March 11, 2024

Luminex Molecular Diagnostics, Inc.
Kate Goscha
Senior Manager, Regulatory Affairs
439 University Avenue
Toronto, Ontario M5G 1Y8
Canada

Re: K231758
Trade/Device Name: NxTAG Respiratory Pathogen Panel v2 (NxTAG RPP v2)
Regulation Number: 21 CFR 866.3981
Regulation Name: Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The SARS-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test
Regulatory Class: Class II
Product Code: QOF
Dated: June 15, 2023
Received: June 16, 2023

Dear Kate Goscha:

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

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

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

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

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

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

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

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

Sincerely,

Joseph Briggs -S

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

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

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

510(k) Number *(if known)*
K231758

Device Name
NxTAG® Respiratory Pathogen Panel v2 (NxTAG® RPP v2)

Indications for Use *(Describe)*
The NxTAG® Respiratory Pathogen Panel v2 (NxTAG® RPP v2) is a multiplexed polymerase chain reaction (PCR) test intended for the simultaneous, qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19.

The following organism types and subtypes are identified and differentiated using the NxTAG RPPv2:

Viral Targets: Influenza A, Influenza A H1, Influenza A H1pdm09, Influenza A H3, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, SARS-CoV-2, Coronavirus 229E, Coronavirus OC43, Coronavirus NL63, Coronavirus HKU1, Human Metapneumovirus, Rhinovirus/Enterovirus, Adenovirus, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4

Bacterial Targets: Chlamydia pneumoniae, Mycoplasma pneumoniae

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

Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by a nasopharyngeal swab specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the NxTAG RPP v2 may not be the definite cause of disease.

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

The NxTAG® Respiratory Pathogen Panel v2 is indicated for use with the Luminex® MAGPIX® Instrument and xPONENT® and SYNCT™ software.

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

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

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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10.0 510(k) Summary

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

Date Prepared: February 27, 2024

A. 510(k) Number:

K231758

B. Purpose for Submission:

Traditional 510(k)

C. Measurand:

The assay detects and identifies nucleic acids of the following respiratory pathogens: Influenza A, Influenza A H1, Influenza A H1pdm09, Influenza A H3, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2), Coronavirus 229E, Coronavirus OC43, Coronavirus NL63, Coronavirus HKU1, Human Metapneumovirus, Rhinovirus/Enterovirus, Adenovirus, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3, Parainfluenza 4, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*.

D. Type of Test:

A multiplexed nucleic acid test intended for use with the Luminex® MAGPIX® Instrument, and xPONENT® and SYNCT™ software, for the qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals with clinical signs and symptoms of a respiratory tract infection, including COVID-19.

E. Applicant:

Kate Goscha
439 University Avenue
Toronto, Ontario M5G 1Y8
Canada
(608) 203-8909
Luminex Molecular Diagnostics, Inc.

F. Proprietary and Established Names:

NxTAG® Respiratory Pathogen Panel v2 (NxTAG® RPP v2)

G. Regulatory Information:

1. Regulation Section:

21 CFR 866.3981

2. Classification:

Class II (special controls)

3. Product Code(s):

QOF

4. Panel:

Microbiology (83)

H. Indications for Use:

1. Indication(s) for use:

The NxTAG® Respiratory Pathogen Panel v2 (NxTAG® RPP v2) is a multiplexed polymerase chain reaction (PCR) test intended for the simultaneous, qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19.

The following organism types and subtypes are identified and differentiated using the NxTAG RPP v2:

Viral Targets	
Influenza A	Coronavirus NL63
Influenza A H1	Coronavirus HKU1
Influenza A H1pdm09	Human Metapneumovirus

Viral Targets	
Influenza A H3	Rhinovirus/Enterovirus
Influenza B	Adenovirus
Respiratory Syncytial Virus A	Parainfluenza 1
Respiratory Syncytial Virus B	Parainfluenza 2
SARS-CoV-2	Parainfluenza 3
Coronavirus 229E	Parainfluenza 4
Coronavirus OC43	
Bacterial Targets	
Chlamydia pneumoniae	*Mycoplasma pneumoniae*

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

Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by a nasopharyngeal swab specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the NxTAG RPP v2 may not be the definite cause of disease.

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

The NxTAG® Respiratory Pathogen Panel v2 is indicated for use with the Luminex® MAGPIX® Instrument and xPONENT® and SYNCT™ software.

2. Special conditions for use statement(s):

For prescription use only.

For *in vitro* diagnostic use.

3. Special instrument requirements:

- Sample Processing:
  - Extraction: bioMérieux NUCLISENS® easyMAG® or EMAG® nucleic acid extraction systems
  - Amplification: IVD-labeled Thermal Cycler required
- Sample Analysis: Luminex® MAGPIX® Instrument using xPONENT® and SYNCT™ software

I. Device Description:

The NxTAG® Respiratory Pathogen Panel v2 (NxTAG® RPP v2) is designed to simultaneously detect and identify 21 different potential pathogens of respiratory tract infections, including the novel coronavirus SARS-CoV-2, from a single NPS specimen in transport medium. NxTAG® RPP v2 is compatible with Luminex's MAGPIX Instrument, and xPONENT® and SYNCT™ software. It incorporates multiplex Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) with the Luminex® proprietary universal tag sorting system on the Luminex platform to easily detect the 21 respiratory pathogen targets.

Samples are extracted using the IVD-labeled bioMérieux NucliSENS® easyMag® or EMAG® extraction systems. Extracted total nucleic acid is then added to the sealed 96-well micro plate by piercing the seal with pipette tips. Each reaction well is pre-plated with two Lyophilized Bead Reagents (LBRs) that contain all the required reagents including primer mixes, bead mix, and enzyme buffer systems. Once the LBRs are resuspended, the reaction wells are re-sealed using the foils provided in the kit. The sealed plate can be placed inside the thermocycler. The reaction is amplified via RT-PCR and the reaction product undergoes near simultaneous bead hybridization within the sealed reaction wells. The hybridized, tagged beads are then sorted and read on the Luminex® MAGPIX® instrument. The MAGPIX® instrument generates a signal in the form of a median fluorescence intensity (MFI) value for each bead population.

The signals are analyzed using the NxTAG® Respiratory Pathogen Panel v2 Assay File for SYNCT™ Software, providing a reliable, qualitative call for each of the 21 targets and internal controls within each reaction well.

J. Substantial Equivalence Information:

1. Predicate device name(s):

BioFire Respiratory Panel 2.1 (RP2.1)

2. Predicate 510(k) number(s):

DEN200031

3. Comparison with predicate:

Device & Predicate Device(s):	K231758	DEN200031
Device Trade Name	NxTAG Respiratory Pathogen Panel v2	BioFire Respiratory Panel 2.1
General Device Characteristic Similarities		
Intended Use/Indications For Use	The Luminex NxTAG Respiratory Pathogen Panel v2 (NxTAG RPP v2) is a multiplexed polymerase chain reaction (PCR) test intended for the simultaneous, qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swab specimens obtained from individuals with signs and symptoms of respiratory tract infection, including COVID-19. The following organism types and subtypes are identified and differentiated using the NxTAG RPP v2: *Viral targets:*	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

	Influenza A Influenza A H1 Influenza A H1pdm09 Influenza A H3 Influenza B Respiratory Syncytial Virus A Respiratory Syncytial Virus B SARS-CoV-2 Coronavirus 229E Coronavirus OC43 Coronavirus NL63 Coronavirus HKU1 Human Metapneumovirus Rhinovirus/Enterovirus Adenovirus Parainfluenza Virus 1 Parainfluenza Virus 2 Parainfluenza Virus 3 Parainfluenza Virus 4 *Bacterial targets:* *Chlamydia pneumoniae* *Mycoplasma pneumoniae* Nucleic acids from the viral and bacterial organisms identified by this test are generally detectable in nasopharyngeal specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals	Coronavirus 229E Coronavirus HKU1 Coronavirus NL63 Coronavirus OC43 Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) Human Metapneumovirus Human Rhinovirus/Enterovirus Influenza A, including subtypes H1, H1-2009, and H3 Influenza B Parainfluenza Virus 1 Parainfluenza Virus 2 Parainfluenza Virus 3 Parainfluenza Virus 4 Respiratory Syncytial Virus *Bordetella parapertussis* (IS1001) *Bordetella pertussis* (prxP) *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* Nucleic acids from the respiratory viral and bacterial organisms identified by this test are generally detectable in NPS specimens during the acute phase of infection. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and/or

	exhibiting signs and/or symptoms of respiratory infection are indicative of the presence of the identified microorganism and aids in diagnosis if used in conjunction with other clinical and epidemiological information, and laboratory findings. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by a nasopharyngeal swab specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the NxTAG RPPv2 may not be the definite cause of disease. Additional laboratory testing (e.g., bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection. The NxTAG Respiratory Pathogen Panel v2 is indicated for use with the Luminex MAGPIX Instrument and xPONENT and SYNCT software.	symptoms of respiratory infection is indicative of the presence of the identified microorganism and aids in the diagnosis of respiratory infection if used in conjunction with other clinical and epidemiological information. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions. Negative results in the setting of a respiratory illness may be due to infection with pathogens that are not detected by this test, or lower respiratory tract infection that may not be detected by an NPS specimen. Positive results do not rule out coinfection with other organisms. The agent(s) detected by the BioFire RP2.1 may not be the definite cause of disease. Additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) may be necessary when evaluating a patient with possible respiratory tract infection.

Specimen Type	Same	Nasopharyngeal swabs
Patient Population	Individuals with signs and symptoms of respiratory tract infection, including COVID-19	Individuals suspected of respiratory tract infections, including COVID-19
Organisms Detected	Same except for: a) addition of assay for differentiation of Respiratory Syncytial Virus A and B b) omission of assays for *Bordetella pertussis* and *Bordetella parapertussis*	*Viruses:* 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 Virus Subtypes: H1, H3, H1-2009 Influenza B Virus Parainfluenza Virus 1 Parainfluenza Virus 2 Parainfluenza Virus 3 Parainfluenza Virus 4 Respiratory Syncytial Virus *Bacteria:* *Bordetella parapertussis* *Bordetella pertussis* *Chlamydia pneumoniae* *Mycoplasma pneumoniae*
Technology	Same	PCR amplification
General Device Characteristic Differences		
System	Separate instruments for nucleic acid extraction, PCR amplification and detection	Integrated nucleic acid extraction, amplification, and detection in a sealed vessel.
Assay Read	MAGPIX Instrument	BIOFIRE FilmARRAY 2.0 or BIOFIRE FilmArray Torch Systems
Detection	Hybridization of amplified products with fluorescently	Array-based melt curve analysis

	labeled beads, sorting of tagged products	

K. Standards/Guidance Documents Referenced:

Guidance

- Highly Multiplexed Microbiological/Medical Countermeasure *In Vitro* Nucleic Acid Based Diagnostic Devices Guidance for Industry and Food and Drug Administration Staff – August 2014
- Respiratory Viral Panel Multiplex Nucleic Acid Assay – Class II Special Controls Guidance for Industry and FDA Staff – October 9, 2009
- Guidance for Industry and FDA Staff Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests, March 13, 2007
- Guidance on Informed Consent for *In Vitro* Diagnostic Device Studies using Leftover Human Specimens that are Not Individually Identifiable, January 2006
- Policy for Coronavirus Disease-2019 Tests during the Public Health Emergency (Revised). Immediately in Effect Guidance for Clinical Laboratories, Commercial Manufacturers, and Food and Drug Administration Staff, May 11, 2020

Standards

- CLSI, EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline – Third Edition
- CLSI, EP07, Interference Testing in Clinical Chemistry; 3rd Edition
- CLSI, EP12-A2, User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition
- CLSI, EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition
- CLSI, EP24-A2, Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline–Second Edition
- CLSI, EP25-A, Evaluation of Stability of *In Vitro* Diagnostic Reagents; Approved Guideline
- CLSI, EP37, Supplemental Tables for Interference Testing in Clinical Chemistry – First Edition
- CLSI, MM03, Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline – Third Edition
- CLSI, MM09-A2, Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine: Approved Guideline -- Second Edition
- CLSI, MM17, Validation and Verification of Multiplex Nucleic Acid Assays – Second Edition

- CLSI, MM18-A, Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline
- ISO 14971:2019, Medical Devices – Application of Risk Management to Medical Devices
- EN ISO 23640:2015, *In vitro* diagnostic medical devices – Evaluation of stability of *in vitro* diagnostics reagents

L. Test Principle:

The NxTAG® Respiratory Pathogen Panel v2 (NxTAG® RPP v2) incorporates multiplex Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) with the Luminex® proprietary universal tag sorting system on the Luminex platform to detect respiratory pathogen targets. Extracted total nucleic acid is added to pre-plated, Lyophilized Bead Reagents (LBRs), and mixed to resuspend the reaction reagents. The reaction is amplified via RT-PCR and the reaction product undergoes near simultaneous microsphere hybridization within the sealed reaction well. The hybridized, tagged microspheres are then sorted and read on the MAGPIX® instrument. The generated signals are analyzed using the NxTAG® Respiratory Pathogen Panel v2 Assay File for SYNCT™ Software, providing a reliable, qualitative call for each of the targets and internal controls within each reaction well.

M. Performance Characteristics:

1. <u>Analytical performance:</u>

a. Precision (Reproducibility and Repeatability):

<u>*Site-to-Site Reproducibility*</u>

A site-to-site reproducibility study was performed to assess the total variability of the NxTAG® Respiratory Pathogen Panel (NxTAG RPP v2) assay across study sites, operators, testing days, and instruments. Two operators at each of the 3 sites tested a 9-member reproducibility panel in 4 replicates on 5 non-consecutive days, for a total of 30 runs (3 sites x 2 operators x 5 days). For each member of the 9-member panel, a total of 120 data points (30 runs x 4 replicates) were generated using 1 assay kit lot. The reproducibility panel comprised of a negative sample and 4 multi-analyte (MA) samples prepared in negative simulated matrix (NSM) at two concentrations, Low Positive (1.5x - 3x LoD) and Moderate Positive (5x – 9x LoD). The test concentration for Influenza A H3 (in sample MA2) is based on the LoD for the subtype. Since the Influenza A H3 matrix LoD is 3-fold less sensitive than the subtype, the expected results for the matrix at 1.5x of the subtype LoD was either “positive” or “negative

(i.e. both results were acceptable based on the respective LoDs of the Influenza A and H3 targets). Therefore, the Influenza A matrix result in sample MA2 could not be assessed and was excluded from data analysis. The results demonstrated reproducibility of the NxTAG RPP v2 assay across 3 sites with an overall percent agreement of 99.90%. The summary of results is shown in Table 1.

