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

I Background Information:

A 510(k) Number

K231758

B Applicant

Luminex Molecular Diagnostics, Inc.

C Proprietary and Established Names

NxTAG Respiratory Pathogen Panel v2 (NxTAG RPP v2)

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
QOF	Class II	21 CFR 866.3981 - Device To Detect And Identify Nucleic Acid Targets In Respiratory Specimens From Microbial Agents That Cause The SARS-Cov-2 Respiratory Infection And Other Microbial Agents When In A Multi-Target Test	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To obtain a substantial equivalence determination for the NxTAG Respiratory Pathogen Panel v2 (NxTAG RPP v2).

B Measurand:

The NxTAG RPP v2 detects and identifies nucleic acids from the following pathogens: Adenovirus, Coronavirus 229E, Coronavirus OC43, Coronavirus NL63, Coronavirus HKU1, Human Metapneumovirus, Influenza A virus with subtyping of Influenza A H1, Influenza A

H1pdm09 and Influenza A H3 (reported separately), Influenza B virus, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Rhinovirus/Enterovirus, Severe Acute Respiratory Syndrome (SARS)-Coronavirus-2, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.

C Type of Test:

Multiplex nucleic acid assay for use with the MAGPIX Instrument for the qualitative detection of viral and/or bacterial pathogens in nasopharyngeal swab specimens from patients with signs and symptoms of respiratory tract infection.

III Intended Use/Indications for Use:

A Intended Use(s):
See Indications for Use below.

B 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
Influenza A H3	Rhinovirus/Enterovirus
Influenza B	Adenovirus
Respiratory Syncytial Virus A	Parainfluenza virus 1
Respiratory Syncytial Virus B	Parainfluenza virus 2
SARS-CoV-2	Parainfluenza virus 3
Coronavirus 229E	Parainfluenza virus 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.

C Special Conditions for Use Statement(s):
Rx - For Prescription Use Only

For *in vitro* diagnostic use

D Special Instrument Requirements:

Luminex MAGPIX Instrument
bioMérieux NUCLISENS easyMAG Instrument or EMAG Instrument
IVD-Labeled Thermal Cycler

IV Device/System Characteristics:

A Device Description:

The NxTAG Respiratory Pathogen Panel v2 (NxTAG RPP v2) is a qualitative reverse-transcription polymerase chain reaction (RT-PCR) *in vitro* diagnostic assay for the simultaneous detection and discrimination of 21 viral and bacterial respiratory pathogens in nasopharyngeal swab specimens from patients with signs and symptoms of respiratory tract infection. The assay uses the proprietary Luminex bead hybridization tag sorting system to discriminate amplified products and is performed on the Luminex MAGPIX Instrument equipped with xPONENT software following nucleic acid extraction and PCR amplification using off-the-shelf instrument systems. Signals obtained with the MAGPIX Instrument are analyzed using the NxTAG RPP v2 Assay File for SYNCT Software.

The NxTAG RPP v2 differs from the original NxTAG Respiratory Pathogen Panel (NxTAG RPP; K152386/K193167) as shown in **Table 1**. Other minor differences include changes to the fluorescence thresholds for certain assays and the Internal Control.

Table 1. Changes to the NxTAG RPP implemented for the NxTAG RPP v2

Target Analyte	Description of Change for NxTAG RPP v2
Influenza A	Modified primers for improved inclusivity
Influenza A H1	Differentiation of subtypes H1 and H1pdm09
Influenza A H3	Modified primers for improved inclusivity
Human Bocavirus	Results not reported
Human Metapneumovirus	New target region for improved inclusivity
Rhinovirus/Enterovirus	Modified formulation for improved specificity
SARS-CoV-2	New analyte

B Principle of Operation:

Nucleic acids are extracted for analysis from nasopharyngeal swab samples in transport medium using either the IVD-labeled bioMérieux NucliSENS easyMAG or EMAG extraction systems. The extracted nucleic acids are added to 96-well plates containing lyophilized PCR primers, hybridization beads and amplification enzymes. Following rehydration, the plates are sealed and loaded into a thermocycler for reverse transcription and PCR amplification. The amplified PCR products hybridize to the tagged beads in near real-time and upon completion of the reaction, the sealed plate is loaded into the MAGPIX Instrument for automated bead sorting and fluorescence reading using xPONENT 4.3u2 software. The data are analyzed using the NxTAG RPP v2 Assay File for SYNCT 1.1u2 software and results for each analyte are reported as “detected”, “not detected” or “invalid”. Details of the method of result interpretation for influenza A and its associated subtypes are provided in **Table 2**.

Table 2. Method of result interpretation for influenza A

Final Result	NxTAG RPP v2 Analyte				Applicable Footnotes
	Influenza A	Influenza A H1	Influenza A H1pdm09	Influenza A H3	
Influenza A Not Detected	Negative	Negative	Negative	Negative	--
Influenza A H1	Positive	Positive	Negative	Negative	--
	Negative	Positive	Negative	Negative	1
Influenza A 2009 H1N1	Positive	Negative	Positive	Negative	--
	Negative	Negative	Positive	Negative	1
Influenza A H1 and Influenza A H1pdm09	Positive	Positive	Positive	Negative	2
	Negative	Positive	Positive	Negative	1, 2
Influenza A H3	Positive	Negative	Negative	Positive	--
	Negative	Negative	Negative	Positive	1
Influenza A H3 and Influenza A H1	Positive	Positive	Negative	Positive	2
	Negative	Positive	Negative	Positive	1, 2
Influenza A H3 and Influenza A H1pdm09	Positive	Negative	Positive	Positive	2
	Negative	Negative	Positive	Positive	1, 2
Influenza A H1, Influenza A H1pdm09 and Influenza A H3	Positive	Positive	Positive	Positive	2
	Negative	Positive	Positive	Positive	1
Influenza A (no sub-type detected)	Positive	Negative	Negative	Negative	3

[1] Detection of influenza A H1, A H1pdm09 or A H3 subtypes without a corresponding positive result for influenza A may occur at low target levels, be due to contamination or be indicative of a mutation in the matrix gene target region used for detection of influenza A. Retesting is recommended prior to reporting test results if clinically indicated or for epidemiological investigation. Further investigation may be warranted if the same result is generated upon retesting.

[2] Co-detection of multiple influenza A subtypes (H1 and/or H1pdm09 and/or H3) may be due to coinfection, false-positive results due to contamination or the presence of a multi-valent influenza virus vaccine in the sample. Retesting is recommended prior to reporting test results. If the same result is obtained upon retest, the presence of multiple subtypes should be confirmed by alternative FDA-cleared methods.

[3] Detection of influenza A without a corresponding positive result for an influenza A subtype may occur at low target levels, be due to contamination or be indicative of the presence of a novel influenza A strain. If a specimen is confirmed positive for influenza A on retesting but produces negative test results for A H1, A H1pdm09 and A H3, the appropriate local, state, or federal public health authorities should be notified to determine necessary measures for verification and to determine whether the specimen contains a novel strain of Influenza A.

C Instrument Description Information:

1. Instrument Name:

   Luminex MAGPIX Instrument
   bioMérieux NUCLISENS easyMAG Instrument or EMAG Instrument
   IVD-labeled thermal cycler

2. Specimen Identification:

   Specimen identification numbers may be entered into the SYNCT Software manually or using the optional barcode reader.

3. Specimen Sampling and Handling:

   The NxTAG RPP v2 assay is intended for use with nasopharyngeal swabs collected in Universal Transport Medium (UTM, Copan Diagnostics) or MicroTest M4RT medium (Remel) using a nylon flocked or polyester swab. Specimens may be stored for up to 7 days at 2-8 °C or up to 12 months at ≤ -70 °C.

   The respective locations of Positive and Negative External Controls and patient samples on the MAGPIX assay plate must be defined in the SYNCT Software. For controls, the expected results for each analyte must also be assigned. Each sample and control within a run must have a unique identifier. The locations of the External Controls should be selected to enable correct orientation of the MAGPIX plate.

   The SYNCT software may be used to select which test results to report for each sample prior to analysis based on the test order. Masked results will not be reported. A default Test Panel that includes all available analytes is provided but, for convenience, users also have the option to create custom Test Panels from within the list of available NxTAG RPP v2 target analytes. Targets that were previously masked, may be unmasked by reanalyzing the data in the SYNCT software upon receipt of an appropriate test order.

4. Calibration:

   Calibration of the MAGPIX Instrument using the MAGPIX Calibration Kit is required at least weekly to normalize the settings for the classification and reporter optical channels. Following calibration, the MAGPIX Performance Verification Kit must be used to verify the integrity of the system. Daily use of the MAGPIX Performance Verification Kit to verify the calibration and optical integrity of the MAGPIX system and the fluidics channels is also recommended. The MAGPIX Calibration Kit and MAGPIX Performance Verification Kit are provided separately from the NxTAG RPP v2.

5. Quality Control:

   Prior to processing, bacteriophage MS2 is added to each patient sample or External Control as an Internal Control to monitor the integrity of nucleic acid extraction, reverse transcription, PCR amplification and detection. External Positive Controls for use with the

NxTAG RPP v2 assay are not provided with the assay kit but are recommended for use in accordance with local, state and/or federal guidelines and good laboratory practices.

Luminex recommends inclusion of a Negative Extraction Control (transport medium) to monitor for contamination. Optionally, a Negative Amplification Control comprised of RNase-free water may be included on each amplification plate to monitor for contamination downstream from the nucleic acid extraction process. The Negative Extraction Control and Negative Amplification Control should produce negative test results for all analytes.

If the External Positive Control, Negative Amplification Control and Negative Extraction Control do not produce the expected results, patient results should not be reported.

V Substantial Equivalence Information:

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

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

C Comparison with Predicate(s):

Device & Predicate Device(s):	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 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	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 and differentiated using the NxTAG RPP v2: *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 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 exhibiting signs and/or symptoms of	identified using the BioFire RP2.1 Adenovirus Coronavirus 229E Coronavirus HKU1 Coronavirus NL63 Coronavirus OC43 Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV-2) Human Metapneumovirus Human Rhinovirus/Enterovirus Influenza A, including subtypes H1, H1-2009, and H3 Influenza B Parainfluenza Virus 1 Parainfluenza Virus 2 Parainfluenza Virus 3 Parainfluenza Virus 4 Respiratory Syncytial Virus *Bordetella parapertussis* (IS1001) *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 symptoms of respiratory infection is indicative of the presence 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.	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:	*Viruses:* Adenovirus

	a) differentiation of Respiratory Syncytial Virus A and B b) omission of assays for *Bordetella pertussis* and *Bordetella parapertussis*	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 labeled beads, sorting of tagged products	Array-based melt curve analysis

VI Standards/Guidance Documents Referenced:

CLSI. *Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline.* 3rd ed. CLSI document EP05-A3. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

CLSI. *Interference Testing in Clinical Chemistry.* 3rd ed. CLSI guideline EP07. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

CLSI. *User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline.* 2nd ed. CLSI document EP12-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

CLSI. *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline.* 2nd ed. CLSI document EP17-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.

CLSI. *Assessment of the Diagnostic Accuracy of Laboratory Tests Using Receiver Operating Characteristic Curves; Approved Guideline.* 2nd ed. CLSI document EP24-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

CLSI. *Evaluation of Stability of* In Vitro *Diagnostic Reagents; Approved Guideline.* CLSI document EP25-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2009.

CLSI. *Supplementary Tables for Interference Testing in Clinical Chemistry*. 1st ed. CLSI supplement EP37. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

ISO 14971: 2019. Medical devices – Application of risk management to medical devices.

BS EN ISO 23640: 2015. *In vitro* diagnostic medical devices – Evaluation of stability of *in vitro* diagnostic reagents.

CLSI. *Molecular Diagnostic Methods for Infectious Diseases.* 3rd ed. CLSI report MM03. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.

CLSI. *Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline.* 2nd ed. CLSI document MM09-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2014.

CLSI. *Validation and Verification of Multiplex Nucleic Acid Assays.* 2nd ed. CLSI guideline MM17. Wayne, PA: Clinical and Laboratory Standards Institute; 2018.

CLSI. Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline. CLSI document MM18-A. Wayne, PA: Clinical and Laboratory Standards Institute; 2008.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Lot-to-Lot and Within-Run Precision

The lot-to-lot precision of the NxTAG RPP v2 was evaluated using three lots of assay kits, each containing unique lots of critical raw materials/sub-components. The panel of samples for analysis comprised simulated nasopharyngeal swab matrix that was spiked with different combinations of all the NxTAG RPP v2 analytes, as well as a negative sample (**Table 3**). Please refer to **Section VII A (6)** for data supporting use of simulated nasopharyngeal swab matrix for Analytical Studies, as well as testing of samples containing multiple analytes.

Each positive panel member was prepared at both low and moderate target levels (1.5X [low] and 5X LoD [moderate], respectively, except where noted). Each panel member at each

concentration was tested a total of 10 times with each lot of reagents (total 30 replicates per panel member per target level). There was close to 100% positive and negative agreement with the expected results for each panel member at each target level with all three lots of reagents (**Table 4**). These results are acceptable.

Table 3. Panel members used to evaluate lot-to-lot assay precision

Sample	Analyte	Strain/Isolate	Source	Catalogue #
1	Influenza A H1pdm09 (subtype) [1, 2, 3]	A/NY/02/09	ZeptoMetrix	0810109CFN
	Respiratory Syncytial Virus A	A2	ATCC	VR-1540
	Rhinovirus	50-525-CV54	ATCC	VR-1195
2	Influenza A H3 (subtype) [1, 4]	Wisconsin/67/05	ZeptoMetrix	0810252CF
	Respiratory Syncytial Virus B	18537	ATCC	VR-1580
3	Influenza B	B/Florida/02/06	ZeptoMetrix	0810037CF
	Parainfluenza Virus 3	C 243	ATCC	VR-93
	Mycoplasma pneumoniae [5]	M129	ZeptoMetrix	0801579
4	SARS-CoV-2	USA-WA1/2020	ATCC	VR-1986HK
	Human Metapneumovirus	hMPV-3, Type B1, Peru2-2002	ZeptoMetrix	0810156CF
	Adenovirus B [2]	Type 14, 2006 isolate	ZeptoMetrix	0810108CF
5	Influenza A H3 (matrix)	Wisconsin/67/05	ZeptoMetrix	0810252CF
	Coronavirus NL63	--	ZeptoMetrix	0810228CF
	Coronavirus HKU1	Genotype B	Clinical	--
6	Influenza A H1 (subtype) [1, 6]	A/Brisbane/59/07	ZeptoMetrix	0810244CF
	Parainfluenza Virus 1	--	ZeptoMetrix	0810014CF
	Chlamydia pneumoniae	TW-183	ATCC	VR-2282
7	Parainfluenza Virus 2	Greer	ATCC	VR-92
	Parainfluenza Virus 4B	CH 19503	ATCC	VR-1377
	Coronavirus 229E	--	ATCC	VR-740
8	Parainfluenza Virus 4A	--	ZeptoMetrix	0810060CF
	Coronavirus OC43	Betacoronavirus 1	ATCC	VR-1558
9	Negative	--	--	--

Samples 1-8 were formulated at 1.5X (Low) and 5X LoD (Moderate), except where noted.

[1] Formulated based on the LoD for the applicable influenza A subtype.

[2] Formulated at approximately 3X and 9X LoD.

[3] Expected results at the Low target level based on the respective analytical sensitivities of the H1pdm09 subtyping and influenza A assays: H1pdm09 subtype "positive", Influenza A "positive".

[4] Expected results at the Low target level based on the respective analytical sensitivities of the H3 subtyping and influenza A assays: H3 subtype "positive", Influenza A "positive" or "negative".

[5] Formulated at approximately 2X and 7X LoD.

[6] Expected results at the Low target level based on the respective analytical sensitivities of the H1 subtyping and influenza A assays: H1 subtype "positive", Influenza A "positive".

Table 4. Lot-to-lot agreement with expected results

Sample	NxTAG RPP v2 Analyte	Level	Percent Agreement				
			Lot 1	Lot 2	Lot 3	Total	
1	Influenza A H1pdm09 (subtype) [1, 2]	Low [3]	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Respiratory Syncytial Virus A	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Rhinovirus/Enterovirus	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	All Other	Low	100 (180/180)	100 (180/180)	100 (180/180)	100 (540/540)	100 (1080/1080)
		Mod	100 (180/180)	100 (180/180)	100 (180/180)	100 (540/540)	
2	Influenza A H3 (subtype) [1]	Low [4]	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Respiratory Syncytial Virus B	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	All Other	Low	100 (190/190)	100 (190/190)	100 (190/190)	100 (570/570)	100 (1140/1140)
		Mod	100 (190/190)	100 (190/190)	100 (190/190)	100 (570/570)	
3	Influenza B	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)

Sample	NxTAG RPP v2 Analyte	Level	Percent Agreement				
			Lot 1	Lot 2	Lot 3	Total	
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Parainfluenza virus 3	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Mycoplasma pneumoniae [5]	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	All Other	Low	100 (180/180)	100 (180/180)	100 (180/180)	100 (540/540)	100 (1080/1080)
		Mod	100 (180/180)	100 (180/180)	100 (180/180)	100 (540/540)	
4	SARS-CoV-2	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Human Metapneumovirus	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Adenovirus [2]	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	All Other	Low	100 (180/180)	100 (180/180)	100 (180/180)	100 (540/540)	100 (1080/1080)
		Mod	100 (180/180)	100 (180/180)	100 (180/180)	100 (540/540)	
5	Influenza A (matrix)	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Coronavirus NL63	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Coronavirus HKU1	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	All Other	Low	100 (180/180)	99.4 (179/180)	100 (180/180)	99.8 (539/540)	99.9 (1079/1080)
		Mod	100 (180/180)	100 (180/180)	100 (180/180)	100 (540/540)	
6	Influenza A H1 (subtype) [1]	Low [6]	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Parainfluenza virus 1	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Chlamydia pneumoniae	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	All Other	Low	100 (180/180)	100 (180/180)	100 (180/180)	100 (540/540)	99.9 (1079/1080)
		Mod	100 (180/180)	99.4 (179/180)	100 (180/180)	99.8 (539/540)	
7	Parainfluenza virus 2	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Parainfluenza virus 4	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Coronavirus 229E	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	All Other	Low	100 (180/180)	100 (180/180)	99.4 (179/180)	99.8 (539/540)	99.9 (1079/1080)
		Mod	100 (180/180)	100 (180/180)	100 (180/180)	100 (540/540)	
8	Parainfluenza virus 4	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	Coronavirus OC43	Low	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	100 (60/60)
		Mod	100 (10/10)	100 (10/10)	100 (10/10)	100 (30/30)	
	All Other	Low	100 (190/190)	100 (190/190)	100 (190/190)	100 (570/570)	100 (1140/1140)
		Mod	100 (190/190)	100 (190/190)	100 (190/190)	100 (570/570)	
9	Negative	N/A	99.0 (208/210)	100 (210/210)	100 (210/210)	99.7 (628/630)	

N/A: Not applicable; Low: 1.5X LoD; Mod (Moderate): 5X LoD, except where noted

[1] Formulated based on the LoD for the applicable influenza A subtype.

