (1.31x10^4 copies/mL) was used. Testing for each target organism (at low concentration) and each potential competitive strain (at high concentration) was performed in triplicate for the 54 co-infection combinations illustrated in **Table 31**. If competitive interference was observed during initial testing, samples were reprepared at the same concentration and retested in triplicate. If competitive interference was not observed during retesting, no additional testing was performed. When this situation was encountered, the original and retesting results were pooled for performance calculations. If competitive interference was observed during retesting, the high concentration analyte concentration was reduced, and additional testing was performed. Results of the study are shown in **Table 31**.

Table 31. Competitive Interference Study Sample Panel Composition & Study 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)
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]

Target 1 (High Conc.)		Target 2 (Low Conc.[1])	% Detected (# Detected/#Tested)	
Organism	**Conc. ($TCID_{50}$/mL[2])**	**Organism**	**Target 1**	**Target 2**
				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)
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)	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**
			Subtype H3: 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 OC43 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.

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).

4. Assay Reportable Range:
   Not applicable; this is a qualitative assay.

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

   a. Controls
   See Section **IV.C.Instrument Descriptive Information.5.Quality Control**.

   b. Sample Stability
   An analytical study was performed to establish the recommended transport and storage conditions for nasopharyngeal swab (NPS) specimens eluted in UVT /UTM that will be tested using the Respiratory *Flex* Assay. A representative panel of five Respiratory *Flex* target organisms (i.e., *Bordetella pertussis*, adenovirus, influenza B, hMPV, and SARS-CoV-2) was evaluated. Positive samples were prepared by co-spiking the five organisms at concentrations of 2x and 5x LoD in natural clinical NPS matrix. Negative samples, consisting of natural clinical NPS matrix, were also included in the study. Initial testing was performed to establish the baseline time point (t=0 hours) for the study, and additional aliquots of the samples were stored at each of the following temperature conditions: (1) room temperature (15-30°C), (2) refrigerated (2-8°C), and (3) Frozen (≤-70°C).

   At the designated time points shown in **Table 32** below, one positive sample at each concentration and a negative sample was tested with the Respiratory *Flex* Assay. The number of replicates performed for each storage condition and sample concentration is illustrated in **Table 33**.

Table 32. Overview of Specimen Stability Storage Conditions and Time Points

Test Time Point	Storage Temperature		
	15-30°C	**2-8°C**	**≤-70°C**
Baseline (0-hours)	X	X	X
5-hours	X	NA	NA
9-hours	X		
27-hours	NA	X	
72-hours		X	
80-hours		X	
36-days		NA	X

"X" indicates the testing time point for each target.
"NA" indicates the time point/storage condition as not part of the testing protocol.

Table 33. Number of Replicates Collected for Each Storage Condition and Sample Concentration

Refrigerated Storage					
Storage Temperature	**Sample Concentration**	**Baseline (0-hours)**	**27-Hours**	**72-Hours**	**80-Hours**
2°C	5x	10	10	10	10
	2x	40	40	40	40
	Negative	10	10	10	10
8°C	5x	10	10	10	10

	2x	40	40	40	40
	Negative	10	10	10	10
Room Temperature Storage					
Storage Temperature	**Sample Concentration**	**Baseline (0-hours)**	**5-Hours**	**9-Hours**	
30°C	5x	20	10	10	
	2x	80	40	40	
	Negative	20	10	10	
Frozen Storage					
Storage Temperature	**Sample Concentration**	**Baseline (0-hours)**	**36-Days**		
≤-70°C	5x	10	10		
	2x	40	40		
	Negative	10	10		

The results of this specimen stability study support the stability claims for the Respiratory *Flex* Assay of clinical NPS specimens in UTM/UVT at the following conditions:

- Up to 8-hours at 15-30°C
- Up to 72-hours at 2-8°C
- Up to 30 days at ≤-70°C

Freeze/Thaw Stability

The performance of the Respiratory *Flex* Assay with fresh and frozen specimens was evaluated by testing 4 sample panels, each consisting of 5-6 target analytes co-spiked into natural clinical NPS matrix (see **Table 34**). For each of the 19 Respiratory *Flex* Assay reportable targets, at least one organism was included in the study. Each positive panel was prepared at both 2x and 5x LoD. In addition, a negative sample was included the study.

Table 34. 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 may undergo up to 2 F/T cycles following storage at -70°C.

c. In-Use Stability

A 'loaded sample' study was evaluated to define the duration that the Respiratory *Flex* Assay cartridges can remain loaded with sample *prior* to being run on a LIAISON PLEX instrument. The loaded sample stability study evaluated 40 cartridges from a single reagent lot using a multi-analyte co-spiked positive target pool, that contained five targets (adenovirus, *B. pertussis*, human metapneumovirus, influenza B, and SARS-CoV-2) each at a final concentration of 5x LoD (for that target) in simulated NPS matrix. Data supporting the use of simulated matrix can be found in section **VII Performance Charactestics.B.2.Matrix Equivalency Study**, later in this document. Twenty valid cartridges were tested immediately following sample addition to the cartridge. Twenty valid cartridges were tested after adding the sample into the cartridge and being allowed to sit at room temperature (between 15°C - 30°C) for a minimum of three hours. The Respiratory *Flex* Assay run time is approximately two hours so performance of a loaded cartridge after three hours will allow a user to delay testing a prepared and loaded cartridge for approximately the duration of a single Respiratory *Flex* Assay run. All cartridges loaded with sample and tested immediately and loaded with sample and tested after three hours at room temperature resulted in 100% positivity for all targets. Results demonstrated that RSP *Flex* Assay cartridges loaded with sample are stable for up to three hours.

6. Detection Limit:

A limit of detection study (LoD) was performed to evaluate the analytical sensitivity of the Respiratory *Flex* Assay. For this study, thirty-eight (38) strains and isolates (**Table 35**) that represent the 19 reportable targets of the Respiratory *Flex* Assay were tested individually by serially diluting each target in NPS matrix. Testing was broken into two parts: preliminary and confirmatory LoD testing. For the preliminary LoD study, a 3-fold dilution series consisting of at least 3 concentrations was tested in replicates of six per dilution. The preliminary LoD for each target was defined as the lowest concentration at which 100% of replicates were positive for the intended reportable target. The confirmed LoD was determined by testing a 3-fold dilution series of concentrations around the preliminary LoD in replicates of 20. The confirmed LoD for each organism 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 LoDs for the Respiratory *Flex* Assay bacterial and viral analytes are illustrated in **Table 36** and **Table 37**, respectively.