Table 1: NxTAG® RPP v2 Site-to-site Reproducibility

Sample	Target	Sample Type	Agreement with Expected Results				
			Site 1	Site 2	Site 3	Overall (All Sites)	
MA1	Influenza A H1pdm09	Low Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	240/240 (100%)
		Moderate Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	
	Respiratory Syncytial Virus A	Low Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	240/240 (100%)
		Moderate Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	
	Rhinovirus[a]	Low Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	240/240 (100%)
		Moderate Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	
	All other targets	Low Positive	719/720 (99.86)	720/720 (100%)	720/720 (100%)	2159/2160 (99.95%)	4316/4320 (99.91%)
		Moderate Positive	718/720 (99.72%)	720/720 (100%)	719/720 (99.86%)	2157/2160 (99.86%)	
MA2	Influenza A H3	Low Positive	39/40 (97.50%)	40/40 (100%)	40/40 (100%)	119/120 (99.17%)	239/240 (99.58%)
		Moderate Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	
	Respiratory Syncytial Virus B	Low Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	240/240 (100%)
		Moderate Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	
	All other targets	Low Positive	717/720 (99.58%)	720/720 (100%)	720/720 (100%)	2157/2160 (99.86%)	4434/4440[b] (99.86%)
		Moderate Positive	757/760 (99.61%)	760/760 (100%)	760/760 (100%)	2277/2280 (99.87%)	
MA3	Influenza B	Low Positive	39/40 (97.50%)	40/40 (100%)	40/40 (100%)	119/120 (99.17%)	239/240 (99.58%)
		Moderate Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	
	Parainfluenza virus 3	Low Positive	39/40 (97.50%)	40/40 (100%)	40/40 (100%)	119/120 (99.17%)	239/240 (99.58%)
		Moderate Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	
	Mycoplasma pneumoniae	Low Positive	39/40 (97.50%)	40/40 (100%)	40/40 (100%)	119/120 (99.17%)	239/240 (99.58%)
		Moderate Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	

Sample	Target	Sample Type	Agreement with Expected Results				
			Site 1	Site 2	Site 3	Overall (All Sites)	
	All other targets	Low Positive	718/720 (99.72%)	720/720 (100%)	720/720 (100%)	2158/2160 (99.91%)	4317/4320 (99.93%)
		Moderate Positive	719/720 (99.86%)	720/720 (100%)	720/720 (100%)	2159/2160 (99.95%)	
MA4	SARS-CoV-2	Low Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	240/240 (100%)
		Moderate Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	
	Human Metapneu-movirus	Low Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	240/240 (100%)
		Moderate Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	
	Adenovirus	Low Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	240/240 (100%)
		Moderate Positive	40/40 (100%)	40/40 (100%)	40/40 (100%)	120/120 (100%)	
	All other targets	Low Positive	716/720 (99.44%)	720/720 (100%)	720/720 (100%)	2156/2160 (99.81%)	4315/4320 (99.88%)
		Moderate Positive	719/720 (99.86%)	720/720 (100%)	720/720 (100%)	2159/2160 (99.96%)	
NEG	Negative	N/A	840/840 (100%)	840/840 (100%)	840/840 (100%)	2520/2520 (100%)	
Overall Agreement with Expected Results (all targets and all test levels)			**7499/7520 (99.72%)**	**7520/7520 (100%)**	**7519/7520 (99.99%)**	**22538/22560 (99.90%)**	

[a] Reported by NxTAG RPP v2 as Rhinovirus/Enterovirus

[b] Excludes results of Influenza A Matrix target at Low Positive concentration.

Lot-to-Lot Reproducibility

A lot-to-lot reproducibility study was performed to assess the total variability of the NxTAG® Respiratory Pathogen Panel (NxTAG RPP v2) assay across 3 assay kit lots with unique lots of critical reagents, including enzymes, buffers, primers, MagPlex microspheres, and dNTPs. One operator at 1 site tested 17-member reproducibility panel in 10 replicates on each of the 3 assay kit lots, for a total of 30 data points (10 replicates x 3 assay lots) for each member. The reproducibility panel comprised of a negative sample and 8 multi-analyte samples prepared in negative simulated matrix (NSM) at two concentrations, Low Positive (1.5x - 3x LoD) and Moderate Positive (5x - 9x LoD). The test concentration for Influenza A H3 (in sample MA2) is based on the LoD for the subtype. Since the Influenza A H3 matrix LoD is 3-fold less sensitive than the subtype, the expected results for the matrix at 1.5x of the subtype LoD was either "positive" or "negative" (i.e. both results were acceptable based on the respective LoDs of the Influenza A and H3 targets). Therefore, the Influenza A matrix result in sample MA2 could not be assessed and was excluded from data analysis. The results demonstrated reproducibility of the NxTAG RPP v2 assay across 3 assay kit lots with an overall percent agreement of 99.95%. The summary of results is shown in Table 2.

Table 2: NxTAG® RPP v2 Lot-to-lot Reproducibility

Sample	Target	Sample Type	Agreement with Expected Results				
			Lot 1	Lot 2	Lot 3	Overall (All Lots)	
MA1	Influenza A 2009 H1N1	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	Respiratory Syncytial Virus A	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	Rhinovirus[a]	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	All other targets	Low Positive	180/180 (100%)	180/180 (100%)	180/180 (100%)	540/540 (100%)	1080/1080 (100%)
		Moderate Positive	180/180 (100%)	180/180 (100%)	180/180 (100%)	540/540 (100%)	
MA2	Influenza A H3	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	Respiratory Syncytial Virus B	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)

Sample	Target	Sample Type	Agreement with Expected Results				
			Lot 1	Lot 2	Lot 3	Overall (All Lots)	
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	All other targets	Low Positive	180/180 (100%)	180/180 (100%)	180/180 (100%)	540/540 (100%)	1110/1110 (100%)[b]
		Moderate Positive	190/190 (100%)	190/190 (100%)	190/190 (100%)	570/570 (100%)	
MA3	Influenza B	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	Parainfluenza virus 3	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	Mycoplasma pneumoniae	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	All other targets	Low Positive	180/180 (100%)	180/180 (100%)	180/180 (100%)	540/540 (100%)	1080/1080 (100%)
		Moderate Positive	180/180 (100%)	180/180 (100%)	180/180 (100%)	540/540 (100%)	
MA4	SARS-CoV-2	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	Human Metapneumovirus	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	Adenovirus	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	All other targets	Low Positive	180/180 (100%)	180/180 (100%)	180/180 (100%)	540/540 (100%)	1080/1080 (100%)
		Moderate Positive	180/180 (100%)	180/180 (100%)	180/180 (100%)	540/540 (100%)	
MA5	Influenza A Matrix	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	Coronavirus NL63	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate	10/10	10/10	10/10	30/30	

Sample	Target	Sample Type	Agreement with Expected Results				
			Lot 1	Lot 2	Lot 3	Overall (All Lots)	
		Positive	(100%)	(100%)	(100%)	(100%)	
	Coronavirus HKU1	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	All other targets	Low Positive	180/180 (100%)	179/180 (99.44%)	180/180 (100%)	539/540 (99.81%)	1079/1080 (99.90%)
		Moderate Positive	180/180 (100%)	180/180 (100%)	180/180 (100%)	540/540 (100%)	
MA6	Influenza A H1	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	Parainfluenza virus 1	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	Chlamydia pneumoniae	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	All other targets	Low Positive	180/180 (100%)	180/180 (100%)	180/180 (100%)	540/540 (100%)	1079/1080 (99.91%)
		Moderate Positive	180/180 (100%)	179/180 (99.44%)	180/180 (100%)	539/540 (99.81%)	
MA7	Parainfluenza virus 2	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	Parainfluenza virus 4 (subtype 4B)[c]	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	Coronavirus 229E	Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	All other targets	Moderate Positive	180/180 (100%)	180/180 (100%)	179/180 (99.44%)	539/540 (99.81%)	1079/1080 (99.91%)
		Low Positive	180/180 (100%)	180/180 (100%)	180/180 (100%)	540/540 (100%)	
MA8	Parainfluenza virus 4 (subtype 4A)[c]	Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	

Sample	Target	Sample Type	Agreement with Expected Results				
			Lot 1	Lot 2	Lot 3	Overall (All Lots)	
	Coronavirus OC43	Moderate Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	60/60 (100%)
		Low Positive	10/10 (100%)	10/10 (100%)	10/10 (100%)	30/30 (100%)	
	All other targets	Moderate Positive	190/190 (100%)	190/190 (100%)	190/190 (100%)	570/570 (100%)	1140/1140 (100%)
		Low Positive	190/190 (100%)	190/190 (100%)	190/190 (100%)	570/570 (100%)	
NEG	Negative	N/A	208/210 (99.04%)	210/210 (100%)	210/210 (100%)	628/630 (99.68%)	
Overall Agreement with Expected Results (all targets and all test levels)			**3558/3560 (99.94%)**	**3558/3560 (99.94%)**	**3559/3560 (99.97%)**	**10675/10680 (99.95%)**	

[a] Reported by NxTAG RPP v2 as Rhinovirus/Enterovirus

[b] Excludes results of Influenza A Matrix target at Low Positive concentration.

[c] NxTAG RPP v2 does not distinguish Parainfluenza virus subtypes 4A and 4B which are both reported as Parainfluenza virus 4.

b. Linearity/assay reportable range:

Not applicable. The NxTAG® RPP v2 assay is a qualitative assay.

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

i) Controls:

(a) Internal Control

Bacteriophage MS2 is the internal control for the assay. This internal positive control is added to each specimen prior to extraction. This internal control allows the user to ascertain whether the assay is functioning properly. Failure to detect the MS2 control indicates a failure at either the extraction step, the reverse-transcription step, the PCR step, or the detection step, and may be indicative of the presence of amplification inhibitors, thereby preventing false negative results.

(b) External Controls

External positive and negative controls should be used in accordance with local, state, federal accrediting organizations, as applicable.

- **Positive Control** – Positive controls are not included in the NxTAG® Respiratory Pathogen Panel v2 assay, but are recommended to be included in every run, as a good laboratory practice.
- **Negative Amplification Control (No Template Control (NTC))** – The negative amplification control is RNase- free water.
- **Negative Extraction Control (NEC)** – The negative extraction control is the sample collection medium that has undergone the entire assay procedure, starting from extraction.

ii) Stability:

(a) Specimen Stability

Stability of raw specimens in Universal Transport Media (UTM), and MicroTest™ M4RT, as well as, stability of extracted specimens were evaluated at temperatures ranging between 2°C to 8°C and -70°C ± 5°C on the NxTAG®

RPP v2 assay. For raw specimens prepared in negative clinical matrix in UTM and extracted specimens, all targets probed by the assay were evaluated with a set of 17 samples: eight multi-analyte samples contrived in negative clinical matrix at two concentrations, Low positive (1.5x – 3x LoD) and Moderate positive (5x – 9x LoD), and a negative sample (negative clinical matrix only). For raw specimens prepared in negative clinical matrix in M4RT, a subset of targets in four multi-analyte samples consisting of representative organisms and genome types, at two concentrations Low positive (1.5x – 3x LoD) and Moderate positive (5x – 9x LoD), as well as a negative sample were evaluated. Ten replicates were tested for each condition.

All samples met the acceptance criteria, and the data support a raw specimen stability claim of 7 days at 2°C to 8°C and 12 months at -70°C ± 5°C for both transport media tested, and an extracted specimen stability claim of 4 hours at 2°C to 8°C and 12 months at -70°C ± 5°C.

(b) Device Stability

A shelf-life study was conducted to evaluate the real-time stability of NxTAG® RPP v2 at the recommended storage conditions of 2 - 8°C. Real-time stability was assessed using Positive Controls that cover all analytes probed by the assay, extracted MS2 (internal control), and no template control. Results of this study demonstrated that NxTAG® RPP v2 is stable for at least 12 months when stored at 2 - 8°C.

In-use, open-pouch/vial stability of NxTAG® RPP v2 was also evaluated by mimicking in-use conditions of the kit, which was stored at the recommended storage conditions of 2 - 8°C. Stability was assessed using Positive Controls that cover all or a subset of analytes probed by the assay, MS2 (Internal Control) and a No Template Control. The study demonstrated 100% agreement between calls made with the NxTAG® RPP v2 assay on a cold block for an hour vs. the baseline and calls made with NxTAG® RPP v2 assay on a cold block that was replaced every 15 to 20 minutes vs. the baseline, and confirmed the stability of the NxTAG® RPP v2 assay after it is opened and re-sealed for 6 cycles over 5 weeks, at the recommended storage conditions.

d. Detection Limit:

The Limit of Detection (LoD) of the NxTAG® RPP v2 assay for each target was assessed by testing simulated samples prepared from high-titer cultured material obtained from commercial suppliers or characterized clinical specimens. For each target, a 3-fold dilution series was prepared in negative clinical matrix (NCM,

pooled negative nasopharyngeal swabs in Universal Transport Medium), extracted using bioMérieux® EMAG® extraction system, and tested with NxTAG® RPP v2 assay. The preliminary LoD for each target was confirmed by preparing and testing 20 replicates. The LoD concentration for each target was defined as the lowest concentration at which ≥ 95% (≥ 19/20) of the replicates can reproducibly be detected. A droplet digital PCR (ddPCR) assay was performed to quantitate clinical specimens and those culture stocks where titer information in copy number was not available. A summary of the confirmed LoD for each target is listed in Table 3. In addition, confirmation of LoD was performed for targets in Multi-Analyte (MA) samples prepared in NCM. Each MA sample consisted of 2 to 4 target analytes and 8 MA samples cover all targets probed by the NxTAG® RPP v2 assay. Confirmation of the single-analyte LoD in MA samples supported the use of MA samples in NxTAG® RPP v2 analytical studies.

Table 3: NxTAG® RPP v2 Limit of Detection

Target	Strain/Isolate	Supplier /Part Number	LoD Concentration		# Detected/ # Tested
			Copies/ mL	In Supplier Unit	
Influenza A (Matrix)	A/Brisbane/59/07	ZeptoMetrix 0810244CF	1.19E+02	2.83E-02 $TCID_{50}$/mL	20/20
	A/NY/02/09	ZeptoMetrix 0810109CFN	3.28E+02	3.74E-02 $TCID_{50}$/mL	20/20
	A/Wisconsin/67/05	ZeptoMetrix 0810252CF	1.68E+02	6.45E-01 $TCID_{50}$/mL	20/20
Influenza A H1 (Subtype)	A/Brisbane/59/07	ZeptoMetrix 0810244CF	1.60E+03	3.82E-01 $TCID_{50}$/mL	20/20
Influenza A H1pdm09 (Subtype)	A/NY/02/09	ZeptoMetrix 0810109CFN	9.84E+02	1.12E-01 $TCID_{50}$/mL	19/20
Influenza A H3 (Subtype)	A/Wisconsin/67/05	ZeptoMetrix 0810252CF	5.60E+01	2.15E-01 $TCID_{50}$/mL	20/20
Influenza B	B/Florida/02/06	ZeptoMetrix 0810037CF	6.33E+01	9.67E-01 $TCID_{50}$/mL	19/20
Respiratory Syncytial Virus A	A2	ATCC VR-1540	4.97E+03	3.77E+01 PFU/mL	19/20
Respiratory Syncytial Virus B	18537	ATCC VR-1580	7.21E+03	3.20E-01 PFU/mL	20/20
Parainfluenza Virus 1	N/A	ZeptoMetrix 0810014CF	6.92E+02	7.64E-01 $TCID_{50}$/mL	20/20
Parainfluenza virus 2	Greer	ATCC VR-92	3.45E+02	7.32E-01 $TCID_{50}$/mL	20/20
Parainfluenza virus 3	C 243	ATCC VR-93	1.01E+03	1.10E+02 $TCID_{50}$/mL	20/20