[2] Formulated at approximately 3X (Low) and 9X LoD (Moderate).

[3] Expected results at the Low target level based on the respective analytical sensitivities of the H1pdm09 subtyping and influenza A assays: H1pdm09 subtype "positive", Influenza A "positive".

[4] Expected results at the Low target level based on the respective analytical sensitivities of the H3 subtyping and influenza A assays: H3 subtype "positive", Influenza A "positive" or "negative"; agreement for Influenza A is included under "All Other" (n = 10 per lot per level).

[5] Formulated at approximately 2X (Low) and 7X LoD (Moderate).

[6] Expected results at the Low target level based on the respective analytical sensitivities of the H1 subtyping and influenza A assays: H1 subtype "positive", Influenza A "positive".

Site-to-Site and Within-Site (Operator-to-Operator) Reproducibility

The site-to-site and within site (operator-to-operator) reproducibility of the NxTAG RPP v2 was evaluated using a panel of samples that were tested by two operators at each of three study sites in replicates of four on five non-consecutive days, using a single reagent lot and unique set of critical equipment at each site (2 operators x 3 sites x 4 replicates x 5 days x 1

lot = 120 replicates per panel member). The panel of samples for analysis comprised simulated nasopharyngeal swab matrix that was spiked with different combinations of a subset of 11 of the NxTAG RPP v2 target analytes, as well as a negative sample (**Table 5**). Each positive panel member was prepared at both low and moderate target levels (1.5X and 5X LoD, respectively, except where noted). Positive and negative agreement for each panel member within and between study sites and operators was close to 100% (**Table 6**). These results are acceptable.

Table 5. Panel members used to evaluate site-to-site and within-site reproducibility

Sample	Analyte	Strain/Isolate	Source	Catalogue #
1	Influenza A H1pdm09 (subtype) [1, 2, 3]	A/NY/02/09	ZeptoMetrix	0810109CFN
	Respiratory Syncytial Virus A	A2	ATCC	VR-1540
	Rhinovirus	50-525-CV54	ATCC	VR-1195
2	Influenza A H3 (subtype) [1, 4]	Wisconsin/67/05	ZeptoMetrix	0810252CF
	Respiratory Syncytial Virus B	18537	ATCC	VR-1580
3	Influenza B	B/Florida/02/06	ZeptoMetrix	0810037CF
	Parainfluenza Virus 3	C 243	ATCC	VR-93
	Mycoplasma pneumoniae [5]	M129	ZeptoMetrix	0801579
4	SARS-CoV-2	USA-WA1/2020	ATCC	VR-1986HK
	Human Metapneumovirus	hMPV-3, Type B1, Peru2-2002	ZeptoMetrix	0810156CF
	Adenovirus B [2]	Type 14, 2006 isolate	ZeptoMetrix	0810108CF
5	Negative	--	--	--

Samples 1-4 were formulated at both 1.5X (Low) and 5X LoD (Moderate), except where noted

[1] Formulated based on the LoD for the applicable influenza A subtype.

[2] Formulated at approximately 3X (Low) and 9X LoD (Moderate).

[3] Expected results based on the relative analytical sensitivities of the H1pdm09 subtyping and influenza A assays: H1pdm09 subtype "positive", Influenza A "positive".

[4] Expected results based on the relative analytical sensitivities of the H3 subtyping and influenza A assays: H3 subtype "positive", Influenza A "positive" or "negative".

[5] Formulated at approximately 2X (Low) and 7X LoD (Moderate).

Table 6. Site-to-site agreement with expected results

Sample	NxTAG RPP v2 Analyte	Panel Level	Percent Agreement						
			Site 1		Site 2		Site 3		Total
			Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2	
1	Influenza A H1pdm09 (subtype) [1, 2, 3]	Low	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
		Mod	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
	Respiratory Syncytial Virus A	Low	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
		Mod	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
	Rhinovirus/ Enterovirus	Low	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
		Mod	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
	All Other	Low	99.7 (359/360)	100 (360/360)	100 (360/360)	100 (360/360)	100 (360/360)	100 (360/360)	**100 (2159/2160)**

Sample	NxTAG RPP v2 Analyte	Panel Level	Percent Agreement						
			Site 1		Site 2		Site 3		Total
			Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2	
			99.9 (719/720)		**100 (720/720)**		**100 (720/720)**		
		Mod	99.7 (359/360)	99.7 (359/360)	100 (360/360)	100 (360/360)	99.7 (359/360)	100 (360/360)	**99.9 (2157/2160)**
			99.7 (718/720)		**100 (720/720)**		**99.9 (719/720)**		
2	Influenza A H3 (subtype) [1,4]	Low	95.0 (19/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**99.2 (119/120)**
			97.5 (39/40)		**100 (40/40)**		**100 (40/40)**		
		Mod	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
	Respiratory Syncytial Virus B	Low	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
		Mod	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
	All Other	Low	99.5 (378/380)	99.7 (379/380)	100 (380/380)	100 (380/380)	100 (380/380)	100 (380/380)	**99.9 (2277/2280)**
			99.6 (757/760)		**100 (760/760)**		**100 (760/760)**		
		Mod	99.7 (379/380)	99.5 (378/380)	100 (380/380)	100 (380/380)	100 (380/380)	100 (380/380)	**99.9 (2277/2280)**
			99.6 (757/760)		**100 (760/760)**		**100 (760/760)**		
3	Influenza B	Low	95.0 (19/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**99.2 (119/120)**
			97.5 (39/40)		**100 (40/40)**		**100 (40/40)**		
		Mod	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
	Parainfluenza virus 3	Low	95.0 (19/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**99.2 (119/120)**
			97.5 (39/40)		**100 (40/40)**		**100 (40/40)**		
		Mod	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
	Mycoplasma pneumoniae [5]	Low	95.0 (19/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**99.2 (119/120)**
			97.5 (39/40)		**100 (40/40)**		**100 (40/40)**		
		Mod	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
	All Other (Negative)	Low	99.5 (358/360)	100 (360/360)	100 (360/360)	100 (360/360)	100 (360/360)	100 (360/360)	**99.9 (2158/2160)**
			99.7 (718/720)		**100 (720/720)**		**100 (720/720)**		
		Mod	99.7 (359/360)	100 (360/360)	100 (360/360)	100 (360/360)	100 (360/360)	100 (360/360)	**100 (2159/2160)**
			99.9 (719/720)		**100 (720/720)**		**100 (720/720)**		
4	SARS-CoV-2	Low	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
		Mod	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**

Sample	NxTAG RPP v2 Analyte	Panel Level	Percent Agreement						
			Site 1		Site 2		Site 3		Total
			Operator 1	Operator 2	Operator 1	Operator 2	Operator 1	Operator 2	
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
	Human Metapneumovirus	Low	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
		Mod	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
	Adenovirus [2]	Low	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
		Mod	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	100 (20/20)	**100 (120/120)**
			100 (40/40)		**100 (40/40)**		**100 (40/40)**		
	All Other (Negative)	Low	99.5 (358/360)	99.5 (358/360)	100 (360/360)	100 (360/360)	100 (360/360)	100 (360/360)	**99.8 (2156/2160)**
			99.5 (716/720)		**100 (720/720)**		**100 (720/720)**		
		Mod	99.7 (359/360)	100 (360/360)	100 (360/360)	100 (360/360)	100 (360/360)	100 (360/360)	**100 (2159/2160)**
			99.9 (719/720)		**100 (720/720)**		**100 (720/720)**		
5	Negative	N/A	100 (420/420)	100 (420/420)	100 (420/420)	100 (420/420)	100 (420/420)	100 (420/420)	**100 (2520/2520)**
			100 (840/840)		**100 (840/840)**		**100 (840/840)**		

Low: 1.5X LoD; Mod (Moderate): 5X LoD, except where noted
Neg.: Negative; N/A: Not applicable
[1] Formulated based on the LoD for the applicable influenza A subtype.
[2] Formulated at approximately 3X (Low) and 9X LoD (Moderate).
[3] Expected results based on the respective analytical sensitivities of the H1pdm09 subtyping and influenza A assays: H1pdm09 subtype "positive", Influenza A "positive".
[4] Expected results based on the relative analytical sensitivities of the H3 subtyping and influenza A assays: H3 subtype "positive", Influenza A "positive" or "negative"; agreement for Influenza A is included under "All Other" (n = 40 per site).
[5] Formulated at approximately 2X (Low) and 7X LoD (Moderate).

2. Linearity:

Not applicable.

3. Analytical Specificity/Interference:

In silico Analysis
An *in silico* BLAST analysis was performed to evaluate the potential for cross-reaction of the NxTAG RPP v2 oligonucleotides with nucleic acids from on- and off-panel species. The analysis was performed with all the primers and probes in the assay mixture against sequences in the GenBank nt sequence database as of April 9, 2023. Prediction of potential cross-reaction/interference was based on the percentage homology with the non-target sequences, the relative locations of potential hybridization (amplicon length), the location of any mismatches and predicted T_m. Based on this analysis, the NxTAG RPP v2 SARS-CoV-2 primers and probe were determined to cross-react with some bat coronavirus and bat-SARS-like coronavirus strains. In addition, the Coronavirus 229E primers and probe are predicted to cross-react with 229E-like coronavirus sequences from bats. This observation was confirmed empirically using synthetic template. The potential for cross-reaction of the Coronavirus

NL63 primers and probes with bat-coronaviruses was demonstrated *in silico* but was not confirmed through functional testing. Overall, *in silico* analysis demonstrated low likelihood for cross-reaction of the NxTAG RPP v2 assay primers and probes with non-target nucleic acids. However, the potential for cross-reaction with some bat SARS-like and 229E-like coronavirus strains is noted as a Limitation in the device labeling.

Laboratory Testing of Analytical Specificity
The analytical specificity of the NxTAG RPP v2 was evaluated by testing high concentrations of potentially cross-reactive on- and off-panel bacteria, fungi and viruses, in addition to pooled nasal wash. No unexpected cross-reaction was observed except with Enterovirus 68 (Species D, US/IL/14-18952), which produced false-positive results for Influenza A H3 (**Tables 7** and **8**). This is noted as a Limitation in the device labeling.

Table 7. Off-panel bacteria, fungi and viruses evaluated for potential cross-reaction

Species	Strain/Isolate	Source/Catalogue No.	Concentration Per mL	Cross-reaction
Bordetella parapertussis	A747	Zeptometrix 0801641	1.00 x 10^6 CFU	None [1]
Bordetella pertussis	A639	Zeptometrix 0801459	1.00 x 10^6 CFU	None
Chlamydia trachomatis	Z054; D-UW3; Serovar D	Zeptometrix 0801775	1.00 x 10^6 IFU	None
Corynebacterium diphtheriae	Z116	Zeptometrix 0801882	1.00 x 10^6 CFU	None
Corynebacterium striatum	FS1	Zeptometrix BAA-1851	1.00 x 10^6 CFU	None
Escherichia coli	O157:H7; EDL 933	Zeptometrix 0801622	1.00 x 10^6 CFU	None
Fusobacterium necrophorum	Z239	Zeptometrix 0804189	1.00 x 10^6 CFU	None
Haemophilus influenzae	Type b; MinnA	Zeptometrix 0801680	1.00 x 10^6 CFU	None
Klebsiella pneumoniae	Z135; IMP-13, CTX-M	Zeptometrix 0801904	1.00 x 10^6 CFU	None
Lactobacillus acidophilus	Z048	Zeptometrix 0801540	1.00 x 10^6 CFU	None
Lactobacillus plantarum	Z524	Zeptometrix 0804436	1.00 x 10^6 CFU	None
Legionella (Tatlockia) micdadei	Tatlock	Zeptometrix 0801576	1.00 x 10^6 CFU	None
Legionella pneumophila	Philadelphia	Zeptometrix 0801645	1.00 x 10^6 CFU	None
Moraxella catarrhalis	Strain NE 11	Zeptometrix 0801509	1.00 x 10^6 CFU	None
Mycobacterium tuberculosis	H37Rx-1	Zeptometrix 0801660	1.00 x 10^6 CFU	None
Mycoplasma genitalium	UMTB-10G	ATCC 49899	1.00 x 10^5 CCU [2]	None
Mycoplasma hominis	Z317	Zeptometrix 0804011	1.00 x 10^6 CCU	None
Neisseria elongate	Z071	Zeptometrix 0801510	1.00 x 10^6 CFU	None
Neisseria gonorrhoeae	Z017	Zeptometrix 0801482	1.00 x 10^6 CFU	None
Neisseria meningitidis	Serotype A	Zeptometrix 0801511	1.00 x 10^6 CFU	None
Pseudomonas aeruginosa	Clinical isolate	Zeptometrix 0801519	1.00 x 10^6 CFU	None
Serratia marcescens	Z053	Zeptometrix 0801723	1.00 x 10^6 CFU	None
Staphylococcus aureus	102-04	Zeptometrix BAA-1765	1.00 x 10^6 CFU	None
Staphylococcus epidermidis	MRSE, RP62A	Zeptometrix 0801651	1.00 x 10^6 CFU	None
Streptococcus agalactiae	Z019	Zeptometrix 0801545	1.00 x 10^6 CFU	None
Streptococcus pneumoniae	Z022	Zeptometrix 0801439	1.00 x 10^6 CFU	None
Streptococcus pyogenes	Z018	Zeptometrix 0801512	1.00 x 10^6 CFU	None
Streptococcus salivarius	Z127	Zeptometrix 0801896	1.00 x 10^6 CFU	None
Cytomegalovirus	Merlin	Zeptometrix 0810500CF	1.00 x 10^5 $TCID_{50}$	None
Epstein Barr Virus	B95-8	Zeptometrix 0810008CF	1.00 x 10^7 copies	None
Herpes Simplex virus Type 1	Macintyre	Zeptometrix 0810005CF	1.00 x 10^5 $TCID_{50}$	None
Human Bocavirus	--	Clinical specimen	1.00 x 10^7 copies	None
Measles Virus	--	Zeptometrix 0810025CF	1.00 x 10^5 $TCID_{50}$	None
MERS-coronavirus	Florida/USA-2 Saudi Arabia 2014	Zeptometrix 0810575CFHI	1.00 x 10^5 $TCID_{50}$	None
Mumps Virus	--	Zeptometrix 0810079CF	1.00 x 10^5 $TCID_{50}$	None
SARS-coronavirus	2003-00592	Zeptometrix NATSARS-ST	3.01 x 10^5 copies	None
Varicella Zoster Virus	Ellen	Zeptometrix 0810171CF	1.00 x 10^5 $TCID_{50}$	None
Aspergillus flavus	Z013	Zeptometrix 0801598	1.00 x 10^6 CFU	None
Aspergillus fumigatus	QM 1981	ATCC 1022	1.00 x 10^6 CFU	None
Candida albicans	Z006	Zeptometrix 0801504	1.00 x 10^6 CFU	None
Pneumocystis carinii	--	ATCC PRA-159	1.00 x 10^6 nuclei	None
Pooled nasal wash	--	--	--	None

CCU: Color Changing Units; CFU: Colony Forming Units; IFU: Inclusion Forming Units; $TCID_{50}$: 50% Tissue Culture Infectious Dose

[1] On initial testing, 1/3 replicates gave a positive result for Rhinovirus/Enterovirus; upon repeat testing of the same samples, 3/3 replicates produced negative results for all analytes. A further 3/3 replicates prepared in negative nasopharyngeal swab matrix also produced negative results for all analytes.

[2] Approximate concentration based on supplier Certificate of Analysis.