Table 35. Strains Included in the LoD Study

Organism (Strain/Isolate)	Vendor	Catalog Number
Bordetella holmesii (F061)	Zeptometrix	0801464
Bordetella parapertussis (C510)	Zeptometrix	0801643
Bordetella pertussis (18323 NCTC 10739)	ATCC	9797
Chlamydia pneumoniae (CM-1)	ATCC	VR-1360
Mycoplasma pneumoniae (M129)	Zeptometrix	0801579
Adenovirus 1 (C)	Zeptometrix	0810050CF
Adenovirus 3 (B)	Zeptometrix	0810062CF
Adenovirus 31 (A)	Zeptometrix	0810073CF
Adenovirus 26 (D)	Zeptometrix	0810117CF
Adenovirus 40 (F)	Zeptometrix	0810084CF
Adenovirus 4 (E)	Zeptometrix	0810070CF
Human Coronavirus HKU1	NA[1]	
Human Coronavirus NL63	Zeptometrix	0810228CF
Human Coronavirus OC43	Zeptometrix	0810024CF

Human Coronavirus 229E	Zeptometrix	0810229CF
SARS-CoV-2 (USA-WA1/2020)	Zeptometrix	0810587CFHI
Echovirus Type 6	Zeptometrix	0810076CF
Rhinovirus (1A)	Zeptometrix	0810012CFN
Rhinovirus (B14)	Zeptometrix	0810284CF
Rhinovirus (C1)	NA[1]	
Human Metapneumovirus 3 (Type B1)	Zeptometrix	0810156CF
Human Metapneumovirus 8 (Type B2)	Zeptometrix	0810159CF
Human Metapneumovirus 9 (Type A1)	Zeptometrix	0810160CF
Human Metapneumovirus 27 (Type A2)	Zeptometrix	0810164CF
Influenza A H3N2 (A/Kansas/14/2017)	Zeptometrix	0810586CF
Influenza A H3N2 (Hongkong/2671-19)	Zeptometrix	0810609CF
Influenza A H3N2 (Singapore/INFIMH-160019/16)	Zeptometrix	0810574CF
Influenza A H1N1 (A/Brisbane/02/2018)	Zeptometrix	0810585CF
Influenza A H1N1 (Guangdong-Maonan/SWL 1536/19)	Zeptometrix	0810610CF
Influenza B (Alabama/2/17, Victoria Lineage)	Zeptometrix	0810572CF
Influenza B (Washington/02/19, Victoria Lineage)	Zeptometrix	0810611CF
Influenza B (Colorado/06/2017, Victoria Lineage)	Zeptometrix	0810573CF
Influenza B (Wisconsin/1/10, Yamagata Lineage)	Zeptometrix	0810241CF
Parainfluenza (Type 1)	Zeptometrix	0810014CF
Parainfluenza (Type 2)	Zeptometrix	0810015CF
Parainfluenza (Type 3)	Zeptometrix	0810016CF
Parainfluenza (Type 4A)	Zeptometrix	0810060CF
RSV A (2006 Isolate)	Zeptometrix	0810040ACF
RSV B (3/2015 Isolate #1)	Zeptometrix	0810479CF

[1]Clinical sample

Table 36. Confirmed LoD Results for Bacterial Targets

Target	Strain/ Isolate	Concentration at LoD		Percent Positive (# Positive/# Tested)
		Copies/mL[1]	CFU/mL[2]	
Bordetella holmesii	F061	$7.29x10^3$	86.8	95.0% (19/20)
Bordetella pertussis	18323 NCTC 10739	$3.80x10^3$	$1.98x10^3$	100% (20/20)
Bordetella parapertussis	C510	$7.90x10^2$	20.6	95.0% (19/20)
Chlamydia pneumoniae	CM-1	$5.68x10^2$	$1.04x10^2$ IFU/mL	100% (20/20)
Mycoplasma pneumoniae	M129	$1.30x10^3$	42.4 CCU/mL	95.0% (19/20)

[1]Concentrations in copies/mL were obtained by digital-droplet PCR.
[2]Concentrations in CFU/mL unless otherwise noted.

Table 37. Confirmed LoD Results for Viral Targets

Target	Strain/ Isolate	Concentration at LoD		Percent Positive (# Positive/# Tested)
		Copies/mL[1]	$TCID_{50}$/mL[2]	
Adenovirus (inclusive to A, B, C, D, E, and F serotypes)	31 (A)	$1.76x10^3$	$1.09x10^{-2}$	100% (20/20
	3 (B)	$6.86x10^2$	$1.69x10^{-1}$	100% (20/20)
	1 (C)	$1.12x10^3$	89.7	95.0% (19/20)
	26 (D)	$7.48x10^2$	$1.10x10^{-2}$	100% (20/20)
	4 (E)	$3.53x10^2$	$1.08x10^{-2}$	95.0% (19/20)
	40 (F)	$4.85x10^2$	$2.29x10^{-2}$	100% (20/20)
Human Coronavirus (inclusive to HKU1,	229E	$4.00x10^2$	$9.15x10^{-2}$	95.0% (19/20)
	HKU1	$1.67x10^3$	NA[3]	100% (20/20)
	NL63	76.4	$1.34x10^{-2}$	95.0% (19/20)

