510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K152386

B. Purpose for Submission:

This is a new 510(k) application for a qualitative Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) assay used with the MAGPIX instrument for the *in vitro* qualitative detection of Influenza A virus (with subtype differentiation), Influenza B virus, Respiratory Syncytial virus (RSV) A and RSV B, Coronaviruses 229E, OC43, NL63 and HKU1, Human Metapneumovirus, Rhinovirus/Enterovirus, Adenovirus, Parainfluenza virus Types 1, 2, 3, and 4, Human Bocavirus, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae* in nasopharyngeal swab (NPS) specimens from symptomatic human patients.

C. Measurand:

Influenza A RNA: Flu A Matrix (M) gene, Flu A H1 (HA) gene, Flu A H3 (HA) gene
Influenza B RNA: Flu B Matrix (M) gene
RSV A and RSV B: RNA L Polymerase gene
Coronaviruses 229E, OC43 and NL63 RNA: Nucleocapsidprotein (N) gene
Coronavirus HKU1: open reading frame 1 ab
Human Metapneumovirus RNA: Phosphoprotein (P) gene
Rhinovirus/Enterovirus RNA: 5'-UTR
Adenovirus DNA: Hexon gene
Parainfluenza virus RNA: Parainfluenza 1 HN gene, Parainfluenza 2 and 3 NP gene, Parainfluenza virus 4 phosphoprotein (P) gene
Human Bocavirus DNA: NP1 gene
Chlamydophila pneumoniae DNA: rpoB gene
Mycoplasma pneumoiae DNA: P1 gene

D. Type of Test:

Real-Time Reverse Transcription Polymerase Chain Reaction (RT-PCR)

E. Applicant:

Luminex Molecular Diagnostics, Inc.

F. Proprietary and Established Names:

NxTAG® Respiratory Pathogen Panel

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3980, Respiratory viral panel multiplex nucleic acid assay

2. Classification:

Class II

3. Product codes:

OCC - Respiratory virus panel nucleic acid assay system
OOI - Real time nucleic acid amplification system
OEM - Human Metapneumovirus RNA assay system
OOU - Parainfluenza multiplex nucleic acid system
OEP - Influenza A Virus subtype differentiation nucleic acid assay
OTG - Non-Sars Coronavirus multiplex nucleic acid assay
OZY - Chlamydophila pneumoniae DNA assay system
OZX - Mycoplasma pneumoniae DNA assay system

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use(s):
NxTAG® Respiratory Pathogen Panel is a qualitative test intended for use on the Luminex® MAGPIX® Instrument for the simultaneous detection and identification of nucleic acids from multiple respiratory viruses and bacteria extracted from nasopharyngeal swabs collected from individuals with clinical signs and symptoms of a respiratory tract infection. The organism types and subtypes detected by the test are Influenza A, Influenza A H1, Influenza A H3, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, 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, Human Bocavirus, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*.

The test is indicated as an aid in the detection and identification of viral and bacterial agents causing respiratory tract infections in symptomatic adult and pediatric patients, who are either hospitalized, admitted to emergency departments or who are outpatients with suspected respiratory tract infection.

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 not detected by this test or lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen. Positive results do not rule out co-infection with other pathogens. The agent detected may not be the definite cause of disease. The use of additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) and clinical presentation must be taken into consideration in order to obtain the final diagnosis of respiratory tract infection.

Performance characteristics for influenza A were established using specimens obtained during the 2013/2014 and 2014/2015 influenza seasons when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

Prescription use only

4. Special instrument requirements:

MAGPIX® Instrument

I. Device Description:

The NxTAG Respiratory Pathogen Panel (RPP) is a qualitative test intended for the simultaneous detection and identification of nucleic acids from multiple respiratory viruses and bacteria extracted from nasopharyngeal swabs collected from individuals with clinical signs and symptoms of respiratory tract infection. It incorporates multiplex Reverse Transcriptase Polymerase Chain Reaction with the Luminex proprietary universal tag sorting system on the Luminex platform to easily detect respiratory pathogen targets.

Samples are extracted using the IVD-labeled bioMérieux NucliSENS® easyMag® system. Extracted total nucleic acid is then added to the sealed 96-well micro plate by piercing the seal with pipette tips. Each reaction well is pre-plated with two Lyophilized Bead Reagents (LBRs) that contain all the required reagents including primer mixes, bead mix, and enzyme buffer systems. Once the LBRs are resuspended,

the reaction wells are re-sealed using the foils provided in the kit. The sealed plate can then be placed inside the thermocycler. The reaction is amplified via RT-PCR and the reaction product undergoes near simultaneous bead hybridization within the sealed reaction wells. The hybridized, tagged beads are then sorted and read on the Luminex MAGPIX instrument. The MAGPIX instrument generates a signal in the form of a median fluorescence intensity (MFI) value for each bead population. The signals are analyzed using the NxTAG Respiratory Pathogen Panel Assay File for SYNCT™ Software, providing a qualitative call for each of the 20 targets and internal controls within each reaction well.

Quality Control

The Luminex MAGPIX Performance Verification Kit is intended to verify the optical calibration of the MAGPIX instrument, and is not provided as part of the NxTAG RPP.

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

RNase-free water is used as a no template control (NTC). Sample collection media used from the starting extraction point functions as a negative extraction control (NEC).

Results Interpretation

The NxTAG RPP Assay has two separate probes for detection of Influenza A H1 (H1 and H1 2009-specific). These have been combined into a single call (positive for either is positive for Influenza A subtype H1) because the Influenza A 2009 H1N1 strain has stabilized and is now considered to be the seasonal strain. Results interpretation for Influenza A and subtypes are listed in Table 1. All other analytes detected by the NxTAG RPP assay are positive if their respective channels are positive in a valid test.

The results are interpreted by the xPONENT software on the MAGPIX Instrument and are exported as a CSV file to the SYNCT software where the results can be viewed by the user on the Results Page.

Table 1 – All possible test results for Influenza A

Final Result	Influenza A	H1-A (H1)	H1-B (2009 H1N1)	H3	Required follow up
Influenza A Not Detected	Negative	Negative	Negative	Negative	None
Influenza A H1	Positive	Positive	Negative	Negative	None
	Positive	Positive	Positive	Negative	
	Positive	Negative	Positive	Negative	
	Negative[2]	Positive	Negative	Negative	
	Negative[2]	Positive	Positive	Negative	

	Negative[2]	Negative	Positive	Negative	
Influenza A H3	Positive	Negative	Negative	Positive	None
	Negative[2]	Negative	Negative	Positive	
Influenza A H1 and Influenca A H3	Positive	Positive	Negative	Positive	None
	Positive	Negative	Positive	Positive	
	Negative[2]	Positive	Negative	Positive	
	Negative[2]	Negative	Positive	Positive	
Influenza A (so subtype detected)	Positive	Negative	Negative	Negative	Retest[1]

[1] If the retest provides the same result for influenza A (no subtype detected), contact local or state public health authorities for confirmatory testing.

[2]Detection of Influenza A/H1 or Influenza A/H3 subtypes without an Influenza A "Positive" result may occur at low titer of the virus in the specimen or may indicate a false positive due to contamination. The result could also indicate potential genetic mutations in the Matrix protein gene among circulating seasonal Influenza A viruses.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   BioFire Diagnostics, LLC FilmArray® Respiratory Panel