Target	Strain/Isolate	Supplier /Part Number	LoD Concentration		# Detected/ # Tested
			Copies/ mL	In Supplier Unit	
Parainfluenza virus 4	Type 4A	ZeptoMetrix 0810060CF	1.69E+04	8.58E-01 $TCID_{50}$/mL	20/20
	Type 4B, CH 19503	ATCC VR-1377	7.15E+03	5.99E+01 $TCID_{50}$/mL	20/20
SARS-CoV-2	USA-WA1/2020	ATCC VR-1986HK	5.00E+02	7.68E+00 $TCID_{50}$/mL	19/20
Coronavirus 229E	N/A	ATCC VR-740	3.81E+02	1.22E-01 $TCID_{50}$/mL	19/20
Coronavirus NL63	N/A	ZeptoMetrix 0810228CF	1.00E+02	6.45E-03 $TCID_{50}$/mL	20/20
Coronavirus OC43	Betacoronavirus 1	ATCC VR-1558	4.55E+03	7.32E-02 $TCID_{50}$/mL	20/20
Coronavirus HKU1	Genotype B	Clinical Sample	4.18E+03	N/A	19/20
Human Metapneumovirus	hMPV-16, Type A1, IA10-2003	ZeptoMetrix 0810161CF	7.15E+01	5.76E-02 $TCID_{50}$/mL	20/20
	hMPV-3, Type B1, Peru2-2002	ZeptoMetrix 0810156CF	2.62E+02	1.78E-02 $TCID_{50}$/mL	20/20
Rhinovirus/ Enterovirus	Rhinovirus 50-525-CV54 [V-192-001-021]	ATCC VR-1195	1.54E+03	6.87E+01 $TCID_{50}$/mL	20/20
	Enterovirus Species D, Type 68 2007 Isolate	ZeptoMetrix 0810237CF	3.53E+03	2.30E+00 $TCID_{50}$/mL	20/20
Adenovirus	Species B, Type 14 2006 isolate	ZeptoMetrix 0810108CF	1.42E+03	1.44E-01 $TCID_{50}$/mL	19/20
	Species C, Type 1	ZeptoMetrix 0810050CF	2.01E+04	9.28E+01 $TCID_{50}$/mL	20/20
	Species E, Type 4	ZeptoMetrix 0810070CF	7.33E+03	1.91E-01 $TCID_{50}$/mL	19/20
Chlamydia pneumoniae	TW-183	ATCC VR-2282	2.38E+02	3.74E+01 IFU/mL	20/20
Mycoplasma pneumoniae	M129	ZeptoMetrix 0801579	3.23E+03	5.56E+01 CCU/mL	19/20

e. Analytical Sensitivity for the First WHO International Standard for SARS-CoV-2

The Analytical Sensitivity of the NxTAG® RPP v2 assay for the WHO standard for SARS-CoV-2 was evaluated.

The preliminary LoD was determined by preparing a 10-fold dilution series in negative clinical matrix (NCM, pooled negative nasopharyngeal swabs in Universal Transport Medium) and testing each dilution level in triplicate. The LoD was confirmed by testing 20 replicates of sample prepared at preliminary LoD, as well as 20 replicates of samples prepared 3-fold above and below this preliminary LoD.

The Analytical Sensitivity was defined as the lowest concentration at which ≥ 95% of the replicates tested generated a positive call. The summary of the results is shown in Table 4.

Table 4. Analytical Sensitivity of NxTAG® RPP v2 for the First WHO International Standard for SARS-CoV-2.

Target	Strain	Supplier /Part Number	Concentration (IU/mL)	# Detected / # tested
SARS-CoV-2	Heat inactivated England/02/2020 isolate	NIBSC 20/146	7.70E+05	20/20 (100%)

f. Analytical Reactivity (Inclusivity)

Analytical Reactivity (Inclusivity) of the NxTAG® RPP v2 assay was assessed by testing a total of 193 pathogen strains/isolates (168 reactivity strains and 25 LoD strains). The strains tested represent the diversity of the targets probed by NxTAG® RPP v2. Each strain was prepared in negative simulated matrix (NSM) and tested in triplicate on the NxTAG RPP v2 assay. A droplet digital PCR (ddPCR) assay was performed to quantitate clinical specimens and those culture stocks where titer information in copy number was not available. For influenza A results, the concentration at which both the influenza A matrix and the subtype of that strain were detected by NxTAG® RPP v2 is shown. When the influenza A matrix and the subtype were detected at different concentrations, the concentration for each target is listed separately. The NxTAG® RPP v2 assay is capable of detecting the Influenza A matrix for all strains, including Influenza A H5, H7, and H9. Specimens containing Influenza A H5, H7, and H9 strains are expected to be reported as “Influenza A” only. The applicable subtype of all strains of influenza A H1, A H1pdm09 and A H3 with the exception of Influenza A A/Denver/1/57 H1 were detected successfully. A summary of the results, including the strain identity and the concentration at which they were detected, are shown in Table 5.

Table 5. Results of NxTAG® RPP v2 Analytical Reactivity

Organism	Strain	Supplier / Part Number	Concentration Tested (Copies/mL)	# Detected /# Tested
Influenza A H1	A/Brisbane/59/07*	ZeptoMetrix 0810244CF	4.81E+03	3/3
	A/New Caledonia/20/99	ZeptoMetrix 0810036CF	4.81E+03	3/3
	A/Solomon Island/03/06	ZeptoMetrix 0810036CFN	4.81E+03	3/3
	A/Taiwan/42/06	ZeptoMetrix 0810036CF (New PN: 0810247CF)	4.81E+03	3/3
	A/Denver/1/57	ATCC VR-546	4.81E+03	3/3 (Matrix)
			5.77E+07[a]	0/3 (H1 Subtype)
Influenza A H1pdm09	A/NY/02/09*	ZeptoMetrix 0810109CFN	5.11E+03	3/3
	A/SwineNY/01/2009 (New name: A/NY/01/09)	ZeptoMetrix 0810109CFN (New PN: 0810248CF)	5.11E+03	3/3
	A/SwineNY/03/2009 (New name: A/NY/03/09)	ZeptoMetrix 0810109CFN (New PN: 0810249CF)	5.11E+03	3/3
	A/Swine/Canada/6294/09	ZeptoMetrix 0810109CFJ	5.11E+03	3/3
	A/California/07/09	ZeptoMetrix 0810165CF	5.11E+03	3/3
	A/Mexico/4108/09	ZeptoMetrix 0810166CF	5.11E+03	3/3
	A/Michigan/45/15	ZeptoMetrix 0810538CF	5.11E+03	3/3
	A/Brisbane/02/18	ZeptoMetrix 0810585CF	5.11E+03	3/3
	A/Virginia/ATCC1/2009	ATCC VR-1736	5.11E+03	3/3
	A/Netherlands/2629/2009	BEI NR-19823	5.11E+03	3/3
	A/Houston/3H/2009	BEI NR-20340	5.11E+03	3/3
	A/Brownsville/31H/2009	BEI NR-20344	5.11E+03	3/3
	A/Dominican Republic/7293/2013	IRR FR-1298	5.11E+03	3/3
	A/Massachusetts/15/2013	IRR FR-1319	5.11E+03	3/3
	A/Swine/1976/31	ATCC VR-99	4.81E+03	3/3 (Matrix)

Organism	Strain	Supplier / Part Number	Concentration Tested (Copies/mL)	# Detected /# Tested
			1.83E+07	3/3 (H1pdm09 Subtype)
	A/Swine/Iowa/15/30	ATCC VR-333	4.81E+03	3/3 (Matrix)
			2.25E+07	3/3 (H1pdm09Subtype)
Influenza A H3	A/Wisconsin/67/05*	ZeptoMetrix 0810252CF	1.68E+02	3/3
	A/Brisbane/10/07	ZeptoMetrix 0810138CF	5.04E+02	3/3 (Matrix)
			1.68E+02	3/3 (H3 Subtype)
	A/Texas/50/12	ZeptoMetrix 0810238CF	5.04E+02	3/3
	A/Perth/16/09	ZeptoMetrix 0810138CF (New PN: 0810251CF)	1.68E+02	3/3
	A/Hong Kong/4801/14	ZeptoMetrix 0810526CF	4.54E+03	3/3 (Matrix)
			1.51E+03	3/3 (H3 Subtype)
	A/Singapore/INFIMH-16-0019/16	ZeptoMetrix 0810574CF	1.51E+03	3/3
	A/Kansas/14/17	ZeptoMetrix 0810586CF	5.04E+02	3/3 (Matrix)
			1.68E+02	3/3 (H3 Subtype)
	A/Hong Kong/8/68	ATCC VR-544	1.68E+02	3/3 (Matrix)
			4.08E+04	3/3 (H3 Subtype)
	A/Alice	ATCC VR-776	1.68E+02	3/3 (Matrix)
			8.04E+03	3/3 (H3 Subtype)
	A/Port Chalmers/1/73	ATCC VR-810	1.51E+03	3/3 (Matrix)
			5.04E+02	3/3 (H3 Subtype)
	A/Sydney/5/1997	BEI NR-12278	4.54E+03	3/3 (Matrix)
			1.68E+02	3/3 (H3 Subtype)
	A/Santiago/7981/2006	IRR FR-336	1.68E+02	3/3
	A/Henan/Jinshui/147/2007	IRR FR-365	1.68E+02	3/3
	A/Brisbane/9/2006	IRR FR-366	1.68E+02	3/3

Organism	Strain	Supplier / Part Number	Concentration Tested (Copies/mL)	# Detected /# Tested
	A/Nepal/921/2006	IRR FR-367	1.68E+02	3/3
	A/Florida/2/2006	IRR FR-368	5.04E+02	3/3 (Matrix)
			1.68E+02	3/3 (H3 Subtype)
	South Australia/55/14	0810512CF	1.68E+02	3/3
	Stockholm/6/14	0810513CF	5.04E+02	3/3 (Matrix)
			1.68E+02	3/3
	Norway/466/14	0810514CF	1.68E+02	3/3
	Hong Kong/2671/19	0810609CF	5.04E+02	3/3
	A/California/2/2014	VR-1938	1.68E+02	3/3
	A/Switzerland/9715293/2013	VR-183	1.68E+02	3/3
	Clinical Sample	500-NEG-161	1.68E+02	3/3
	Clinical Sample	500-NEG-199	5.04E+02	3/3
Influenza A H5[b]	A/Anhui/01/2005 (H5N1)-PR8-IBCDC-RG6	IRR FR-735	5.04E+02	3/3 (Matrix only)
	A/Egypt/N03072/2010 (H5N1)-PR8-IDCDC-RG29	IRR FR-1065	5.04E+02	3/3 (Matrix only)
	A/pheasant/New Jersey /1355/1998(H5N2)-PR8-IBCDC-4	IRR FR-771	5.04E+02	3/3 (Matrix only)
	A/Hubei/1/2010 (H5N1)-PR8-IDCDC-RG30	IRR FR-1066	5.04E+02	3/3 (Matrix only)
Influenza A H7[b]	A/turkey/Virginia/4529/2002 (H7N2) x PR8-IBCDC-5	IRR FR-772	5.04E+02	3/3 (Matrix only)
	A/mallard/Netherlands/12/2000 (H7N7)/PR8-IBCDC-1	IRR FR-773	5.04E+02	3/3 (Matrix only)
Influenza A H9[b]	A/Hong Kong/33982/2009 (H9N2)-PR8-IDCDC-RG26	IRR FR-1068	5.04E+02	3/3 (Matrix only)
Influenza B[c] (Yamagata Lineage)	B/Florida/02/06*	ZeptoMetrix 0810037CF	1.90E+02	3/3
	B/Massachusetts/2/12	ZeptoMetrix 0810239CF	1.90E+02	3/3
	B/Wisconsin/1/10	ZeptoMetrix 0810241CF	1.90E+02	3/3
	B/Florida/04/06	ZeptoMetrix 0810037CF (New PN: 0810255CF)	1.90E+02	3/3

Organism	Strain	Supplier / Part Number	Concentration Tested (Copies/mL)	# Detected /# Tested
	B/Florida/07/04	ZeptoMetrix 0810037CF (New PN: 0810256CF)	1.90E+02	3/3
	B/Panama/45/90	ZeptoMetrix 0810037CF (New PN: 0810259CF)	1.90E+02	3/3
	B/Phuket/3073/13	ZeptoMetrix 0810515CF	1.90E+02	3/3
	B/Bangladesh/5972/2007	IRR FR-450	1.90E+02	3/3
	B/Hubei-Wujiagang/158/2009	IRR FR-469	1.90E+02	3/3
Influenza B[c] (Victoria Lineage)	B/Brisbane/33/08	ZeptoMetrix 0810037CF (New PN: 0810253CF)	1.90E+02	3/3
	B/Brisbane/60/08	ZeptoMetrix 0810037CF (New PN: 0810254CF)	1.90E+02	3/3
	B/Malaysia/2506/04	ZeptoMetrix 0810258CF	1.90E+02	3/3
	B/Colorado/06/17	ZeptoMetrix 0810573CF	5.69E+02	3/3
	B/Hong Kong/259/2010	IRR FR-663	1.90E+02	3/3
	B/New Jersey/1/2012	IRR FR-1270	1.90E+02	3/3
	B/Texas/02/2013	IRR FR-1302	1.90E+02	3/3
RSVA	A2*	ATCC VR-1540	1.49E+04	3/3
	2006 Isolate	ZeptoMetrix 0810040ACF	1.49E+04	3/3
	Long	ATCC VR-26	1.49E+04	3/3
RSVB	18537*	ATCC VR-1580	2.16E+04	3/3
	CH93(18)-18	ZeptoMetrix 0810040CF	2.16E+04	3/3
	9320	ATCC VR-955	2.16E+04	3/3
	B WV/14617/85	ATCC VR-1400	2.16E+04	3/3
	B1	BEI NR-4052	2.16E+04	3/3
PIV1	Type 1*	ZeptoMetrix 0810014CF	2.08E+03	3/3

Organism	Strain	Supplier / Part Number	Concentration Tested (Copies/mL)	# Detected /# Tested
	C35	ATCC VR-94	2.08E+03	3/3
PIV2	Greer*	ATCC VR-92	1.03E+03	3/3
	Type 2	ZeptoMetrix 0810015CF	1.03E+03	3/3
PIV3	C 243*	ATCC VR-93	3.04E+03	3/3
	Type 3	ZeptoMetrix 0810016CF	3.04E+03	3/3
	ATCC-2011-5	ATCC VR-1782	3.04E+03	3/3
	NIH 47885	BEI NR-3233	3.04E+03	3/3
PIV4	Type 4A*	ZeptoMetrix 0810060CF	5.08E+04	3/3
	M-25; Type 4A	ATCC VR-1378	5.08E+04	3/3
	CH 19503; Type 4B*	ATCC VR-1377	2.14E+04	3/3
	Type 4B	ZeptoMetrix 0810060BCF	2.14E+04	3/3
SARS-CoV-2	2019-nCoV/USA-WA1/2020*	ATCC VR-1986HK	1.50E+03	3/3
	HongKong/VM2000106/2020	ZeptoMetrix 0810590CFHI	1.50E+03	3/3
	USA-WA1/2020	ZeptoMetrix 0810587CFHI	1.50E+03	3/3
	BetaCoV/Germany/BavPat1/2020p.1[d]	EVAg 026N-03889	2.73E+03	3/3
	2019-nCoV/Italy-INMI1[d]	EVAg 008N-03894	2.73E+03	3/3
	England/02/2020[d]	BEI NR-52499	2.73E+03[e]	3/3
	Singapore/2/20202[d]	BEI NR-52501	2.73E+03[e]	3/3
	USA-IL1/2020[d]	BEI NR-52503	2.73E+03[e]	3/3
	USA-CA1/2020[d]	BEI NR-52504	2.73E+03[e]	3/3
	USA-AZ1/2020[d]	BEI NR-52505	2.73E+03[e]	3/3
	USA-WI1/2020[d]	BEI NR-52506	2.73E+03[e]	3/3
	USA-CA3/2020[d]	BEI NR-52507	2.73E+03[e]	3/3
	USA-CA4/2020[d]	BEI NR-52508	2.73E+03[e]	3/3