229E, NL63, and OC43)	OC43		$9.48x10^3$	$9.58x10^{-1}$	100% (20/20)
Human Metapneumovirus	hMPV-9 (A1)		$2.13x10^3$	$2.09x10^{-1}$	95.0% (19/20)
	hMPV-27 (A2)		$2.04x10^3$	$2.14x10^{-1}$	95.0% (19/20)
	hMPV-3 (B1)		$5.00x10^3$	$4.31x10^{-1}$	95.0% (19/20)
	hMPV-8 (B2)		$1.50x10^4$	1.66	95.0% (19/20)
Influenza A / Subtype H1	Brisbane	Matrix	$1.35x10^4$	3.97	100.0% (20/20)
		H1	$1.50x10^3$	$4.41x10^{-1}$	95.0% (19/20)
	Guangdong	Matrix	$1.37x10^4$	5.86	100.0% (20/20)
		H1	$1.37x10^4$	5.86	95.0% (19/20)
Influenza A / Subtype H3	Hong Kong	Matrix	$1.59x10^5$	15.0	95.0% (19/20)
		H3	$5.30x10^4$	4.98	100.0% (20/20)
	Kansas	Matrix	$1.96x10^3$	5.58	95.0% (19/20)
		H3	$1.96x10^3$	5.58	100.0% (20/20)
	Singapore	Matrix	$4.55x10^3$	11.0	100.0% (20/20)
		H3	$4.55x10^3$	11.0	100.0% (20/20)
Influenza B	Alabama/2/17 (Victoria lineage)		$3.35x10^2$	$7.30x10^{-1}$	95.0% (19/20)
	Washington/02/19 (Victoria lineage)		$3.02x10^3$	27.9	100.0% (20/20)
	Colorado/6/17 (Victoria lineage)		$3.02x10^3$	$6.64x10^{-1}$	100.0% (20/20)
	Wisconsin/1/10 (Yamagata lineage)		$1.01x10^3$	$3.23x10^{-1}$	95.0% (19/20)
Parainfluenza 1	NA		$7.61x10^2$	10.6	95.0% (19/20)
Parainfluenza 2	NA		$8.46x10^3$	15.5	95.0% (19/20)
Parainfluenza 3	NA		$1.93x10^3$	3.18	100.0% (20/20)
Parainfluenza 4	A		$5.76x10^3$	66.5	95.0% (19/20)
RSV (inclusive to RSV A and RSV B)	A (2006 Isolate)		$3.83x10^3$	1.11	95.0% (19/20)
	B (3/2015 Isolate #1)		$1.61x10^4$	$7.48x10^{-1}$	100.0% (20/20)
Enterovirus/ Rhinovirus	Human Rhinovirus 1A		$8.19x10^3$	$4.99x10^{-1}$	100.0% (20/20)
	Human Rhinovirus B14		$8.18x10^3$	11.0	100.0% (20/20)
	Human Rhinovirus C1		$1.92x10^4$	NA[3]	100.0% (20/20)
	Human Enterovirus Echovirus Type 6		$2.25x10^4$	30.0	95.0% (19/20)
SARS-CoV-2	USA-WA1/2020		$8.00x10^3$	40.4	95.0% (19/20)

[1]Concentrations in copies/mL were obtained by digital-droplet PCR.
[2]Concentrations in $TCID_{50}$/mL unless otherwise noted.
[3]Clinical sample without a titer in $TCID_{50}$/mL.

The LoD for co-analyte spiked samples was also evaluated and shown to be equivalent to single analyte spiked samples.

LoD 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. The WHO SARS-CoV-2 standard was reconstituted then serially diluted in NP matrix. As with the LoD study described above, testing was broken into two parts: preliminary and confirmatory LoD testing. For the preliminary LoD study, a 10-fold dilution series consisting of nine concentrations was tested in triplicate per dilution. 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.

7. Assay Cut-Off:
The Respiratory *Flex* Assay determines the presence/absence of each target analyte (and control) by comparing the mean intensity of capture spots to pre-defined thresholds. In addition to the capture spot thresholds, a set of assay-dependent parameters are used to evaluate the validity of the run. To determine the threshold and parameter values, a sequential, multi-step approach was employed.

   In the first step, a threshold training set consisting of 1905 samples from analytical testing was used to estimate the thresholds and assay-dependent parameters. Proposed threshold and assay-dependent parameter values were determined by their ability to distinguish between true and false target calls while minimizing the no call rate. The proposed thresholds were determined by the negative signal distribution, where the target is absent from the samples.

   In the second step of threshold determination, the performance of the proposed parameter and threshold values from step 1 were evaluated using the threshold test set, which consisted of 736 contrived/clinical samples. The clinical samples included in the Assay Cut-Off Study are independent from those used to demonstrate clinical performance of the Respiratory *Flex* Assay. The true call vs. no call rate for performance parameters and controls, and reportable target sensitivity and specificity were tabulated for presentation to a technical review team.

   In the third step, a technical review team consisting of subject matter experts of the assay and chemistry determined the final threshold values through an assessment of the results for each performance parameter, control, and reportable target. As part of this analysis, an ROC curve was generated for each spot group with the final established thresholds marked to illustrate the expected clinical performance of the Respiratory *Flex* Assay. The specific assay parameters and thresholds are not reported in this document, as they are considered confidential and proprietary.

8. Accuracy (Instrument):
Not applicable.

9. Carryover:
An analytical study was performed to assess potential carryover or cross-contamination in the Respiratory *Flex* Assay by testing high positive and negative samples in an alternating fashion on the LIAISON PLEX instrument. The high positive samples consisted of a representative panel of five assay targets (i.e., *B. pertussis*, adenovirus, influenza B, hMPV, and SARS-CoV-2). Contrived positive samples were prepared by spiking *B. pertussis* at $1x10^6$ CFU/mL and the viral targets at $1x10^5$ $TCID_{50}$/mL into simulated NPS matrix. Data supporting the use of simulated matrix can be found in section **VII Performance Charactestics.B.2.Matrix Equivalency Study**, later in this document.

   The study was performed by two operators, each using one of two LIAISON PLEX instruments, each containing six modules. Alternating high positive and negative samples were evaluated per the format summarized in **Table 38**. In total, 30 high positive and 30 negative samples were evaluated. All high positive samples yielded positive results for the five analytes in the sample, while all negative samples were negative. These results confirm that there is no evidence of carryover from samples tested with the Respiratory *Flex* Assay.

Table 38. Overview of Carryover/Cross-contamination Study Design

Operator	LIAISON PLEX Instrument	Test Round	Module					
			1	2	3	4	5	6
1	1	1	POS	NEG	POS	NEG	POS	NEG
		2	NEG	POS	NEG	POS	NEG	POS
		3	POS	NEG	POS	NEG	POS	NEG
		4	NEG	POS	NEG	POS	NEG	POS
		5	POS	NEG	POS	NEG	POS	NEG
2	2	1	NEG	POS	NEG	POS	NEG	POS
		2	POS	NEG	POS	NEG	POS	NEG
		3	NEG	POS	NEG	POS	NEG	POS
		4	POS	NEG	POS	NEG	POS	NEG
		5	NEG	POS	NEG	POS	NEG	POS

POS-Positive; NEG-Negative

B Comparison Studies:

1. Method Comparison with Predicate Device:
   Not applicable.

2. Matrix Comparison:

   Matrix Equivalency Study
   This study was designed to demonstrate the equivalence of simulated NPS matrix used in analytical studies, with clinically-derived natural NPS matrix. Simulated NPS matrix was formulated to resemble the content of a clinical NPS specimen collected in VTM, such that the simulated matrix would not artificially alter the performance of the test. The simulated NPS matrix consisted of $2x10^3$ HeLa cells/mL in UTM. The natural clinical NPS matrix consisted of NPS samples collected in UTM, which were negative for all Respiratory *Flex* Assay targets.