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

   K120267

3. Comparison with predicate:

Table 2 – Assay Comparison with Predicate Device

Item	Subject Device (K152386) NxTAG RPP	Predicate (K120267) FilmArray Respiratory Panel
510(k) Number	K152386	K120267
Regulation	866.3980	Same
Product Code	OCC, OEM, OOU, OEP, OTG, OOI, OZY, OZX	OCC, OEM, OOU, OEP, OTG, NXD, OOI, OZZ, OZY, OZX
Device Class	II	Same
Technology Principle of Operation	Multiplex real time RT-PCR followed by detection of fluorescently labeled products coupled to magnetic beads	Multiplex real time RT-PCR followed by high resolution melting analysis to confirm identity of amplified product
Intended Use	NxTAG® Respiratory Pathogen Panel is a qualitative test intended for use on the Luminex® MAGPIX® Instrument for the simultaneous detection and identification of nucleic acids from multiple respiratory viruses and bacteria	FilmArray Respiratory Panel (RP) is a multiplexed nucleic acid test intended for use with the FilmArray instrument for the simultaneous qualitative detection and identification of multiple respiratory viral and bacterial nucleic acids in nasopharyngeal swabs (NPS)

	extracted from nasopharyngeal swabs collected from individuals with clinical signs and symptoms of a respiratory tract infection. The organism types and subtypes detected by the test are Influenza A, Influenza A H1, Influenza A H3, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, 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, Human Bocavirus, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*. The test is indicated as an aid in the detection and identification of viral and bacterial agents causing respiratory tract infections in symptomatic adult and pediatric patients, who are either hospitalized, admitted to emergency departments or who are outpatients with suspected respiratory tract infection. 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 not detected by this test or lower respiratory tract infection that is not detected by a nasopharyngeal swab specimen. Positive results do not rule out co-infection with other pathogens. The agent detected may not be the definite cause of disease. The use of additional laboratory testing (e.g. bacterial and viral culture, immunofluorescence, and radiography) and clinical presentation must be taken into consideration in order to obtain the final diagnosis of respiratory tract infection. Performance characteristics for influenza A were established using specimens obtained during the	obtained from individuals suspected of respiratory tract infections. The following organism types and subtypes are identified using the FilmArray RP: Adenovirus, Coronavirus 229E, Coronavirus HKU1, Coronavirus NL63, Coronavirus OC43, Human Metapneumovirus, Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza A subtype 2009 H1, Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Parainfluenza Virus 4, Rhinovirus/Enterovirus, Respiratory Syncytial Virus, Bordetella pertussis, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*. The detection and identification of specific viral and bacterial nucleic acids from individuals exhibiting signs and symptoms of a respiratory infection 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 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 is not detected by a nasopharyngeal swab specimen. Positive results do not rule out co-infection with other organisms: the agent(s) detected by the Film Array RP 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. Due to the small number of positive specimens collected for certain organisms during the prospective clinical study, performance characteristics for Bordetella pertussis, Coronavirus 229E, Coronavirus OC43, Influenza A/H1, Influenza A/H3, Influenza A/2009 H1, Influenza B, Mycoplasma pneumoniae Parainfluenza Virus 1, Parainfluenza Virus 2, and Parainfluenza Virus 4 were established primarily with retrospective clinical specimens. Performance characteristics for

	2013/2014 and 2014/2015 influenza seasons when influenza A/H3 and A/H1 were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.	Chlamydophila pneumoniae were established primarily using contrived clinical specimens. Due to the genetic similarity between human Rhinovirus and Enterovirus, the FilmArray RP cannot reliably differentiate them. A positive FilmArray RP Rhinovirus/Enterovirus result should be followed-up using an alternate method (e.g., cell culture or sequence analysis). The FilmArray RP detects Adenovirus species C serotype 2 and serotype 6 with reduced sensitivity. It is recommended that specimens found to be negative for Adenovirus after examination using FilmArray RP be confirmed by an alternate method (e.g., FDA cleared molecular test or cell culture). The FilmArray RP assay for coronavirus OC43 may cross-react with some isolates of Coronavirus HKU1. A dual positive result may be due to cross-reactivity or may indicate a co-infection. Performance characteristics for influenza A were established when influenza A/2009 H1N1, A/H1, and A/H3 were the predominant influenza A viruses in circulation. Performance of detecting influenza A may vary if other influenza A strains are circulating or a novel influenza A virus emerges. If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
Indication for Use	Same as device Intended Use	Same as device Intended Use
Specimen Types	Nasopharyngeal swab specimens (NPS)	Same
Nucleic Acid Extraction	Yes	Same

Extraction Methods	bioMérieux NucliSENS easyMag system	FilmArray RP assay
Assay Results	Qualitative	Same
Instrument System	MAGPIX Instrument	FilmArray Instrument

K. Standard/Guidance Document Referenced (if applicable):

1. FDA Guidance: Highly Multiplexed Microbiological/Medical Countermeasure *In Vitro* Nucleic Acid Based Diagnostic Devices; issued Auguest 27, 2014.
2. FDA Guidance: Respiratory Viral Panel Multiplex Nucleic Acid Assay; issued Oct 9, 2009.
3. FDA Guidance: Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices; issued May 11, 2005.
4. FDA Guidance: Format for Traditional and Abbreviated 510(k); issued August 12, 2005.
5. FDA Guidance: Content of Premarket Submissions for Management of Cybersecurity in Medical Devices; issued October 2, 2014
6. FDA Guidance: The New 510(k) Paradigm - Alternate Approaches to Demonstrating Substantial Equivalence in Premarket Notifications; issued March 20, 1998.
7. FDA Guidance: The 510(k) Program: Evaluating Substantial Equivalence in Premarket Notifications [510(k)]; issued December 27, 2011
8. EP05-A3: Evaluation of Precision Performance of Quantitative Measurement Methods (3rd ed.); August 14, 2015
9. EP07-A2: Interference Testing in Clinical Chemistry (2nd ed.); May 21, 2007
10. EP12-A2: User Protocol for Evaluation of Qualitative Test Performance (2nd ed.); January 30, 2014
11. EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; January 15, 2013
12. ISO 14971: Application of Risk Management to Medical Devices
13. MM03-A2: Molecular Diagnostic Methods for Infectious Diseases (2nd ed.); September 9, 2008
14. MM13-A: Collection, Transport, Preparation and Storage of Specimens; March 18, 2009
15. MM18-A Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; March 18, 2009

L. Test Principle:

The NxTAG RPP Assay utilizes automated real-time reverse-transcription polymerase chain reaction (RT-PCR) for unique gene-specific sequence amplification and hybridization of labeled probes to magnetic beads for sorting and detection. Samples are extracted using the IVD-labeled bioMérieux NucliSENS easyMag system. Extracted total nucleic acid is then added to the sealed 96-well micro plate by piercing the seal with pipette tips. Each reaction well is pre-plated with two Lyophilized Bead Reagents (LBRs) that

contain all the required reagents including primer mixes, bead mix, and enzyme buffer systems. For each sample, extracted viral or bacterial nucleic acid (RNA or DNA) is amplified in a single multiplex RT-PCR reaction. During amplification the product undergoes near simultaneous bead hybridization within the sealed reaction wells. The hybridized, tagged beads are then sorted and their mean fluorescence intensity (MFI) signals are read on the Luminex MAGPIX instrument using xPONENT 4.2 software. The signals are analyzed using the NxTAG RPP Assay File for SYNCT Software.

For each analyte in a sample, the multi-dimension detection (MDD) value is a measure resulting from the subtraction of the median MFI signal of all analytes within the sample from the signal of that particular analyte. The result is a measure that has been adjusted for the noise within the sample. The NxTAG RPP data analysis algorithm uses the MFI measure to determine the validity of a sample, followed by the MDD measure to make a target call of positive or negative for a valid sample. As such, during cut-off determination, both MFI and MDD thresholds are set for each target; however, only MDD cut-offs are used to determine the presence of a target.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Reproducibility

   A reproducibility study was performed to assess the total variability of the NxTAG RPP assay across operators, study sites, testing days, and instruments. The assay was evaluated at 3 sites. Two operators at each of the 3 sites tested a 17-member reproducibility panel in triplicate on 5 non-consecutive days, for a total of 30 batch runs (2 operators × 5 days × 3 sites). For each member of the 17-member panel, a total of 90 data points (30 batch runs x 3 replicates per batch run) were generated. The reproducibility panel comprised a negative sample, 8 low positive multi-analyte samples (1x limit of detection) and 8 medium positive multi-analyte samples (3x LoD). All sample dilutions for this study were prepared using UTM as sample matrix. All samples were extracted using bioMérieux NucliSENS easyMAG System. This information is summarized in Tables 3 through 22 below.