Organism	Strain	Supplier / Part Number	Concentration Tested (Copies/mL)	# Detected /# Tested
	USA-CA2/2020[d]	BEI NR-52509	2.73E+03[e]	3/3
	Chile/Santiago_op4d1/2020[d]	BEI NR-52510	2.73E+03[e]	3/3
	New York-PV08410/2020[d]	BEI NR-53518	2.73E+03[e]	3/3
	USA/CA_CDC_5574/2020, Heat Inactivated	BEI NR-55245	1.50E+03[e]	3/3
	Alpha (B1.1.7)/UK Variant	Clinical Specimen	1.50E+03	3/3
	Epsilon (B1.429)/California Variant	Clinical Specimen	1.50E+03	3/3
	Epsilon (B1.429)/California Variant	Clinical Specimen	1.50E+03	3/3
	Delta (B.1.617.2)	Clinical Specimen	1.50E+03	3/3
	Delta (B.1.617.2)	Clinical Specimen	1.50E+03	3/3
	Delta (B.1.617.2)	Clinical Specimen	1.50E+03	3/3
	Delta (B.1.617.2)	Clinical Specimen	1.50E+03	3/3
	Delta (B.1.617.2)	Clinical Specimen	1.50E+03	3/3
	Omicron (B.1.1.529 and BA lineages)	Clinical Specimen	1.50E+03	3/3
	Omicron (B.1.1.529 and BA lineages)	Clinical Specimen	1.50E+03	3/3
	Omicron (B.1.1.529 and BA lineages)	Clinical Specimen	1.50E+03	3/3
	Omicron (B.1.1.529 and BA lineages)	Clinical Specimen	1.50E+03	3/3
	Omicron (B.1.1.529 and BA lineages)	Clinical Specimen	1.50E+03	3/3
Coronavirus 229E	229E*	ATCC VR-740	1.14E+03	3/3
	229E	ZeptoMetrix 0810229CF	1.14E+03	3/3
Coronavirus NL63	NL63*	ZeptoMetrix 0810228CF	3.00E+02	3/3
	NL63	BEI NR-470	3.00E+02	3/3
Coronavirus OC43	Betacoronavirus 1*	ATCC VR-1558	1.36E+04	3/3
	OC43	ZeptoMetrix 0810024CF	1.36E+04	3/3

Organism	Strain	Supplier / Part Number	Concentration Tested (Copies/mL)	# Detected /# Tested
Coronavirus HKU1	HKU1, Genotype B*	Clinical Specimen	1.25E+04	3/3
	HKU1, Genotype B	Clinical Specimen	1.25E+04	3/3
	HKU1, Genotype A	Clinical Specimen	1.25E+04	3/3
	HKU1, Genotype A	Clinical Specimen	1.25E+04	3/3
Human Metapneumovirus	Type A1, IA10-2003, hMPV-16*	ZeptoMetrix 0810161CF	2.15E+02	3/3
	Type A1, IA3-2002, hMPV-9	ZeptoMetrix 0810160CF	2.15E+02	3/3
	Type A2, IA27-2004, hMPV-27	ZeptoMetrix 0810164CF	2.15E+02	3/3
	Type A2, DHI 26583	Clinical Specimen	2.15E+02	3/3
	Type B1, Peru2-2002, hMPV-3*	ZeptoMetrix 0810156CF	7.85E+02	3/3
	Type B1, Peru3-2003, hMPV-5	ZeptoMetrix 0810158CF	7.85E+02	3/3
	Type B2, Peru1-2002, hMPV-4	ZeptoMetrix 0810157CF	7.85E+02	3/3
	Type B2, Peru6-2003, hMPV-8	ZeptoMetrix 0810159CF	7.85E+02	3/3
	Type B2, IA18-2003, hMPV-18	ZeptoMetrix 0810162CF	7.85E+02	3/3
Rhinovirus[f]	Species A, Type 85, strain 50-525-CV54 [V-192-001-021]*	ATCC VR-1195	4.61E+03	3/3
	Species A, Type 1A	ZeptoMetrix 0810012CFN	4.61E+03	3/3
	Species A, Type 2, strain HGP	ATCC VR-482	4.61E+03	3/3
	Species A, Type 7, strain 68-CV11	ATCC VR-1601	4.61E+03	3/3
	Species A, Type 16	ZeptoMetrix 0810285CF	4.61E+03	3/3
	Species A, Type 34, strain 137-3	ATCC VR-1365	4.61E+03	3/3
	Species A, Type 39, strain 209	ATCC VR-340	4.61E+03	3/3
	Species A, Type 54, strain FO1-3774	ATCC VR-1661	4.61E+03	3/3
	Species A, Type 57, strain Ch47	ATCC VR-1600	4.61E+03	3/3

Organism	Strain	Supplier / Part Number	Concentration Tested (Copies/mL)	# Detected /# Tested
	Species A, Type 77, strain 130-63 [V-185-001-021]	ATCC VR-1187	4.61E+03	3/3
	Species B, Type 3, strain FEB	ATCC VR-483	4.61E+03	3/3
	Species B, Type 14, strain 1059	ATCC VR-284	4.61E+03	3/3
	Species B, Type 17, strain 33342	ATCC VR-1663	4.61E+03	3/3
	Species B, Type 27, strain 5870 [5870-CV28] (NIAID V-144-001-021)	ATCC VR-1137	4.61E+03	3/3
	Species B, Type 42, strain 56822	ATCC VR-338	4.61E+03	3/3
	Species B, Type 83, strain Baylor 7 [V-190-001-021]	ATCC VR-1193	4.61E+03	3/3
	Type C	Clinical Specimen	4.61E+03	3/3
	Type C	Clinical Specimen	4.61E+03	3/3
	Type C	Clinical Specimen	4.61E+03	3/3
	Type C	Clinical Specimen	4.61E+03	3/3
	Type C	Clinical Specimen	4.61E+03	3/3
Enterovirus[f]	Species D, Type 68, 2007 isolate*	ZeptoMetrix 0810237CF	1.06E+04	3/3
	Species A, Human Enterovirus 71, strain H	ATCC VR-1432	1.06E+04	3/3
	Species A, Human Coxsackie A10, strain M.K. (Kowalik)	ATCC VR-168	1.06E+04	3/3
	Species B, Human Coxsackievirus B1, strain Conn-5	ATCC VR-28	1.06E+04	3/3
	Species B, Human Coxsackievirus B4, strain J.V.B. (Benschoten)	ATCC VR-184	1.06E+04	3/3
	Species B, Human Echovirus 11, strain Gregory	ATCC VR-41	2.86E+05	3/3
	Species B, Human Echovirus 13, strain Del Carmen	ATCC VR-1054 (New PN: VR-43)	1.06E+04	3/3
	Species B, Enterovirus Type 69, strain Toluca-1 [V-068-001-021]	ATCC VR-1077	1.06E+04	3/3
	Species C, Human coxsackievirus A21, strain Kuykendall	ATCC VR-850	1.06E+04	3/3

Organism	Strain	Supplier / Part Number	Concentration Tested (Copies/mL)	# Detected /# Tested
	Species C, Human coxsackievirus A24, strain DN-19	ATCC VR-1662	1.06E+04	3/3
	Species D, Type 68, 2014 Isolate	ZeptoMetrix 0810300CF	1.06E+04	3/3
	Species D, Type 68, strain US/MO/14-18947	ATCC VR-1823	1.06E+04	3/3
	Species D, Type 68, strain US/IL/14-18952	ATCC VR-1824	1.06E+04	3/3
	Species D, Type 68, strain US/KY/14-18953	ATCC VR-1825	1.06E+04	3/3
	Species D, Type 68, strain Fermon	ATCC VR-1076 (New PN: VR-1826)	1.06E+04	3/3
	Species D, Type 70, strain J670/71	ATCC VR-836	1.06E+04	3/3
Adenovirus	Species B, Type 14, 2006 isolate*	ZeptoMetrix 0810108CF	7.38E+03	3/3
	Species B, Type 3	ZeptoMetrix 0810062CF	7.38E+03	3/3
	Species B, Type 7, strain Gomen	ATCC VR-7	7.38E+03	3/3
	Species B, Type 7A	ZeptoMetrix 0810021CF	7.38E+03	3/3
	Species B, Type 21, AV-1645 [128]	ATCC VR-1098 (New PN: VR-256)	7.38E+03	3/3
	Species C, Type 1*	ZeptoMetrix 0810050CF	6.02E+04	3/3
	Species C, Type 1, strain Adenoid 71	ATCC VR-1	6.02E+04	3/3
	Species C, Type 2	ZeptoMetrix 0810110CF	6.02E+04	3/3
	Species C, Type 5	ZeptoMetrix 0810020CF	6.02E+04	3/3
	Species C, Type 6	ZeptoMetrix 0810111CF	6.02E+04	3/3
	Species E, Type 4*	ZeptoMetrix 0810070CF	2.20E+04	3/3
C. pneumoniae	TW-183*	ATCC VR-2282	7.13E+02	3/3
	TWAR (CDC/CWL-029)	ATCC VR-1310	7.13E+02	3/3

Organism	Strain	Supplier / Part Number	Concentration Tested (Copies/mL)	# Detected /# Tested
	TWAR 2023	ATCC VR-1356	7.13E+02	3/3
	AR-39	ATCC 53592	7.13E+02	3/3
M. pneumoniae	M129*	ZeptoMetrix 0801579	1.29E+04	3/3
	[M52]	ATCC 15293	1.29E+04	3/3
	FH strain of Eaton Agent [NCTC 10119]	ATCC 15531-TTR	1.29E+04	3/3
	Mutant 22	ATCC 39505	1.29E+04	3/3

* Indicates a LoD strain.
[a] Highest possible concentration.
[b] NxTAG RPP v2 does not differentiate Influenza A H5, H7 or H9, all of which are reported as Influenza A
[c] NxTAG RPP v2 does not differentiate the Yamagata and Victoria lineages, both of which are reported as influenza B
[d] Samples were obtained as RNA. The RNA was diluted in extracted negative simulated matrix to a concentration that represented 1.50E+03 copies/mL in a raw sample.
[e] Concentration units for these strains are Genome equivalents/mL.
[f] Reported by NxTAG RPP v2 as Rhinovirus/Enterovirus

Analytical Reactivity In Silico Analysis

Based on *in silico* inclusivity analysis, it is predicted that the SARS-CoV-2 sequences available from GISAID EpiCoV database as of November 30, 2023, including sequences from all defined variants of concern or interest, are 100% detectable by NxTAG® Respiratory Pathogen Panel v2 (NxTAG RPP v2) assay.

Influenza A and B inclusivity was assessed with sequences available from the GISAID EpiFlu database between January 1, 2017 and May 5, 2023, as well as between January 1, 2000 and December 31, 2008. The assay oligos for Influenza A, Influenza A H1 (including H1pdm09), Influenza A H3, and Influenza B are predicted to have ~99% inclusivity against the analyzed sequences.

For all targets other than SARS-CoV-2 and the influenza viruses, *in silico* inclusivity analysis was performed with sequences available from the GenBank® Nucleotide (nt) database as of April 8, 2023. Based on this analysis, ≥96% of sequences of each analyte were predicted to be detected by NxTAG® RPP v2, except for Parainfluenza Virus 2 (~92%) and untyped strains of Parainfluenza Virus 4 (~94%), which exhibited lower homology.

g. Analytical Specificity

i) Cross-Reactivity

Analytical Specificity (Exclusivity) of the NxTAG® Respiratory Pathogen Panel v2 (NxTAG® RPP v2) assay was assessed with pathogens that cause respiratory infections or those that may be found in respiratory specimens. Sixty-three (63) organisms (82 strains total) were tested, including 41 pathogens that are not detected by NxTAG® RPP v2, and pooled nasal wash (referred to as "Off-panel organisms" – Table 6) and 22 that are detected by the assay (referred to as "On-Panel organisms" – Table 7). Each strain was prepared in negative simulated matrix (NSM) to reach the high positive concentration and tested in triplicate on the NxTAG® RPP v2 assay. None of the off-panel or on-panel organisms tested showed cross-reactivity, with the exception of one strain: Enterovirus (Species D, Type 68, US/IL/14-18952). This strain generated a false positive call for Influenza A H3 when it was tested at ≥ 1.00E+03 $TCID_{50}$/mL, although the influenza A matrix gene target was negative. No false positive call was generated when the strain was tested at 1.00E+02 $TCID_{50}$/mL and five other isolates of Enterovirus D showed no evidence of cross-reaction.

Table 6: NxTAG® RPP v2 Analytical Specificity (Off-Panel Organisms)

Off-Panel Organisms	Concentration Tested		Cross-Reactivity Detected
Aspergillus flavus	1.00E+06	CFU/mL	None
Aspergillus fumigatus	1.00E+06	CFU/mL	None
Bordetella parapertussis	1.00E+06	CFU/mL	None
Bordetella pertussis	1.00E+06	CFU/mL	None
Candida albicans	1.00E+06	CFU/mL	None
Chlamydia trachomatis	1.00E+06	IFU/mL	None
Corynebacterium diphtheriae	1.00E+06	CFU/mL	None
Corynebacterium striatum	1.00E+06	CFU/mL	None
Cytomegalovirus	1.00E+05	$TCID_{50}$/mL	None
Epstein Barr Virus	1.00E+07	Copies/mL	None
Escherichia coli	1.00E+06	CFU/mL	None
Fusobacterium necrophorum	1.00E+06	CFU/mL	None
Haemophilus influenzae	1.00E+06	CFU/mL	None
Herpes Simplex virus Type 1	1.00E+05	$TCID_{50}$/mL	None
Human Bocavirus	1.00E+07	Copies/mL	None
Klebsiella pneumoniae	1.00E+06	CFU/mL	None
Lactobacillus acidophilus	1.00E+06	CFU/mL	None
Lactobacillus plantarum	1.00E+06	CFU/mL	None
Legionella (Tatlockia) micdadei	1.00E+06	CFU/mL	None

Off-Panel Organisms	Concentration Tested		Cross-Reactivity Detected
Legionella pneumophila	1.00E+06	CFU/mL	None
Measles Virus	1.00E+05	$TCID_{50}$/mL	None
MERS-coronavirus	1.00E+05	$TCID_{50}$/mL	None
Moraxella catarrhalis	1.00E+06	CFU/mL	None
Mumps Virus	1.00E+05	$TCID_{50}$/mL	None
Mycobacterium tuberculosis	1.00E+06	CFU/mL	None
Mycoplasma genitalium	1.00E+05[1]	CCU/mL (Approximate)	None
Mycoplasma hominis	1.00E+06	CCU/mL	None
Neisseria elongata	1.00E+06	CFU/mL	None
Neisseria gonorrhoeae	1.00E+06	CFU/mL	None
Neisseria meningitidis	1.00E+06	CFU/mL	None
Pneumocystis carinii	1.00E+06	nuclei/mL	None
Pseudomonas aeruginosa	1.00E+06	CFU/mL	None
Serratia marcescens	1.00E+06	CFU/mL	None
Staphylococcus aureus	1.00E+06	CFU/mL	None
Staphylococcus epidermidis	1.00E+06	CFU/mL	None
Streptococcus agalactiae	1.00E+06	CFU/mL	None
Streptococcus pneumoniae	1.00E+06	CFU/mL	None
Streptococcus pyogenes	1.00E+06	CFU/mL	None
Streptococcus salivarius	1.00E+06	CFU/mL	None
SARS-coronavirus	3.01E+05[1]	Copies/mL	None
Varicella Zoster Virus	1.00E+05	$TCID_{50}$/mL	None
N/A (Pooled Nasal Wash)	N/A	N/A	None

[1]Highest concentration based on the available stock.