   For this study, a representative panel of five assay targets (i.e., *B. pertussis*, adenovirus, influenza B, hMPV, and SARS-CoV-2) were co-spiked into pooled negative natural clinical NPS matrix and simulated NPS matrix at <1x LoD, 2x LoD, and 5x LoD. Aliquots of negative matrices were also included in the evaluation. Positive samples spiked at <1x LoD and 5x LoD were tested in replicates of 20 per condition, while positive samples prepared at 2x LoD were tested in replicates of 60 per condition. Negative samples in natural clinical NPS matrix were tested in replicates of 22 per condition, while negative samples in simulated NPS matrix were tested in replicates of 20 per condition. The results of the study are shown in **Table 39**.

Table 39. Matrix Equivalency Study Results

Target	% Positivity (# Tested/# Positive)							
	5x LoD		2x LoD		<1x LoD		Negative	
	Simulated	Clinical	Simulated	Clinical	Simulated	Clinical	Simulated	Clinical
Adenovirus	95.0% (19/20)	95.0% (19/20)	95.0% (57/60)	96.7% (58/60)	30.0% (6/20)	35.0% (7/20)	0.0% (0/20)	0.0% (0/22)
B. pertussis	100% (20/20)	100% (20/20)	100% (60/60)	98.3% (59/60)	65.0% (13/20)	70.0% (14/20)	0.0% (0/20)	0.0% (0/22)
Influenza B	100% (20/20)	100% (20/20)	100% (60/60)	98.3% (59/60)	60.0% (12/20)	60.0% (12/20)	0.0% (0/20)	0.0% (0/22)

hMPV	100% (20/20)	100% (20/20)	100% (60/60)	100% (60/60)	55.0% (11/20)	30.0% (6/20)	0.0% (0/20)	0.0% (0/22)
SARS-CoV-2	100% (20/20)	100% (20/20)	100% (60/60)	98.3% (59/60)	70.0% (14/20)	80.0% (16/20)	0.0% (0/20)	0.0% (0/22)

All samples spiked at 5x LoD and 2x LoD met the acceptance criteria of 100% agreement and 95% agreement with expected results, respectively, except for adenovirus at 5x LoD, which was detected in 95% (19/20) of replicates in both simulated and natural clinical matrices. Since the results were adenovirus at 5x LoD were equivalent between matrices, they were deemed acceptable. As expected, detection varied for samples prepared at <1x LoD. All negative samples were negative, as expected. The results from the study indicate that performance of the Respiratory *Flex* Assay is equivalent with a representative panel of analytes seeded into natural clinical NPS matrix in UTM and simulated NPS matrix in UTM and thus support use of simulated NPS matrix in select analytical validation studies.

C Clinical Studies:

Prospective Clinical Study

The clinical performance of the LIAISON PLEX Respiratory *Flex* Assay was established in a multi-center study conducted with nasopharyngeal swab (NPS) specimens in BD UVT and Copan UTM collected from individuals with signs and symptoms of respiratory infection. Specimens were prospectively collected (i.e., all comers between two time points that met the clinical study inclusion criteria) during the 2022-2023 respiratory illness season (i.e., October 2022 thru April 2023). Specimens were stored refrigerated at 2-8°C 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°C (Category II specimens). In total, six geographically distinct sites in the U.S. were involved in the prospective collection of NPS specimens. Of these six sites, three sites conducted LIAISON PLEX Respiratory *Flex* Assay testing. A fourth site (Luminex), which was not involved in the prospective collection, also performed Respiratory *Flex* Assay testing.

A total of 1911 NPS specimens were enrolled in the study. Of these 1911 specimens, 68 were excluded due to protocol deviations (e.g., specimen not stored appropriately, insufficient volume, etc.). This left 1843 clinical specimens for evaluation. Of these 1843 specimens, 66.3% (1221/1843) were tested fresh (Category I specimens), while 33.7% (622/1843) were tested frozen (Category II specimens). Patient demographic information for the 1843 prospectively collected NPS specimens is presented in **Table 40**.

Table 40. Demographic Data for Prospectively Collected Specimens

		Overall	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site 5**	**Site 6**
Sex	Male	839 (45.5%)	271 (52.9%)	43 (37.7%)	143 (43.2%)	354 (44.5%)	18 (35.3%)	10 (25.0%)
	Female	1004 (54.5%)	241 (47.1%)	71 (62.3%)	188 (56.8%)	441 (55.5%)	33 (64.7%)	30 (75.0%)
	Total	**1843 (100%)**	**512 (100%)**	**114 (100%)**	**331 (100%)**	**795 (100%)**	**51 (100%)**	**40 (100%)**
Age (Years)	0-1	350 (19.0%)	205 (40.0%)	4 (3.5%)	62 (18.7%)	75 (9.4%)	4 (7.8%)	0 (0.0%)
	>1-5	274 (14.9%)	146 (28.5%)	7 (6.1%)	49 (14.8%)	61 (7.7%)	11 (21.6%)	0 (0.0%)
	>5-21	447	158	36	57	165	24	7

		(24.3%)	(30.9%)	(31.6%)	(17.2%)	(20.8%)	(47.1%)	(17.5%)
	>21-65	535 (29.0%)	3 (0.6%)	63 (55.3%)	126 (38.1%)	303 (38.1%)	12 (23.5%)	28 (70.0%)
	>65	237 (12.9%)	0 (0.0%)	4 (3.5%)	37 (11.2%)	191 (24.0%)	0 (0.0%)	5 (12.5%)
	Total	**1843 (100%)**	**512 (100%)**	**114 (100%)**	**331 (100%)**	**795 (100%)**	**51 (100%)**	**40 (100%)**

The LIAISON PLEX Respiratory *Flex* Assay was evaluated for 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 41**). The FDA-cleared comparator assays were performed in accordance with their respective package inserts.