Table 3 - Influenza A calls - Reproducibility

Influenza A			Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive	H3 Strain (0.749 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	270	12.9
		Site 2	30/30 (100.0%)	88.7-100%	244.5	17.2
		Site 3	30/30 (100.0%)	88.7-100%	255	20.9
		Overall	90/90 (100.0%)	96.0-100%	258.5	17.8
	H1 Strain (9.24	Site 1	30/30 (100.0%)	88.7-100%	276.3	5.7

	$TCID_{50}$/mL)	Site 2	30/30 (100.0%)	88.7-100%	261.5	16.7
		Site 3	30/30 (100.0%)	88.7-100%	275.5	8
		Overall	90/90 (100.0%)	96.0-100%	271	11.6
	2009 H1N1 Strain (1.66 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	282	6.4
		Site 2	30/30 (100.0%)	88.7-100%	252.5	7.2
		Site 3	30/30 (100.0%)	88.7-100%	293	11.9
		Overall	90/90 (100.0%)	96.0-100%	271.3	10.8
Low Positive	H3 Strain (0.250 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	159.5	33.1
		Site 2	30/30 (100.0%)	88.7-100%	114	41.1
		Site 3	30/30 (100.0%)	88.7-100%	138.8	44.1
		Overall	90/90 (100.0%)	96.0-100%	140.3	39.8
	H1 Strain (3.08 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	266.5	15.7
		Site 2	30/30 (100.0%)	88.7-100%	220	16.1
		Site 3	30/30 (100.0%)	88.7-100%	240.3	24.7
		Overall	90/90 (100.0%)	96.0-100%	243	20.3
	2009 H1N1 Strain (.553 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	259.3	21.1
		Site 2	29/30 (96.7%)	83.3-99.4%	229	22.5
		Site 3	30/30 (100.0%)	88.7-100%	218	25.7
		Overall	89/90 (98.9%)	94.0-100%	240	23.6
Negative	Negative	Site 1	30/30 (100.0%)	88.7-100%	0	N/A
		Site 2	30/30 (100.0%)	88.7-100%	0	N/A
		Site 3	30/30 (100.0%)	88.7-100%	0	N/A
		Overall	90/90 (100.0%)	96.0-100%	0	N/A

Table 4 - Influenza A H1 calls - Reproducibility

Influenza A H1			Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive	H1 Strain (9.24 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	333.3	11
		Site 2	29/30 (96.7%)	83.3-99.4%	309.5	26
		Site 3	30/30 (100.0%)	88.7-100%	279.3	17.8
		Overall	89/90 (98.9%)	94.0-100%	315.3	19.9
	2009 H1N1 Strain (1.66 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	241	18.3
		Site 2	30/30 (100.0%)	88.7-100%	232	14.2
		Site 3	30/30 (100.0%)	88.7-100%	239	24
		Overall	90/90 (100.0%)	96.0-100%	239.5	19.5
Low Positive	H1 Strain (3.08 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	229.5	26.9
		Site 2	30/30 (100.0%)	88.7-100%	164	28.8
		Site 3	29/30 (96.7%)	83.3-99.4%	156.5	47.8
		Overall	89/90 (98.9%)	94.0-100%	181	38.9
	2009 H1N1 Strain (.553 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	126.3	33.9
		Site 2	29/30 (96.7%)	83.3-99.4%	119.3	38.1
		Site 3	30/30 (100.0%)	88.7-100%	106.5	44
		Overall	89/90 (98.9%)	94.0-100%	118	39.2

Negative	Negative	Site 1	30/30 (100.0%)	88.7-100%	2	N/A
		Site 2	30/30 (100.0%)	88.7-100%	2	N/A
		Site 3	30/30 (100.0%)	88.7-100%	3.3	N/A
		Overall	90/90 (100.0%)	96.0-100%	2	N/A
	Negative	Site 1	30/30 (100.0%)	88.7-100%	-1	N/A
		Site 2	30/30 (100.0%)	88.7-100%	-1	N/A
		Site 3	30/30 (100.0%)	88.7-100%	-1	N/A
		Overall	90/90 (100.0%)	96.0-100%	-1	N/A

Table 5 - Influenza A H3 calls - Reproducibility

Influenza A H3		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (2.81E-01 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	303.5	7.7
	Site 2	30/30 (100.0%)	88.7-100%	282	8.6
	Site 3	30/30 (100.0%)	88.7-100%	305.8	12
	Overall	90/90 (100.0%)	96.0-100%	298.5	10.9
Low Positive (9.36E-02 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	270	21.6
	Site 2	29/30 (96.7%)	83.3-99.4%	224.8	27.9
	Site 3	30/30 (100.0%)	88.7-100%	208.5	29.5
	Overall	89/90 (98.9%)	94.0-100%	237.3	27
Negative	Site 1	30/30 (100.0%)	88.7-100%	-1	N/A
	Site 2	30/30 (100.0%)	88.7-100%	-1	N/A
	Site 3	30/30 (100.0%)	88.7-100%	-1	N/A
	Overall	90/90 (100.0%)	96.0-100%	-1	N/A

Table 6 - Influenza B calls - Reproducibility

Influenza B		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (1.74 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	294.5	11.8
	Site 2	30/30 (100.0%)	88.7-100%	254.5	9.5
	Site 3	30/30 (100.0%)	88.7-100%	280.5	12
	Overall	90/90 (100.0%)	96.0-100%	277	12.7
Low Positive (5.81E-01 $TCID_{50}$/mL)	Site 1	29/30 (96.7%)	83.3-99.4%	168.8	36.5
	Site 2	30/30 (100.0%)	88.7-100%	145.3	31.8
	Site 3	30/30 (100.0%)	88.7-100%	146.3	27
	Overall	90/90 (100.0%)	94.0-100%	154	32.5
Negative	Site 1	30/30 (100.0%)	88.7-100%	-1	N/A
	Site 2	30/30 (100.0%)	88.7-100%	-0.5	N/A
	Site 3	30/30 (100.0%)	88.7-100%	-1	N/A
	Overall	90/90 (100.0%)	96.0-100%	-1	N/A

Table 7 - RSV A calls - Reproducibility

RSV A		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (6.44 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	270.5	17.4
	Site 2	30/30 (100.0%)	88.7-100%	240	21.6
	Site 3	30/30 (100.0%)	88.7-100%	244.8	17.8
	Overall	90/90 (100.0%)	96.0-100%	254.8	19.6
Low Positive (2.15 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	163	35.6
	Site 2	30/30 (100.0%)	88.7-100%	128.5	26.6
	Site 3	30/30 (100.0%)	88.7-100%	114.3	38.5
	Overall	90/90 (100.0%)	96.0-100%	129.5	36.1
Negative	Site 1	30/30 (100.0%)	88.7-100%	0	N/A
	Site 2	30/30 (100.0%)	88.7-100%	0	N/A
	Site 3	30/30 (100.0%)	88.7-100%	0	N/A
	Overall	90/90 (100.0%)	96.0-100%	0	N/A

Table 8 - RSV B calls - Reproducibility

RSV B		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (4.07 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	293.8	18
	Site 2	30/30 (100.0%)	88.7-100%	240.5	21.4
	Site 3	30/30 (100.0%)	88.7-100%	262	29.6
	Overall	90/90 (100.0%)	96.0-100%	262.8	24.1
Low Positive (1.36 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	142.5	33
	Site 2	30/30 (100.0%)	88.7-100%	117.3	58.4
	Site 3	30/30 (100.0%)	88.7-100%	134.8	55.7
	Overall	90/90 (100.0%)	96.0-100%	128.3	49.7
Negative	Site 1	30/30 (100.0%)	88.7-100%	0	N/A
	Site 2	30/30 (100.0%)	88.7-100%	0	N/A
	Site 3	30/30 (100.0%)	88.7-100%	0.3	N/A
	Overall	90/90 (100.0%)	96.0-100%	0	N/A

Table 9 - Coronavirus 229E calls - Reproducibility

Coronavirus 229E		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (3.22E-02 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	375.5	6.7
	Site 2	30/30 (100.0%)	88.7-100%	352.5	4.7
	Site 3	30/30 (100.0%)	88.7-100%	383.8	9.6
	Overall	90/90 (100.0%)	96.0-100%	370.5	8.3
Low Positive (1.07E-02 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	301.3	32.6
	Site 2	30/30 (100.0%)	88.7-100%	304	27.5
	Site 3	29/30 (96.7%)	83.3-99.4%	227.5	49.5
	Overall	89/90 (98.9%)	94.0-100%	289.5	37
Negative	Site 1	30/30 (100.0%)	88.7-100%	0	N/A
	Site 2	30/30 (100.0%)	88.7-100%	0	N/A
	Site 3	30/30 (100.0%)	88.7-100%	0.3	N/A
	Overall	90/90 (100.0%)	96.0-100%	0	N/A