Table 7: NxTAG® RPP v2 Analytical Specificity (On-Panel Organisms)

On-Panel Organisms (strain/subtype)	Concentration Tested		Cross-Reactivity Detected
Influenza A H1N1 (Brisbane/59/07)	1.00E+05	$TCID_{50}$/mL	None
Influenza A 2009 H1N1 (A/NY/02/09)	1.00E+05	$TCID_{50}$/mL	None
Influenza A H3N2 (Wisconsin/67/05)	1.00E+05	$TCID_{50}$/mL	None
Influenza B (Florida/02/06) (LN: 325286)	1.00E+05	$TCID_{50}$/mL	None
Influenza B (Florida/02/06) (LN: 325345)	1.00E+05	$TCID_{50}$/mL	None
Influenza B (Florida/02/06) (LN: 307551)	1.00E+05	$TCID_{50}$/mL	None
Influenza B (B/Brisbane/33/2008)	1.00E+05	$TCID_{50}$/mL	None
Influenza B (B/Massachusetts/2/12)	1.00E+05	$TCID_{50}$/mL	None
Influenza B (B/Wisconsin/1/2010)	1.00E+05	$TCID_{50}$/mL	None

On-Panel Organisms (strain/subtype)	Concentration Tested		Cross-Reactivity Detected
Influenza B (B/Brigit (B/Russia/69))	1.00E+05	$CEID_{50}$/mL	None
Influenza B (B/Hong Kong/5/72)	1.00E+05	$CEID_{50}$/mL	None
Influenza B (B/Russia/69)	1.00E+05	$CEID_{50}$/mL	None
Influenza B (B/GreLakes/1739/1954)	1.00E+05	$CEID_{50}$/mL	None
Influenza B (B/Bangladesh/5972/2007)	1.00E+05	$TCID_{50}$/mL	None
Influenza B (B/Hong Kong/259/2010)	1.00E+05	$CEID_{50}$/mL	None
Influenza B (B/Texas/02/2013)	1.00E+05	$TCID_{50}$/mL	None
Influenza B (B/Hubei-Wujiagang/158/2009)	1.00E+05	$CEID_{50}$/mL	None
Influenza B (B/New Jersey/1/2012)	1.00E+05	$TCID_{50}$/mL	None
Influenza B (B/Brisbane/3/2007)	1.00E+05	$CEID_{50}$/mL	None
Human Respiratory Syncytial Virus A (A2)	1.00E+05	PFU/mL	None
Human Respiratory Syncytial Virus B (18537)	7.00E+04[a]	PFU/mL	None
SARS-CoV-2 (USA-WA-1/2020)	1.00E+07	Copies/mL	None
Coronavirus 229E	1.00E+05	$TCID_{50}$/mL	None
Coronavirus OC43	1.00E+05	$TCID_{50}$/mL	None
Coronavirus NL63	1.00E+05	$TCID_{50}$/mL	None
Coronavirus HKU1	1.00E+06[a]	Copies/mL	None
Human Metapneumovirus (hMPV-16, Type A1, IA10-2003)	1.00E+05	$TCID_{50}$/mL	None
Human Metapneumovirus (hMPV-2, Type B1, Peru2-2002)	3.89E+04[a]	$TCID_{50}$/mL	None
Rhinovirus (Type 85, 50-525-CV54 [V-192-001-021])	1.00E+05	$TCID_{50}$/mL	None
Enterovirus (Species D, Type 68, 2007 Isolate)	1.00E+05	$TCID_{50}$/mL	None
Enterovirus (Species D, Type 68, US/MO/14-18947)	1.00E+05	$TCID_{50}$/mL	None
Enterovirus (Species D, Type 68, US/IL/14-18952)	1.00E+03	$TCID_{50}$/mL	Influenza A H3
Enterovirus (Species D, Type 68, US/KY/14-18953)	1.00E+05	$TCID_{50}$/mL	None
Enterovirus (Species D, Type 68, Fermon)	1.00E+05	$TCID_{50}$/mL	None
Enterovirus (68, 2014 Isolate 1)	1.00E+05	$TCID_{50}$/mL	None
Adenovirus (Species B; Type 14, 2006 isolate)	1.00E+05	$TCID_{50}$/mL	None
Parainfluenza virus 1 (Type 1)	1.00E+05	$TCID_{50}$/mL	None
Parainfluenza virus 2 (Greer)	1.00E+05	$TCID_{50}$/mL	None
Parainfluenza virus 3 (C243)	1.00E+05	$TCID_{50}$/mL	None
Parainfluenza virus 4 (Subtype A)	1.00E+05	$TCID_{50}$/mL	None
Parainfluenza virus 4 (Subtype B; CH 19503)	1.00E+05	$TCID_{50}$/mL	None

On-Panel Organisms (strain/subtype)	Concentration Tested		Cross-Reactivity Detected
Chlamydia pneumoniae (TW-183)	1.00E+06	IFU/mL	None
Mycoplasma pneumoniae (M129)	1.00E+06	CCU/mL	None

[a]Highest concentration possible based on the available stock.

Analytical Cross-Reactivity In Silico Analysis

For *in silico* exclusivity assessment of the assay oligos against on-panel and off-panel organisms listed in Table 8 below, based on analysis of sequences available in the GenBank® Nucleotide (nt) database as of April 9, 2023, the following potential cross-reactivity is predicted:

- SARS-CoV-2 oligos are likely to detect some SARS-related coronavirus strains, as well as some bat coronavirus and bat SARS-like coronavirus strains.
- One bat 229E-like coronavirus sequence (KT253270) is likely to be detected by the Coronavirus 229E oligos at high viral titer.

Table 8. Potential Cross-Reactive Organisms assessed in the *In Silico* Exclusivity Analysis

On-Panel Organisms	Off-Panel Organisms	
Adenovirus	Bat SARS-like Coronavirus	*Chlamydia psittaci*
Enterovirus	Bat SARS-like Coronavirus HKU5	*Coxiella burnetii*
Human coronavirus 229E	Herpes Simplex Virus 2 (HSV2)	*Cryptococcus neoformans*
Human coronavirus HKU1	Human Bocavirus	*Fusobacterium necrophorum*
Human coronavirus NL63	Human Herpes Virus 6 (HHV6)	*Haemophilus influenza*
Human coronavirus OC43	Human Parechovirus (HPeV)	*Histoplasma capsulatum*
Human metapneumovirus (hMPV)	Influenza C	*Klebsiella (Enterobacter) aerogenes*
Influenza A	MERS-coronavirus	*Klebsiella oxytoca*
Influenza B	SARS-coronavirus	*Legionella pneumophila*
Parainfluenza virus 1	*Acinetobacter calcoaceticus*	*Leptospira interrogans*
Parainfluenza virus 2	*Arcanobacterium haemolyticum*	*Mycobacterium tuberculosis*
Parainfluenza virus 3	*Aspergillus fumigatus*	*Mycoplasma orale*
Parainfluenza virus 4	*Aspergillus flavus*	*Pneumocystis jirovecii* (PJP)
Respiratory syncytial virus A	*Bacillus anthracis*	*Proteus mirabilis*
Respiratory syncytial virus B	*Blastomyces dennatitidis*	*Pseudomonas aeruginosa*
Rhinovirus	*Bordetella avium*	*Staphylococcus epidermidis*
SARS-CoV-2	*Bordetella bronchiseptica*	*Stenotrophomonas maltophilia*
Chlamydia pneumoniae	*Bordetella hinzii*	*Streptococcus dysgalactiae*
Mycoplasma pneumoniae	*Bordetella holmesii*	*Streptococcus pneumonia*
	Bordetella pertussis	*Streptococcus pyogenes*
	Burkholderia cepacia	*Streptococcus salivarius*

On-Panel Organisms	Off-Panel Organisms	
	Candida albicans	*Ureaplasma urealyticum*

ii) Microbial Interference

The performance of NxTAG® RPP v2 in the presence of potentially interfering off-panel organisms (i.e., pathogens that are not detected by NxTAG® RPP v2) was evaluated by testing 11 off-panel organisms against 22 on-panel organisms detected by the assay. The samples were prepared in negative simulated matrix (NSM), with on-panel targets in multi-analyte samples at Low-Moderate Positive concentration (3x-6x LoD) and pools of off-panel organisms at the high positive concentration. Each combination was tested in triplicate on the NxTAG® RPP v2 assay. No off-panel organism present at high positive concentration interfered with the detection of any on-panel organism present at low positive concentration. The summary of results is shown in Table 9.

Table 9: NxTAG® RPP v2 Microbial Interference

Off-Panel Organisms	Strain	Concentration Tested	Microbial Interference Detected
Bordetella pertussis	A639	1.00E+06 CFU/mL	None
Cytomegalovirus	Merlin	1.00E+05 $TCID_{50}$/mL	None
Corynebacterium diphtheriae	Z116	1.00E+06 CFU/mL	None
Haemophilus influenzae	Type b; MinnA	1.00E+06 CFU/mL	None
Measles Virus	N/A	1.00E+05 $TCID_{50}$/mL	None
Moraxella catarrhalis	Strain NE 11	1.00E+06 CFU/mL	None
Mumps Virus	N/A	1.00E+05 $TCID_{50}$/mL	None
Neisseria meningitides	Serotype A	1.00E+06 CFU/mL	None
Pseudomonas aeruginosa	Clinical isolate	1.00E+06 CFU/mL	None
Staphylococcus aureus	102-04	1.00E+06 CFU/mL	None
Streptococcus pneumoniae	Z022	1.00E+06 CFU/mL	None

iii) Competitive Interference (Co-infection)

The performance of NxTAG® RPP v2 assay in the presence of potentially interfering on-panel organisms (i.e., pathogens that are detected by NxTAG® RPP v2) was

evaluated by testing samples that contain multiple on-panel targets, which included clinically relevant co-infections that occur in respiratory samples. The samples were prepared in negative simulated matrix (NSM), with on-panel organisms (in either single-analyte or multi-analyte samples) at Low-Moderate Positive concentration (3x-6x LoD), and the potentially interfering on-panel organisms at high positive concentration. Each combination was tested in triplicate on the NxTAG® RPP v2 assay. No on-panel organism present at high positive concentration interfered with the detection of any other on-panel organism present at low-moderate positive concentration. The summary of results is shown in Table 10.

Table 10: NxTAG® RPP v2 Competitive Interference

High Positive		Low-Moderate Positive	Competitive Interference Detected
Target	**Concentration**	**Target**	
Influenza A H1pdm09 (strain: A/NY/02/09)	1.00E+05 $TCID_{50}$/mL	Respiratory Syncytial Virus A	None
		Rhinovirus	None
		Respiratory Syncytial Virus B	None
		Influenza A H3	None
		Influenza B	None
		Parainfluenza virus 3	None
		Mycoplasma pneumoniae	None
		SARS-CoV-2	None
		Human Metapneumovirus	None
		Adenovirus	None
Influenza A H3 (Strain: Wisconsin/67/05)	1.00E+05 $TCID_{50}$/mL	Influenza A H1pdm09	None
		Respiratory Syncytial Virus A	None
		Rhinovirus	None
		Respiratory Syncytial Virus B	None
		Influenza B	None
		Parainfluenza virus 3	None
		Mycoplasma pneumoniae	None
		SARS-CoV-2	None
		Human Metapneumovirus	None
		Adenovirus	None
Influenza B	1.00E+05	Influenza A H1pdm09	None

High Positive		Low-Moderate Positive	Competitive Interference Detected
Target	**Concentration**	**Target**	
(Strain: Florida/02/06)	$TCID_{50}$/mL	Respiratory Syncytial Virus A	None
		Rhinovirus	None
		Respiratory Syncytial Virus B	None
		Influenza A H3	None
		Parainfluenza virus 3	None
		Mycoplasma pneumoniae	None
		SARS-CoV-2	None
		Human Metapneumovirus	None
		Adenovirus	None
Respiratory Syncytial Virus B (Strain: B/18537)	6.30E+04[a] PFU/mL	Influenza A H1pdm09	None
		Respiratory Syncytial Virus A	None
		Rhinovirus	None
		Influenza A H3	None
		Influenza B	None
		Parainfluenza virus 3	None
		Mycoplasma pneumoniae	None
		SARS-CoV-2	None
		Human Metapneumovirus	None
		Adenovirus	None
Respiratory Syncytial Virus B (Strain: B/WV/14617/85)	1.00E+05 $TCID_{50}$/mL	Influenza A H1pdm09	None
		Respiratory Syncytial Virus A	None
		Rhinovirus	None
		Influenza A H3	None
		Influenza B	None
		Parainfluenza virus 3	None
		Mycoplasma pneumoniae	None
		SARS-CoV-2	None
		Human Metapneumovirus	None
		Adenovirus	None
SARS-CoV-2 (Strain: USA-WA-	1.00E+07 Copies/mL	Influenza A H1pdm09	None
		Respiratory Syncytial Virus A	None

High Positive		Low-Moderate Positive	Competitive Interference Detected
Target	**Concentration**	**Target**	
1/2020)		Rhinovirus	None
		Respiratory Syncytial Virus B	None
		Influenza A H3	None
		Influenza B	None
		Parainfluenza virus 3	None
		Mycoplasma pneumoniae	None
		Human Metapneumovirus	None
		Adenovirus	None
Coronavirus NL63 (Strain: NL63)	1.00E+05 $TCID_{50}$/mL	Influenza A H1pdm09	None
		Respiratory Syncytial Virus A	None
		Rhinovirus	None
		Respiratory Syncytial Virus B	None
		Influenza A H3	None
		Influenza B	None
		Parainfluenza virus 3	None
		Mycoplasma pneumoniae	None
		SARS-CoV-2	None
		Human Metapneumovirus	None
		Adenovirus	None
Human Metapneumovirus (Strain: hMPV-16, Type A1, IA10-2003)	1.00E+05 $TCID_{50}$/mL	Influenza A H1pdm09	None
		Respiratory Syncytial Virus A	None
		Rhinovirus	None
		Respiratory Syncytial Virus B	None
		Influenza A H3	None
		Influenza B	None
		Parainfluenza virus 3	None
		Mycoplasma pneumoniae	None
		SARS-CoV-2	None
		Human Metapneumovirus	None
		Adenovirus	None
Human	3.50E+04[a]	Influenza A H1pdm09	None

High Positive		Low-Moderate Positive	Competitive Interference Detected
Target	**Concentration**	**Target**	
Metapneumovirus (Strain: hMPV-3, Type B1, Peru2-2002)	$TCID_{50}$/mL	Respiratory Syncytial Virus A	None
		Rhinovirus	None
		Respiratory Syncytial Virus B	None
		Influenza A H3	None
		Influenza B	None
		Parainfluenza virus 3	None
		Mycoplasma pneumoniae	None
		SARS-CoV-2	None
		Adenovirus	None
Human Metapneumovirus (Strain: hMPV-5, Type B1, Peru3-2003 G gene)	1.00E+05 $TCID_{50}$/mL	Influenza A H1pdm09	None
		Respiratory Syncytial Virus A	None
		Rhinovirus	None
		Respiratory Syncytial Virus B	None
		Influenza A H3	None
		Influenza B	None
		Parainfluenza virus 3	None
		Mycoplasma pneumoniae	None
		SARS-CoV-2	None
		Adenovirus	None
Rhinovirus (Strain: Type 85, 50-525-CV54 [V-192-001-021])	1.00E+05 $TCID_{50}$/mL	Influenza A H1pdm09	None
		Respiratory Syncytial Virus A	None
		Respiratory Syncytial Virus B	None
		Influenza A H3	None
		Influenza B	None
		Parainfluenza virus 3	None
		Mycoplasma pneumoniae	None
		SARS-CoV-2	None
		Human Metapneumovirus	None
		Adenovirus	None
Adenovirus B		Influenza A H1pdm09	None

High Positive		Low-Moderate Positive	Competitive Interference Detected
Target	Concentration	Target	
(Strain: Type 14, 2006 isolate)	1.00E+05 $TCID_{50}$/mL	Respiratory Syncytial Virus A	None
		Rhinovirus	None
		Respiratory Syncytial Virus B	None
		Influenza A H3	None
		Influenza B	None
		Parainfluenza virus 3	None
		Mycoplasma pneumoniae	None
		SARS-CoV-2	None
		Human Metapneumovirus	None

[a] The highest possible concentration was tested.