Table 41. 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 testing, 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. The final number of evaluable results for each assay target is shown in **Table 42**.

Table 42. Total Evaluable Results for Prospective Specimens, Stratified by Analyte

Target	Comparator Method	Total Specimens	Respiratory *Flex* Invalid Results[1]	Respiratory *Flex* Valid AND Comparator Results Unavailable[2]	Total Evaluable Results
Adenovirus	FDA-cleared molecular respiratory panel	1843	11	12	1820
Bordetella holmesii	FA & PCR/BDS	1843	11	102	1730
Bordetella parapertussis		1843	11	59	1773
Bordetella pertussis		1843	11	79	1753
Chlamydia pneumoniae	FDA-cleared molecular respiratory panel	1843	11	12	1820
Human Coronavirus		1843	11	12	1820
Enterovirus/Rhinovirus		1843	11	12	1820
hMPV		1843	11	12	1820
Influenza A		1843	11	12	1820
Influenza A subtype H1		1843	11	12	1820
Influenza A subtype H3		1843	11	12	1820
Influenza B		1843	11	12	1820
Mycoplasma pneumoniae		1843	11	12	1820
Parainfluenza 1		1843	11	12	1820
Parainfluenza 2		1843	11	12	1820
Parainfluenza 3		1843	11	12	1820
Parainfluenza 4		1843	11	12	1820
RSV		1843	11	12	1820
SARS-CoV-2	FDA-cleared molecular SARS-CoV-2 assay	1843	11	52	1780

[1]Eleven specimens were invalid after repeat testing with the Respiratory *Flex* Assay. Of these 11 specimens, 6 specimens remained invalid on repeat, while 5 specimens were not retested with the Respiratory *Flex* Assay.
[2]Unavailable indicates a specimen that was invalid by the comparator method or not tested with the comparator method.

A summary of the LIAISON PLEX Respiratory *Flex* Assay prospective clinical study performance is provided in **Table 43**. 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 an FDA-cleared molecular respiratory panel or PCR/BDS for investigation.

Table 43. 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	82.1-94.7	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***
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

Analyte		Positive Percent Agreement			Negative Percent Agreement		
		TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI
	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.

Positive Percent Agreement (PPA) ranged from 80.0%-100%, depending on the analyte. The only analytes for which PPA was lower than expected were *B. parapertussis* with a PPA of 80.0% (4/5) and parainfluenza 4 with a PPA of 88.9% (8/9). The limited number of positive NPS specimens for these pathogens required supplementation with archived (Category III specimens) to obtain accurate PPA point estimates.

Negative Percent Agreement (NPA) ranged from 95.7-100%.

The Respiratory *Flex* Assay reported multiple organism detections (coinfections) in a total of 176 prospectively collected NPS specimens. Of these 176 specimens, 3 specimens lacked comparator results for least one organism identified in the coinfection, and thus were excluded from further analysis. The remaining 173 specimens with coinfections represent 14.6% (173/1187) of all positive prospective specimens and 9.4% (173/1840) of all prospective specimens. Most coinfections contained two organisms (87.3%, 151/173) while 11.6% (20/173) contained three organisms and 1.2% (2/173) contained four organisms. Out of the 173 specimens with coinfections, 44.5% (77/173) contained one or more organisms that were not detected by the comparator methods (**Table 44**). Coinfections identified by the comparator methods which were not reported by the LIAISON PLEX Respiratory *Flex* Assay are illustrated in **Table 45**.

Table 44. Coinfections Reported by the LIAISON PLEX Respiratory *Flex* Assay in the Prospective Study

Analyte 1	Analyte 2	Analyte 3	Analyte 4	Number of Specimens		Respiratory *Flex* Assay False Positive Analyte(s)[1]
				Total	False Positive	
Adenovirus	*Bordetella parapertussis*	Enterovirus/ Rhinovirus		2	2	Adenovirus (2), *Bordetella parapertussis* (1)
Adenovirus	Coronavirus			10	5	Adenovirus (5)
Adenovirus	Coronavirus	Enterovirus/ Rhinovirus		1	1	Adenovirus (1)
Adenovirus	Coronavirus	hMPV		1	1	hMPV (1)
Adenovirus	Coronavirus	PIV-3		1	0	--
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 (1)
Adenovirus	Enterovirus/ Rhinovirus	PIV-1		1	1	Adenovirus (1)
Adenovirus	Enterovirus/ Rhinovirus	RSV		1	1	Adenovirus (1), Enterovirus/ Rhinovirus (1)
Adenovirus	Enterovirus/	SARS-CoV-2		1	0	--

Analyte 1	Analyte 2	Analyte 3	Analyte 4	Number of Specimens		Respiratory *Flex* Assay False Positive Analyte(s)[1]
				Total	False Positive	
	Rhinovirus					
Adenovirus	hMPV			7	4	Adenovirus (4)
Adenovirus	hMPV	PIV-2		1	0	--
Adenovirus	hMPV	SARS-CoV-2		1	1	Adenovirus (1)
Adenovirus	Influenza A & Influenza A (subtype H1)			2	2	Adenovirus (2)
Adenovirus	Influenza A & Influenza A (subtype H3)			2	2	Adenovirus (2)
Adenovirus	Influenza B			1	1	Adenovirus (1)
Adenovirus	PIV-1	RSV		1	1	Adenovirus (1)
Adenovirus	PIV-2			1	1	Adenovirus (1)
Adenovirus	PIV-3			5	4	Adenovirus (4)
Adenovirus	RSV			4	3	Adenovirus (3)
Adenovirus	SARS-CoV-2			2	2	Adenovirus (2)
Bordetella parapertussis	Coronavirus			1	1	*Bordetella parapertussis* (1)
Bordetella parapertussis	Enterovirus/ Rhinovirus			2	1	*Bordetella parapertussis* (1)
Bordetella parapertussis	hMPV			1	0	--
Bordetella parapertussis	PIV-3			1	0	--
Coronavirus	Enterovirus/ Rhinovirus			8	2	Coronavirus (1), Enterovirus/ Rhinovirus (1)
Coronavirus	Enterovirus/ Rhinovirus	hMPV		1	0	--
Coronavirus	Enterovirus/ Rhinovirus	SARS-CoV-2		1	0	--
Coronavirus	hMPV			6	2	Coronavirus (2)
Coronavirus	Influenza A & Influenza A (subtype H1)			1	0	--
Coronavirus	PIV-3			3	0	--
Coronavirus	RSV			2	0	--
Coronavirus	SARS-CoV-2			4	1	Coronavirus (1)
Enterovirus/ Rhinovirus	hMPV			11	1	Enterovirus/ Rhinovirus (1)
Enterovirus/ Rhinovirus	hMPV	Influenza A (subtype H3)	SARS-CoV-2	1	1	Enterovirus/ Rhinovirus (1), Influenza A (subtype H3) (1), SARS-CoV-2 (1)
Enterovirus/ Rhinovirus	hMPV	SARS-CoV-2		1	1	Enterovirus/ Rhinovirus (1)
Enterovirus/ Rhinovirus	Influenza A & Influenza A (subtype H1)			2	0	--
Enterovirus/ Rhinovirus	Influenza A & Influenza A (subtype H3)			7	2	Enterovirus/ Rhinovirus (2)