Table 10 - Coronavirus OC43 calls - Reproducibility

Coronavirus OC43		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (2.15E-01 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	395.5	20.3
	Site 2	30/30 (100.0%)	88.7-100%	357	13.4
	Site 3	30/30 (100.0%)	88.7-100%	395.8	21.2
	Overall	90/90 (100.0%)	96.0-100%	381	18.8
Low Positive (7.15E-02 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	196.5	39.6
	Site 2	30/30 (100.0%)	88.7-100%	154.8	55.7
	Site 3	28/30 (93.3%)	78.7-98.2%	128.5	61.9
	Overall	88/90 (97.8%)	92.2-99.7%	165	53.3
Negative	Site 1	30/30 (100.0%)	88.7-100%	-1	N/A
	Site 2	30/30 (100.0%)	88.7-100%	-1	N/A
	Site 3	30/30 (100.0%)	88.7-100%	-1	N/A
	Overall	90/90 (100.0%)	96.0-100%	-1	N/A

Table 11 - Coronavirus NL63 calls - Reproducibility

Coronavirus NL63		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (1.01E-02 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	305.5	13.1
	Site 2	30/30 (100.0%)	88.7-100%	287.3	9
	Site 3	30/30 (100.0%)	88.7-100%	289.5	11.8
	Overall	90/90 (100.0%)	96.0-100%	293.5	11.5
Low Positive (3.37E-03 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	183	32
	Site 2	30/30 (100.0%)	88.7-100%	170.8	26.8
	Site 3	30/30 (100.0%)	88.7-100%	154.5	24.9
	Overall	90/90 (100.0%)	96.0-100%	169.8	28.6
Negative	Site 1	30/30 (100.0%)	88.7-100%	0	N/A
	Site 2	30/30 (100.0%)	88.7-100%	0	N/A
	Site 3	30/30 (100.0%)	88.7-100%	0	N/A
	Overall	90/90 (100.0%)	96.0-100%	0	N/A

Table 12 - Coronavirus HKU1 calls - Reproducibility

Coronavirus HKU1		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (4.71E+04 copies/mL)	Site 1	30/30 (100.0%)	88.7-100%	291.3	12.2
	Site 2	30/30 (100.0%)	88.7-100%	284.3	4.5
	Site 3	30/30 (100.0%)	88.7-100%	278.3	18.3
	Overall	90/90 (100.0%)	96.0-100%	284.8	13
Low Positive (1.57E+04 copies/mL)	Site 1	30/30 (100.0%)	88.7-100%	153	34
	Site 2	30/30 (100.0%)	88.7-100%	161.8	25.6
	Site 3	27/30 (90.0%)	74.4-96.5%	112.3	44.8
	Overall	87/90 (96.7%)	90.6-99.3%	145	37.5
Negative	Site 1	29/30 (96.7%)	83.3-99.4%	1	N/A
	Site 2	30/30 (100.0%)	88.7-100%	1	N/A
	Site 3	30/30 (100.0%)	88.7-100%	1	N/A
	Overall	89/90 (98.9%)	94.0-100%	1	N/A

Table 13 - Human Metapneumovirus calls – Reproducibility

Human Metapneumovirus		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (4.15E-01 $TCID_{50}$/mL)	Site 1	29/30 (96.7%)	83.3-99.4%	169.5	16.5
	Site 2	30/30 (100.0%)	88.7-100%	154.8	11.3
	Site 3	30/30 (100.0%)	88.7-100%	169.5	17.2
	Overall	89/90 (98.9%)	94.0-100%	166	15.8
Low Positive (1.38E-01 $TCID_{50}$/mL)	Site 1	28/30 (93.3%)	78.7-98.2%	165	27.6
	Site 2	30/30 (100.0%)	88.7-100%	149.5	19.9
	Site 3	30/30 (100.0%)	88.7-100%	149	26.2
	Overall	88/90 (97.8%)	92.2-99.7%	153	24.6
Negative	Site 1	30/30 (100.0%)	88.7-100%	1	N/A
	Site 2	30/30 (100.0%)	88.7-100%	1	N/A
	Site 3	30/30 (100.0%)	88.7-100%	1	N/A
	Overall	90/90 (100.0%)	96.0-100%	1	N/A

Table 14 - Rhinovirus/Enterovirus calls - Reproducibility

Rhinovirus/Enterovirus		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (1.55 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	181	9.1
	Site 2	30/30 (100.0%)	88.7-100%	192	16.6
	Site 3	30/30 (100.0%)	88.7-100%	196.3	6.5
	Overall	90/90 (100.0%)	96.0-100%	190.5	12
Low Positive (5.18E-01 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	112.5	13
	Site 2	30/30 (100.0%)	88.7-100%	119.5	11.9
	Site 3	30/30 (100.0%)	88.7-100%	121.5	17.1
	Overall	90/90 (100.0%)	96.0-100%	117.5	14.2
Negative	Site 1	30/30 (100.0%)	88.7-100%	2	N/A
	Site 2	30/30 (100.0%)	88.7-100%	2	N/A
	Site 3	30/30 (100.0%)	88.7-100%	4	N/A
	Overall	90/90 (100.0%)	96.0-100%	3	N/A

Table 15 - Adenovirus calls - Reproducibility

Adenovirus		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (9.76 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	220.5	13.6
	Site 2	30/30 (100.0%)	88.7-100%	186.8	12.8
	Site 3	30/30 (100.0%)	88.7-100%	199	17.7
	Overall	90/90 (100.0%)	96.0-100%	201.5	16.6
Low Positive (3.25 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	149.5	22.7
	Site 2	30/30 (100.0%)	88.7-100%	113	22.5
	Site 3	30/30 (100.0%)	88.7-100%	118.5	36.1
	Overall	90/90 (100.0%)	96.0-100%	127.3	28.6
Negative	Site 1	30/30 (100.0%)	88.7-100%	0	N/A
	Site 2	30/30 (100.0%)	88.7-100%	0	N/A
	Site 3	30/30 (100.0%)	88.7-100%	0.3	N/A
	Overall	90/90 (100.0%)	96.0-100%	0	N/A

Table 16 - Parainfluenza virus 1 calls - Reproducibility

Parainfluenza virus 1		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (8.46E+01 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	324.5	14.3
	Site 2	30/30 (100.0%)	88.7-100%	272	12.7
	Site 3	30/30 (100.0%)	88.7-100%	291	20.3
	Overall	90/90 (100.0%)	96.0-100%	298	16.7
Low Positive (2.82E+01 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	132.5	31.2
	Site 2	28/30 (93.3%)	78.7-98.2%	109.8	43.3
	Site 3	27/30 (90.0%)	74.4-96.5%	107.5	57.2
	Overall	85/90 (94.4%)	87.5-98.2%	122.5	44.9
Negative	Site 1	30/30 (100.0%)	88.7-100%	1	N/A
	Site 2	30/30 (100.0%)	88.7-100%	1	N/A
	Site 3	30/30 (100.0%)	88.7-100%	1	N/A
	Overall	90/90 (100.0%)	96.0-100%	1	N/A

Table 17 - Parainfluenza virus 2 calls - Reproducibility

Parainfluenza virus 2		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive(1.61 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	460.3	19.6
	Site 2	30/30 (100.0%)	88.7-100%	440.3	14.8
	Site 3	30/30 (100.0%)	88.7-100%	440.8	22.4
	Overall	90/90 (100.0%)	96.0-100%	449.8	19
Low Positive (5.36E-01 $TCID_{50}$/mL)	Site 1	28/30 (93.3%)	78.7-98.2%	260	50.5
	Site 2	30/30 (100.0%)	88.7-100%	217.5	42.8
	Site 3	29/30 (96.7%)	83.3-99.4%	240	49.7
	Overall	87/90 (96.7%)	90.6-99.3%	240.3	47.4
Negative	Site 1	30/30 (100.0%)	88.7-100%	-1	N/A
	Site 2	30/30 (100.0%)	88.7-100%	-0.8	N/A
	Site 3	30/30 (100.0%)	88.7-100%	-1	N/A
	Overall	90/90 (100.0%)	96.0-100%	-1	N/A