Additional competitive interference was evaluated for SARS-CoV-2. Low Positive SARS-CoV-2 (3x LoD) was tested against the remaining 13 NxTAG® RPP v2 assay targets that were not tested with the SARS-CoV-2 containing multi-analyte combinations. High Positive targets were tested at 1.00E+06 CCU/mL or IFU/mL for bacteria; 1.00E+05 $TCID_{50}$/mL, PFU/mL, or 1.00E+07 Copies/mL, or highest possible concentration for virus. Samples were prepared in negative simulated matrix and each combination was tested in triplicate with the assay. No interference of SARS-CoV-2 low positive detection was observed (Table 11).

Table 11: NxTAG® RPP v2 Competitive Interference Study – Additional Tests Performed with SARS-CoV-2

High Positive		Low Positive	Competitive Interference Detected
Target	Concentration	Target	
Influenza A H1	1.00E+05 $TCID_{50}$/mL	SARS-CoV-2	None
Respiratory Syncytial Virus A	1.00E+05 PFU/mL		None
Parainfluenza virus 1	1.00E+05 $TCID_{50}$/mL		None
Parainfluenza virus 2	1.00E+05 $TCID_{50}$/mL		None
Parainfluenza virus 3	1.00E+05 $TCID_{50}$/mL		None

Parainfluenza virus 4A[a]	1.00E+05 $TCID_{50}$/mL		None
Parainfluenza virus 4B[a]	1.00E+05 $TCID_{50}$/mL		None
Coronavirus 229E	1.00E+05 $TCID_{50}$/mL		None
Coronavirus OC43	1.00E+05 $TCID_{50}$/mL		None
Coronavirus HKU1	1.00E+06[b] Copies/mL		None
Enterovirus[c]	1.00E+05 $TCID_{50}$/mL		None
Chlamydia pneumoniae	1.00E+06 IFU/mL		None
Mycoplasma pneumoniae	1.00E+06 CCU/mL		None

[a] Reported by NxTAG RPP as Parainfluenza virus 4
[b] The highest possible concentration was tested
[c] Reported by NxTAG RPP v2 as Rhinovirus/Enterovirus

iv) Interfering Substances:

The performance of NxTAG® RPP v2 in the presence of potentially interfering substances was assessed. Twenty non-microbial substances commonly found in respiratory specimens were tested on the assay alone or in the presence of pathogens detected by the assay in multi-analyte samples. The samples were prepared in negative simulated matrix (NSM), with the on-panel organisms at Low-Moderate Positive concentration (3x-6x LoD) and the potentially interfering substance at the concentration listed in Table 14. All samples were tested in triplicate on the NxTAG® RPP v2 assay. None of the substances tested interfered with the detection of on-panel organisms present in the sample, with the exception of menthol and FluMist®. Menthol interfered with the detection of Coronavirus OC43 at 1% w/v; no interference was observed when menthol was tested at 0.5% w/v. FluMist® generated positive calls for Influenza A (matrix), Influenza A H1pdm09, Influenza A H3, and Influenza B for all replicates it was present in. These positive calls are expected as FluMist® contains attenuated Influenza A H1N1, Influenza A H3N2, and Influenza B strains. Positive influenza results obtained in a patient who received FluMist® prior to sample collection may be due to detection of vaccine virus and may mask a true positive result due to infection by one or more of these analytes. The list of potentially interfering substances and the concentrations tested are shown in Table 12.

Table 12: NxTAG® RPP v2 Interfering Substances

Potential Interferent	Active Ingredient	Concentration Tested
Human Whole Blood	N/A	5% (v/v)

Potential Interferent	Active Ingredient	Concentration Tested
Human Genomic DNA	N/A	20 ng/µL
Mucin	Mucin	100 µg/mL
Phenylephrine	Phenylephrine	0.03 µg/mL
Beclomethasone dipropionate	Beclomethasone dipropionate	25 µg/mL
Dexamethasone	Dexamethasone	12 µg/mL
Flunisolide	Flunisolide	5 µg/mL
Triamcinolone acetonide	Triamcinolone acetonide	22 µg/mL
Budesonide	Budesonide	6.30E-03 µg/mL
Mometasone furoate	Mometasone furoate	4.50E-04 µg/mL
Fluticasone proprionate	Fluticasone	1.26E-03 µg/mL
Drixoral®	Oxymetaxoline	10% (v/v)
		15% (v/v)
ZICAM® Allergy Relief	Galphimia Glauca Histaminum Hydrochloricum Luffa operculata Sulfur	1% (v/v)
		5% (v/v)
Salinex®	Sodium Chloride	1% (v/v)
		15% (v/v)
Mupirocin	Mupirocin	1.5 µg/mL
Tobramycin	Tobramycin	33 µg/mL
		600 µg/mL
Zanamivir	Zanamivir	100 µg/mL
FluMist®	Influenza A H1N1, Influenza A H3N2, Influenza B Yamagata lineage, Influenza B Victoria Lineage	0.5% (v/v) [a]
Benzocaine	Benzocaine	10% (w/v)
Menthol	Menthol	1% (w/v) [b]
		0.5% (w/v)
Leukocyte	Leukocyte	1.00E+03 cells/µL
Early Defence Nasal Spray®	Zinc	5% (v/v)

[a] FluMist® demonstrated interference with the detection of Influenza A (matrix), Influenza A H1pdm09, Influenza A H3, and Influenza B at 0.5% v/v.

[b] Menthol demonstrated interference with the detection of Coronavirus OC43 at 1% w/v; no interference was observed at 0.5% w/v.

v) <u>Carry-Over/Cross-Contamination:</u>

The risk of carry-over and cross-contamination events for the NxTAG® RPP v2 assay was assessed by testing samples at high titer in alternating pattern with negative samples. Two representative targets, SARS-CoV-2 (viral) and *Mycoplasma pneumoniae* (bacterial), were each extracted in an alternating arrangement with the negative sample across two easyMAG instruments. The extracts were then tested in an alternating arrangement with the NxTAG® RPP v2 assay. Two false positives were observed for the SARS-CoV-2 target; one after a negative sample and the other after a high titer sample. The samples were re-run to determine whether the extracts were contaminated, and the re-run results confirmed the contamination of the extracts. Following this, the samples were re-prepared and re-tested, generating the expected results.

h. Assay cut-off

Thresholds for the NxTAG® RPP v2 assay are provided in Table 13.

Table 13. MFI and MDD Thresholds (Cut-Off Values) for NxTAG® RPP v2

Analyte	MFI Threshold	MDD Threshold
Influenza A	45	35
Influenza A H1 (H1-A)	90	75
Influenza A H1pdm09 (H1-B)	55	45
Influenza A H3	80	50
Influenza B	60	40
RSV A	50	45
RSV B	45	35
Parainfluenza 1	65	50
Parainfluenza 2	70	55
Parainfluenza 3	60	50
Parainfluenza 4A [a]	70	55
Parainfluenza 4B [a]	60	45
SARS-CoV-2 (ORF1ab) [b]	35	30
SARS-CoV-2 (M) [b]	35	30
Coronavirus 229E	60	50
Coronavirus NL63	75	60
Coronavirus OC43	70	55
Coronavirus HKU1	60	40
Metapneumovirus	55	40
Rhinovirus/Enterovirus	50	40
Adenovirus	75	65
Chlamydia pneumoniae	45	40

Analyte	MFI Threshold	MDD Threshold
Mycoplasma pneumoniae	40	30
Internal Control	120	100

[a] NxTAG RPP v2 does not differentiate Parainfluenza virus 4A and 4B, both of which are reported as Parainfluenza virus 4

[b] NxTAG RPP v2 reports a single combined result for the SARS-CoV-2 ORF1ab and M gene targets

2. Comparison Studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix and Multi-Analyte Sample Comparison:

Equivalency between multi-analyte (MA) and single target samples, and NCM and negative simulated matrix (NSM) were assessed to demonstrate the validity of using MA samples and/or NSM in applicable analytical studies. Eight (8) MA samples, covering all targets probed by NxTAG® RPP v2, were evaluated. Each MA sample consisted of 2-3 targets and was prepared in both NCM and NSM at or near the LoD concentration (1x – 2x LoD) confirmed with single target samples in NCM. Twenty (20) replicates of each MA were tested, and all targets in MA samples generated ≥ 95% positivity. The results demonstrate equivalency between single-analyte and multi-analyte samples as well as between NCM and NSM.

The multi-analyte sample composition and the equivalency test results are shown in Table 14.

Table 14: NxTAG® RPP v2 Multi-Analyte Vs. Single Analyte, and Negative Clinical Matrix Vs. Negative Simulated Matrix Equivalency

Multi-analyte Sample	Organism	Confirmed LoD (Copies/mL)	Positivity (%)	
			MA Sample in NCM	MA Sample in NSM
MA-1	Influenza A 2009 H1N1 (subtype)	9.84E+02	20/20 (100%)	20/20 (100%)
	Respiratory Syncytial Virus A	4.97E+03	19/20 (95%)	20/20 (100%)
	Rhinovirus	1.54E+03	20/20 (100%)	20/20 (100%)
MA-2	Influenza A H3 (subtype)	5.60E+01	20/20 (100%)	20/20 (100%)
	Respiratory Syncytial Virus B	7.21E+03	20/20 (100%)	20/20 (100%)

Multi-analyte Sample	Organism	Confirmed LoD (Copies/mL)	Positivity (%)	
			MA Sample in NCM	MA Sample in NSM
MA-3	Influenza B	6.33E+01	20/20 (100%)	19/20 (95%)
	Parainfluenza virus 3	1.01E+03	20/20 (100%)	20/20 (100%)
	Mycoplasma pneumoniae	3.23E+03	20/20 (100%)	20/20 (100%)
MA-4	SARS-CoV-2	5.00E+02	20/20 (100%)	20/20 (100%)
	Human Metapneumovirus	2.62E+02	20/20 (100%)	19/20 (95%)
	Adenovirus	1.42E+03	20/20 (100%)	20/20 (100%)
MA-5	Influenza A H3 (Matrix)	1.68E+02	20/20 (100%)	20/20 (100%)
	Coronavirus NL63	1.00E+02	20/20 (100%)	20/20 (100%)
	Coronavirus HKU1	4.18E+03	20/20 (100%)	19/20 (95%)
MA-6	Influenza H1 (subtype)	1.60E+03	20/20 (100%)	19/20 (95%)
	Parainfluenza virus 1	6.92E+02	20/20 (100%)	20/20 (100%)
	Chlamydia pneumoniae	2.38E+02	20/20 (100%)	20/20 (100%)
MA-7	Parainfluenza virus 2	3.45E+02	19/20 (95%)	19/20 (95%)
	Parainfluenza virus 4B	7.15E+03	20/20 (100%)	20/20 (100%)
	Coronavirus 229E	3.81E+02	20/20 (100%)	19/20 (95%)
MA-8	Parainfluenza virus 4A	1.69E+04	20/20 (100%)	20/20 (100%)
	Coronavirus OC43	4.55E+03	20/20 (100%)	20/20 (100%)

c. Specimen Collection Device Comparison:

The equivalency between three swab types (swabs with a nylon flocked tip, polyester tip, and rayon tip) for the collection of samples for testing on the NxTAG® RPP v2 was evaluated. The study assessed the transfer of samples from the swab to the collection media. Four multi-analyte (MA) samples consisting of targets detected by the NxTAG® RPP v2 assay were prepared in negative simulated matrix (NSM) and tested at a final concentration of 3x-6x LoD, along with a negative sample.

Samples prepared using swabs with a nylon flocked tip and a polyester tip generated 100% positivity for all targets present in the respective multi-analyte samples and 0% positivity for all targets for the negative sample.

Samples prepared using swabs with a rayon tip only generated 100% positivity for all targets present in the MA1 and MA2 samples, and 0% positivity for all targets for the negative sample. For the MA3 sample, two of three replicates generated negative results for all targets (Influenza B, Parainfluenza virus 3, and *M. pneumoniae*) and for the MA4 sample, one of three replicates generated a negative result for Adenovirus.

The results demonstrate equivalency between swabs with nylon flocked tip and polyester tip (Table 15). Based on the results of this study, rayon-tipped swabs should not be used to collect specimens for use with the NxTAG RPP v2 assay.

Table 15: NxTAG® RPP v2 Assay Swab Equivalency

Sample	Organism	Target Positivity		
		Nylon Flocked	Polyester	Rayon
MA1	Influenza A 2009 H1N1 (subtype)	100% (3/3)	100% (3/3)	100% (3/3)
	Respiratory Syncytial Virus A	100% (3/3)	100% (3/3)	100% (3/3)
	Rhinovirus	100% (3/3)	100% (3/3)	100% (3/3)
MA2	Influenza A H3 (subtype)	100% (3/3)	100% (3/3)	100% (3/3)
	Respiratory Syncytial Virus B	100% (3/3)	100% (3/3)	100% (3/3)
MA3	Influenza B	100% (3/3)	100% (3/3)	33% (1/3)
	Parainfluenza virus 3	100% (3/3)	100% (3/3)	33% (1/3)
	Mycoplasma pneumoniae	100% (3/3)	100% (3/3)	33% (1/3)
MA4	SARS-CoV-2	100% (3/3)	100% (3/3)	100% (3/3)
	Human Metapneumovirus	100% (3/3)	100% (3/3)	100% (3/3)
	Adenovirus	100% (3/3)	100% (3/3)	67% (2/3)
NEG	N/A	0% (0/3)	0% (0/3)	0% (0/3)

d. Specimen Collection Media and Extraction Comparison:

Equivalency between collection media; Universal Transport Media (UTM), and Remel MicroTest™ M4RT (M4RT), and between two extraction systems (bioMérieux's NucliSENS® easyMAG® and EMAG®) was assessed to verify their compatibility with the NxTAG® RPP v2 assay.