Analyte 1	Analyte 2	Analyte 3	Analyte 4	Number of Specimens		Respiratory *Flex* Assay False Positive Analyte(s)[1]
				Total	False Positive	
Enterovirus/ Rhinovirus	Influenza A & Influenza A (subtype H3)	RSV		2	2	Influenza A (2), Influenza A (subtype H3) (1)
Enterovirus/ Rhinovirus	PIV-1			3	1	Enterovirus/ Rhinovirus (1)
Enterovirus/ Rhinovirus	PIV-3			5	2	Enterovirus/ Rhinovirus (2)
Enterovirus/ Rhinovirus	PIV-4			4	1	PIV-4 (1)
Enterovirus/ Rhinovirus	RSV			9	0	--
Enterovirus/ Rhinovirus	SARS-CoV-2			5	2	Enterovirus/ Rhinovirus (2)
hMPV	RSV			1	1	hMPV (1)
Influenza A & Influenza A (subtype H1)	Influenza A (subtype H3)			1	0	--
Influenza A & Influenza A (subtype H1)	SARS-CoV-2			1	1	SARS-CoV-2 (1)
Influenza A & Influenza A (subtype H3)	RSV			1	1	Influenza A (1)
PIV-2	SARS-CoV-2			1	0	--
PIV-3	RSV			1	0	--
PIV-3	SARS-CoV-2			1	1	SARS-CoV-2 (1)
Total				**173**	**77**	
Total Double Infections				**151**	**62**	
Total Triple Infections				**20**	**13**	
Total Quadruple Infections				**2**	**2**	

[1]Based on comparator test results

Thirty-four (34) specimens were positive for two or more analytes (i.e., coinfected) by the comparator methods but were reported as negative for one of the analytes using the LIAISON PLEX Respiratory *Flex* Assay (**Table 45**).

Table 45. Coinfections Identified by the Comparator Methods which were Not Reported by the LIAISON PLEX Respiratory *Flex* Assay in the Prospective Study

Analyte 1	Analyte 2	Analyte 3	Number of Specimens		Respiratory *Flex* Assay False Negative Analyte(s)[1]
			Total	False Negative	
Adenovirus	Coronavirus		7	1	Coronavirus (1)
Adenovirus	Enterovirus/ Rhinovirus		18	2	Enterovirus/ Rhinovirus (2)
Adenovirus	Enterovirus/ Rhinovirus	PIV-2	1	1	PIV-2 (1)
Adenovirus	hMPV		3	1	hMPV (1)
Adenovirus	hMPV	SARS-CoV-2	1	1	SARS-CoV-2 (1)
Adenovirus	SARS-CoV-2		1	1	SARS-CoV-2 (1)
Coronavirus	Enterovirus/ Rhinovirus		8	1	Coronavirus (1)
Coronavirus	Enterovirus/	SARS-CoV-2	2	1	Coronavirus (1)

	Rhinovirus				
Coronavirus	hMPV		6	2	Coronavirus (2)
Coronavirus	Influenza A & Influenza A (subtype H3)		1	1	Coronavirus (1)
Coronavirus	PIV-3		5	2	Coronavirus (1), PIV-3 (1)
Coronavirus	RSV		3	1	Coronavirus (1)
Influenza A & Influenza A (subtype H3)	PIV-4		1	1	PIV-4 (1)
Influenza A & Influenza A (subtype H3)	RSV		1	1	RSV (1)
hMPV	SARS-CoV-2		4	1	hMPV (1)
RSV	hMPV		1	1	hMPV (1)
RSV	SARS-CoV-2		1	1	RSV (1)
Enterovirus/ Rhinovirus	*Bordetella Parapertussis*		3	1	*Bordetella parapertussis* (1)
Enterovirus/ Rhinovirus	hMPV		11	1	hMPV (1)
Enterovirus/ Rhinovirus	Influenza A & Influenza A (subtype H1)		6	3	Enterovirus/Rhinovirus (3)
Enterovirus/ Rhinovirus	Influenza A (subtype H3)		1	1	Influenza A (subtype H3) (1)
Enterovirus/ Rhinovirus	Influenza A & Influenza A (subtype H3)	SARS-CoV-2	1	1	SARS-CoV-2 (1)
Enterovirus/ Rhinovirus	RSV		13	3	Enterovirus/Rhinovirus (3)
Enterovirus/ Rhinovirus	SARS-CoV-2		5	3	Enterovirus/Rhinovirus (2), SARS-CoV-2 (1)
PIV-3	Enterovirus/ Rhinovirus	RSV	1	1	PIV-3 (1), Enterovirus/ Rhinovirus (1)
		Total	**105**	**34**	
		Total Double Infections	**99**	**29**	
		Total Triple Infections	**6**	**5**	

[1]Based on comparator test results

Retrospective Clinical Study

Several analytes included on the Respiratory *Flex* Assay were not encountered during the Prospective Clinical Study in sufficient numbers to demonstrate assay performance. Therefore, the Prospective Clinical Study was supported by additional testing that was performed at three U.S. study sites (including one internal) on frozen, archived specimens (Category III specimens) obtained from four clinical laboratories in the U.S. These specimens were selected for inclusion in the study based solely on the historic qualitative result.

A total of 256 archived NPS specimens were evaluated, of which 66 negatives were included for blinding and randomization purposes. A summary of the demographic information of the tested retrospective specimens is provided in **Table 46** below.