Table 18 - Parainfluenza virus 3 calls - Reproducibility

Parainfluenza virus 3		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (4.83E+01 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	342.3	11
	Site 2	30/30 (100.0%)	88.7-100%	314.8	10.6
	Site 3	30/30 (100.0%)	88.7-100%	333	12.3
	Overall	90/90 (100.0%)	96.0-100%	328.8	11.8
Low Positive (1.61E+01 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	186.5	29.4
	Site 2	30/30 (100.0%)	88.7-100%	188.5	22.8
	Site 3	29/30 (96.7%)	83.3-99.4%	148.5	28.9
	Overall	89/90 (98.9%)	94.0-100%	168.8	29.4
Negative	Site 1	30/30 (100.0%)	88.7-100%	-1	N/A
	Site 2	30/30 (100.0%)	88.7-100%	-1	N/A
	Site 3	30/30 (100.0%)	88.7-100%	-1	N/A
	Overall	90/90 (100.0%)	96.0-100%	-1	N/A

Table 19 - Parainfluenza virus 4 calls - Reproducibility

Parainfluenza virus 4		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Parainfluenza 4A Moderate Positive (7.63 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	380.8	8.7
	Site 2	30/30 (100.0%)	88.7-100%	351.8	6.6
	Site 3	30/30 (100.0%)	88.7-100%	384.3	8.5
	Overall	90/90 (100.0%)	96.0-100%	370.5	9.7
Parainfluenza 4B Moderate Positive (1.83 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	362	6.3
	Site 2	30/30 (100.0%)	88.7-100%	343	9.3
	Site 3	30/30 (100.0%)	88.7-100%	372.5	18.4
	Overall	90/90 (100.0%)	96.0-100%	355	12.8
Parainfluenza 4A Low Positive (2.54 $TCID_{50}$/mL)	Site 1	28/30 (93.3%)	78.7-98.2%	310.8	45.2
	Site 2	29/30 (96.7%)	83.3-99.4%	182.8	48.9
	Site 3	30/30 (100.0%)	88.7-100%	251.3	44
	Overall	87/90 (96.7%)	90.6-99.3%	249	46.5
Parainfluenza 4B Low Positive (6.09E-01 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	269.3	42.6
	Site 2	29/30 (96.7%)	83.3-99.4%	160.3	57.9
	Site 3	30/30 (100.0%)	88.7-100%	178.3	54.3
	Overall	89/90 (98.9%)	94.0-100%	204	52
Negative	Site 1	30/30 (100.0%)	88.7-100%	0	N/A
	Site 2	30/30 (100.0%)	88.7-100%	0	N/A
	Site 3	30/30 (100.0%)	88.7-100%	0	N/A
	Overall	90/90 (100.0%)	96.0-100%	0	N/A
Negative	Site 1	30/30 (100.0%)	88.7-100%	0	N/A
	Site 2	30/30 (100.0%)	88.7-100%	0	N/A
	Site 3	30/30 (100.0%)	88.7-100%	0	N/A
	Overall	90/90 (100.0%)	96.0-100%	0	N/A

Table 20 - Human Bocavirus calls - Reproducibility

Human Bocavirus		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (1.17E+03 copies/mL)	Site 1	30/30 (100.0%)	88.7-100%	464	10.6
	Site 2	30/30 (100.0%)	88.7-100%	414.3	12.2
	Site 3	30/30 (100.0%)	88.7-100%	394	17.6
	Overall	90/90 (100.0%)	96.0-100%	418	14.5
Low Positive (3.91E+02 copies/mL)	Site 1	29/30 (96.7%)	83.3-99.4%	195	35.3
	Site 2	30/30 (100.0%)	88.7-100%	228	36.8
	Site 3	30/30 (100.0%)	88.7-100%	190.5	48
	Overall	89/90 (98.9%)	94.0-100%	206	39.9
Negative	Site 1	30/30 (100.0%)	88.7-100%	1	N/A
	Site 2	30/30 (100.0%)	88.7-100%	1	N/A
	Site 3	30/30 (100.0%)	88.7-100%	0.8	N/A
	Overall	90/90 (100.0%)	96.0-100%	1	N/A

Table 21 - *Chlamydophila pneumoniae* calls - Reproducibility

Chlamydophila pneumoniae		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (1.93E-01 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	340.3	11.5
	Site 2	30/30 (100.0%)	88.7-100%	287.5	17.4
	Site 3	30/30 (100.0%)	88.7-100%	313.8	24.7
	Overall	90/90 (100.0%)	96.0-100%	314.8	19.5
Low Positive (6.43E-02 $TCID_{50}$/mL)	Site 1	30/30 (100.0%)	88.7-100%	168	37.2
	Site 2	30/30 (100.0%)	88.7-100%	145	28.4
	Site 3	30/30 (100.0%)	88.7-100%	151	31.9
	Overall	90/90 (100.0%)	96.0-100%	153	34.8
Negative	Site 1	30/30 (100.0%)	88.7-100%	-1	N/A
	Site 2	30/30 (100.0%)	88.7-100%	-1	N/A
	Site 3	30/30 (100.0%)	88.7-100%	-1	N/A
	Overall	90/90 (100.0%)	96.0-100%	-1	N/A

Table 22 - *Mycoplasma pneumoniae* calls - Reproducibility

Mycoplasma pneumoniae		Agreement with Expected Result (%)	95% CI	Median Percentile MDD	%CV
Moderate Positive (4.25E+02 CCU/mL)	Site 1	30/30 (100.0%)	88.7-100%	343.5	24.3
	Site 2	30/30 (100.0%)	88.7-100%	257.3	29.9
	Site 3	30/30 (100.0%)	88.7-100%	333	21.4
	Overall	90/90 (100.0%)	96.0-100%	321.5	27.2
Low Positive (1.42E+02 CCU/mL)	Site 1	29/30 (96.7%)	83.3-99.4%	177.5	58.7
	Site 2	30/30 (100.0%)	88.7-100%	192.8	49.2
	Site 3	30/30 (100.0%)	88.7-100%	228.5	55.7
	Overall	89/90 (98.9%)	94.0-100%	200.8	54.7
Negative	Site 1	30/30 (100.0%)	88.7-100%	-1	N/A
	Site 2	30/30 (100.0%)	88.7-100%	-1	N/A
	Site 3	30/30 (100.0%)	88.7-100%	-1	N/A
	Overall	90/90 (100.0%)	96.0-100%	-1	N/A

Repeatability

Repeatability was assessed for each NxTAG RPP analyte using multi-analyte (MA) samples (each comprised of two to four analytes) prepared by spiking cultured organisms into UTM. Nasopharyngeal swabs collected from anonymous asymptomatic volunteers were used as negative samples. The MA samples were assessed at two concentrations: low positive (LP) (the limit of detection level) and moderate positive (MP) (3x the limit of detection level). Each sample set was tested in 20 replicates starting from sample extraction, and assessed in a single run by the same operator using the same set of instruments.