Table 8. On-panel viruses and bacteria evaluated for potential cross-reaction

Analyte	Strain/Isolate	Source/Catalogue No.	Concentration per mL	Cross-reaction
Adenovirus B	Type 14, 2006 isolate	Zeptometrix 0810300CF	1.00 x 10^5 $TCID_{50}$	None
Coronavirus 229E	--	ATCC VR-740	1.00 x 10^5 $TCID_{50}$	None
Coronavirus HKU1	Genotype A	Clinical	1.00 x 10^6 copies	None
Coronavirus NL63	--	Zeptometrix 0810228CF	1.00 x 10^5 $TCID_{50}$	None
Coronavirus OC43	--	ATCC VR-1558	1.00 x 10^5 $TCID_{50}$	None
Enterovirus	Species D, Type 68, 2014 Isolate 1	Zeptometrix 0810300CF	1.00 x 10^5 $TCID_{50}$	None
Enterovirus	Species D, Type 68, 2007 Isolate	Zeptometrix 0810237CF	1.00 x 10^5 $TCID_{50}$	None
Enterovirus	Species D, Type 68, Fermon	ATCC VR-1826	1.00 x 10^5 $TCID_{50}$	None
Enterovirus	Species D, Type 68, US/IL/14-18952	ATCC VR-1824	1.00 x 10^3 $TCID_{50}$	Influenza A H3 [1]
Enterovirus	Species D, Type 68, US/KY/14-18953	ATCC VR-1825	1.00 x 10^5 $TCID_{50}$	None
Enterovirus	Species D, Type 68, US/MO/14-18947	ATCC VR-1823	1.00 x 10^5 $TCID_{50}$	None
Human Metapneumovirus	16, Type A1, IA10-2003	Zeptometrix 0810161CF	1.00 x 10^5 $TCID_{50}$	None
Human Metapneumovirus	3, Type B1, Peru2-2002	Zeptometrix 0810156CF	3.89 x 10^4 $TCID_{50}$ [2]	None
Influenza A H1pdm09	A/NY/02/09	Zeptometrix 0810109CFN	1.00 x 10^5 $TCID_{50}$	None
Influenza A H1	A/Brisbane/59/07	Zeptometrix 0810244CF	1.00 x 10^5 $TCID_{50}$	None
Influenza A H3	A/Wisconsin/67/05	Zeptometrix 0810252CF	1.00 x 10^5 $TCID_{50}$	None
Influenza B	B/Bangladesh/5972/2007	IRR FR-450	1.00 x 10^5 $TCID_{50}$	None
Influenza B	B/Brigit (B/Russia/69)	ATCC VR-786	1.00 x 10^5 $CEID_{50}$	None
Influenza B	B/Brisbane/3/2007	IRR FR-18	1.00 x 10^5 $CEID_{50}$	None
Influenza B	B/Brisbane/33/2008	Zeptometrix 0810253CF	1.00 x 10^5 $TCID_{50}$	None
Influenza B	B/GreLakes/1739/1954	BEI NR-3179	1.00 x 10^5 $CEID_{50}$	None
Influenza B	B/Hong Kong/259/2010	IRR FR-663	1.00 x 10^5 $CEID_{50}$	None
Influenza B	B/Hong Kong/5/72	ATCC VR-823	1.00 x 10^5 $CEID_{50}$	None
Influenza B	B/Hubei-Wujiagang/158/2009	IRR FR-469	1.00 x 10^5 $CEID_{50}$	None
Influenza B	B/Massachusetts/2/12	Zeptometrix 0810239CF	1.00 x 10^5 $TCID_{50}$	None
Influenza B	B/New Jersey/1/2012	IRR FR-1270	1.00 x 10^5 $TCID_{50}$	None
Influenza B	B/Russia/69	ATCC VR-790	1.00 x 10^5 $CEID_{50}$	None
Influenza B	B/Texas/02/2013	IRR FR-1302	1.00 x 10^5 $TCID_{50}$	None
Influenza B	B/Wisconsin/1/2010	Zeptometrix 0810241CF	1.00 x 10^5 $TCID_{50}$	None
Influenza B	B/Florida/02/06	Zeptometrix 0810037CF	1.00 x 10^5 $TCID_{50}$	None [3]
Parainfluenza virus 1	Type 1	Zeptometrix 0810014CF	1.00 x 10^5 $TCID_{50}$	None
Parainfluenza virus 2	Greer	ATCC VR-92	1.00 x 10^5 $TCID_{50}$	None
Parainfluenza virus 3	C 243	ATCC VR-93	1.00 x 10^5 $TCID_{50}$	None
Parainfluenza virus 4A	--	Zeptometrix 0810060CF	1.00 10^5 $TCID_{50}$	None
Parainfluenza virus 4B	CH 19503	ATCC VR-1377	1.00 x 10^5 $TCID_{50}$	None
Respiratory Syncytial Virus A	A2	ATCC VR-1540	1.00 x 10^5 PFU	None
Respiratory Syncytial Virus B	18537	ATCC VR-1580	7.00 x 10^4 PFU	None
Rhinovirus	Type 85, 50-525-CV54 [V-192-001-021]	ATCC VR-1195	1.00 x 10^5 $TCID_{50}$	None
SARS-CoV-2	USA-WA-1/2020	ATCC VR-1986HK	1.00 x 10^7 copies	None
Chlamydia pneumoniae	TW-183	ATCC VR-2282	1.00 x 10^6 IFU	None
Mycoplasma pneumoniae	M129	Zeptometrix 0801579	1.00 x 10^6 CCU	None

CCU: Color Changing Units; $CEID_{50}$; 50% Chicken Embryo Infectious Dose; IFU: Inclusion Forming Units; PFU: Plaque Forming Units; $TCID_{50}$: 50% Tissue Culture Infectious Dose

[1] Cross-reaction observed at levels ≥ 1.00 x 10^3 $TCID_{50}$/mL; no cross-reaction observed at 1.00 x 10^2 $TCID_{50}$/mL; this is noted as a Limitation in the device labeling.

[2] Highest attainable concentration.

[3] 3/9 replicates produced false-positive results for Coronavirus 229E due to suspected contamination of the viral stock; positive results were not obtained with any other strains of Influenza B.

Off-Panel Microbial Interference

The potential for interference with the NxTAG RPP by off-panel microorganisms that may be present in nasopharyngeal swab specimens was evaluated by testing 11 potentially cross-reactive species (**Table 9**) at high concentration in the presence of low-moderate levels of 22 on-panel target analytes, including different subtypes of influenza A. Testing was performed with combinations of two or three off-panel analytes at high concentration in the presence of different combinations of three on-panel analytes, each at ~3-6X LoD. The expected positive

results were obtained for each of the on-panel analytes, with no evidence of interference from the off-panel species.

Table 9. Off-panel species evaluated for potential interference with the NxTAG RPP v2

Species/	Strain	Source/Catalogue No.	Concentration per mL	Interference
Bordetella pertussis	A639	Zeptometrix 0801459	1.00 x 10^6 CFU	None
Corynebacterium diphtheriae	Z116	Zeptometrix 0801882	1.00 x 10^6 CFU	None [1]
Haemophilus influenzae	Type b; MinnA	Zeptometrix 0801680	1.00 x 10^6 CFU	None
Moraxella catarrhalis	Strain NE 11	Zeptometrix 0801509	1.00 x 10^6 CFU	None
Neisseria meningitidis	Serotype A	Zeptometrix 0801511	1.00 x 10^6 CFU	None
Pseudomonas aeruginosa	Clinical	Zeptometrix 0801519	1.00 x 10^6 CFU	None
Staphylococcus aureus	102-04	Zeptometrix BAA-1765	1.00 x 10^6 CFU	None
Streptococcus pneumoniae	Z022	Zeptometrix 0801439	1.00 x 10^6 CFU	None
Cytomegalovirus	Merlin	Zeptometrix 0810500CF	1.00 x 10^5 $TCID_{50}$	None
Measles Virus	--	Zeptometrix 0810025CF	1.00 x 10^5 $TCID_{50}$	None
Mumps Virus	--	Zeptometrix 0810079CF	1.00 x 10^5 $TCID_{50}$	None

CFU: Colony Forming Units; $TCID_{50}$: 50% Tissue Culture Infectious Dose

[1] On initial testing, 1/3 samples containing high levels of *Corynebacterium diphtheriae* produced false negative results for Coronavirus NL63 and Coronavirus HKU1 but produced the expected positive results for both analytes upon repeat.

On-Panel Competitive Interference

The potential for competitive interference between viral and bacterial species targeted by the NxTAG RPP v2 assay was evaluated by testing combinations of on panel analytes at asymmetric concentrations. Analytes at low-moderate concentration (3-6X LoD) were combined into four groups (**Table 10**) that were each tested in the presence of a single high concentration analyte (**Table 11**) in simulated nasopharyngeal swab matrix. In all cases (n = 3 ea.), each analyte was successfully detected irrespective of the concentration tested, demonstrating the absence of assay interference.

Table 10. Combinations of on-panel analytes at 3-6X LoD used to evaluate potential competitive interference

Sample	Analyte	Strain	Source/Catalogue No.
1	Influenza A H1pdm09 (subtype) [1]	A/NY/02/09	Zeptometrix 0810109CFN
	Respiratory Syncytial Virus A	A2	ATCC VR-1540
	Rhinovirus	50-525-CV 54	ATCC VR-1195
2	Respiratory Syncytial Virus B	18537	ATCC VR-1580
	Influenza A H3 (subtype) [1]	A/Wisconsin/67/05	Zeptometrix 0810252CF
3	Influenza B	B/Florida/02/06	Zeptometrix 0810037CF
	Parainfluenza virus 3	C 243	ATCC VR-93
	Mycoplasma pneumoniae	M129	Zeptometrix 0801579
4	Adenovirus B	Type 14	Zeptometrix 0810108CF
	Human Metapneumovirus B1	hMPV-3	Zeptometrix 0810156CF
	SARS-CoV-2	USA-WA1/2020	ATCC VR-1986HK

CCU: Color Changing Units; PFU: Plaque Forming Units; $TCID_{50}$: 50% Tissue Culture Infectious Dose

[1] Target level based on the LoD for the applicable subtype.

Table 11. On-panel analytes tested at high concentration to evaluate potential competitive interference

Analyte	Strain	Source/Catalogue No.	Concentration per mL
Adenovirus B	Type 14	Zeptometrix 0810108CF	1.00 x 10^5 $TCID_{50}$
Coronavirus NL63	--	Zeptometrix 0810228CF	1.00 x 10^5 $TCID_{50}$
Influenza A H1pdm09	A/NY/02/09	Zeptometrix 0810109CFN	1.00 x 10^5 $TCID_{50}$
Influenza A H3	A/Wisconsin/67/05	Zeptometrix 0810252CF	1.00 x 10^5 $TCID_{50}$
Influenza B	B/Florida/02/06	Zeptometrix 0810037CF	1.00 x 10^5 $TCID_{50}$
Human Metapneumovirus	hMPV-16, Type A1, IA10-2003	Zeptometrix 0810161CF	1.00 x 10^5 $TCID_{50}$
Human Metapneumovirus	hMPV-3, Type B1, Peru2-2002	Zeptometrix 0810156CF	3.50 x 10^4 $TCID_{50}$
Human Metapneumovirus	hMPV-5, Type B1, Peru3-2003 G gene	Zeptometrix 0810158CF	1.00 x 10^5 $TCID_{50}$
Respiratory Syncytial Virus B	B/18537	ATCC VR-1580	6.30 x 10^4 PFU
Respiratory Syncytial Virus B	B/WV/14617/85	ATCC VR-1400	1.00 x 10^5 $TCID_{50}$
Rhinovirus	50-525-CV 54 [V-192-001-021]	ATCC VR-1195	1.00 x 10^5 $TCID_{50}$
SARS-CoV-2	USA-WA1/2020	VR-1986HK	1.00 x 10^7 copies

PFU: Plaque Forming Units; $TCID_{50}$: 50% Tissue Culture Infectious Dose

Because there is limited information with respect to co-infections involving SARS-CoV-2, additional evaluation of the potential for competitive interference was performed with low concentrations of SARS-CoV-2 (3X LoD) in the presence of high concentrations of 13 on-panel analytes that were not included in **Table 11** (**Table 12**). In all cases (n =3 ea.), positive results were reported for both SARS-CoV-2 and the high concentration analyte, demonstrating the absence of competitive interference.

Table 12. On-panel analytes tested at high concentration to evaluate potential competitive interference with the detection of SARS-CoV-2

Analyte	Strain	Source/Catalogue No.	Concentration per mL
Coronavirus 229E	--	ATCC VR-740	1.00 x 10^5 $TCID_{50}$
Coronavirus OC43	Betacoronavirus 1	ATCC VR-1558	1.00 x 10^5 $TCID_{50}$
Coronavirus HKU1	Clinical specimen	--	1.00 x 10^6 copies
Enterovirus	Type 68, 2007 isolate	Zeptometrix 0810237CF	1.00 x 10^5 PFU
Influenza A H1	A/Brisbane/59/07	Zeptometrix 0810244CF	1.00 x 10^5 $TCID_{50}$
Parainfluenza virus 1	Type 1	Zeptometrix 0810014CF	1.00 x 10^5 $TCID_{50}$
Parainfluenza virus 2	Greer	ATCC VR-92	1.00 x 10^5 $TCID_{50}$
Parainfluenza virus 3	C 243	ATCC VR-93	1.00 x 10^5 $TCID_{50}$
Parainfluenza virus 4A	Type 4A	Zeptometrix 0810060CF	1.00 x 10^5 $TCID_{50}$
Parainfluenza virus 4B	CH 19503	ATCC VR-1377	1.00 x 10^5 $TCID_{50}$
Respiratory Syncytial Virus A	A2	ATCC VR-1540	1.00 x 10^5 PFU
Chlamydia pneumoniae	TW-183	ATCC VR-2282	1.00 x 10^6 IFU
Mycoplasma pneumoniae	M129	Zeptometrix 0801579	1.00 x 10^6 CCU

CCU: Color Changing Units; IFU: Inclusion Forming Units; PFU: Plaque Forming Units; $TCID_{50}$: 50% Tissue Culture Infectious Dose

Interfering Substances

Testing was conducted to evaluate the potential for interference with the NxTAG RPP v2 assay by endogenous and exogenous substances that may be present in nasopharyngeal swab specimens. Eight multi-analyte samples (**Table 13**) comprised of simulated nasopharyngeal swab matrix containing on-panel analytes at 3-6X LoD were tested in triplicate in the presence of 22 potentially interfering substances (**Table 14**). Each substance was also added to negative simulated nasopharyngeal matrix to evaluate the potential for false positive results.

The expected results were obtained in all cases, except in the presence of menthol and FluMist influenza vaccine. Menthol 1% (w/v) was shown to interfere with the detection of Coronavirus

OC43, although no interference was observed at lower concentration (0.5% w/v). Positive results for Influenza A, Influenza A H1pdm09, Influenza A H3 and Influenza B were obtained in the presence of FluMist because the vaccine contains attenuated strains of these analytes that are detectable at the levels used in this study. The potential for false results with menthol and FluMist is noted in the Limitations section of the device labeling.

Table 13. Multi-analyte panels used to evaluate the potential for assay interference by endogenous and exogenous substances

Sample	Analyte [1]	Strain	Source	Catalogue No.
1	Influenza A H1pdm09 (subtype) [2]	A/NY/02/09	Zeptometrix	0810109CFN
	Respiratory Syncytial Virus A	A2	ATCC	VR-1540
	Rhinovirus	50-525-CV54	ATCC	VR-1195
2	Influenza A H3 (subtype) [2]	A/Wisconsin/67/05	Zeptometrix	0810252CF
	Respiratory Syncytial Virus B	CH 19503	ATCC	VR-1377
3	Influenza B	B/Florida/02/06	Zeptometrix	0810037CF
	Parainfluenza virus 3	C 243	ATCC	VR-93
	Mycoplasma pneumoniae	M129	Zeptometrix	0801579
4	SARS-CoV-2	USA-WA1/2020	ATCC	VR-1986HK
	Human Metapneumovirus B1	hMPV-3	Zeptometrix	0810156CF
	Adenovirus B	Type 14	Zeptometrix	0810108CF
5	Influenza A H3 (matrix)	A/Wisconsin/67/05	Zeptometrix	0810252CF
	Coronavirus NL63	--	Zeptometrix	0810228CF
	Coronavirus HKU1	Clinical specimen	--	--
6	Influenza H1 (subtype) [2]	A/Brisbane/59/07	Zeptometrix	0810244CF
	Parainfluenza virus 1	--	Zeptometrix	0810014CF
	Chlamydia pneumoniae	TW-183	ATCC	VR-2282
7	Parainfluenza virus 2	Greer	ATCC	VR-92
	Parainfluenza virus 4B	CH 19503	ATCC	VR-1377
	Coronavirus 229E	--	Zeptometrix	0810228CF
8	Parainfluenza virus 4A	--	Zeptometrix	0810060CF
	Coronavirus OC43	Betacoronavirus 1	ATCC	VR-1558
9	Negative	--	--	--

[1] Each analyte was tested at 3-6X LoD.

[2] Target level based on the LoD for the applicable subtype.

Table 14. Endogenous and exogenous substances evaluated for potential interference with the NxTAG RPP v2

Source	Substance	Active Ingredient	Concentration	Interference
Endogenous	Human whole blood	--	5% (v/v/)	None [1]
	Human Genomic DNA	--	20 µg/mL	None [2]
	Leukocytes		1.00 x 10^3 cells/µL	None
	Mucin	--	100 µg/mL	None
Exogenous	Beclomethasone dipropionate	--	25 µg/mL	None
	Benzocaine		10% (w/v)	None
	Budesonide	--	6.30 x 10^{-3} µg/mL	None
	Dexamethasone	--	12 µg/mL	None
	Early Defense Nasal Spray	Zinc	5% (v/v)	None
	FluMist	Influenza A H1N1 Influenza A H3N2 Influenza B (Yamagata) Influenza B (Victoria)	0.5% (v/v)	Yes [3]
	Flunisolide	--	5 µg/mL	None
	Fluticasone propionate	--	1.26 x 10^{-3} µg/mL	None
	Menthol	--	1% (w/v)	Yes [4]
			0.5% (w/v)	None
	Mometasone furoate	--	4.50 x 10^{-4} µg/mL	None
	Mupirocin	--	1.5 mg/mL	None
	Drixoral	Oxymetaxoline	10% (v/v)	None
			15% (v/v)	None
	Phenylephrine	--	0.03 µg/mL	None
	Salinex	Sodium chloride	1% (v/v)	None
			15% (v/v)	None
	Tobramycin	--	33 µg/mL	None
			600 µg/mL	None
	Triamcinolone acetonide	--	22 µg/mL	None
	Zanamivir		100 µg/mL	None
	ZICAM Allergy Relief	Galphimia glauca, Histaminum hydrochoricum, Luffa operculate, Sulfur	1% (v/v)	None
			5% (v/v)	None

[1] On initial testing, 1/3 replicates with Sample 5 from **Table 13** produced false negative results for Coronavirus NL63 and Coronavirus HKU1 and the result was confirmed upon retesting; however, upon testing with new samples 3/3 replicates gave the expected positive results.

[2] On initial testing, 1/3 replicates with Sample 2 from **Table 13** produced a false negative result for RSV B; upon re-testing of the same sample, the expected result was obtained.

[3] All samples containing FluMist produced positive results for influenza A, influenza A H1pdm09, influenza A H3 and influenza B; this is noted as a Limitation in the device labeling.

[4] 3/3 replicates in the presence of 1% (w/v) menthol produced false negative results for Coronavirus OC43; interference by menthol was alleviated at lower concentration (0.5% w/v); this is noted as a Limitation in the device labeling.

4. Assay Reportable Range:

Not applicable.