Table 46. Demographic Data for Retrospective Specimens

		Overall
Sex	Male	117 (45.7%)
	Female	124 (48.4%)
	Unknown	15 (5.9%)
	Total	**256 (100%)**
Age	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%)**

The performance of the LIAISON PLEX Respiratory *Flex* Assay was determined by comparing to an FDA-cleared molecular respiratory panel for all analytes, except the following: *B. holmesii*, *B. parapertussis*, and *B. pertussis*. As noted previously in this Decision Summary, performance for the denoted *Bordetella* species was based on comparison to analytically validated Fragment Analysis (FA) assays followed by PCR/Bi-Directional Sequencing (PCR/BDS) assays.

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).

For the 256 pre-selected specimens, the final number of evaluable results varied by target based on valid comparator results obtained. The final number of evaluable results for each target is shown in **Table 47**.

Table 47. Total Evaluable Results for Retrospective Specimens, Stratified by Analyte

Target	Comparator Method	Total Specimens	Respiratory *Flex* Invalid Results[1]	Respiratory *Flex* Valid AND Comparator Results Unavailable[1]	Total Evaluable Results
Adenovirus	FDA-cleared molecular respiratory panel	256	0	0	256
Bordetella holmesii	FA & PCR/BDS	256	0	22	234
Bordetella parapertussis		256	0	12	244
Bordetella pertussis		256	0	16	240
Chlamydia pneumoniae	FDA-cleared molecular respiratory panel	256	0	0	256
Human Coronavirus		256	0	0	256
Enterovirus/Rhinovirus		256	0	0	256
hMPV		256	0	0	256
Influenza A		256	0	0	256
Influenza A subtype H1		256	0	0	256

Influenza A subtype H3		256	0	0	256
Influenza B		256	0	0	256
Mycoplasma pneumoniae		256	0	0	256
Parainfluenza 1		256	0	0	256
Parainfluenza 2		256	0	0	256
Parainfluenza 3		256	0	0	256
Parainfluenza 4		256	0	0	256
RSV		256	0	0	256

[1]Unavailable indicates a specimen that was invalid by the comparator method or not tested with the comparator method.

A summary of the LIAISON PLEX Respiratory *Flex* Assay retrospective clinical study performance, expressed as positive percent and negative percent agreements against the comparator method, are presented in **Table 48**.

Table 48. Retrospective Clinical Performance of the Respiratory *Flex* Assay vs. the Comparator Assay

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 Clinical Study

Some respiratory pathogens are so rare that both prospective and archived specimen collection efforts were insufficient to demonstrate clinical performance. To supplement the prospective clinical study and retrospective archived study data, an evaluation of contrived specimens was performed for five pathogens: *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. These contrived clinical specimens were prepared using unique negative clinical NPS specimens. Contrived specimens were prepared by spiking representative strains at concentrations of 2x, 10x, and 100x LoD. Fifty total positive samples for each pathogen were prepared, interspersed with negative samples, and randomized before testing at two clinical sites.

A total of 300 contrived positive samples were tested along with pre-selected archived specimens in a randomized, blinded fashion. Out of the 300 specimens included in the contrived study analysis, 291 specimens (97.0%) generated valid Respiratory *Flex* Assay results 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). The results of contrived specimen testing with the Respiratory *Flex* Assay are presented in **Table 49** below.

Table 49. 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 pneumoniae	2x	25/25	100	86.7-100	125/125	100	97.0-100
	10x	13/13	100	77.2-100	65/65	100	97.0-100
	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

Analyte	Target Conc. (xLoD)	Positive Percent Agreement			Negative Percent Agreement		
		TP/ (TP+FN)	%	95% CI	TN/ (TN+FP)	%	95% CI
	Combined	***49/50***	***98.0***	***89.5-99.6***	***249/250***	***99.6***	***97.8-99.9***

1. Clinical Specificity:
   See section **C.Clinical Studies** above.

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

D Clinical Cut-Off:

Not applicable.

E Expected Values/Reference Range:

The LIAISON PLEX Respiratory *Flex* Assay prospective clinical study included a total of 1843 prospectively collected NPS specimens, of which 1780 were evaluable for SARS-CoV-2, 1730 were evaluable for *Bordetella holmesii*, 1773 were evaluable for *Bordetella parapertussis*, 1753 were evaluable for *Bordetella pertussis*, and 1820 were evaluable for all remaining target analytes. The number and percentage of cases positive for each analyte, as determined by LIAISON PLEX Respiratory *Flex* Assay, are presented below, stratified by collection site and age group.

Table 50. LIAISON PLEX Respiratory *Flex* Assay - Expected Values Stratified by Specimen Collection Site

Target	Expected Values						
	Site 1 (N=508)	Site 02 (N=113)	Site 03 (N=326)	Site 4 (N=789)	Site 5 (N=51)	Site 16 (N=39)	Overall (N=1820)
Adenovirus	14.8% (75/508)	1.8% (2/112)	12.1% (39/321)	4.6% (36/789)	27.5% (14/51)	5.1% (2/39)	9.2% (168/1820)
Bordetella holmesii	0.0% (0/447)	0.0% (0/113)	0.0% (0/320)	0.0% (0/778)	0.0% (0/34)	0.0% (0/38)	0.0% (0/1730)
Bordetella parapertussis	0.0% (0/477)	0.0% (0/113)	0.6% (2/322)	0.6% (5/788)	0.0% (0/34)	0.0% (0/39)	0.4% (7/1773)
Bordetella pertussis	0.0% (0/463)	0.0% (0/113)	0.0% (0/320)	0.0% (0/784)	0.0% (0/34)	0.0% (0/39)	0.0% (0/1753)
Chlamydia pneumoniae	0.0% (0/508)	0.0% (0/112)	0.0% (0/321)	0.0% (0/789)	0.0% (0/51)	0.0% (0/39)	0.0% (0/1820)
Human Coronavirus	10.4% (53/508)	3.6% (4/112)	2.8% (9/321)	6.3% (50/789)	9.8% (5/51)	10.3% (4/39)	6.9% (125/1820)
Enterovirus/Rhinovirus	31.1% (158/508)	6.3% (7/112)	19.0% (61/321)	12.0% (95/789)	33.3% (17/51)	15.4% (6/39)	18.9% (344/1820)
Human Metapneumovirus	14.8% (75/508)	1.8% (2/112)	4.4% (14/321)	3.4% (27/789)	15.7% (8/51)	12.8% (5/39)	7.2% (131/1820)
Influenza A	2.2% (11/508)	0.9% (1/112)	3.1% (10/321)	15.3% (121/789)	3.9% (2/51)	0.0% (0/39)	8.0% (145/1820)
Influenza A H1	0.8% (4/508)	0.9% (1/112)	1.2% (4/321)	3.4% (27/789)	3.9% (2/51)	0.0% (0/39)	2.1% (38/1820)
Influenza A H3	1.6% (8/508)	0.0% (0/112)	1.9% (6/321)	11.9% (94/789)	0.0% (0/51)	0.0% (0/39)	5.9% (108/1820)
Influenza B	0.0% (0/508)	0.0% (0/112)	2.5% (8/321)	0.0% (0/789)	0.0% (0/51)	0.0% (0/39)	0.4% (8/1820)
Mycoplasma pneumoniae	0.0% (0/508)	0.0% (0/112)	0.0% (0/321)	0.0% (0/789)	0.0% (0/51)	0.0% (0/39)	0.0% (0/1820)