To assess collection media equivalency, four multi-analyte samples consisting of representative targets were contrived in pooled negative nasopharyngeal swabs (NPS) collected in UTM (NCM), and in pooled negative NPS collected in M4RT (NCM-M4RT). The samples, prepared at 3 dilution levels: Above LoD (5x -9x LoD), At/Near LoD (1x-2x LoD), and Below LoD (1/3x-2/3x LoD), along with a negative sample (negative clinical matrix alone), were extracted using the EMAG system and tested on the NxTAG® RPP v2 assay.

All samples prepared in NCM and extracted using easyMAG generated ≥ 95% positivity for all targets at concentrations that were ≤ 2x LoD concentration confirmed in using the EMAG in the LoD study. The results demonstrate equivalency between extractions by EMAG and easyMAG for use with NxTAG® RPP v2. Results are summarized in Table 16.

Table 16: NxTAG® RPP v2 Collection Media Equivalency

Organism	Strain information	Concentration (Copies/mL)	Sample Type (LoD)	Target Positivity (%)	
				NCM (UTM)	NCM-M4RT
Influenza A 2009 H1N1	A/NY/02/09	8.52E+03	Above	10/10(100%)	10/10 (100%)
		1.70E+03	At/Near	30/30 (100%)	30/30 (100%)
		5.68E+02	Below	10/10 (100%)	7/10 (70%)
Respiratory Syncytial Virus A	A2	2.48E+04	Above	10/10 (100%)	10/10 (100%)
		4.97E+03	At/Near	30/30 (100%)	30/30 (100%)
		1.66E+03	Below	6/10 (60%)	5/10 (50%)
Rhinovirus	50-525-CV54	7.68E+03	Above	10/10 (100%)	10/10 (100%)
		1.54E+03	At/Near	30/30 (100%)	30/30 (100%)
		5.12E+02	Below	10/10 (100%)	9/10 (90%)
Influenza A H3	A/Wisconsin/67/05	2.80E+02	Above	10/10 (100%)	10/10 (100%)
		5.60E+01	At/Near	30/30 (100%)	30/30 (100%)
		1.87E+01	Below	4/10 (40%)	4/10 (40%)
Respiratory Syncytial Virus B	18537	3.60E+04	Above	10/10 (100%)	10/10 (100%)
		7.21E+03	At/Near	30/30 (100%)	30/30 (100%)
		2.40E+03	Below	6/10 (60%)	3/10 (30%)
Influenza B	B/Florida/02/06	3.16E+02	Above	10/10 (100%)	10/10 (100%)
		6.33E+01	At/Near	29/30 (97%)	29/30 (97%)
		2.11E+01	Below	5/10 (50%)	4/10 (40%)
Parainfluenza virus	C 243	5.07E+03	Above	10/10 (100%)	10/10 (100%)

Organism	Strain information	Concentration (Copies/mL)	Sample Type (LoD)	Target Positivity (%)	
				NCM (UTM)	NCM-M4RT
3		2.03E+03	At/Near	30/30 (100%)	29/30 (97%)
		1.01E+03	At/Near	26/30 (87%)	22/30 (73%)
		3.38E+02	Below	0/10 (0%)	2/10 (20%)
Mycoplasma pneumoniae	M129	2.15E+04	Above	10/10 (100%)	10/10 (100%)
		4.30E+03	At/Near	30/30 (100%)	29/30 (97%)
		1.43E+03	Below	3/10 (30%)	4/10 (40%)
SARS-CoV-2	USA-WA1/2020	2.50E+03	Above	10/10 (100%)	10/10 (100%)
		5.00E+02	At/Near	30/30 (100%)	30/30 (100%)
		1.67E+02	Below	10/10 (100%)	7/10 (70%)
Human Metapneumovirus	hMPV-3, Type B1, Peru2-2002	1.31E+03	Above	10/10 (100%)	10/10 (100%)
		2.62E+02	At/Near	29/30 (97%)	30/30 (100%)
		8.73E+01	Below	5/10 (50%)	7/10 (70%)
Adenovirus	Type 14	1.23E+04	Above	10/10 (100%)	10/10 (100%)
		3.69E+03	At/Near	30/30 (100%)	
		2.46E+03	At/Near	28/30 (93%)	30/30 (100%)
		8.20E+02	Below	4/10 (40%)	0/10 (0%)
N/A (Negative Sample)	N/A	N/A	N/A	0/10(0%)	0/10 (0%)

To demonstrate extractor equivalency, eight multi-analyte samples covering all targets probed by NxTAG® RPP v2 were tested. Multi-analyte samples were contrived in NCM, extracted on the easyMAG system, and tested on the NxTAG® RPP v2 assay.

All samples prepared in NCM and extracted using easyMAG generated ≥ 95% positivity for all targets at concentrations that were within 2x LoD of the confirmed concentration in multi-analyte samples prepared in NCM and extracted using the EMAG. The results demonstrate equivalency between extractions by EMAG and easyMAG for use with NxTAG® RPP v2. Results are summarized in Table 17.

Table 17: NxTAG® RPP v2 Extractor Equivalency

Multi-analyte Sample	Organism	Strain information	easyMAG (in NCM)		
			Concentration (Copies/mL)	Sample Type (LoD)	Positivity (%)
MA1	Influenza A 2009 H1N1	A/NY/02/09	1.70E+03	At/Near	20/20 (100%)
	Respiratory Syncytial Virus A	A2	4.97E+03	At/Near	20/20 (100%)
	Rhinovirus	50-525-CV54	5.12E+02	Below	20/20 (100%)
MA2	Influenza A H3	A/Wisconsin/67/05	5.60E+01	At/Near	20/20 (100%)
	Respiratory Syncytial Virus B	18537	7.21E+03	At/Near	20/20 (100%)
MA3	Influenza B	B/Florida/02/06	6.33E+01	At/Near	20/20 (100%)
	Parainfluenza virus 3	C 243	1.01E+03	At/Near	20/20 (100%)
	Mycoplasma pneumoniae	M129	4.30E+03	At/Near	20/20 (100%)
MA4	SARS-CoV-2	USA-WA1/2020	5.00E+02	At/Near	20/20 (100%)
	Human Metapneumovirus	hMPV-3, Type B1, Peru2-2002	2.62E+02	At/Near	20/20 (100%)
	Adenovirus	Type 14	2.46E+03	At/Near	20/20 (100%)
MA5	Influenza A Matrix	A/Wisconsin/67/05	1.68E+02	At/Near	20/20 (100%)
	Coronavirus NL63	N/A	1.00E+02	At/Near	20/20 (100%)
	Coronavirus HKU1	Type B	4.18E+03	At/Near	20/20 (100%)
MA6	Influenza H1	A/Brisbane/59/07	5.35E+02	Below	19/20 (95%)
	Parainfluenza virus 1	N/A	6.92E+02	Below	20/20 (100%)
	Chlamydia pneumoniae	TW-183	7.93E+01	Below	20/20 (100%)
MA7	Parainfluenza virus 2	Greer	3.45E+02	At/Near	19/20 (95%)
	Parainfluenza virus 4B	CH 19503	7.15E+03	At/Near	20/20 (100%)
	Coronavirus 229E	N/A	3.81E+02	At/Near	20/20 (100%)
MA8	Parainfluenza virus 4A	N/A	5.65E+03	Below	19/20 (95%)
	Coronavirus OC43	Betacoronavirus 1	4.55E+03	At/Near	20/20 (100%)

3. <u>Clinical Performance:</u>

A multi-site clinical study established the clinical performance of the NxTAG® RPP v2 assay for the detection and identification of nucleic acids from multiple respiratory viruses and bacteria extracted from upper respiratory tract specimens collected from individuals with clinical signs and symptoms of a respiratory tract infection. The clinical performance of the NxTAG® RPP v2 assay was evaluated using clinical specimens prospectively collected between October 2022 and April 2023 from five geographically diverse clinical sites within the United States. The clinical study utilized leftover, de-

identified specimens collected from pediatric and adult patients exhibiting clinical signs and symptoms of a respiratory tract infection.

The NxTAG® RPP v2 results were compared to those obtained with an FDA-cleared molecular assay and PCR/bi-directional sequencing for influenza A subtyping. The two-step PCR analysis followed by BDS assays employed two independent sets of validated PCR assays. The primers used for PCR analysis and sequencing assays, where possible, were designed to amplify distinct regions from the investigational device. PCR analysis composite positive specimens were confirmed by BDS assays. See Table 18 for comparator method testing by target.

Table 18: Prospective Comparator Method Algorithm

NxTAG® RPP v2 Target	Comparator Method
Adenovirus	FDA cleared molecular assay
Enterovirus/Rhinovirus	
Influenza A	
Influenza A H3	
Influenza B	
Respiratory Syncytial Virus A	
Respiratory Syncytial Virus B	
Parainfluenza 1	
Parainfluenza 2	
Parainfluenza 3	
Parainfluenza 4	
Coronavirus 229E	
Coronavirus NL63	
Coronavirus OC43	
Coronavirus HKU1	
Human Metapneumovirus	
Chlamydia pneumoniae	
Mycoplasma pneumoniae	
Influenza A H1	FDA cleared molecular assay followed by composite of PCR followed by BDS NAATs*
Influenza A H1pdm09	
SARS-CoV-2	FDA cleared molecular assay

* NAAT – Nucleic Acid Amplification Test

A total of 1844 prospective specimens, collected from five geographically diverse US sites were initially enrolled in the study, of which 19 were excluded from the analysis of performance (duplicate specimen, not from a unique subject (11), absence of signs and symptoms (5), improper labeling (2), operator error (1)). Nineteen of the remaining 1825 specimens initially produced invalid NxTAG RPP v2 results, of which 14 resolved upon repeat testing. Therefore, a total of 1820 prospectively collected specimens

generated valid NxTAG RPP v2 results after allowing for a single retest. Certain additional specimens were excluded from performance calculations for specific analytes based on the availability of valid results for the applicable comparator method. Clinical runs and re-runs using the NxTAG® RPP v2 assay were tested on the MAGPIX System by trained operators at three sites. Prospective specimen (Arm 1) testing occurred between March 2023 and April 2023.

For targets that exhibited low prevalence rates in the prospective study cohort, the prospective specimen set was supplemented with 320 pre-selected left-over, de-identified specimens (Arm 2) sourced from six sites in the United States. Pre-selected specimens were identified by Standard of Care (SoC) results and confirmed by BDS testing prior to enrollment in the study. Of the preselected specimens, 11 were excluded from the analysis of performance because confirmatory testing could not be completed. In addition, 3 specimens initially produced invalid NxTAG RPP v2 results, of which 2 resolved upon repeat testing. Therefore, a total of 308 preselected specimens were included in the analysis of performance. To minimize bias, pre-selected specimens were tested in a randomized, blinded manner with negative specimens at four sites. Pre-selected specimen (Arm 2) testing occurred between December 2021 and May 2023.

To supplement the number of clinical specimens positive for *Chlamydia pneumoniae*, RSV B, pre-2009 pandemic Influenza A H1N1, and Coronavirus 229E in the prospective and pre-selected arms of the study, additional testing was performed using contrived specimens. Contrived specimens were prepared by spiking representative strains into unique negative human nasopharyngeal specimens at 2x Limit of Detection (LoD), 10x LoD, and 100x LoD, for all strains except influenza A H1N1 which was prepared at 2x and 10x LoD. A total of 199 specimens were contrived and tested as part of Arm 3.

To minimize bias, contrived specimens were blinded, randomized, and tested along with Arm 2 positive and negative clinical specimens at two testing sites between December 2021 and January 2022. Results from contrived specimens were analyzed separately from the prospective and pre-selected data sets.

Out of the 199 specimens included in the contrived study analysis, 198 (99.50%) generated valid NxTAG® RPP v2 Assay results (i.e., Positive or Negative) on the first attempt. There was one specimen (0.50%) with an invalid result on the initial run. This specimen generated a valid result after a single retest for a final success rate of 100%.

The invalid rate for prospective, pre-selected, and contrived specimens combined was 0.98% (23/2344) after the initial run. Of the 23 specimens with initial invalid results, 17 (0.73%) specimens generated valid NxTAG® RPP v2 results after a single retest, three (0.13%) specimens remained invalid on repeat, and three (0.13%) specimens were not retested due to volume limitations.

For each target in the NxTAG® RPP v2 Assay, the performance (Positive Percent Agreement, Negative Percent Agreement, and 95% confidence interval) of the NxTAG® RPP v2 Assay as compared to the reference method are summarized in Tables 19 and 20 for prospective and pre-selected specimen analysis, respectively. The performance of the NxTAG® RPP v2 Assay for contrived specimens is presented separately in Table 21. For each of the contrived specimens (n = 199), negative results were obtained for all other analytes included on the panel that are not listed in Table 21.

Table 19: NxTAG® RPP v2 Performance for the Prospective Data Set

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
		TP / (TP+FN)	PPA (%)	95% CI	TN / (TN+FP)	NPA (%)	95% CI
Viruses							
Adenovirus	Fresh	39/39	100%	91%-100%	733/736	99.6%	99%-100%
	Frozen	55/59	93.2%	84%-97%	976/980	99.6%	99%-100%
	Overall	**94/98[a]**	**95.9%**	**90%-98%**	**1709/1716[b]**	**99.6%**	**99%-100%**
Coronavirus 229E	Fresh	2/2	100%	34%-100%	773/773	100%	100%-100%
	Frozen	5/5	100%	57%-100%	1033/1034	99.9%	99%-100%
	Overall	**7/7**	**100%**	**65%-100%**	**1806/1807**	**99.9%**	**100%-100%**
Coronavirus HKU1	Fresh	8/8	100%	68%-100%	767/767	100%	100%-100%
	Frozen	12/13	92.3%	67%-99%	1026/1026	100%	100%-100%
	Overall	**20/21[c]**	**95.2%**	**77%-99%**	**1793/1793**	**100%**	**100%-100%**
Coronavirus NL63	Fresh	25/27	92.6%	77%-98%	748/748	100%	99%-100%
	Frozen	23/25	92%	75%-98%	1014/1014	100%	100%-100%
	Overall	**48/52**	**92.3%**	**82%-97%**	**1762/1762**	**100%**	**100%-100%**
Coronavirus OC43	Fresh	10/10	100%	72%-100%	765/765	100%	100%-100%
	Frozen	29/29	100%	88%-100%	1010/1010	100%	100%-100%
	Overall	**39/39**	**100%**	**91%-100%**	**1775/1775**	**100%**	**100%-100%**
Human Metapneumovirus	Fresh	87/87	100%	96%-100%	680/688	98.8%	98%-99%
	Frozen	70/70	100%	95%-100%	963/969	99.4%	99%-100%
	Overall	**157/157**	**100%**	**98%-100%**	**1643/1657[d]**	**99.2%**	**99%-99%**
Influenza A	Fresh	20/20	100%	84%-100%	753/755	99.7%	99%-100%
	Frozen	54/54	100%	93%-100%	984/985	99.9%	99%-100%
	Overall	**74/74**	**100%**	**95%-100%**	**1737/1740[e]**	**99.8%**	**99%-100%**
Influenza A H1pdm09	Fresh	9/9	100%	70%-100%	765/765	100%	100%-100%
	Frozen	22/22	100%	85%-100%	1017/1017	100%	100%-100%
	Overall	**31/31**	**100%**	**89%-100%**	**1782/1782**	**100%**	**100%-100%**
Influenza A H1	Fresh	0/0	N/A	N/A	774/774	100%	100%-100%
	Frozen	0/0	N/A	N/A	1039/1039	100%	100%-100%
	Overall	**0/0**	**N/A**	**N/A**	**1813/1813**	**100%**	**100%-100%**
Influenza A H3	Fresh	11/11	100%	74%-100%	764/764	100%	99%-100%
	Frozen	34/36	94.4%	82%-98%	1002/1003	99.9%	99%-100%