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

Controls

Bacteriophage MS2 is added to each sample to be tested with the NxTAG RPP v2 prior to nucleic acid extraction as an internal control to monitor process and reagent integrity. Failure to detect the MS2 control (in conjunction with failure to detect any of the target analytes) is indicative of failure of one or more processes: nucleic acid extraction, reverse transcription PCR amplification and/or detection.

External Controls
External Positive and Negative Controls are not provided with in the NxTAG RPP v2 assay kit but are recommended for inclusion in every assay run in accordance with good laboratory practice and applicable local, state and federal accrediting organizations. External Controls will be reported as "Pass", "Fail" or "Invalid" in accordance with the expected values entered by the user in the SYNCT Software.

External Positive and Negative Controls were included in each run of the NxTAG RPP v2 assay in the Clinical Studies described in **Section VII C**. On each day of testing, the External Positive and Negative Controls were required to produce the expected results for the results obtained with clinical specimens to be considered acceptable.

Specimen Stability
To evaluate the stability of the NxTAG RPP v2 target analytes in nasopharyngeal swab matrix, separate studies were performed with samples prepared in Universal Transport Medium (UTM, Copan Diagnostics) and MicroTest M4RT (M4RT, Remel). All the analytes targeted by the NxTAG RPP v2 assay were included in the analysis of performance with UTM, whereas only a representative subset was included in evaluating the stability of specimens prepared in M4RT (**Table 15**). The positive samples for testing were prepared at two concentrations (1.5X [Low] and 5X LoD [Moderate], unless otherwise noted in **Table 15**) and stored at 2-8 °C or -70 ± 5 °C prior to testing. The results of the study support the claimed stability of the target analytes in nasopharyngeal swab matrix collected in UTM or M4RT for up to 7 days at 2-8 °C or 12 months at -70 ± 5 °C.

Additional testing also demonstrated the stability of extracted nucleic acid after storage at 2-8 °C for up to 4 hours or -70 ± 5 °C for up to 12 months.

Table 15. Multi-analyte samples used to evaluate specimen stability

Sample	Analyte	Strain	Source	Catalogue No.
1 [1]	Influenza A H1pdm09 (subtype) [2, 3]	A/NY/02/09	Zeptometrix	0810109CFN
	Respiratory Syncytial Virus A	A2	ATCC	VR-1540
	Rhinovirus	50-525-CV54	ATCC	VR-1195
2 [1]	Influenza A H3 (subtype) [2]	A/Wisconsin/67/05	Zeptometrix	0810252CF
	Respiratory Syncytial Virus B	CH 19503	ATCC	VR-1377
3 [1]	Influenza B [2]	B/Florida/02/06	Zeptometrix	0810037CF
	Parainfluenza virus 3	C 243	ATCC	VR-93
	Mycoplasma pneumoniae [4]	M129	Zeptometrix	0801579
4 [1]	SARS-CoV-2	USA-WA1/2020	ATCC	VR-1986HK
	Human Metapneumovirus B1	hMPV-3	Zeptometrix	0810156CF
	Adenovirus B [2, 3]	Type 14	Zeptometrix	0810108CF
5	Influenza A H3 (matrix)	A/Wisconsin/67/05	Zeptometrix	0810252CF
	Coronavirus NL63	--	Zeptometrix	0810228CF
	Coronavirus HKU1	Clinical specimen	--	--
6	Influenza H1 (subtype) [2]	A/Brisbane/59/07	Zeptometrix	0810244CF
	Parainfluenza virus 1	--	Zeptometrix	0810014CF
	Chlamydia pneumoniae	TW-183	ATCC	VR-2282
7	Parainfluenza virus 2	Greer	ATCC	VR-92
	Parainfluenza virus 4B	CH 19503	ATCC	VR-1377
	Coronavirus 229E	--	Zeptometrix	0810228CF
8	Parainfluenza virus 4A	--	Zeptometrix	0810060CF
	Coronavirus OC43	Betacoronavirus 1	ATCC	VR-1558
9 [1]	Negative	--	--	--

[1] Panel member included in evaluation of the stability of specimens in M4RT medium.
[2] Target level based on the LoD for the applicable strain/subtype.
[3] Formulated at approximately 3X (Low) and 9X LoD (Moderate).
[4] Formulated at approximately 2X (Low) and 7X LoD (Moderate).

Reagent Stability

A Real-time Stability Study to evaluate the stability of the NxTAG RPP v2 reagents under the recommended storage conditions of 2-8 °C is currently on-going. The study includes three independent kit lots that were manufactured with unique lots of critical reagents. Each kit lot will be tested at 3-month intervals over the course of the study using a panel of Positive Controls that collectively include target nucleic acids for each assay on the panel, in addition to the MS2 bacteriophage Internal Control (n = 3 replicates per assay per kit lot at each test point). To-date, testing has been completed that supports the stability of NxTAG RPP v2 kits for up to 12 months from the date of manufacture when stored under the recommended conditions.

In-use Reagent Stability

Lyophilized NxTAG RPP v2 assay reagents are supplied in foil-sealed 8-well strips that are packaged in sealed pouches of 96 reactions and stored at 2-8 °C. Testing was performed to demonstrate the stability of the prefilled wells after removal from the original packaging, prior to sample addition. Additional testing was also conducted to support resealing of the original reagent pouch and storage of unused foil-sealed wells if less than a full 96 well plate of reactions is consumed. Under all conditions tested, the expected results were obtained, supporting the in-use stability of NxTAG RPP v2 reagents in accordance with the Instructions For Use.

6. Detection Limit:

Limit of Detection

The analytical sensitivity of the NxTAG RPP v2 was evaluated by testing contrived specimens that were prepared by adding quantified, diluted stocks of representative viral and bacterial strains/isolates of each target analyte to pooled negative nasopharyngeal swab matrix in Universal Transport Medium (UTM) that was processed using the bioMérieux EMAG Extraction System. Each analyte was tested individually in a three-fold dilution series to estimate the limit of detection (LoD) which was then confirmed by testing an additional 20 replicates at or near the concentration that yielded 3/3 positive replicates in the preliminary titration. The confirmed LoD for each strain/isolate was defined as the lowest concentration that yielded ≥ 19/20 positive replicates (i.e., ≥ 95% proportion positive) (**Table 16**).

Table 16. Analytical sensitivity of the NxTAG RPP v2 with representative strains/isolates of the target analytes (tested individually using the EMAG Extraction System)

NxTAG RPP v2 Analyte	Strain/Isolate Information	Source	Catalogue No.	LoD Concentration per mL	
				Copies [1]	Supplier Units
Adenovirus	Type 14, Species B, 2006 isolate	ZeptoMetrix	0810108CF	1,420	0.144 $TCID_{50}$
	Type 1, Species C	ZeptoMetrix	0810050CF	20,100	92.8 $TCID_{50}$
	Type 4, Species E	ZeptoMetrix	0810070CF	7,330	0.191 $TCID_{50}$
Coronavirus 229E	--	ATCC	VR-740	381	0.122 $TCID_{50}$
Coronavirus HKU1	Genotype A	Clinical	68972	4,180	--
Coronavirus NL63	--	ZeptoMetrix	0810228CF	100	0.00645 $TCID_{50}$
Coronavirus OC43	--	ATCC	VR-1558	4,550	0.0732 $TCID_{50}$
Human Metapneumovirus	hMPV-16, Type A1, IA10-2003	ZeptoMetrix	0810161CF	71.5	0.0576 $TCID_{50}$
	hMPV-3, Type B1, Peru2-2002	ZeptoMetrix	0810156CF	262	0.0178 $TCID_{50}$
Influenza A (matrix)	A/Brisbane/59/07 (H1)	ZeptoMetrix	0810244CF	119	0.0283 $TCID_{50}$
	A/NY/02/09 (H1pdm09)	ZeptoMetrix	0810109CFN	328	0.0374 $TCID_{50}$
	A/Wisconsin/67/05 (H3)	ZeptoMetrix	0810252CF	168	0.645 $TCID_{50}$
Influenza A H1 (subtype)	A/Brisbane/59/07	ZeptoMetrix	0810244CF	1,600	0.382 $TCID_{50}$
Influenza A H1pdm09 (subtype)	A/NY/02/09	ZeptoMetrix	0810109CFN	984	0.112 $TCID_{50}$
Influenza A H3 (subtype)	A/Wisconsin/67/05	ZeptoMetrix	0810252CF	56	0.215 $TCID_{50}$
Influenza B	B/Florida/02/06	ZeptoMetrix	0810037CF	63.3	0.967 $TCID_{50}$
Parainfluenza Virus	Serotype 1, N/A	ZeptoMetrix	0810014CF	692	0.764 $TCID_{50}$
	Serotype 2, Greer	ATCC	VR-92	345	0.732 $TCID_{50}$
	Serotype 3, C 243	ATCC	VR-93	1,010	110 $TCID_{50}$
	Serotype 4A	ZeptoMetrix	0810060CF	16,900	0.858 $TCID_{50}$
	Serotype 4B, CH 19503	ATCC	VR-1377	7,150	59.9 $TCID_{50}$
Respiratory Syncytial Virus A	A2	ATCC	VR-1540	4,970	37.7 PFU
Respiratory Syncytial Virus B	18537	ATCC	VR-1580	7,205	0.320 PFU
Rhinovirus/Enterovirus	Rhinovirus, 50-525-CV54 (V-192-001-021)	ATCC	VR-1195	1,536	68.7 $TCID_{50}$
	Enterovirus Type 68, 2007 Isolate	ZeptoMetrix	0810237CF	3,526	2.30 $TCID_{50}$
SARS-CoV-2	USA-WA1/2020	ATCC	VR-1986HK	500	7.68 $TCID_{50}$

NxTAG RPP v2 Analyte	Strain/Isolate Information	Source	Catalogue No.	LoD Concentration per mL	
				Copies [1]	Supplier Units
Chlamydia pneumoniae	TW-183	ATCC	VR-2282	238	37.4 IFU
Mycoplasma pneumoniae	M129	ZeptoMetrix	0801579	3,230	55.6 CCU

CCU: Color Changing Units; Copies: copies of nucleic acid target as determined by quantitative PCR; IFU: Inclusion Forming Units; PFU: Plaque Forming Units; $TCID_{50}$: 50% Tissue Culture Infectious Dose

Limit of Detection with Multi-analyte Samples and Simulated Nasopharyngeal Swab Matrix
Additional testing was performed to evaluate the analytical sensitivity of the NxTAG RPP v2 assay with samples containing multiple target analytes and to evaluate use of simulated nasopharyngeal swab matrix in analytical studies. Samples containing combinations of two or three target species at concentrations equivalent to 1-2X the confirmed single target LoD in either negative clinical or simulated nasopharyngeal swab matrix were tested in parallel. The analyte combinations evaluated are shown in **Table 17**. Twenty replicates of each analyte combination were included in the study and all analytes were successfully detected (≥ 95% proportion positive), demonstrating the absence of interference from the presence of multiple analytes at levels close to the LoD of assay and supporting use simulated nasopharyngeal swab matrix in other analytical studies to evaluate device performance.

Table 17. Multi-analyte samples used to evaluate the analytical sensitivity

Sample	Analyte	Strain	Source	Catalogue No.	% Detected (n = 20)	
					NCM	NSM
1	Influenza A H1pdm09 (subtype) [1, 2]	A/NY/02/09	ZeptoMetrix	0810109CFN	100	100
	Respiratory Syncytial Virus A	A2	ATCC	VR-1540	95	100
	Rhinovirus	50-525-CV54	ATCC	VR-1195	100	100
2	Influenza A H3 (subtype) [1]	A/Wisconsin/67/05	ZeptoMetrix	0810252CF	100	100
	Respiratory Syncytial Virus B	18537	ATCC	VR-1580	100	100
3	Influenza B	B/Florida/02/06	ZeptoMetrix	0810037CF	100	95
	Parainfluenza Virus 3	C 243	ATCC	VR-93	100	100
	M. pneumoniae [2]	M129	ZeptoMetrix	0801579	100	100
4	SARS-CoV-2	USA-WA1/2020	ATCC	VR-1986HK	100	100
	Human Metapneumovirus	hMPV-3, Type B1, Peru2-2002	ZeptoMetrix	0810156CF	100	95
	Adenovirus B [2]	Type 14, 2006 isolate	ZeptoMetrix	0810108CF	100	100
5	Influenza A H3 (matrix)	A/Wisconsin/67/05	ZeptoMetrix	0810252CF	100	100
	Coronavirus NL63	NL63	ZeptoMetrix	0810228CF	100	100
	Coronavirus HKU1	Genotype B	Clinical	HKU1-20210301	100	95
6	Influenza A H1 (subtype) [1]	A/Brisbane/59/07	ZeptoMetrix	0810244CF	100	95
	Parainfluenza Virus 1	--	ZeptoMetrix	0810014CF	100	100
	C. pneumoniae	TW-183	ATCC	VR-2282	100	100
7	Parainfluenza Virus 2	Greer	ATCC	VR-92	95	95
	Parainfluenza Virus 4B	CH 19503	ATCC	VR-1377	100	100
	Coronavirus 229E	--	ATCC	VR-740	100	95
8	Parainfluenza Virus 4A	--	ZeptoMetrix	0810060CF	100	100
	Coronavirus OC43	--	ATCC	VR-1558	100	100

NCM: Nasopharyngeal Clinical Matrix; NSM: Nasopharyngeal Simulated Matrix
[1] Target level based on the LoD for the influenza A subtype.
[2] Formulated at 1-2X LoD; all other analytes tested at 1X LoD.

Comparison of Alternative Methods of Nucleic Acid Extraction

The analytical sensitivity of the NxTAG RPP v2 assay was established using samples that were processed using the bioMérieux EMAG Extraction System and NUCLISENS extraction reagents. Additional testing was performed to evaluate the analytical sensitivity of the assay with the alternative NUCLISENS easyMAG Extraction System, which uses the same extraction reagents as the EMAG instrument. Representative strains/isolates of each of the NxTAG RPP v2 target analytes were diluted in negative nasopharyngeal swab matrix in UTM at levels close to the LoD established using the bioMérieux EMAG Extraction System (**Table 16**). The combinations of strains/isolates used was the same as that described in **Table 15**, above. For each of the analytes, ≥ 95% of replicates produced positive results at ≤ 2X the established LoD of the NxTAG RPP v2 assay (**Table 18**), demonstrating that the analytical sensitivity is similar with both nucleic acid extraction systems.

Table 18. Analytical sensitivity of the NxTAG RPP v2 with samples processed using the NUCLISENS easyMAG Extraction System

NxTAG RPP v2 Analyte	Concentration (copies/mL)	X LoD [1]	Positive/Tested (%)
Adenovirus	**2,460**	**1.73**	**20/20 (100)**
	820	0.58	13/20 (65)
Coronavirus 229E	**381**	**1**	**20/20 (100)**
	127	0.33	14/20 (70)
Coronavirus HKU1	**4,180**	**1**	**20/20 (100)**
	1,390	0.33	15/20 (75)
Coronavirus NL63	**100**	**1**	**20/20 (100)**
	33	0.33	18/20 (90)
Coronavirus OC43	**4,550**	**1**	**20/20 (100)**
	1,520	0.33	16/20 (80)
Human Metapneumovirus	**262**	**1**	**20/20 (100)**
	87	0.33	15/20 (75)
Influenza A (matrix)	**168**	**1**	**20/20 (100)**
	56	0.33	17/20 (85)
Influenza A H1pdm09 (subtype)	**1,700**	**1.73**	**20/20 (100)**
	567	0.58	17/20 (85)
Influenza A H3 (subtype)	**56**	**1**	**20/20 (100)**
	19	0.33	16/20 (80)
Influenza H1 (subtype)	1,630	1	20/20 (100)
	535	**0.33**	**19/20 (95)**
Influenza B	**63**	**1**	**20/20 (100)**
	21	0.33	11/20 (55)
Parainfluenza Virus 1	692	1	20/20 (100)
	231	**0.33**	**20/20 (100)**
	77	0.11	8/20 (40)
Parainfluenza Virus 2	345	1	19/20 (95)
Parainfluenza Virus 3	**1,010**	**1**	**20/20 (100)**
	338	0.33	9/20 (45)
Parainfluenza Virus 4A [2]	16,900	1	20/20 (100)
	5,650	**0.33**	**19/20 (95)**
Parainfluenza Virus 4B [2]	**7,150**	**1**	**20/20 (100)**
	2,380	0.33	15/20 (75)
Respiratory Syncytial Virus A	**4,970**	**1**	**20/20 (100)**
	1,660	0.33	16/20 (80)
Respiratory Syncytial Virus B	**7,210**	**1**	**20/20 (100)**
	2,400	0.33	6/20 (30)
Rhinovirus [3]	1,540	1	20/20 (100)
	512	**0.33**	**20/20 (100)**
	171	0.11	9/20 (45)

NxTAG RPP v2 Analyte	Concentration (copies/mL)	X LoD [1]	Positive/Tested (%)
SARS-CoV-2	500	1	20/20 (100)
	167	0.33	14/20 (70)
Chlamydia pneumoniae	238	1	20/20 (100)
	79	**0.33**	**20/20 (100)**
	26	0.11	7/20 (35)
Mycoplasma pneumoniae	**4,300**	**1.33**	**20/20 (100)**
	1,430	0.44	15/20 (75)

[1] Multiple of the LoD established with samples processed using the bioMérieux EMAG Extraction System (**Table 16**).
[2] Reported by NxTAG RPP v2 as Parainfluenza virus 4.
[3] Reported by NxTAG RPP v2 as Rhinovirus/Enterovirus.

Analytical Sensitivity with the WHO International Standard for SARS-CoV-2
The analytical sensitivity of the NxTAG RPP v2 was also evaluated using the WHO International Standard for SARS-CoV-2 (NIBSC 20/146: Heat inactivated England/02/2020). Serial dilutions of the standard were prepared in pooled nasopharyngeal swab matrix and tested. The LoD with the WHO International Standard, defined as the lowest concentration that produced ≥ 95% positive replicates, was determined to be 7.70×10^5 IU/mL.