Target	Expected Values						
	Site 1 (N=508)	Site 02 (N=113)	Site 03 (N=326)	Site 4 (N=789)	Site 5 (N=51)	Site 16 (N=39)	Overall (N=1820)
Parainfluenza 1	1.2% (6/508)	0.9% (1/112)	0.6% (2/321)	0.3% (2/789)	0.0% (0/51)	0.0% (0/39)	0.6% (11/1820)
Parainfluenza 2	0.2% (1/508)	1.8% (2/112)	0.0% (0/321)	1.0% (8/789)	0.0% (0/51)	2.6% (1/39)	0.7% (12/1820)
Parainfluenza 3	4.5% (23/508)	0.0% (0/112)	2.8% (9/321)	0.4% (3/789)	11.8% (6/51)	2.6% (1/39)	2.3% (42/1820)
Parainfluenza 4	0.6% (3/508)	0.0% (0/112)	0.3% (1/321)	0.5% (4/789)	2.0% (1/51)	0.0% (0/39)	0.5% (9/1820)
Respiratory Syncytial Virus (RSV)	6.1% (31/508)	0.0% (0/112)	5.0% (16/321)	8.7% (69/789)	3.9% (2/51)	0.0% (0/39)	6.5% (118/1820)
SARS-CoV-2	5.5% (28/507)	40.0% (42/105)	16.9% (55/326)	15.6% (119/765)	7.3% (3/41)	19.4% (7/36)	14.3% (254/1780)

Table 51. LIAISON PLEX Respiratory *Flex* Assay - Expected Values Stratified by Age Group

Target	Expected Values					
	0-1 years (N=347)	>1-5 years (N=273)	>5-21 years (N=439	>21-65 years (N=528)	> 65 years (N=237	Overall (N=1820)
Adenovirus	15.3% (53/347)	22.0% (60/273)	10.0% (44/439)	2.1% (11/528)	0.0% (0/233)	9.2% (168/1820)
Bordetella holmesii	0.0% (0/316)	0.0% (0/247)	0.0% (0/411)	0.0% (0/522)	0.0% (0/234)	0.0% (0/1730)
Bordetella parapertussis	0.6% (2/329)	1.6% (4/257)	0.2% (1/422)	0.0% (0/528)	0.0% (0/237)	0.4% (7/1773)
Bordetella pertussis	0.0% (0/322)	0.0% (0/254)	0.0% (0/416)	0.0% (0/525)	0.0% (0/236)	0.0% (0/1753)
Chlamydia pneumoniae	0.0% (0/347)	0.0% (0/273)	0.0% (0/439)	0.0% (0/528)	0.0% (0/233)	0.0% (0/1820)
Human Coronavirus	10.7% (37/347)	10.3% (28/273)	6.6% (29/439)	4.5% (24/528)	3.0% (7/233)	6.9% (125/1820)
Rhinovirus/Enterovirus	30.5% (106/347)	32.6% (89/273)	20.3% (89/439)	8.7% (46/528)	6.0% (14/233)	18.9% (344/1820)
Human Metapneumovirus	11.5% (40/347)	13.9% (38/273)	6.4% (28/439)	3.8% (20/528)	2.1% (5/233)	7.2% (131/1820)
Influenza A	4.3% (15/347)	2.2% (6/273)	13.2% (58/439)	7.8% (41/528)	10.7% (25/233)	8.0% (145/1820)
Influenza A H1	1.4% (5/347)	0.4% (1/273)	2.7% (12/439)	2.7% (14/528)	2.6% (6/233)	2.1% (38/1820)
Influenza A H3	3.2% (11/347)	1.8% (5/273)	10.7% (47/439)	4.9% (26/528)	8.2% (19/233)	5.9% (108/1820)
Influenza B	0.0% (0/347)	1.1% (3/273)	0.7% (3/439)	0.4% (2/528)	0.0% (0/233)	0.4% (8/1820)
Mycoplasma pneumoniae	0.0% (0/347)	0.0% (0/273)	0.0% (0/439)	0.0% (0/528)	0.0% (0/233)	0.0% (0/1820)
Parainfluenza 1	1.7% (6/347)	1.1% (3/273)	0.2% (1/439)	0.2% (1/528)	0.0% (0/233)	0.6% (11/1820)
Parainfluenza 2	0.6% (2/347)	0.7% (2/273)	0.5% (2/439)	0.9% (5/528)	0.4% (1/233)	0.7% (12/1820)
Parainfluenza 3	4.0% (14/347)	6.2% (17/273)	1.1% (5/439)	1.1% (6/528)	0.0% (0/233)	2.3% (42/1820)
Parainfluenza 4	0.3% (1/347)	0.7% (2/273)	1.1% (5/439)	0.2% (1/528)	0.0% (0/233)	0.5% (9/1820)
Respiratory Syncytial Virus (RSV)	17.3% (60/347)	8.4% (23/273)	2.5% (11/439)	2.1% (11/528)	5.6% (13/233)	6.5% (118/1820)
SARS-CoV-2	8.4% (29/345)	5.2% (14/270)	9.2% (39/426)	21.9% (112/512)	26.4% (60/227)	14.3% (254/1780)

F Other Supportive Instrument Performance Characteristics Data:
Not applicable.

VIII Proposed Labeling:

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

IX Conclusion:

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