Table 23 - Summary of Repeatability Results

NxTAG RPP Target	Strain Assessed	Low Positive (LoD)	Moderate Positive (3x LoD)
Influenza A Matrix	A/Brisbane/59/07	20/20 POS	20/20 POS
	A/SwineNY/03/2009	20/20 POS	20/20 POS
	A/Wisconsin/67/05	20/20 POS	20/20 POS
Influenza A H1 Subtype	A/Brisbane/59/07	19/20 POS	20/20 POS
	A/SwineNY/03/2009	19/20 POS	20/20 POS
Influenza A H3 Subtype	A/Wisconsin/67/05	20/20 POS	20/20 POS
Influenza B	B/Florida/04/2006	20/20 POS	20/20 POS
Respiratory Syncytial Virus A	A2	20/20 POS	20/20 POS
Respiratory Syncytial Virus B	18537	20/20 POS	20/20 POS
Coronavirus 229E	OC229E	20/20 POS	20/20 POS
Coronavirus OC43	Betacoronavirus 1	20/20 POS	20/20 POS
Coronavirus NL63	NL63	20/20 POS	20/20 POS

NxTAG RPP Target	Strain Assessed	Low Positive (LoD)	Moderate Positive (3x LoD)
Coronavirus HKU1	Clinical Specimen	20/20 POS	20/20 POS
Human Metapneumovirus	Human Metapneumovirus	19/20 POS	20/20 POS
Rhinovirus/Enterovirus	Rhinovirus type 1A	20/20 POS	20/20 POS
Adenovirus	C, type 1	20/20 POS	20/20 POS
Parainfluenza 1	C35	20/20 POS	20/20 POS
Parainfluenza 2	Greer	20/20 POS	19/20 POS
Parainfluenza 3	C 243	20/20 POS	20/20 POS
Parainfluenza 4A	Type 4A	20/20 POS	20/20 POS
Parainfluenza 4B	CH19503	20/20 POS	20/20 POS
Human Bocavirus	Type 1	20/20 POS	20/20 POS
Chlamydophila pneumoniae	TW-183	20/20 POS	20/20 POS
Mycoplasma pneumonia	M129	20/20 POS	20/20 POS
N/A (RPP negative samples)	N/A (pooled RPP negative nasopharyngeal swabs)	20/20 NEG for all targets	

b. Linearity/assay reportable range:

N/A

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

External Controls

None. The assay contains an internal control to ensure proper functionality of the test from extraction to results interpretation.

Shipping and Storage Stability

See “fresh versus frozen equivalency” section for stability information.

d. Detection limit:

Limit of Detection (LoD)

The LoD for each of the NxTAG RPP targets was assessed by analyzing serial dilutions of simulated samples made from high-titer stocks from commercial

suppliers or clinical specimens when the target pathogen was not commercially available. All sample dilutions were prepared using UTM. The LoD titer for each target was defined as the lowest concentration at which ≥95% (≥19/20) of samples tested generated positive calls, as listed in Table 24. In addition, the confirmed LoD of each target was evaluated in Multi-Analyte (MA) samples where two to four analytes were included in one contrived sample (Table 25). Confirmation of the single analyte LoD in MA samples supports the use of MA samples in other analytical studies.

Table 25 – Composition of Multi-Analyte Samples

MA #	Analyte-1	Analyte-2	Analyte-3
MA-1	Influenza A H1	Rhinovirus	Respiratory Syncytial Virus A
MA-2	Influenza A H3 (for matrix)	Adenovirus C	N/A
MA-3	Influenza A 2009 H1N1	Parainfluenza 1	*Chlamydophila pneumoniae*
MA-4	Influenza A H3 (for subtype)	Respiratory Syncytial Virus B	Human Bocavirus
MA-5	Parainfluenza 3	Coronavirus OC43	
MA-6	Influenza B	Parainfluenza 4A	*Mycoplasma pneumoniae*
MA-7	Coronavirus NL63	Human Metapneumovirus	Coronavirus HKU1
MA-8	Parainfluenza 4B	Parainfluenza 2	Coronavirus 229E

Table 24 – LoD Results on the NxTAG RPP Assay

NxTAG RPP Target	Strain	Concentration	LoD	LoD in MA samples
Influenza A Matrix	A/Brisbane/59/07	3.08E+00 $TCID_{50}$/mL	20/20 POS	20/20 POS
	A/SwineNY/03/2009	5.53E - 01 $TCID_{50}$/mL	20/20 POS	20/20 POS
	A/Wisconsin/67/05	2.50E - 01 $TCID_{50}$/mL	20/20 POS	20/20 POS
Influenza A H1 Subtype	A/Brisbane/59/07	3.08E+00 $TCID_{50}$/mL	20/20 POS	19/20 POS
	A/SwineNY/03/2009	5.53E - 01 $TCID_{50}$/mL	20/20 POS	19/20 POS
Influenza A H3 Subtype	A/Wisconsin/67/05	9.36E - 02 $TCID_{50}$/mL	20/20 POS	20/20 POS
Influenza B	B/Florida/04/2009	5.81E - 01 $TCID_{50}$/mL	20/20 POS	20/20 POS
Respiratory Syncytial Virus A	A2	2.15E+00 $TCID_{50}$/mL	20/20 POS	20/20 POS
Respiratory Syncytial Virus B	18537	1.36E+00 $TCID_{50}$/mL	20/20 POS	20/20 POS
Coronavirus 229E	229E	1.07E - 02 $TCID_{50}$/mL	20/20 POS	20/20 POS
Coronavirus OC43	Betacoronavirus 1	7.15E - 02 $TCID_{50}$/mL	19/20 POS	20/20 POS
Coronavirus NL63	NL63	3.37E - 03 $TCID_{50}$/mL	20/20 POS	20/20 POS
Coronavirus HKU1	Clinical Specimen	1.57E+04 Copies/mL	20/20 POS	20/20 POS
Human Metapneumovirus	Human Metapneumovirus	1.38 - 01 $TCID_{50}$/mL	20/20 POS	19/20 POS
Rhinovirus/Enterovirus	Rhinovirus type 1A	5.18E-01 $TCID_{50}$/mL	20/20 POS	20/20 POS
	Enterovirus D68	3.34E+00 $TCID_{50}$/mL	20/20 POS	N/A
Adenovirus	C, type 1	3.25E+00 $TCID_{50}$/mL	20/20 POS	20/20 POS
	B, type 14	1.52E - 01 $TCID_{50}$/mL	20/20 POS	N/A
	E, type 4	6.91E - 02 $TCID_{50}$/mL	20/20 POS	N/A
Parainfluenza 1	C35	2.82E+01 $TCID_{50}$/mL	20/20 POS	20/20 POS
Parainfluenza 2	Greer	5.36E - 01 $TCID_{50}$/mL	19/20 POS	20/20 POS
Parainfluenza 3	C 243	1.61E+01 $TCID_{50}$/mL	20/20 POS	20/20 POS
Parainfluenza 4A	Type 4A	2.54E+00 $TCID_{50}$/mL	20/20 POS	20/20 POS
Parainfluenza 4B	CH19503	6.09E - 01 $TCID_{50}$/mL	20/20 POS	20/20 POS
Human Bocavirus	Type 1	3.91E+02 Copies/mL	20/20 POS	20/20 POS
Chlamydophila pneumoniae	TW-183	6.43E - 02 $TCID_{50}$/mL	20/20 POS	20/20 POS
Mycoplasma pneumonia	M129	1.42E+02 CCU/mL	20/20 POS	20/20 POS

e. Analytical reactivity:

Analytical Reactivity

The analytical reactivity of the NxTAG RPP Assay was evaluated against multiple strains of each analyte detected by the assay. The concentration detected in the table represents the lowest concentration for a particular analyte which could be detected 100% of the time (3/3 positive). A total of 201 strains were tested: including 75 influenza A, 24 influenza B, 8 RSV, 13 Parainfluenza virus, 9 Coronavirus, 10 human Metapneumovirus, 12 Rhinovirus, 15 Enterovirus, 18 Adenovirus, 8 *Chlamydia pneumoniae*, and 10 *Mycoplasma pneumoniae* strains. Three replicates were tested for each strain. Results are shown in Table 26. Inclusivity testing results showed broad coverage of all the analytes detected in the NxTAG RPP assay.