Inclusivity

In silico Analysis
The inclusivity of the NxTAG RPP v2 SARS-CoV-2 primers and probes was evaluated using 5,859,379 SARS-CoV-2 sequences available in the GISAID EpiCoV database as of November 30, 2023, including sequences from all defined variants of concern or interest. The NxTAG RPP v2 assay is predicted to detect all the SARS-CoV-2 sequences evaluated.

The inclusivity of the NxTAG RPP v2 primers and probes for influenza A and B was evaluated using sequences in the GISAID EpiFlu database from two time periods: January 1, 2000 to December 31, 2008 for influenza A H1 and January 1, 2017 to May 5, 2023 for influenza A H1pdm09, influenza A H3 and influenza B. The NxTAG RPP v2 primers and probes for influenza A, the influenza A subtypes H1, H1pdm09 and H3, and influenza B were predicted to detect approximately 97-100% of the respective target sequences analyzed.

The inclusivity of the NxTAG RPP v2 primers and probes for all other analytes was evaluated using sequences of the target analytes that were deposited in the NCBI GenBank nt database as of April 8, 2023. Based on this analysis, ≥ 96% of sequences of each analyte were predicted to be detected, except for Parainfluenza Virus 2 (~92%) and untyped strains of Parainfluenza Virus 4 (~94%), which exhibited increased potential for mismatches with the corresponding NxTAG RPP v2 primers and probes which could affect analytical and/or clinical sensitivity. The potential for false-negative results with certain strains of Parainfluenza Virus 2 and 4 based on *in silico* analysis is noted as Limitation in the device labeling.

Laboratory Testing of Inclusivity
The inclusivity (analytical reactivity) of the NxTAG RPP v2 assay was evaluated using a panel of representative strains of each of the analytes on the panel. Testing of each strain/isolate was performed in at least triplicate by adding a quantified, diluted stock to simulated or natural nasopharyngeal swab matrix at a final concentration equivalent to 3X the claimed LoD for the analyte. If negative results were obtained with one or more

replicates, additional testing was performed at higher concentrations. The results of the study are summarized in **Tables 19 – 30** and are acceptable.

Table 19. Strains of Adenovirus evaluated for inclusivity

Species [1]	Type	Strain/Isolate	Source	Catalogue No.	Concentration Detected (copies/mL)
B	**14**	**2006 isolate**	**ZeptoMetrix**	**0810108CF**	**7,380**
	3	--	ZeptoMetrix	0810062CF	7,380
	7	Gomen	ATCC	VR-7	7,380
	7A	AV-1645 [128]	ZeptoMetrix	0810021CF	7,380
	21	--	ATCC	VR-256	7,380
C	**1**	**--**	**ZeptoMetrix**	**0810050CF**	**60,200**
	1	Adenoid 71	ATCC	VR-1	60,200
	2	--	ZeptoMetrix	0810110CF	60,200
	3	--	ZeptoMetrix	0810020CF	60,200
	6	--	ZeptoMetrix	0810111CF	60,200
E	**4**	**--**	**ZeptoMetrix**	**0810070CF**	**22,000**

Bold text: strain/isolate used in the LoD Study
[1] The NxTAG RPP v2 assay reports a single test result for all Adenovirus species combined (undifferentiated).

Table 20. Strains of the common human coronaviruses evaluated for inclusivity

Species [1]	Genotype	Source	Catalogue or Lot No.	Concentration Detected (copies/mL)
229E	**--**	**ATCC**	**VR740**	**1,140**
	--	Zeptometrix	0810229CF	1,140
NL63	**--**	**Zeptometrix**	**0810228CF**	**300**
	--	BEI Resources	NR-470	300
OC43	**--**	**ATCC**	**VR-1558**	**13,600**
	--	Zeptometrix	0810024CF	13,600
HKU1	**B**	**Clinical Specimen**	**HKU1-20210301**	**12,500**
	B	Clinical Specimen	HKU1-176622	12,500
	A	Clinical Specimen	68972	12,500
	A	Clinical Specimen	69174	12,500

Bold text: strain/isolate used in the LoD Study
[1] The NxTAG RPP v2 assay reports separate results for each Coronavirus species.

Table 21. Strains of Human Metapneumovirus evaluated for inclusivity

Clade [1]	Serotype	Strain/Isolate	Source	Catalogue No.	Concentration Detected (copies/mL)
A1	**16**	**IA10-2003**	**Zeptometrix**	**0810161CF**	**215**
	9	IA3-2002	Zeptometrix	0810160CF	215
A2	27	IA27-2004	Zeptometrix	0810164CF	215
	--	DHI 26583	SJH 030209	DHI 26583	215
B1	**3**	**Peru2-2002**	**Zeptometrix**	**0810156CF**	**785**
	5	Peru3-2003	Zeptometrix	0810158CF	785
B2	4	Peru1-2002	Zeptometrix	0810157CF	785
	8	Peru6-2003	Zeptometrix	0810159CF	785
	18	IA18-2003	Zeptometrix	0810162CF	785

Bold text: strain/isolate used in the LoD Study
[1] The NxTAG RPP v2 assay reports a single test result for all clades of Human Metapneumovirus combined (undifferentiated).

Table 22. Strains of Influenza A evaluated for inclusivity [1]

Subtype	Strain/Isolate	Source	Catalogue No.	Concentration Detected (copies/mL)	
				Influenza A	Subtype
H1	**A/Brisbane/59/07**	**ZeptoMetrix**	**0810244CF**	**4,810**	**4,810**
	A/New Caledonia/20/99	ZeptoMetrix	0810036CF	4,810	4,810
	A/Solomon Island/03/06	ZeptoMetrix	0810036CFN	4,810	4,810
	A/Taiwan/42/06	ZeptoMetrix	0810247CF	4,810	4,810
	A/Denver/1/57	ATCC	VR-546	4,810	Negative [2]
H1pdm09	**A/NY/02/09**	**ZeptoMetrix**	**0810109CFN**	**5,110**	**5,110**
	A/Swine/NY/01/2009 (A/NY/01/2009)	ZeptoMetrix	0810248CF	5,110	5,110
	A/Swine/NY/03/09 (A/NY/03/2009)	ZeptoMetrix	0810249CF	5,110	5,110
	A/Swine/Canada/6294/09	ZeptoMetrix	0810109CFJ	5,110	5,110
	A/California/07/09	ZeptoMetrix	0810165CF	5,110	5,110
	A/Mexico/4108/09	ZeptoMetrix	0810166CF	5,110	5,110
	A/Michigan/45/15	ZeptoMetrix	0810538CF	5,110	5,110
	A/Brisbane/02/18	ZeptoMetrix	0810585CF	5,110	5,110
	A/Virginia/ATCC1/2009	ATCC	VR-1736	5,110	5,110
	A/Netherlands/2629/2009	BEI Resources	NR-19823	5,110	5,110
	A/Houston/3H/2009	BEI Resources	NR-20340	5,110	5,110
	A/Brownsville/31H/2009	BEI Resources	NR-20344	5,110	5,110
	A/Dominican Republic/7293/2013	IRR	FR-1298	5,110	5,110
	A/Massachusetts/15/2013	IRR	FR-1319	5,110	5,110
	A/Swine/Iowa/15/30	ATCC	VR-333	4,810	2.25×10^7
	A/Swine/1976/31	ATCC	VR-99	4,810	1.83×10^7
H3	**A/Wisconsin/67/05**	**ZeptoMetrix**	**0810252CF**	**168**	**168**
	A/Brisbane/10/07	ZeptoMetrix	0810138CF	504	168
	A/Texas/50/2012	ZeptoMetrix	0810238CF	504	504
	A/Perth/16/09	ZeptoMetrix	0810251CF	168	168
	A/Hong Kong/4801/14	ZeptoMetrix	0810526CF	4,540	1,510
	A/Singapore/INFIMH-16-0019/16	ZeptoMetrix	0810574CF	1,510	1,510
	A/Kansas/14/17	ZeptoMetrix	0810586CF	504	168
	A/Hong Kong/8/68	ATCC	VR-544	168	40,800 [3]
	A/Alice/1973	ATCC	VR-776	168	8,040 [3]
	A/Port Chalmers/1/73	ATCC	VR-810	1,510	504 [3]
	A/Sydney/5/97	BEI Resources	NR-12278	4,540	168
	A/Santiago/7981/2006	IRR	FR-336	168	168
	A/Henan/Jinshui/147/07	IRR	FR-365	168	168
	A/Brisbane/9/2006	IRR	FR-366	168	168
	A/Nepal/921/2006	IRR	FR-367	168	168
	A/Florida/2/2006	IRR	FR-368	504	168
	A/South Australia/55/14	Zeptometrix	0810512CF	168	168
	A/Stockholm/6/14	ZeptoMetrix	0810513CF	504	168
	A/Norway/466/14	ZeptoMetrix	0810514CF	168	168
	A/Hong Kong/2671/19	ZeptoMetrix	0810609CF	504	504
	A/California/2/2014	ATCC	VR-1938	168	168
	A/Switzerland/9715293/2013	ATCC	VR-183	168	168
	Clinical Sample	--	500-NEG-161	168	168
	Clinical Sample	--	500-NEG-199	504	504
H5	A/Anhui/01/2005 (H5N1)	IRR	FR-735	540	Not Applicable
	A/Egypt/N03072/2010 (H5N1)	IRR	FR-1065	540	Not Applicable
	A/Hubei/1/2010 (H5N1)	IRR	FR-1066	540	Not Applicable
	A/pheasant/New Jersey/1355/1998 (H5N2)	IRR	FR-771	540	Not Applicable
H7	A/turkey/Virginia/4529/2002 (H7N2)	IRR	FR-772	540	Not Applicable
	A/mallard/Netherlands/12/2000 (H7N7)	IRR	FR-773	540	Not Applicable
H9	A/Hong Kong/33982/2009 (H9N2)	IRR	FR-1068	540	Not Applicable

Bold text: strain/isolate used in the LoD Study

[1] The NxTAG RPP v2 assay reports separate results for influenza A, influenza A H1, influenza A H1pdm09 and influenza A H3.

[2] Reported negative for Influenza A H1 at 5.77 x 10^7 copies/mL, the highest concentration attainable.
[3] Sequence analysis identified 1 or more mismatches with the labeled NxTAG primer; however, strains exhibiting these mutations are not currently in circulation and therefore negligible effect on assay performance is predicted.

Table 23. Strains of Influenza B evaluated for inclusivity

Lineage [1]	Strain/Isolate	Source	Catalogue No.	Concentration Detected (copies/mL)
Yamagata	**B/Florida/02/06**	**ZeptoMetrix**	**0810037CF**	**190**
	B/Massachusetts/2/2012	ZeptoMetrix	0810239CF	190
	B/Florida/04/2006	ZeptoMetrix	0810255CF	190
	B/Florida/07/04	ZeptoMetrix	0810256CF	190
	B/Panama/45/90	ZeptoMetrix	0810259CF	190
	B/Phuket/3073/13	ZeptoMetrix	0810515CF	190
	B/Bangladesh/5972/07	IRR	FR-450	190
	B/Hubei-Wujiagang/158/2009	IRR	FR-469	190
	B/Wisconsin/1/2010	ZeptoMetrix	0810241CF	190
Victoria	B/Brisbane/33/08	ZeptoMetrix	0810253CF	190
	B/Brisbane/60/08	ZeptoMetrix	0810254CF	190
	B/Malaysia/2506/04	ZeptoMetrix	0810258CF	190
	B/Colorado/06/17	ZeptoMetrix	0810573CF	569
	B/Hong Kong/259/2010	IRR	FR-663	190
	B/New Jersey/1/2012	IRR	FR-1270	190
	B/Texas/02/2013	IRR	FR-1302	190

Bold text: strain/isolate used in the LoD Study
[1] The NxTAG RPP v2 assay reports a single result for both lineages of influenza B (undifferentiated).

Table 24. Strains of Parainfluenza Virus evaluated for inclusivity

Serotype [1]	Strain/Isolate	Source	Catalogue No.	Concentration Detected (copies/mL)
1	**Type 1**	**ZeptoMetrix**	**0810014CF**	**2,080**
	C35	ATCC	VR-94	2,080
2	**Greer**	**ATCC**	**VR-92**	**1,030**
	Type 2	ZeptoMetrix	0810015CF	1,030
3	**C 243**	**ATCC**	**VR-93**	**3,040**
	Type 3	ZeptoMetrix	0810016CF	3,040
	ATCC-2011-5	ATCC	VR-1782	3,040
	NIH47885	BEI	NR-3233	3,040
4A	**Type 4A**	**ZeptoMetrix**	**0810060CF**	**50,800**
	M-25	ATCC	VR-1378	50,800
4B	**CH 19503**	**ATCC**	**VR-1377**	**21,400**
	Type 4B	ZeptoMetrix	0810060BCF	21,400

Bold text: strain/isolate used in the LoD Study
[1] The NxTAG RPP v2 assay reports separate results for RSV serotypes 1-4 (serotypes 4A and 4B are not differentiated).

Table 25. Strains of Respiratory Syncytial Virus evaluated for inclusivity

Subtype [1]	Strain/Isolate	Source	Catalogue No.	Concentration Detected (copies/mL)
A	**A2**	**ATCC**	**VR-1540**	**14,900**
	2006 Isolate	ZeptoMetrix	0810040ACF	14,900
	Long	ATCC	VR-26	14,900
B	**18537**	**ATCC**	**VR-1580**	**21,600**
	CH93(18)-18	ZeptoMetrix	0810040CF	21,600
	9320	ATCC	VR-955	21,600
	B WV/14617/85	ATCC	VR-1400	21,600
	B1	BEI Resources	NR-4052	21,600

Bold text: strain/isolate used in the LoD Study
[1] The NxTAG RPP v2 assay reports separate results for RSV subtypes A and B.

Table 26. Strains of Rhinovirus evaluated for inclusivity [1]

Species	Type	Strain/Isolate	Source	Catalogue No.	Concentration Detected (copies/mL)
A	**85**	**50-525-CV54 (V-192-001-021)**	**ATCC**	**VR-1195**	**4,610**
	1A	--	Zeptometrix	0810012CFN	4,610
	2	--	ATCC	VR-482	4,610
	7	68-CV11	ATCC	VR-1601	4,610
	16	--	Zeptometrix	0810285CF	4,610
	34	137-3	ATCC	VR-1365	4,610
	39	209	ATCC	VR-340	4,610
	54	FO1-3774	ATCC	VR-1661	4,610
	57	Ch47	ATCC	VR-1600	4,610
	77	130-63 (V-185-001-021)	ATCC	VR-1187	4,610
B	3	FEB	ATCC	VR-483	4,610
	14	1059	ATCC	VR-284	4,610
	17	33342	ATCC	VR-1663	4,610
	27	5870 (5870-CV28) (NIAID V-144-001-021)	ATCC	VR-1137	4,610
	42	56822	ATCC	VR-338	4,610
	83	Baylor 7 (V-190-001-021)	ATCC	VR-1193	4,610
C	--	SARS-COV2-NEG017	Clinical Specimen	--	4,610
	--	NPS/UTM NEG 178	Clinical Specimen	--	4,610
	--	75466-1	Clinical Specimen	--	4,610
	--	SARS-COV2-NEG004	Clinical Specimen	--	4,610
	--	SARS-COV2-NEG012	Clinical Specimen	--	4,610

Bold text: strain/isolate used in the LoD Study

[1] The NxTAG RPP v2 assay reports a single test result for Rhinovirus/Enterovirus (undifferentiated). Strains of Enterovirus evaluated for inclusivity are shown in **Table 27**.

Table 27. Strains of Enterovirus evaluated for inclusivity [1]

Species	Serotype	Strain/Isolate	Source	Catalogue No.	Concentration Detected (copies/mL)
A	Enterovirus 71	Strain H	ATCC	VR-1432	10,600
	Coxsackievirus A10	M.K. (Kowalik)	ATCC	VR-168	10,600
B	Coxsackievirus B1	Conn-5	ATCC	VR-28	10,600
	Coxsackievirus B4	J.V.B. (Benschoten)	ATCC	VR-184	10,600
	Echovirus 11	Gregory	ATCC	VR-41	286,000
	Echovirus 13	Del Carmen	ATCC	VR-43	10,600
	Enterovirus Type 69	Toluca-1 [V- 068-001-021]	ATCC	VR-1077	10,600
C	Coxsackievirus A21	Kuykendall	ATCC	VR-850	10,600
	Coxsackievirus A24	DN-19	ATCC	VR-1662	10,600
D	**Enterovirus 68**	**US/IL/14-18952**	**ZeptoMetrix**	**0810237CF**	**10,600**
	Enterovirus 68	2014 Isolate	ZeptoMetrix	0810300CF	10,600
	Enterovirus 68	US/MO/14-18947	ATCC	VR-1823	10,600
	Enterovirus 68	US/KY/14-18953	ATCC	VR-1825	10,600
	Enterovirus 68	Fermon	ATCC	VR-1826	10,600
	Enterovirus 70	J670/71	ATCC	VR-836	10,600

Bold text: strain/isolate used in the LoD Study

[1] The NxTAG RPP v2 assay reports a single test result for Rhinovirus/Enterovirus (undifferentiated). Strains/isolates of Rhinovirus evaluated for inclusivity are shown in **Table 26**.