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
		TP / (TP+FN)	PPA (%)	95% CI	TN / (TN+FP)	NPA (%)	95% CI
	Overall	**45/47[f]**	**95.7%**	**86%-99%**	**1766/1767**	**99.9%**	**100%-100%**
Influenza B	Fresh	5/5	100%	57%-100%	770/770	100%	100%-100%
	Frozen	6/6	100%	61%-100%	1033/1033	100%	100%-100%
	Overall	**11/11**	**100%**	**74%-100%**	**1803/1803**	**100%**	**100%-100%**
Parainfluenza 1	Fresh	7/7	100%	65%-100%	768/768	100%	100%-100%
	Frozen	11/11	100%	74%-100%	1028/1028	100%	100%-100%
	Overall	**18/18**	**100%**	**82%-100%**	**1796/1796**	**100%**	**100%-100%**
Parainfluenza 2	Fresh	4/5	80%	38%-96%	770/770	100%	100%-100%
	Frozen	5/5	100%	57%-100%	1034/1034	100%	100%-100%
	Overall	**9/10**	**90%**	**60%-98%**	**1804/1804**	**100%**	**100%-100%**
Parainfluenza 3	Fresh	19/19	100%	83%-100%	756/756	100%	99%-100%
	Frozen	23/23	100%	86%-100%	1015/1016	99.9%	99%-100%
	Overall	**42/42**	**100%**	**92%-100%**	**1771/1772**	**99.9%**	**100%-100%**
Parainfluenza 4	Fresh	2/3	66.7%	21%-94%	770/772	99.7%	99%-100%
	Frozen	11/12	91.7%	65%-99%	1026/1027	99.9%	99%-100%
	Overall	**13/15[g]**	**86.7%**	**62%-96%**	**1796/1799[h]**	**99.8%**	**100%-100%**
RSV A	Fresh	10/10	100%	72%-100%	764/765	99.9%	99%-100%
	Frozen	45/45	100%	92%-100%	992/994	99.8%	99%-100%
	Overall	**55/55**	**100%**	**93%-100%**	**1756/1759[i]**	**99.8%**	**99%-100%**
RSV B	Fresh	3/3	100%	44%-100%	772/772	100%	100%-100%
	Frozen	17/17	100%	82%-100%	1022/1022	100%	100%-100%
	Overall	**20/20**	**100%**	**84%-100%**	**1794/1794**	**100%**	**100%-100%**
Rhinovirus / Enterovirus	Fresh	123/132	93.2%	88%-96%	643/643	100%	99%-100%
	Frozen	228/237	96.2%	93%-98%	801/802	99.9%	99%-100%
	Overall	**351/369[j]**	**95.1%**	**92%-97%**	**1444/1445**	**99.9%**	**100%-100%**
SARS-CoV-2	Fresh	103/106	97.2%	92%-99%	656/660	99.4%	98%-100%
	Frozen	126/128	98.4%	94%-100%	902/909	99.2%	98%-100%
	Overall	**229/234[k]**	**97.9%**	**95%-99%**	**1558/1569[l]**	**99.3%**	**99%-100%**
Bacteria							
Chlamydia pneumoniae	Fresh	0/0	N/A	N/A	775/775	100%	100%-100%
	Frozen	0/0	N/A	N/A	1039/1039	100%	100%-100%
	Overall	**0/0**	**N/A**	**N/A**	**1814/1814**	**100%**	**100%-100%**
Mycoplasma pneumoniae	Fresh	0/0	N/A	N/A	775/775	100%	100%-100%
	Frozen	0/0	N/A	N/A	1039/1039	100%	100%-100%
	Overall	**0/0**	**N/A**	**N/A**	**1814/1814**	**100%**	**100%-100%**

[a]Two of the four Adenovirus False Negatives were negative by BDS and two were not tested due to volume limitations.
[b]Four of the seven Adenovirus False Positives were positive by the molecular SoC assay.
[c]The one prospective Coronavirus HKU1 False Negative was negative by the molecular SoC assay.
[d]Two of the fourteen Human Metapneumovirus False Positives were positive by the molecular SoC assay. Seven samples could not be tested due to volume limitations.
[e]The Influenza A subtype was detected by the reference method for three of the three Influenza A False Positives.
[f]One of the two Influenza A H3 False Negatives was negative by the molecular SoC assay.
[g]One of the two Parainfluenza 4 False Negatives was negative by the molecular SoC assay.

[h]Two of the three Parainfluenza 4 False Positives were positive by the molecular SoC assay.
[i]One of the three RSV A False Positives was positive by the molecular SoC assay.
[j]Eight of the eighteen Rhinovirus/Enterovirus False Negatives were negative by BDS, and three False Negatives were negative by the molecular SoC assay. Five samples could not be tested due to volume limitations.
[k]Two of the five SARS-CoV-2 False Negatives were negative by the molecular SoC assay.
[l]Six of the eleven SARS-CoV-2 False Positives were positive by the molecular SoC assay.

Table 20: NxTAG® RPP v2 Performance for the Pre-Selected Data Set

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
		TP / (TP+FN)	PPA (%)	95% CI	TN / (TN+FP)	NPA (%)	95% CI
Viruses							
Adenovirus	Pre-Selected	1/1	100%	21%-100%	307/307	100%	99%-100%
Coronavirus 229E	Pre-Selected	11/11	100%	74%-100%	297/297	100%	99%-100%
Coronavirus HKU1	Pre-Selected	30/32	93.8%	80%-98%	276/276	100%	99%-100%
Coronavirus NL63	Pre-Selected	0/0	N/A	N/A	308/308	100%	99%-100%
Coronavirus OC43	Pre-Selected	0/0	N/A	N/A	308/308	100%	99%-100%
Human Metapneumovirus	Pre-Selected	0/0	N/A	N/A	308/308	100%	99%-100%
Influenza A	Pre-Selected	30/30	100%	89%-100%	277/278	99.6%	98%-100%
Influenza A H1pdm09	Pre-Selected	29/30	96.7%	83%-99%	278/278	100%	99%-100%
Influenza A H1	Pre-Selected	0/0	N/A	N/A	277/278	99.6%	98%-100%
Influenza A H3	Pre-Selected	0/0	N/A	N/A	278/278	100%	99%-100%
Influenza B	Pre-Selected	30/30	100%	89%-100%	278/278	100%	99%-100%
Parainfluenza 1	Pre-Selected	29/29	100%	88%-100%	279/279	100%	99%-100%
Parainfluenza 2	Pre-Selected	30/30	100%	89%-100%	278/278	100%	99%-100%
Parainfluenza 3	Pre-Selected	0/0	N/A	N/A	307/307	100%	99%-100%
Parainfluenza 4	Pre-Selected	15/16	93.8%	72%-99%	292/292	100%	99%-100%
RSV A	Pre-Selected	0/0	N/A	N/A	305/307	99.3%	98%-100%
RSV B	Pre-Selected	0/0	N/A	N/A	306/307	99.7%	98%-100%
Rhinovirus / Enterovirus	Pre-Selected	1/1	100%	21%-100%	302/306	98.7%	97%-99%
SARS-CoV-2	Pre-Selected	0/0	N/A	N/A	0/0	N/A	N/A
Bacteria							
Chlamydia pneumoniae	Pre-Selected	14/14	100%	78%-100%	293/294	99.7%	98%-100%

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
		TP / (TP+FN)	PPA (%)	95% CI	TN / (TN+FP)	NPA (%)	95% CI
Viruses							
Mycoplasma pneumoniae	Pre-Selected	48/52	92.3%	82%-97%	256/256	100%	99%-100%

Table 21: NxTAG® RPP v2 Performance for the Contrived Data Set

Pathogen Target		Positive Percent Agreement			Negative Percent Agreement		
		TP / (TP+FN)	PPA (%)	95% CI	TN / (TN+FP)	NPA (%)	95% CI
Analyte	**LoD**	**Viruses**					
Coronavirus 229E	2x	25/25	100%	87%-100%	N/A	N/A	N/A
	10x	12/12	100%	76%-100%	N/A	N/A	N/A
	100x	12/12	100%	76%-100%	N/A	N/A	N/A
	Combined	**49/49**	**100%**	**93%-100%**	**150/150**	**100%**	**98%-100%**
Influenza A (matrix)	2x	26/26	100%	87%-100%	N/A	N/A	N/A
	10x	24/24	100%	86%-100%	N/A	N/A	N/A
	100x	0/0	N/A	N/A	N/A	N/A	N/A
	Combined	**50/50**	**100%**	**93%-100%**	**149/149**	**100%**	**97%-100%**
Influenza A H1 (subtype)	2x	26/26	100%	87%-100%	N/A	N/A	N/A
	10x	24/24	100%	86%-100%	N/A	N/A	N/A
	100x	0/0	N/A	N/A	N/A	N/A	N/A
	Combined	**50/50**	**100%**	**93%-100%**	**149/149**	**100%**	**97%-100%**
RSV B	2x	24/25	96.0%	80%-99%	N/A	N/A	N/A
	10x	13/13	100%	77%-100%	N/A	N/A	N/A
	100x	12/12	100%	76%-100%	N/A	N/A	N/A
	Combined	**49/50**	**98.0%**	**90%-100%**	**148/149**	**99.3%**	**96%-100%**
Analyte	**LoD**	**Bacteria**					
Chlamydia pneumoniae	2x	25/25	100%	87%-100%	N/A	N/A	N/A
	10x	12/13	92.3%	67%-99%	N/A	N/A	N/A
	100x	12/12	100%	76%-100%	N/A	N/A	N/A
	Combined	**49/50**	**98.0%**	**90%-100%**	**149/149**	**100%**	**97%-100%**

The study results demonstrate that the diagnostic accuracy of the NxTAG® RPP v2 assay is acceptable for the detection and identification of respiratory bacteria and viruses from NPS specimens collected from patients exhibiting clinical signs and symptoms of RTI.

4. Expected values/Reference range:

Table 22: NxTAG® RPP v2 Expected Values for Prospective Specimens by Age

	0-1 years		>1-5 years		>5-21 years		>21-65 years		> 65 years		Unknown		Overall	
Target (Analyte)	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**
Adenovirus	34	10.1% (34/337)	40	15.8% (40/253)	22	6.7% (22/329)	5	0.8% (5/641)	0	0.0% (0/242)	0	0.0% (0/12)	101	5.6% (101/1814)
Chlamydia pneumoniae	0	0.0% (0/337)	0	0.0% (0/253)	0	0.0% (0/329)	0	0.0% (0/641)	0	0.0% (0/242)	0	0.0% (0/12)	0	0.0% (0/1814)
Coronavirus 229E	1	0.3% (1/337)	1	0.4% (1/253)	0	0.0% (0/329)	5	0.8% (5/641)	1	0.4% (1/242)	0	0.0% (0/12)	8	0.4% (8/1814)
Coronavirus HKU1	3	0.9% (3/337)	5	2.0% (5/253)	4	1.2% (4/329)	8	1.2% (8/641)	0	0.0% (0/242)	0	0.0% (0/12)	20	1.1% (20/1814)
Coronavirus NL63	17	5.0% (17/337)	12	4.7% (12/253)	8	2.4% (8/329)	9	1.4% (9/641)	2	0.8% (2/242)	0	0.0% (0/12)	48	2.6% (48/1814)
Coronavirus OC43	16	4.7% (16/337)	8	3.2% (8/253)	7	2.1% (7/329)	6	0.9% (6/641)	2	0.8% (2/242)	0	0.0% (0/12)	39	2.1% (39/1814)
Human Metapneumovirus	45	13.4% (45/337)	41	16.2% (41/253)	29	8.8% (29/329)	39	6.1% (39/641)	14	5.8% (14/242)	3	25% (3/12)	171	9.4% (171/1814)
Influenza A	7	2.1% (7/337)	8	3.2% (8/253)	25	7.6% (25/329)	27	4.2% (27/641)	10	4.1% (10/242)	0	0.0% (0/12)	77	4.2% (77/1814)
Influenza A H1pdm09	3	0.9% (3/337)	3	1.2% (3/253)	8	2.4% (8/329)	14	2.2% (14/641)	3	1.2% (3/242)	0	0.0% (0/12)	31	1.7% (31/1813)
Influenza A H1	0	0.0% (0/337)	0	0.0% (0/253)	0	0.0% (0/328)	0	0.0% (0/641)	0	0.0% (0/242)	0	0.0% (0/12)	0	0.0% (0/1813)
Influenza A H3	4	1.2% (4/337)	6	2.4% (6/253)	18	5.5% (18/329)	11	1.7% (11/641)	7	2.9% (7/242)	0	0.0% (0/12)	46	2.5% (46/1814)
Influenza B	0	0.0% (0/337)	3	1.2% (3/253)	3	0.9% (3/329)	5	0.8% (5/641)	0	0.0% (0/242)	0	0.0% (0/12)	11	0.6% (11/1814)

	0-1 years		>1-5 years		>5-21 years		>21-65 years		> 65 years		Unknown		Overall	
Target (Analyte)	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**	**#Pos**	**(%)**
Mycoplasma pneumoniae	0	0.0% (0/337)	0	0.0% (0/253)	0	0.0% (0/329)	0	0.0% (0/641)	0	0.0% (0/242)	0	0.0% (0/12)	0	0.0% (0/1814)
Parainfluenza 1	6	1.8% (6/337)	3	1.2% (3/253)	1	0.3% (1/329)	7	1.1% (7/641)	1	0.4% (1/242)	0	0.0% (0/12)	18	1.0% (18/1814)
Parainfluenza 2	1	0.3% (1/337)	1	0.4% (1/253)	3	0.9% (3/329)	1	0.2% (1/641)	3	1.2% (3/242)	0	0.0% (0/12)	9	0.5% (9/1814)
Parainfluenza 3	15	4.5% (15/337)	16	6.3% (16/253)	5	1.5% (5/329)	7	1.1% (7/641)	0	0.0% (0/242)	0	0.0% (0/12)	43	2.4% (43/1814)
Parainfluenza 4	7	2.1% (7/337)	1	0.4% (1/253)	4	1.2% (4/329)	4	0.6% (4/641)	0	0.0% (0/242)	0	0.0% (0/12)	16	0.9% (16/1814)
RSV A	28	8.3% (28/337)	9	3.6% (9/253)	2	0.6% (2/329)	11	1.7% (11/641)	8	3.3% (8/242)	0	0.0% (0/12)	58	3.2% (58/1814)
RSV B	10	3.0% (10/337)	4	1.6% (4/253)	1	0.3% (1/329)	4	0.6% (4/641)	1	0.4% (1/242)	0	0.0% (0/12)	20	1.1% (20/1814)
Rhinovirus/ Enterovirus	113	33.5% (113/337)	75	29.6% (75/253)	75	22.8% (75/329)	65	10.1% (65/641)	20	8.3% (20/242)	4	33.3% (4/12)	352	19.4% (352/1814)
SARS-CoV-2	32	9.5% (32/336)	12	4.8% (12/251)	21	6.5% (21/325)	118	18.4% (118/640)	56	23.3% (56/240)	1	9.1% (1/11)	240	13.3% (240/1803)

N. Proposed Labeling:

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

O. Conclusion:

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