Table 26 – NxTAG RPP Results on Influenza A strains

Organism	Strain	Source	Matrix or Subtype	Concentration	
				Detected	Unit
Flu A H1	A/Brisbane/59/07 H1	ZeptoMetrix 0810036CF	FluA matrix	3.80E+06	$TCID_{50}$/mL
			H1 subtype	3.80E+06	$TCID_{50}$/mL
			FluA matrix	5.04E+01	Copies/mL
			H1 subtype	5.04E+01	Copies/mL
	A/NewCaledonia/20/99	ZeptoMetrix 0810036CF	FluA matrix	3.08E+00	$TCID_{50}$/mL
			H1 subtype	7.70E-01	$TCID_{50}$/mL
	A/SolomanIsland/3/06	ZeptoMetrix 0810036CFN	FluA matrix	1.48E+02	$TCID_{50}$/mL
			H1 subtype	3.70E+01	$TCID_{50}$/mL
	A/Beijing/262/95	BEI NR-12277	FluA matrix	3.70E+01	$CEID_{50}$/mL
			H1 subtype	1.48E+02	$CEID_{50}$/mL
	A/Fujian Gulou/1896/2009	IRR FR-468	FluA matrix	1.00E+03	$CEID_{50}$/mL
			H1 subtype	2.36E+03	$CEID_{50}$/mL
	A/Florida/3/2006	IRR FR-364	FluA matrix	5.91E+02	$CEID_{50}$/mL
			H1 subtype	3.70E+01	$CEID_{50}$/mL
	A/FM/1/47	ATCC VR-97	FluA matrix	5.91E+02	$CEID_{50}$/mL
			H1 subtype	1.00E+06	$CEID_{50}$/mL
	A/South Dakota/6/2007	IRR FR-3	FluA matrix	5.91E+02	$CEID_{50}$/mL
			H1 subtype	1.48E+02	$CEID_{50}$/mL
	A/Kentucky/2/2006	IRR FR-332	FluA matrix	2.36E+03	$CEID_{50}$/mL
			H1 subtype	1.48E+02	$CEID_{50}$/mL
	A/Chelyabinsk/1/2006	IRR FR-333	FluA matrix	5.91E+02	$CEID_{50}$/mL
			H1 subtype	1.48E+02	$CEID_{50}$/mL
	A/Fukushima/141/2006	IRR FR-334	FluA matrix	5.91E+02	$CEID_{50}$/mL
			H1 subtype	5.91E+02	$CEID_{50}$/mL
	A/St.Petersburg/8/2006	IRR FR-335	FluA matrix	5.91E+02	$CEID_{50}$/mL
			H1 subtype	3.70E+01	$CEID_{50}$/mL
	A/Hong Kong/2652/2006	IRR FR-363	FluA matrix	5.91E+02	$CEID_{50}$/mL

Organism	Strain	Source	Matrix or Subtype	Concentration	
				Detected	Unit
			H1 subtype	1.48E+02	$CEID_{50}$/mL
	A/Jiangxi/134/2005	IRR FR-405	FluA matrix	1.48E+02	$TCID_{50}$/mL
			H1 subtype	9.24E+00	$TCID_{50}$/mL
	A/Mexico/949/2007	IRR FR-452	FluA matrix	3.70E+01	$TCID_{50}$/mL
			H1 subtype	9.24E+00	$TCID_{50}$/mL
	A/Victoria/504/2005	IRR FR-453	FluA matrix	5.91E+02	$CEID_{50}$/mL
			H1 subtype	1.48E+02	$CEID_{50}$/mL
	A/Hawaii/31/2007	IRR FR-5	FluA matrix	9.24E+00	$TCID_{50}$/mL
			H1 subtype	9.24E+00	$TCID_{50}$/mL
	A/Paraguay/61/2009	IRR FR-585	FluA matrix	1.48E+02	$TCID_{50}$/mL
			H1 subtype	1.48E+02	$TCID_{50}$/mL
	A/Bangladesh/7286/2007	IRR FR-586	FluA matrix	3.70E+01	$TCID_{50}$/mL
			H1 subtype	9.24E+00	$TCID_{50}$/mL
	A/Cambodia/0371/2007	IRR FR-7	FluA matrix	5.91E+02	$CEID_{50}$/mL
			H1 subtype	1.48E+02	$CEID_{50}$/mL
	A/Mal/302/54	ATCC VR-98	FluA matrix	9.24E+00	$CEID_{50}$/mL
			H1 subtype	5.91E+02	$CEID_{50}$/mL
	A/NWS/33(H1N1)	ATCC VR-219	FluA matrix	3.70E+01	$CEID_{50}$/mL
			H1 subtype	5.91E+02	$CEID_{50}$/mL
	A/Taiwan/42/06	ZeptoMetrix 0810036CF	FluA matrix	9.24E+00	$TCID_{50}$/mL
			H1 subtype	9.24E+00	$TCID_{50}$/mL
	A/WS/33	ATCC VR-1520	FluA matrix	9.24E+00	$TCID_{50}$/mL
			H1 subtype	5.91E+02	$TCID_{50}$/mL
	A/Denver/1/57	ATCC VR-546	FluA matrix	5.91E+02	$CEID_{50}$/mL
			H1 subtype	ND* at 1.00E+05	$CEID_{50}$/mL
	A/PR/8/34	ATCC VR-1469	FluA matrix	3.70E+01	$TCID_{50}$/mL
			H1 subtype	ND* at 1.00E+05	$TCID_{50}$/mL
	A/Weiss/43	ATCC VR-96	FluA matrix	1.00E+04	$CEID_{50}$/mL
			H1 subtype	ND* at 1.00E+05	$CEID_{50}$/mL
	A/SwineNY/03/2009	ZeptoMetrix 0810109CFN	FluA matrix	5.53E-01	$TCID_{50}$/mL
			H1 subtype	5.53E-01	$TCID_{50}$/mL
	A/Brownsville/31H/2009	BEI NR-20344	FluA matrix	1.66E+00	$TCID_{50}$/mL
			H1 subtype	1.66E+00	$TCID_{50}$/mL
	A/California/04/2009	BEI NR-13658	FluA matrix	1.66E+00	$TCID_{50}$/mL
			H1 subtype	1.66E+00	$TCID_{50}$/mL
	A/California/7/2009	ZeptoMetrix 0810165CF	FluA matrix	1.66E+00	$TCID_{50}$/mL
			H1 subtype	1.66E+00	$TCID_{50}$/mL
	A/Dominican Republic/7293/2013	IRR FR-1298	FluA matrix	1.66E+00	$TCID_{50}$/mL
			H1 subtype	1.66E+00	$TCID_{50}$/mL

Organism	Strain	Source	Matrix or Subtype	Concentration	
				Detected	Unit
	A/Houston/3H/2009	BEI NR-20340	FluA matrix	1.66E+00	$TCID_{50}$/mL
			H1 subtype	1.66E+00	$TCID_{50}$/mL
	A/Massachusetts/15/2013	IRR FR-1319	FluA matrix	1.06E+02	$CEID_{50}$/mL
			H1 subtype	4.24E+02	$CEID_{50}$/mL
	A/Mexico/4108/09	ZeptoMetrix 0810166CF	FluA matrix	1.66E+00	$TCID_{50}$/mL
			H1 subtype	1.66E+00	$TCID_{50}$/mL
	A/Netherlands/2629/2009	BEI NR-19823	FluA matrix	1.66E+00	$TCID_{50}$/mL
			H1 subtype	6.63E+00	$TCID_{50}$/mL
	A/New Jersey/8/76	ATCC VR-897	FluA matrix	9.24E+00	$CEID_{50}$/mL
			H1 subtype	1.00E+04	$CEID_{50}$/mL
	A/Swine/1976/31	ATCC VR-99	FluA matrix	3.70E+01	$CEID_{50}$/mL
			H1 subtype	5.00E+04	$CEID_{50}$/mL
	A/Swine/Canada/6294/09	ZeptoMetrix 0810109CFJ	FluA matrix	1.66E+00	$TCID_{50}$/mL
			H1 subtype	1.66E+00	$TCID_{50}$/mL
	A/Swine/Iowa/15/30	ATCC VR-333	FluA matrix	2.36E+03	$CEID_{50}$/mL
			H1 subtype	1.00E+06	$CEID_{50}$/mL
	A/Virginia/ATCC1/2009	ATCC VR-1736	FluA matrix	1.66E+00	pfu/mL
			H1 subtype	6.63E+00	pfu/mL
Flu A H3	A/Wisconsin/67/05	ZeptoMetrix 0810138CF	FluA matrix	2.50E-01	$TCID_{50}$/mL
			H3 subtype	9.36E-02	$TCID_{50}$/mL
	A/PortChalmers/1/73	ATCC VR-810	FluA matrix	9.59E+02	$CEID_{50}$/mL
			H3 subtype	4.79E+02	$CEID_{50}$/mL
	A/New York/39/2012 (H3N2)	IRR FR-1307	FluA matrix	7.49E-01	$TCID_{50}$/mL
			H3 subtype	7.49E-01	$TCID_{50}$/mL
	A/Aichi/2/68	ATCC VR-547	FluA matrix	1.20E+01	$CEID_{50}$/mL
			H3 subtype	1.00E+04	$CEID_{50}$/mL
	A/Alice	ATCC VR-776	FluA matrix	1.20E+01	$CEID_{50}$/mL
			H3 subtype	1.92E+02	$CEID_{50}$/mL
	A/Uruguay/716/2007 (H3N2)	IRR FR-10	FluA matrix	1.92E+02	$CEID_{50}$/mL
			H3 subtype	4.79E+01	$CEID_{50}$/mL
	A/Taiwan/760/2007 (H3N2)	IRR FR-12	FluA matrix	3.00E+00	$TCID_{50}$/mL
			H3 subtype	7.49E-01	$TCID_{50}$/mL
	A/Texas/71/2007 (H3N2)	IRR FR-13	FluA matrix	7.49E-01	$TCID_{50}$/mL
			H3 subtype	7.49E-01	$TCID_{50}$/mL
	A/Santiago/7981/2006 (H3N2)	IRR FR-336	FluA matrix	4.79E+01	$CEID_{50}$/mL
			H3 subtype	1.20E+01	$CEID_{50}$/mL
	A/Brisbane/9/2006(H3N2)	IRR FR-366	FluA matrix	1.92E+02	$CEID_{50}$/mL
			H3 subtype	4.79E+01	$CEID_{50}$/mL
	A/Nepal/921/2006 (H3N2)	IRR FR-367	FluA matrix	3.00E+00	$CEID_{50}$/mL
			H3 subtype	7.49E-01	$CEID_{50}$/mL
	A/Florida/2/2006 (H3N2)	IRR FR-368	FluA matrix	4.79E+01	$CEID_{50}$/mL