Table 28. Strains of SARS-CoV-2 evaluated for inclusivity

Strain/Isolate	Source	Catalogue or Lot No.	Material	Concentration Detected (copies/mL)
2019-nCoV/USA WA1/2020	**ATCC**	**VR-1986HK**	**Heat inactivated**	**1,500**
Hong Kong/VM2000106/2020	ZeptoMetrix	0810590CFHI	Heat inactivated	1,500
Isolate USA-WA1/2020	ZeptoMetrix	0810587CFHI	Heat inactivated	1,500
Strain BetaCoV/Germany/BavPat1/2020	EVAg	026N-03889	RNA	2,730 [1]
Strain 2019- nCoV/Italy-INMI1	EVAg	008N-03894	RNA	2,730 [1]
England/02/2020	BEI Resources	NR-52499	RNA	2,730 [1]
Singapore/2/2020	BEI Resources	NR-52501	RNA	2,730 [1]
USA-IL1/2020	BEI Resources	NR-52503	RNA	2,730 [1]
USA-CA1/2020	BEI Resources	NR-52504	RNA	2,730 [1]
USA-AZ1/2020	BEI Resources	NR-52505	RNA	2,730 [1]
USA-WI1/2020	BEI Resources	NR-52506	RNA	2,730 [1]
USA-CA3/2020	BEI Resources	NR-52507	RNA	2,730 [1]
USA-CA4/2020	BEI Resources	NR-52508	RNA	2,730 [1]
USA-CA2/2020	BEI Resources	NR-52509	RNA	2,730 [1]
Chile/Santiago_op4d1/2020	BEI Resources	NR-52510	RNA	2,730 [1]
New York-PV08410/2020	BEI Resources	NR-53518	RNA	2,730 [1]
USA/CA_CDC_5574/2020	BEI Resources	NR-55245	Heat inactivated	1,500
Alpha (B1.1.7)/UK Variant	Clinical	RP-1760	--	1,500
Epsilon (B1.429)/California Variant	Clinical	RP-1877	--	1,500
Epsilon (B1.429)/California Variant	Clinical	RP-1878	--	1,500
Delta (B.1.617.2)	Clinical	CoV-P-UTM-384	--	1,500
Delta (B.1.617.2)	Clinical	CoV-P-UTM-385	--	1,500
Delta (B.1.617.2)	Clinical	CoV-P-UTM-385	--	1,500
Delta (B.1.617.2)	Clinical	CoV-P-UTM-392	--	1,500
Delta (B.1.617.2)	Clinical	CoV-P-UTM-394	--	1,500
Omicron (B.1.1.529 and BA lineages)	Clinical	COV-053	--	1,500
Omicron (B.1.1.529 and BA lineages)	Clinical	COV-054	--	1,500
Omicron (B.1.1.529 and BA lineages)	Clinical	COV-055	--	1,500
Omicron (B.1.1.529 and BA lineages)	Clinical	COV-058	--	1,500
Omicron (B.1.1.529 and BA lineages)	Clinical	COV-059	--	1,500

Bold text: strain/isolate used in the LoD Study

[1] RNA diluted in extracted negative simulated matrix to a concentration equivalent to 1,500 copies/mL of raw sample.

Table 29. Strains of the *Chlamydia pneumoniae* evaluated for inclusivity

Strain	Source	Catalogue No.	Concentration Detected (copies/mL)
TW-183	**ATCC**	**VR-2282**	**713**
TWAR (CDC/CWL-029)	ATCC	VR-1310	713
TWAR 2023	ATCC	VR-1356	713
AR-39	ATCC	53592	713

Bold text: strain/isolate used in the LoD Study

Table 30. Strains of the *Mycoplasma pneumoniae* evaluated for inclusivity

Strain/Isolate	Source	Catalogue No.	Concentration Detected (copies/mL)
M129	**ZeptoMetrix**	**0801579**	**12,900**
[M52]	ATCC	15293	12,900
FH strain of Eaton Agent [NCTC 10119]	ATCC	15531-TTR	12,900
Mutant 22	ATCC	39505	12,900

Bold text: strain/isolate used in the LoD Study

7. Assay Cut-Off:

The design of the NxTAG RPP v2 assay was based on that of the previously FDA-cleared NxTAG RPP (K152386/K193167), with various modifications aimed to improve inclusivity and specificity, for the addition of SARS-CoV-2 as a target analyte and for discrimination of Influenza A H1pdm09. Because of these changes, a new threshold analysis was performed using a combination of clinical and contrived specimens, as well as negative controls.

The thresholds for the NxTAG RPP v2 assay were established in a three-step optimization process:

a) Selection of an initial range of threshold values for each analyte;

b) Determination of performance for each analyte using the range of potential threshold values;

c) Optimization of the thresholds through Receiver Operating Characteristic (ROC) curve analysis.

Discrete data sets were used for preliminary threshold selection, performance evaluation (optimization) and validation. The final optimized thresholds were used to analyze the data obtained in the Analytical and Clinical Studies described in **Sections VII A**, **B** and **C**.

8. Accuracy (Instrument):

Refer to **Section VII C Clinical Studies**.

9. Carry-Over:

The potential for contamination within and between runs of the NxTAG RPP v2 assay was evaluated by testing high concentrations of two representative analytes (SARS-CoV-2 and *M. pneumoniae* at 10^5 $TCID_{50}$ and 10^6 CCU/mL, respectively) in simulated nasopharyngeal swab matrix, in conjunction with negative samples. Five MAGPIX runs were performed on two instruments using an alternating pattern of high positive and negative samples (n = 12 ea.) that were processed on two bioMérieux NucliSENS easyMAG extraction systems. On initial testing, 2/12 negative samples in one MAGPIX run gave positive results for SARS-CoV-2 (**Table 31**). Repreparation, extraction and testing of 12 additional positive and 12 additional negative samples on the same easyMAG and MAGPIX instruments produced the expected results. Overall, in conjunction with the results of the Prospective Clinical Study described in **Section VIII ©**, the likelihood of false positive results with the NxTAG RPP v2 assay due to cross-contamination was considered acceptable.

Table 31. Results from evaluation of run-to-run and sample-to-sample contamination

MAGPIX Run	Analyte	Sample	Agreement (%)
1	SARS-CoV-2	Positive	12/12 (100)
		Negative	12/12 (100)
2	*M. pneumoniae*	Positive	12/12 (100)
		Negative	12/12 (100)
3	SARS-CoV-2	Positive	12/12 (100)
		Negative	10/12 (83.3) [1]
4	*M. pneumoniae*	Positive	12/12 (100)
		Negative	12/12 (100)
5	SARS-CoV-2	Positive	12/12 (100)
		Negative	12/12 (100)
Total	SARS-CoV-2	Positive	36/36 (100)
		Negative	34/36 (94.4)
	M. pneumoniae	Positive	24/24 (100)
		Negative	24/24 (100)

SARS-CoV-2 Positive: 10^5 $TCID_{50}$/mL
M. pneumoniae Positive: 10^6 CCU/mL
[1] Repreparation, extraction and testing of 12 additional positive and 12 additional negative samples produced the expected results (100% agreement for both positive and negative samples).

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Not applicable.

2. Matrix Comparison:

   Comparison of Natural and Simulated Nasopharyngeal Swab Matrix
   Please refer to **Section VII A (6)** regarding use of simulated nasopharyngeal swab matrix in Analytical Studies to evaluate the performance of the NxTAG RPP v2 assay.

   Comparison of Alternative Transport Media
   The compatibility of the NxTAG RPP v2 assay with nasopharyngeal swabs collected in alternative transport media was evaluated by testing contrived specimens composed of negative nasopharyngeal swab matrix containing representative NxTAG RPP v2 target analytes at levels above and below the LoD established with Universal Transport Medium (UTM, Copan Diagnostics) (**Table 32**). The NxTAG RPP V2 assay demonstrated similar analytical sensitivity (≥ 95% detection at 1 - 3X LoD) with specimens prepared in both transport media evaluated, supporting the use of these media for specimen collection (**Table 33**).

Table 32. Representative NxTAG RPP v2 analytes used to evaluate alternative transport media

Sample	Analyte	Strain	Source	Catalogue No.
1	Influenza A H1pdm09 (subtype) [1]	A/NY/02/09	ZeptoMetrix	0810109CFN
	Respiratory Syncytial Virus A	A2	ATCC	VR-1540
	Rhinovirus	50-525-CV54	ATCC	VR-1195
2	Influenza A H3 (subtype) [1]	A/Wisconsin/67/05	ZeptoMetrix	0810252CF
	Respiratory Syncytial Virus B	18537	ATCC	VR-1580
3	Influenza B	B/Florida/02/06	ZeptoMetrix	0810037CF
	Parainfluenza Virus 3	C 243	ATCC	VR-93
	Mycoplasma pneumoniae	M129	ZeptoMetrix	0801579
4	SARS-CoV-2	USA-WA1/2020	ATCC	VR-1986HK
	Human Metapneumovirus	hMPV-3, Type B1, Peru2-2002	ZeptoMetrix	0810156CF
	Adenovirus B	Type 14, 2006 isolate	ZeptoMetrix	0810108CF
5	Negative	--	--	--

[1] Target level based on the LoD for the applicable subtype.

Table 33. Comparison of analytical sensitivity with alternative transport media

NxTAG RPP v2 Analyte	Concentration (copies/mL)	X LoD	Positive/Tested (%)	
			UTM	M4RT
Adenovirus	12,300	8.7	10/10 (100)	10/10 (100)
	3,690	**2.6**	**30/30 (100)**	
	2,460	**1.7**	28/30 (93)	**30/30 (100)**
	820	0.6	4/10 (40)	0/10 (0)
Human Metapneumovirus	1,310	5	10/10 (100)	10/10 (100)
	262	**1**	**29/30 (97)**	**30/30 (100)**
	87	0.3	5/10 (50)	7/10 (70)
Influenza A H1pdm09 (subtype)	8,520	8.7	10/10 (100)	10/10 (100)
	1,700	**1.7**	30/30 (100)	**30/30 (100)**
	568	**0.6**	**10/10 (100)**	7/10 (70)
Influenza A H3 (subtype)	280	5	10/10 (100)	10/10 (100)
	56	**1**	**30/30 (100)**	**30/30 (100)**
	19	0.3	4/10 (40)	4/10 (40)
Influenza B	316	5	10/10 (100)	10/10 (100)
	63	**1**	**29/30 (97)**	**29/30 (97)**
	21	0.3	5/10 (50)	4/10 (40)
Parainfluenza Virus 3	5,070	5	10/10 (100)	10/10 (100)
	2,030	**2**	**30/30 (100)**	**29/30 (97)** [1]
	1,010	1	26/30 (87)	22/30 (73)
	338	0.3	0/10 (0)	2/10 (20)
Respiratory Syncytial Virus A	24,800	5	10/10 (100)	10/10 (100)
	4,970	**1**	**30/30 (100)**	**30/30 (100)**
	1,660	0.3	6/10 (60)	5/10 (50)
Respiratory Syncytial Virus B	36,000	5	10/10 (100)	10/10 (100)
	7,210	**1**	**30/30 (100)**	**30/30 (100)**
	2,400	0.3	6/10 (60)	3/10 (30)
Rhinovirus/Enterovirus	7,680	5	10/10 (100)	10/10 (100)
	1,540	**1**	30/30 (100)	**30/30 (100)**
	512	**0.3**	**10/10 (100)**	9/10 (90)
SARS-CoV-2	2,500	5	10/10 (100)	10/10 (100)
	500	**1**	30/30 (100)	**30/30 (100)**
	167	**0.3**	**10/10 (100)**	7/10 (70)
Mycoplasma pneumoniae	21,500	6.7	10/10 (100)	10/10 (100)
	4,300	**1.3**	**30/30 (100)**	**29/30 (97)**
	1,430	0.4	3/10 (30)	4/10 (40)
Negative	--	--	0/10 (0)	0/10 (0)

UTM: Universal Transport Medium (Copan Diagnostics); M4RT: MicroTest M4RT (Remel)

[1] 1 sample that produced a negative result exhibited an unexpectedly high signal for the MS2 Internal Control and was retested. The retest result was positive and was included in the analysis.

Comparison of Alternative Swabs for Specimen Collection

The compatibility of the NxTAG RPP v2 assay with alternative types of collection swab was evaluated by testing flocked nylon, polyester and rayon swabs that were seeded with simulated nasopharyngeal swab matrix containing representative target analytes (**Table 34**). Swabs of each type were prepared and expressed in 1 mL Universal Transport Medium prior to testing with the NxTAG RPP v2 assay. All results for flocked nylon and polyester swabs were as expected. However, diminished recovery/detection from rayon swabs was observed for multiple analytes (**Table 35**). As a result, the device labeling includes a Limitation indicating that rayon nasopharyngeal swabs are not compatible with the NxTAG RPP v2 assay.

Table 34. Representative NxTAG RPP v2 analytes used to evaluate alternative collection swab for specimen collection

Sample	Analyte	Strain	Source	Catalogue No.
1	Influenza A H1pdm09dm (subtype) [1]	A/NY/02/09	ZeptoMetrix	0810109CFN
	Respiratory Syncytial Virus A	A2	ATCC	VR-1540
	Rhinovirus	50-525-CV54	ATCC	VR-1195
2	Influenza A H3 (subtype) [1]	A/Wisconsin/67/05	ZeptoMetrix	0810252CF
	Respiratory Syncytial Virus B	18537	ATCC	VR-1580
3	Influenza B	B/Florida/02/06	ZeptoMetrix	0810037CF
	Parainfluenza Virus 3	C 243	ATCC	VR-93
	Mycoplasma pneumoniae	M129	ZeptoMetrix	0801579
4	SARS-CoV-2	USA-WA1/2020	ATCC	VR-1986HK
	Human Metapneumovirus	hMPV-3, Type B1, Peru2-2002	ZeptoMetrix	0810156CF
	Adenovirus B	Type 14, 2006 isolate	ZeptoMetrix	0810108CF
5	Negative	--	--	--

[1] Target level based on the LoD for the applicable subtype.

Table 35. Results obtained from evaluation of alternative collection swabs

Sample	NxTAG RPP v2 Analyte	Agreement (%)		
		Nylon Flocked	Polyester	Rayon
1	Influenza A H1pdm09 (subtype)	3/3 (100)	3/3 (100)	3/3 (100)
	Respiratory Syncytial Virus A	3/3 (100)	3/3 (100)	3/3 (100)
	Rhinovirus/Enterovirus	3/3 (100)	3/3 (100)	3/3 (100)
2	Influenza A H3 (subtype)	3/3 (100)	3/3 (100)	3/3 (100)
	Respiratory Syncytial Virus B	3/3 (100)	3/3 (100)	3/3 (100)
3	Influenza B	3/3 (100)	3/3 (100)	1/3 (33) [1]
	Parainfluenza Virus 3	3/3 (100)	3/3 (100)	1/3 (33) [1]
	Mycoplasma pneumoniae	3/3 (100)	3/3 (100)	1/3 (33) [1]
4	SARS-CoV-2	3/3 (100)	3/3 (100)	3/3 (100)
	Human Metapneumovirus	3/3 (100)	3/3 (100)	3/3 (100)
	Adenovirus	3/3 (100)	3/3 (100)	2/3 (67) [2]
5	Negative	3/3 (100)	3/3 (100)	3/3 (100)

[1] Negative were obtained results for all 3 analytes (Influenza B, Parainfluenza Virus 3 and *M. pneumoniae*) from 2 rayon swabs.

[2] A negative result for Adenovirus was obtained from a single rayon swab.

C Clinical Studies:

1. Clinical Sensitivity:

Study Design
The performance of the NxTAG RPP v2 was evaluated in a multi-site Clinical Study using a combination of nasopharyngeal swab specimens that were either:

a) prospectively collected and tested fresh (Category I specimens), or after freezing at ≤ -70 °C and thawing if they could not be processed within 7 days (Category II specimens), or

b) preselected, archived specimens (Category III specimens), or

c) contrived simulated specimens (Category IV specimens).

For inclusion in the study, Category I, II or III specimens were obtained from patients exhibiting signs and symptoms of respiratory tract infection. Samples for testing with the NxTAG RPP v2 assay were processed using either the bioMérieux easyMAG or EMAG instruments.

Prospectively Collected Specimens (Category I/II)
A total of 1,844 nasopharyngeal swab specimens from five geographically diverse sites in the United States were initially enrolled in the Prospective Clinical Study between October 2022 and April 2023. The performance of the NxTAG RPP v2 was compared to that of an analyte-specific molecular assay for SARS-CoV-2 that was cleared by FDA under 21 CFR 866.3981 (Product Code QQX) and an FDA-cleared molecular respiratory panel for all other analytes. Specimens that were reported positive for influenza A H1 by the comparator method were further characterized by PCR/bidirectional sequencing to distinguish the pandemic strain A H1pdm09 from other influenza A H1 strains. The prospectively collected specimens were tested using the NxTAG RPP v2 assay at three study sites.

Nineteen specimens were excluded from the analysis of performance as follows: duplicate specimen, not from a unique patient (11), not collected from the intended use population with signs and symptoms of respiratory tract infection (5), improper labeling (2) or NxTAG RPP v2 operator error (1). Nineteen of the remaining 1,825 specimens (1.0%) produced invalid NxTAG results on initial testing. Sixteen were retested, of which 14 produced valid positive or negative results and 2 were again reported as “invalid”. In total, 5 specimens (0.3%) were excluded from the analysis of performance due to invalid NxTAG RPP v2 test results. Additional specimens were also excluded from analysis of performance for specific analytes due to failure to perform the applicable comparator test method(s) or generation of invalid comparator test results.

The demographic characteristics of the 1,820 patients from whom specimens with valid NxTAG RPP v2 results were obtained are provided in **Table 36**.

Table 36. Characteristics of patients and specimens included in the Prospective Clinical Study

Category		Number	Percent
Gender	Male	817	44.9
	Female	1,003	55.1
	Total	**1,820**	**100**
Age	0-1	337	18.5
	> 1 - 5	253	13.9
	> 5 - 21	331	18.2
	> 21- 65	645	35.4
	> 65	242	13.3
	Not Known	12	0.7
	Total	**1,820**	**100**
Patient Status	Outpatient	356	19.6
	Hospitalized	304	16.7
	Emergency Room	1,146	63.0
	Not Known	14	0.8
	Total	**1,820**	**100**
Location	California	447	24.6
	Florida	826	45.4
	Texas	40	2.2
	Wisconsin	507	27.9
	Total	**1,820**	**100**
Transport Medium	UTM	566	31.1
	UVT	1,254	68.9
	Total	**1,820**	**100**
Specimen Category	Category I - Fresh	776	42.6
	Category II - Frozen	1,044	57.4
	Total	**1,820**	**100**

UTM: Universal Transport Medium (Copan Diagnostics); UVT: Universal Viral Transport (BD)

Preselected, Archived Specimens (Category III)

For analytes that exhibited low prevalence in the Prospective Clinical Study, 320 preselected, archived specimens that were initially reported as positive based on standard of care test results were obtained from six sites in the United States. The microbial content of each specimen was confirmed by PCR/bidirectional sequencing prior to enrollment in the study. Eleven specimens were excluded from the analysis of performance because confirmatory testing could not be completed. To avoid bias, testing of the remaining 309 archived specimens with the NxTAG RPP v2 assay was performed in a randomized, blinded manner at four study sites without further preselection based on their microbial content. Three specimens (0.9%) produced invalid NxTAG RPP v2 results on initial testing, of which 2 gave valid positive/negative results upon repeat, for a final invalid rate of 0.3% (1/309) and a final total of 308 preselected specimens for inclusion in the analysis of performance.

Contrived Specimens (Category IV)

Positive clinical specimens could not be obtained in sufficient numbers to meet study requirements for some analytes for which additional testing was performed using contrived specimens. These were prepared by spiking known analyte-negative individual clinical specimens in Universal Transport Medium (UTM; Copan Diagnostics) using previously characterized bacterial or viral stocks of representative strains at 2X, 10X or 100X the established LoD. Testing of the contrived specimens with the NxTAG RPP v2 assay was performed at two study sites in a blinded manner. Results were compared to the known analyte content of each specimen. On initial testing 1/199 contrived specimens

(0.5%) produced an invalid NxTAG RPP v2 test result. A valid result was obtained for this specimen upon retest.

Clinical Performance
A summary of the results from testing prospectively collected and archived, preselected nasopharyngeal swabs is shown in **Table 37**.

Valid NxTAG RPP v2 test results were obtained from a total of 1,820 prospectively collected nasopharyngeal swab specimens. Positive Percent Agreement (PPA) ranged from 90.0-100% for prospectively collected specimens depending on the analyte, except for Parainfluenza Virus 4 for which the number of specimens tested was small (n = 15). For archived, preselected specimens, PPA ranged from 92.3-100%, depending on the analyte.

Negative Percent Agreement (NPA) ranged from 99.7-100% for prospectively collected specimens and from 99.3-100% for archived specimens.

Table 37. Clinical performance of the NxTAG RPP v2 with prospectively collected and archived (preselected) specimens

Viruses					
NxTAG RPP v2 Analyte	**Study**	**Positive Agreement**		**Negative Agreement**	
		Percent (95% CI)	**TP/(TP+FN)**	**Percent (95% CI)**	**TN/(TN+FP)**
Adenovirus	Prospective (Fresh)	100 (91.0-100)	39/39	99.6 (98.8-99.9)	733/736
	Prospective (Frozen)	93.2 (83.8-97.3)	55/59	99.6 (99.0-99.8)	976/980
	Prospective Total	**95.9 (90.0-98.4)**	**94/98**	**99.6 (99.2-99.8)**	**1709/1716**
	Archived	**100 (20.7-100)**	**1/1**	**100 (98.8-100)**	**307/307**
Coronavirus 229E	Prospective (Fresh)	100 (34.2-100)	2/2	100 (99.5-100)	773/773
	Prospective (Frozen)	100 (56.6-100)	5/5	99.9 (99.5-100)	1033/1034
	Prospective Total	**100 (64.6-100)**	**7/7**	**99.9 (99.7-100)**	**1806/1807**
	Archived	**100 (74.1-100)**	**11/11**	**100 (98.7-100)**	**297/297**
Coronavirus HKU1	Prospective (Fresh)	100 (67.6-100)	8/8	100 (99.5-100)	767/767
	Prospective (Frozen)	92.3 (66.7-98.6)	12/13	100 (99.6-100)	1026/1026
	Prospective Total	**95.2 (77.3-99.2)**	**20/21**	**100 (99.8-100)**	**1793/1793**
	Archived	**93.8 (79.9-98.3)**	**30/32**	**100 (98.6-100)**	**276/276**
Coronavirus NL63	Prospective (Fresh)	92.6 (76.6-97.9)	25/27	100 (99.5-100)	748/748

Viruses					
NxTAG RPP v2 Analyte	**Study**	**Positive Agreement**		**Negative Agreement**	
		Percent (95% CI)	**TP/(TP+FN)**	**Percent (95% CI)**	**TN/(TN+FP)**
	Prospective (Frozen)	92.0 (75.0-97.8)	23/25	100 (99.6-100)	1014/1014
	Prospective Total	**92.3 (81.8-97.0)**	**48/52**	**100 (99.8-100)**	**1762/1762**
	Archived	**Not Applicable**	**Not Applicable**	**100 (98.8-100)**	**308/308**
Coronavirus OC43	Prospective (Fresh)	100 (72.3-100)	10/10	100 (99.5-100)	765/765
	Prospective (Frozen)	100 (88.3-100)	29/29	100 (99.6-100)	1010/1010
	Prospective Total	**100 (91.0-100)**	**39/39**	**100 (99.8-100)**	**1775/1775**
	Archived	**Not Applicable**	**Not Applicable**	**100 (98.8-100)**	**308/308**
Human Metapneumovirus	Prospective (Fresh)	100 (95.8-100)	87/87	98.8 (97.7-99.4)	680/688
	Prospective (Frozen)	100 (94.8-100)	70/70	99.4 (98.7-99.7)	963/969
	Prospective Total	**100 (97.6-100)**	**157/157**	**99.2 (98.6-99.5)**	**1643/1677**
	Archived	**Not Applicable**	**Not Applicable**	**100 (98.8-100)**	**308/308**
Influenza A (matrix)	Prospective (Fresh)	100 (83.9-100)	20/20	99.7 (99.0-99.9)	753/755
	Prospective (Frozen)	100 (93.4-100)	54/54	99.9 (9.4-100)	984/985
	Prospective Total	**100 (95.1-100)**	**74/74**	**99.8 (99.5-99.9)**	**1737/1740**
	Archived	**100 (88.7-100)**	**30/30**	**99.6 (98.0-99.9)**	**277/278**
Influenza A H1 (subtype)	Prospective (Fresh)	Not Applicable	Not Applicable	100 (99.5-100)	774/774
	Prospective (Frozen)	Not Applicable	Not Applicable	100 (99.6-100)	1039/1039
	Prospective Total	**Not Applicable**	**Not Applicable**	**100 (99.8-100)**	**1813/1813**
	Archived	**Not Applicable**	**Not Applicable**	**99.6 (98.0-99.9)**	**277/278**
Influenza A H1pdm09 (subtype)	Prospective (Fresh)	100 (70.1-100)	9/9	100 (99.5-100)	765/765
	Prospective (Frozen)	100 (85.1-100)	22/22	100 (99.6-100)	1017/1017
	Prospective Total	**100 (89.0-100)**	**31/31**	**100 (99.8-100)**	**1782/1782**
	Archived	**96.7 (83.3-99.4)**	**29/30**	**100 (98.6-100)**	**278/278**

Viruses					
NxTAG RPP v2 Analyte	**Study**	**Positive Agreement**		**Negative Agreement**	
		Percent (95% CI)	**TP/(TP+FN)**	**Percent (95% CI)**	**TN/(TN+FP)**
Influenza A H3 (subtype)	Prospective (Fresh)	100 (74.1-100)	11/11	100 (99.5-100)	764/764
	Prospective (Frozen)	94.4 (81.9-98.5)	34/36	99.9 (99.4-100)	1002/1003
	Prospective Total	**95.7 (85.8-98.8)**	**45/47**	**99.9 (99.7-100)**	**1766/1767**
	Archived	**Not Applicable**	**Not Applicable**	**100 (98.6-100)**	**278/278**
Influenza B	Prospective (Fresh)	100 (56.6-100)	5/5	100 (99.5-100)	770/770
	Prospective (Frozen)	100 (61.0-100)	6/6	100 (99.6-100)	1033/1033
	Prospective Total	**100 (74.1-100)**	**11/11**	**100 (99.8-100)**	**1803/1803**
	Archived	**100 (88.7-100)**	**30/30**	**100 (98.6-100)**	**278/278**
Parainfluenza Virus 1	Prospective (Fresh)	100 64.6-100)	7/7	100 (99.5-100)	768/768
	Prospective (Frozen)	100 (74.1-100)	11/11	100 (99.6-100)	1028/1028
	Prospective Total	**100 (82.4-100)**	**18/18**	**100 (99.8-100)**	**1796/1796**
	Archived	**100 (88.3-100)**	**29/29**	**100 (98.6-100)**	**279/279**
Parainfluenza Virus 2	Prospective (Fresh)	80.0 (37.6-96.4)	4/5	100 (99.5-100)	770/770
	Prospective (Frozen)	100 (56.6-100)	5/5	100 (99.6-100)	1034/1034
	Prospective Total	**90.0 (59.6-98.2)**	**9/10**	**100 (99.8-100)**	**1804/1804**
	Archived	**100 (88.7-100)**	**30/30**	**100 (98.6-100)**	**278/278**
Parainfluenza Virus 3	Prospective (Fresh)	100 (83.2-100)	19/19	100 (99.5-100)	756/756
	Prospective (Frozen)	100 (85.7-100)	23/23	99.9 (99.4-100)	1015/1016
	Prospective Total	**100 (91.6-100)**	**42/42**	**99.9 (99.7-100)**	**1771/1772**
	Archived	**Not Applicable**	**Not Applicable**	**100 (98.8-100)**	**307/307**
Parainfluenza Virus 4	Prospective (Fresh)	66.7 (20.8-93.9)	2/3	99.7 (99.1-99.9)	770/772
	Prospective (Frozen)	91.7 (64.6-98.5)	11/12	99.9 (99.5-100)	1026/1027
	Prospective Total	**86.7 (62.1-96.3)**	**13/15**	**99.8 (99.5-99.9)**	**1796/1799**

Viruses					
NxTAG RPP v2 Analyte	**Study**	**Positive Agreement**		**Negative Agreement**	
		Percent (95% CI)	**TP/(TP+FN)**	**Percent (95% CI)**	**TN/(TN+FP)**
	Archived	**93.8 (71.7-98.9)**	**15/16**	**100 (98.7-100)**	**292/292**
Respiratory Syncytial Virus A	Prospective (Fresh)	100 (72.3-100)	10/10	99.9 (99.3-100)	764/765
	Prospective (Frozen)	100 (92.1-100)	45/45	99.8 (99.3-99.9)	992/994
	Prospective Total	**100 (93.5-100)**	**55/55**	**99.8 (99.5-99.9)**	**1756/1759**
	Archived	**Not Applicable**	**Not Applicable**	**99.3 (97.7-99.8)**	**305/307**
Respiratory Syncytial Virus B	Prospective (Fresh)	100 (43.9-100)	3/3	100 (99.5-100)	772/772
	Prospective (Frozen)	100 (81.6-100)	17/17	100 (99.6-100)	1022/1022
	Prospective Total	**100 (83.9-100)**	**20/20**	**100 (99.8-100)**	**1794/1794**
	Archived	**Not Applicable**	**Not Applicable**	**99.7 (98.2-99.9)**	**306/307**
Rhinovirus/Enterovirus	Prospective (Fresh)	93.2 (87.6-96.4)	123/132	100 (99.4-100)	643/643
	Prospective (Frozen)	96.2 (92.9-98.0)	228/237	99.9 (99.3-100)	801/802
	Prospective Total	**95.1 (92.4-96.9)**	**351/369**	**99.9 (99.6-100)**	**1444/1445**
	Archived	**100 (20.7-100)**	**1/1**	**98.7 (96.7-99.5)**	**302/306**
SARS-CoV-2	Prospective (Fresh)	97.2 (92.0-99.0)	103/106	99.4 (98.5-99.8)	656/660
	Prospective (Frozen)	98.4 (94.5-99.6)	126/128	99.2 (98.4-99.6)	902/909
	Prospective Total	**97.9 (95.1-99.1)**	**229/234**	**99.3 (98.7-99.6)**	**1558/1569**
	Archived	**Not Applicable**	**Not Applicable**	**Not Applicable**	**Not Applicable**

Bacteria					
NxTAG RPP v2 Analyte	**Study**	**Positive Agreement**		**Negative Agreement**	
		Percent (95% CI)	**TP/(TP+FN)**	**Percent (95% CI)**	**TN/(TN+FP)**
Chlamydia pneumoniae	Prospective (Fresh)	Not Applicable	Not Applicable	100 (99.5-100)	775/775
	Prospective (Frozen)	Not Applicable	Not Applicable	100 (99.6-100)	1039/1039
	Prospective Total	**Not Applicable**	**Not Applicable**	**100 (99.8-100)**	**1814/1814**

Bacteria					
NxTAG RPP v2 Analyte	**Study**	**Positive Agreement**		**Negative Agreement**	
		Percent (95% CI)	**TP/(TP+FN)**	**Percent (95% CI)**	**TN/(TN+FP)**
	Archived	**100 (78.5-100)**	**14/14**	**99.7 (98.1-99.9)**	**293/294**
Mycoplasma pneumoniae	Prospective (Fresh)	Not Applicable	Not Applicable	100 (99.5-100)	775/775
	Prospective (Frozen)	Not Applicable	Not Applicable	100 (99.6-100)	1039/1039
	Prospective Total	**Not Applicable**	**Not Applicable**	**100 (99.8-100)**	**1814/1814**
	Archived	**92.3 (81.8-97.0)**	**48/52**	**100 (98.5-100)**	**256/256**

FN: False Negative; FP: False Positive; TN: True Negative; TP: True Positive

Contrived Specimens

Because some analytes exhibited low prevalence in the Prospective Clinical Study and insufficient archived positive specimens were available to demonstrate acceptable positive agreement, additional testing was performed with contrived specimens that were prepared by spiking quantified viral or bacterial stocks into unique individual nasopharyngeal swab specimens that were known to be negative for the NxTAG panel analytes (**Table 38**). The results of the study are described in **Table 39** and show acceptable positive and negative agreement.

Table 38. Strains of bacteria and viruses used to prepare contrived specimens

Analyte	Strain	Source	Catalog No.
Coronavirus 229E	229E	Zeptometrix	0810229CF
Influenza A H1	A/Brisbane/59/07	Zeptometrix	0810244CF
Human Bocavirus	Recombinant	Zeptometrix	0830039
Respiratory Syncytial Virus B	CH93(18)-18	Zeptometrix	0810040CF
Chlamydia pneumoniae	TW-183	ATCC	VR-2282

Table 39. Results from testing contrived specimens

Viruses			
NxTAG RPP v2 Analyte	**Level**	**Agreement**	
		Percent	**Number**
Adenovirus	Negative	100	199/199
Coronavirus 229E	100X	100	12/12
	10X	100	12/12
	2X	100	25/25
	Negative	100	149/149
Coronavirus HKU1	Negative	100	199/199
Coronavirus NL63	Negative	100	199/199
Coronavirus OC43	Negative	100	199/199
Human Metapneumovirus	Negative	100	199/199
Influenza A (matrix)	10X	100	24/24
	2X	100	26/26
	Negative	100	149/149
Influenza A H1 (subtype)	10X	100	24/24
	2X	100	26/26
	Negative	100	149/149
Influenza A H1pdm09 (subtype)	Negative	100	199/199
Influenza A H3 (subtype)	Negative	100	199/199
Influenza B	Negative	100	199/199
Parainfluenza Virus 1	Negative	100	199/199
Parainfluenza Virus 2	Negative	99.5	198/199
Parainfluenza Virus 3	Negative	100	199/199
Parainfluenza Virus 4	Negative	100	199/199
Respiratory Syncytial Virus A	Negative	100	199/199
Respiratory Syncytial Virus B	100X	100	13/13
	10X	100	13/13
	2X	96.0	24/25
	Negative	99.3	148/149
Rhinovirus/Enterovirus	Negative	100	199/199
SARS-Coronavirus	Negative	100	199/199

Bacteria			
NxTAG RPP v2 Analyte	**Level**	**Agreement**	
		Percent	**Number**
Chlamydia pneumoniae	100X	100	12/12
	10X	92.3	12/13
	2X	100	25/25
	Negative	100	149/149
Mycoplasma pneumoniae	Negative	100	199/199

Coinfections

The NxTAG RPP v2 assay reported detection of two or more target analytes from 125/1820 specimens (6.9%) in the Prospective Clinical Study (**Table 40**). Of these, 116 (92.8%) were positive for two viral pathogens while 9 specimens (7.2%) were positive for three different viruses.

Table 40. Coinfections detected by the NxTAG RPP v2 assay in the Prospective Clinical Study

NxTAG RPP v2 Positive Result				False NxTAG RPP v2 Results vs Comparator	
Analyte 1	**Analyte 2**	**Analyte 3**	***N***	**Positive**	**Negative**
Adenovirus	CoV HKU1		1	--	--
Adenovirus	CoV NL63		2	--	--
Adenovirus	CoV OC43	hMPV	1	hMPV (1)	
Adenovirus	hMPV		4	Adenovirus (1)	SARS-CoV-2 (1)
Adenovirus	Influenza A H3		1	Influenza A H3 (1)	--
Adenovirus	PIV 2	hMPV	1	--	--
Adenovirus	PIV 3		1	--	--
Adenovirus	PIV 3	CoV NL63	1	--	--
Adenovirus	RSV A		1	--	--
Adenovirus	Rh/EV		18	Adenovirus (2)	
Adenovirus	SARS-CoV-2		1	--	--
CoV 229E	RSV A		1	RSV A (1)	--
CoV 229E	Rh/EV		2		CoV NL63, SARS-CoV-2 (1)
CoV HKU1	hMPV		3	--	--
CoV NL63	CoV HKU1		1	--	--
CoV NL63	CoV OC43		1	--	--
CoV NL63	hMPV		3	--	--
CoV NL63	Rh/EV		6	--	--
CoV OC43	hMPV		1	--	--
CoV OC43	hMPV	Rh/EV	1	--	--
CoV OC43	RSV B		1	--	--
CoV OC43	Rh/EV		2	--	--
hMPV	RSV A		2	hMPV (1)	--
hMPV	Rh/EV		17	hMPV (1)	Adenovirus (1)
hMPV	Rh/EV	SARS-CoV-2	1	--	CoV NL63 (1)
Influenza A H3	CoV 229E		1	CoV 229E (1)	--
Influenza A H3	RSV A		1	RSV A (1)	--
Influenza A H3	Rh/EV		3	--	--
Influenza A H3	SARS-CoV-2	Rh/EV	1	--	--
Influenza A H1pdm09	CoV HKU1		1	--	--
Influenza A H1pdm09	Rh/EV		5	--	--
PIV 1	Rh/EV		2	--	--
PIV 2	RSV A		1	--	--
PIV 3	CoV NL63		1	--	--
PIV 3	CoV NL63	hMPV	1	hMPV (1)	--
PIV 3	CoV OC43		2	--	--
PIV 3	hMPV		1	--	--
PIV 3	RSV B		1	--	--
PIV 3	Rh/EV		3	PIV 3 (1)	--
PIV 4	Rh/EV		5	PIV 4 (2)	--
PIV 4	SARS-CoV-2		1	PIV 4 (1)	--
PIV 4	SARS-CoV-2	Rh/EV	1	--	--
Rh/EV	RSV A		8	--	--
Rh/EV	RSV B		1	--	--
SARS-CoV-2	CoV HKU1		1	--	--
SARS-CoV-2	CoV OC43		4	SARS-CoV-2 (1)	--
SARS-CoV-2	CoV OC43	Rh/EV	1	--	--
SARS-CoV-2	RSV A		1	RSV A (1)	--
SARS-CoV-2	Rh/EV		4	SARS-CoV-2 (2)	--

CoV: Coronavirus; hMPV: Human Metapneumovirus; PIV: Parainfluenza Virus; Rh/EV: Rhinovirus/Enterovirus; RSV: Respiratory Syncytial Virus

2. Clinical Specificity:

   Refer to **Section VII C (1)**, above.

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

   Not applicable.

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

The rates of positive results for the NxTAG RPP v2 analytes in the Prospective Clinical Study described in **Section VII C** are presented in **Tables 41** and **42** stratified by study site and patient age, respectively.

Table 41. Expected values and prevalence of analytes in prospectively collected nasopharyngeal swab specimens, as determined by the NxTAG RPP v2 and comparator methods, respectively, stratified by site

NxTAG RPP v2 Analyte	Test Method	Percent Positive Stratified by Study Site					
		All	Site 1	Site 2	Site 3	Site 4	Site 5
Adenovirus	NxTAG	5.6 (101/1814)	8.7 (39/446)	0 (0/40)	6.2 (51/822)	3.0 (10/334)	0.6 (1/172)
	Comparator	5.4 (98/1814)	8.7 (39/446)	0 (0/40)	5.8 (48/822)	3.0 (10/334)	0.6 (1/172)
Coronavirus HKU1	NxTAG	1.1 (20/1814)	1.3 (6/446)	0 (0/40)	0.7 (6/822)	1.5 (5/334)	1.7 (3/172)
	Comparator	1.2 (21/1814	1.3 (6/446)	0 (0/40)	0.9 (7/822)	1.5 (5/334)	1.7 (3/172)
Coronavirus NL63	NxTAG	2.6 (48/1814)	5.2 (23/446)	5.0 (2/40)	0.9 (7/822)	3.0 (13//334)	1.7 (3/172)
	Comparator	2.9 (52/1814)	5.6 (25/446)	5.0 (2/40)	0.9 (7/822)	4.2 (14/334)	2.3 (4/172)
Coronavirus OC43	NxTAG	2.1 (39/1814)	4.5 (20/446)	0 (0/40)	1.3 (11/822)	2.4 (8/334)	0 (0/172)
	Comparator	2.1 (39/1814)	4.5 (20/446)	0 (0/40)	1.3 (11/822))	2.4 (8/334)	0 (0/172)
Coronavirus 229E	NxTAG	0.4 (8/1814)	0.2 (1/446)	0 (0/40)	0.6 (5/822)	0.3 (1/334)	0.6 (1/172)
	Comparator	0.4 (7/1814)	0.2 (1/446)	0 (0/40)	0.5 (4/822)	0.3 (1/334)	0.6 (1/172)
Influenza A (matrix)	NxTAG	4.2 (77/1814)	0.9 (4/446)	0 (0/40)	7.7 (63/822)	2.7 (9/334)	0.6 (1/172)
	Comparator	4.1 (74/1814)	0.9 (4/446)	0 (0/40)	7.3 (60/822)	2.7 (9/334)	0.6 (1/172)
Influenza A H1 (subtype)	NxTAG	0 (0/1813)	0 (0/446)	0 (0/40)	0 (0/821)	0 (0//334)	0 (0/172)
	Comparator	0 (0/1813)	0 (0/446)	0 (0/40)	0 (0/821)	0 (0/334)	0 (0/172)
Influenza A H1pdm09 (subtype)	NxTAG	1.7 (31/1813)	0.7 (3/446)	0 (0/40)	2.3 (19/821)	2.4 (8/334)	0.6 (1/172)
	Comparator	1.7 (31/1813)	0.7 (3/446)	0 (0/40)	2.3 (19/821)	2.4 (8/334)	0.6 (1/172)
Influenza A H3 (subtype)	NxTAG	2.5 (46/1814)	0.4 (2/446)	0 (0/40)	5.4 (44/822)	0 (0/334)	0 (0/172)
	Comparator	2.6 (47/1814)	0.7 (3/446)	0 (0/40)	5.4 (44/822)	0 (0/334)	0 (0/172)
Influenza B	NxTAG	0.6 (11/1814)	0 (0/446)	0 (0/40)	1.3 (11/822)	0 (0/334)	0 (0/172)
	Comparator	0.6 (11/1814)	0 (0/446)	0 (0/40)	1.3 (11/822)	0 (0/334)	0 (0/172)
Human Metapneumovirus	NxTAG	9.4 (171/1814)	17.3 (77/446)	2.5 (1/40)	4.6 (38/822)	7.8 (26/334)	16.9 (29/172)
	Comparator	8.7 (157/1814)	15.7 (70/446)	2.5 (1/40)	4.5 (37/822)	6.6 (22/334)	15.7 (27/172)
Parainfluenza Virus 1	NxTAG	1.0 (18/1814)	1.6 (7/446)	0 (0/40)	1.1 (9/822)	0 (0/334)	1.2 (2/172)
	Comparator	1.0	1.6	0	1.1	0	1.2

NxTAG RPP v2 Analyte	Test Method	Percent Positive Stratified by Study Site					
		All	Site 1	Site 2	Site 3	Site 4	Site 5
		(18/1814)	(7/446)	(0/40)	(9/822)	(0/334)	(2/172)
Parainfluenza Virus 2	NxTAG	0.5 (9/1814)	0 (0/446)	0 (0/40)	0.5 (4/822)	1.5 (5/334)	0 (0/172)
	Comparator	0.6 (10/1814)	0 (0/446)	0 (0/40)	0.5 (4/822)	1.8 (6/334)	0 (0/172)
Parainfluenza Virus 3	NxTAG	2.4 (43/1814)	4.9 (22/446)	0 (0/40)	1.6 (13/822)	0.9 (3/334)	2.9 (5/172)
	Comparator	2.3 (42/1814)	4.9 (22/446)	0 (0/40)	1.5 (12/822)	0.9 (3/334)	2.9 (5/172)
Parainfluenza Virus 4	NxTAG	0.9 (16/1814)	0.4 (2/446)	0 (0/40)	1.6 (13/822)	0.3 (1/334)	0 (0/172)
	Comparator	0.8 (15/1814)	0.4 (2/446)	0 (0/40)	1.5 (12/822)	0.3 (1/334)	0 (0/172)
Respiratory Syncytial Virus A	NxTAG	3.2 (58/1814)	3.6 (16/446)	0 (0/40)	4.5 (37/822)	1.5 (5/334)	0 (0/172)
	Comparator	3.0 (55/1814)	3.6 (16/446)	0 (0/40)	4.1 (34/822)	1.5 (5/334)	0 (0/172)
Respiratory Syncytial Virus B	NxTAG	1.1 (20/1814)	2.2 (10/446)	0 (0/40)	0.7 (6/822)	0.9 (3/334)	0.6 (1/172)
	Comparator	1.1 (20/1814)	2.2 (10/446)	0 (0/40)	0.7 (6/822)	0.9 (3/334)	0.6 (1/172)
Rhinovirus / Enterovirus	NxTAG	19.4 (352/1814)	30.5 (136/446)	2.5 (1/40)	18.0 (148/822)	12.6 (42/334)	14.5 (25/172)
	Comparator	20.3 (369/1814)	30.7 (137/446)	5.0 (2/40)	19.1 (157/822)	13.2 (44/334)	16.9 (29/172)
SARS-CoV-2	NxTAG	13.3 (240/1803)	5.4 (24/444)	60.0 (24/40)	13.6 (112/824)	17.9 (59/329)	12.7 (21/166)
	Comparator	13.0 (234/1803)	5.2 (23/444)	60.0 (24/40)	13.0 (107/824)	17.9 (59/329)	12.7 (21/166)
Chlamydia pneumoniae	NxTAG	0 (0/1814)	0 (0/446)	0 (0/40)	0 (0/822)	0 (0/334)	0 (0/172)
	Comparator	0 (0/1814)	0 (0/446)	0 (0/40)	0 (0/822)	0 (0/334)	0 (0/172)
Mycoplasma pneumoniae	NxTAG	0 (0/1814)	0 (0/446)	0 (0/40)	0 (0/822)	0 (0/334)	0 (0/172)
	Comparator	0 (0/1814)	0 (0/446)	0 (0/40)	0 (0/822)	0 (0/334)	0 (0/172)

Table 42. Expected values and prevalence of analytes in prospectively collected nasopharyngeal swab specimens, as determined by the NxTAG RPP v2 and comparator methods, respectively, stratified by patient age

NxTAG RPP v2 Analyte	Test Method	Number Positive (%) Stratified by Patient Age (years)						
		All	0 to 1	> 1 to 5	> 5 to 21	> 21 to 65	> 65	Unknown
Adenovirus	NxTAG	5.6 (101/1814)	10.1 (34/337)	15.8 (40/253)	6.7 (22/329)	0.8 (5/641)	0 (0/242)	0 (0/12)
	Comparator	5.4 (98/1814	9.5 (32/337)	15.0 (38/253)	7.0 (23/329)	0.8 (5/641)	0 (0/242)	0 (0/12)
Coronavirus HKU1	NxTAG	1.1 (20/1814)	0.9 (3/337)	2.0 (5/253)	1.2 (4/329)	1.2 (8/641)	0 (0/242)	0 (0/12)
	Comparator	1.2 (21/1814	0.9 (3/337)	2.0 (5/253)	1.2 (4/329)	1.2 (8/641)	0.4 (1/242)	0 (0/12)
Coronavirus NL63	NxTAG	2.6 (48/1814)	5.0 (17/337)	4.0 (12/253)	2.4 (8/329)	1.4 (9/641)	0.8 (2/242)	0 (0/12)
	Comparator	2.9 (52/1814)	5.0 (17/337)	5.1 (13/253)	2.7 (9/329)	1.6 (10/641)	0.8 (2/242)	8.3 (1/12)
Coronavirus OC43	NxTAG	2.1 (39/1814)	4.7 (16//337)	3.2 (8/253)	2.1 (7/329)	0.9 (6/641)	0.8 (2/242)	0 (0/12)
	Comparator	2.1 (39/1814)	4.7 (16/337)	3.2 (8/253)	2.1 (7/329)	0.9 (6/641)	0.8 (2/242)	0 (0/12)
Coronavirus 229E	NxTAG	0.4 (8/1814)	0.3 (1/337)	0.4 (1/253)	0 (0/329)	0.8 (5/641)	0.4 (1/242)	0 (0/12)
	Comparator	0.4 (7/1814)	0.3 (1/337)	0.4 (1/253)	0 (0/329)	0.8 (5/641)	0 (0/242)	0 (0/12)
Influenza A (matrix)	NxTAG	4.2	2.1	3.2	7.6	4.2	4.1	0

NxTAG RPP v2 Analyte	Test Method	Number Positive (%) Stratified by Patient Age (years)						
		All	0 to 1	> 1 to 5	> 5 to 21	> 21 to 65	> 65	Unknown
		(77/1814)	(7/337)	(8/253)	(25/329)	(27/641)	(10/242)	(0/12)
	Comparator	4.1 (74/1814)	2.1 (7/337)	3.2 (8/253)	7.6 (25/329)	3.9 (25/641)	3.7 (9/242)	0 (0/12)
Influenza A H1 (subtype)	NxTAG	0 (0/1813)	0 (0/337)	0 (0/253)	0 (0/328)	0 (0/641)	0 (0/242)	0 (0/12)
	Comparator	0 (0/1813)	0 (0/337)	0 (0/253)	0 (0/328)	0 (0/641)	0 (0/242)	0 (0/12)
Influenza A H1pdm09 (subtype)	NxTAG	1.7 (31/1813)	0.9 (3/337)	1.2 (3/253)	2.4 (8/328)	2.2 (14/641)	1.2 (3/242)	0 (0/12)
	Comparator	1.7 (31/1813)	0.9 (3/337)	1.2 (3/253)	2.4 (8/328)	2.2 (14/641)	1.2 (3/242)	0 (0/12)
Influenza A H3 (subtype)	NxTAG	2.5 (46/1814)	1.2 (4/337)	2.4 (6/253)	5.5 (18/329)	1.7 (11/641)	2.9 (7/242)	0 (0/12)
	Comparator	2.6 (47/1814)	1.3 (5/337)	2.0 (5/253)	5.5 (18/329)	1.7 (11/641)	3.3 (8/242)	0 (0/12)
Influenza B	NxTAG	0.6 (11/1814)	0 (0/337)	1.2 (3/253)	0.9 (3/329)	0.8 (5/641)	0 (0/242)	0 (0/12)
	Comparator	0.6 (11/1814)	0 (0/337)	1.2 (3/253)	0.9 (3/329)	0.8 (5/641)	0 (0/242)	0 (0/12)
Human Metapneumovirus	NxTAG	9.4 (171/1814)	13.4 (45/337)	16.2 (41/253)	8.8 (29/329)	6.1 (39/641)	5.8 (14/242)	25.0 (3/12)
	Comparator	8.7 (157/1814)	11.9 (40/337)	15.4 (39/253)	8.2 (27/329)	5.9 (38/641)	4.1 (10/242)	25.0 (3/12)
Parainfluenza Virus 1	NxTAG	1.0 (18/1814)	1.8 (6/337)	1.2 (3/253)	0.3 (1/329)	1.1 (7/641)	0.4 (1/242)	0 (0/12)
	Comparator	1.0 (18/1814)	1.8 (6/337)	1.2 (3/253)	0.3 (1/329)	1.1 (7/641)	0.4 (1/242)	0 (0/12)
Parainfluenza Virus 2	NxTAG	0.5 (9/1814)	0.3 (1/337)	0.4 (1/253)	0.9 (3/329)	0.2 (1/641)	1.2 (3/242)	0 (0/12)
	Comparator	0.6 (10/1814)	0.3 (1/337)	0.8 (2/253)	0.9 (3/329)	0.2 (1/641)	1.2 (3/242)	0 (0/12)
Parainfluenza Virus 3	NxTAG	2.4 (43/1814)	4.5 (15/337)	6.3 (16/253)	1.5 (5/329)	1.1 (7/641)	0 (0/242)	0 (0/12)
	Comparator	2.3 (42/1814)	4.2 (14/337)	6.3 (16/253)	1.5 (5/329)	1.1 (7/641)	0 (0/242)	0 (0/12)
Parainfluenza Virus 4	NxTAG	0.9 (16/1814)	2.1 (7/337)	0.4 (1/253)	1.2 (4/329)	0.6 (4/641)	0 (0/242)	0 (0/12)
	Comparator	0.8 (15/1814)	2.1 (7/337)	0.4 (1/253)	1.2 (4/329)	0.5 (3/641)	0 (0/242)	0 (0/12)
Respiratory Syncytial Virus A	NxTAG	3.2 (58/1814)	8.3 (28/337)	3.6 (9/253)	0.6 (2/329)	1.7 (11/641)	3.3 (8/242)	0 (0/12)
	Comparator	3.0 (55/1814)	8.3 (28/337)	3.6 (9/253)	0.6 (2/329)	1.2 (8/641)	3.3 (8/242)	0 (0/12)
Respiratory Syncytial Virus B	NxTAG	1.1 (20/1814)	3.0 (10/337)	1.6 (4/253)	0.3 (1/329)	0.6 (4/641)	0.4 (1/242)	0 (0/12)
	Comparator	1.1 (20/1814)	3.0 (10/337)	1.6 (4/253)	0.3 (1/329)	0.6 (4/641)	0.4 (1/242)	0 (0/12)
Rhinovirus / Enterovirus	NxTAG	19.4 (352/1814)	33.5 (113/337)	29.6 (75/253)	22.8 (75/329)	10.1 (65/641)	8.3 (20/242)	33.3 (4/12)
	Comparator	20.3 (369/1814)	34.7 (117/337)	31.2 (79/253)	23.7 (78/329)	10.8 (69/641)	8.7 (21/242)	41.7 (5/12)
SARS-CoV-2	NxTAG	13.3 (240/1803)	9.5 (32/336)	4.8 (12/251)	6.5 (21/325)	18.4 (118/640)	23.3 (56/240)	9.1 (1/11)
	Comparator	13.0 (234/1803)	8.9 (30/336)	4.8 (12/251)	6.5 (21/325)	18.4 (118/640)	21.7 (52/240)	9.1 (1/11)
Chlamydia pneumoniae	NxTAG	0 (0/1814)	0 (0/337)	0 (0/253)	0 (0/329)	0 (0/641)	0 (0/242)	0 (0/12)
	Comparator	0 (0/1814)	0 (0/337)	0 (0/253)	0 (0/329)	0 (0/641)	0 (0/242)	0 (0/12)
Mycoplasma pneumoniae	NxTAG	0 (0/1814)	0 (0/337)	0 (0/253)	0 (0/329)	0 (0/641)	0 (0/242)	0 (0/12)
	Comparator	0 (0/1814)	0 (0/337)	0 (0/253)	0 (0/329)	0 (0/641)	0 (0/242)	0 (0/12)

F Other Supportive Instrument Performance Characteristics Data:

Not applicable.

VIII Proposed Labeling:

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

IX Conclusion:

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