Organism	Strain	Source	Matrix or Subtype	Concentration	
				Detected	Unit
			H3 subtype	1.20E+01	$CEID_{50}$/mL
	A/Wisconsin/15/2009 (H3N2)	IRR FR-369	FluA matrix	3.00E+00	$CEID_{50}$/mL
			H3 subtype	7.49E-01	$CEID_{50}$/mL
	A/Victoria/210/2009 (H3N2)	IRR FR-643	FluA matrix	1.92E+02	$CEID_{50}$/mL
			H3 subtype	4.79E+01	$CEID_{50}$/mL
	A/Rhode Island/01/2010 (H3N2)	IRR FR-662	FluA matrix	4.79E+01	$CEID_{50}$/mL
			H3 subtype	1.92E+02	$CEID_{50}$/mL
	A/Minnesota/11/2010 (H3N2)	IRR FR-945	FluA matrix	7.67E+02	$CEID_{50}$/mL
			H3 subtype	1.20E+01	$CEID_{50}$/mL
	A/Henan/Jinshui/147/2007 (H3N2)	IRR FR-365	FluA matrix	4.79E+01	$CEID_{50}$/mL
			H3 subtype	1.20E+01	$CEID_{50}$/mL
	A/HongKong/8/68	ATCC VR-544	FluA matrix	3.00E+00	$CEID_{50}$/mL
			H3 subtype	1.00E+03	$CEID_{50}$/mL
	A/Indiana/08/2011	IRR FR-944	FluA matrix	7.49E-01	$TCID_{50}$/mL
			H3 subtype	7.49E-01	$TCID_{50}$/mL
	MRC 2	ATCC VR-777	FluA matrix	4.79E+01	$CEID_{50}$/mL
			H3 subtype	1.92E+02	$CEID_{50}$/mL
	A/Ohio/02/2012	IRR FR-1143	FluA matrix	4.79E+01	$CEID_{50}$/mL
			H3 subtype	4.79E+01	$CEID_{50}$/mL
	A/Perth/16/09	ZeptoMetrix 0810138CF	FluA matrix	7.49E-01	$TCID_{50}$/mL
			H3 subtype	7.49E-01	$TCID_{50}$/mL
	A/Sydney/5/97 (H3N2)	BEI NR-12278	FluA matrix	3.84E+02	$CEID_{50}$/mL
			H3 subtype	1.20E+01	$CEID_{50}$/mL
	A/Victoria/3/75	ATCC VR-822	FluA matrix	3.00E+00	$CEID_{50}$/mL
			H3 subtype	4.79E+01	$CEID_{50}$/mL
Flu A H5	A/Egypt/N03072/2010 (H5N1)	IRR FR-1065	FluA H5 matrix	1.51E+02	Copies/mL
	A/Hubei/1/2010 (H5N1)	IRR FR-1066	FluA H5 matrix	1.51E+02	Copies/mL
	A/Anhui/01/2005 (H5N1)	IRR FR-735	FluA H5 matrix	1.51E+02	Copies/mL
	A/India/NIV/2006 (H5N1)	IRR FR-763	FluA H5 matrix	1.51E+02	Copies/mL
	A/chicken/Vietnam/NCVD-016/2008 (H5N1)	IRR FR-766	FluA H5 matrix	1.51E+02	Copies/mL
	A/pheasant/New Jersey/1355/1998 (H5N2)	IRR FR-771	FluA H5 matrix	1.51E+02	Copies/mL
Flu A H7	A/turkey/Virginia/4529/2002 (H7N2)	IRR FR-772	FluA H7 matrix	1.51E+02	Copies/mL
	A/mallard/Netherlands/12/2000 (H7N7)	IRR FR-773	FluA H7 matrix	1.51E+02	Copies/mL
Flu A H9	A/Hong Kong/33982/2009 (H9N2)	IRR FR-1068	FluA H9 matrix	1.00E+02	$CEID_{50}$/mL
	A/chicken/Hong Kong/G9/1997 (H9N2)	IRR FR-732	FluA H9 matrix	1.00E+02	$CEID_{50}$/mL

*ND = Not Detected.

Table 27 – NxTAG RPP Results on Influenza B strains

Strain	Source	Concentration	
		Detected	Unit
B/Florida/04/2006	ZeptoMetrix 0810037CF Lot 305764	5.81E-01	$TCID_{50}$/mL
		9.53E+06	Copies/mL
B/Taiwan/2/62	ATCC VR-295	1.39E+01	$TCID_{50}$/mL
B/Allen/45	ATCC VR-102	6.97E+00	$CEID_{50}$/mL
B/Brigit	ATCC VR-786	1.00E+02	$CEID_{50}$/mL
B/Brisbane/33/2008	ZeptoMetrix 0810037CF Lot 307548	1.74E+00	$TCID_{50}$/mL
B/Brisbane/60/08	ZeptoMetrix 0810037CF Lot 308390	1.74E+00	$TCID_{50}$/mL
B/Florida/02/06	ZeptoMetrix 0810037CF Lot 307550	1.74E+00	$TCID_{50}$/mL
B/Florida/07/04	ZeptoMetrix 0810037CF Lot 308487	6.97E+00	$TCID_{50}$/mL
B/Texas/06/2011 (Yamagata Lineage)	IRR FR-1062	2.79E+01	$CEID_{50}$/mL
B/New Jersey/1/2012 (Victoria Lineage)	IRR FR-1270	6.97E+00	$TCID_{50}$/mL
B/Texas/02/2013 (Victoria Lineage)	IRR FR-1302	1.74E+00	$TCID_{50}$/mL
B/Bangladesh/5972/2007 (Yamagata Lineage)	IRR FR-450	1.74E+00	$TCID_{50}$/mL
B/Hubei Wujiagang/158/2009 (Yamagata Lineage)	IRR FR-469	6.97E+00	$CEID_{50}$/mL
B/Hong Kong/259/2010 (Victoria Lineage)	IRR FR-663	2.79E+01	$CEID_{50}$/mL
B/GL/1739/54	ATCC VR-103	1.00E+04	$CEID_{50}$/mL
B/HongKong/5/72	ATCC VR-823	2.79E+01	$CEID_{50}$/mL
B/Lee/40	ATCC VR-1535	1.12E+02	$CEID_{50}$/mL
B/Malaysia/2506/04	ZeptoMetrix 0810037CF Lot 307680	1.74E+00	$TCID_{50}$/mL
B/Maryland/1/59	ATCC VR-296	1.74E+00	$CEID_{50}$/mL
B/Mass/3/66	ATCC VR-523	6.97E+00	$CEID_{50}$/mL
B/Panama/45/90	ZeptoMetrix 0810037CF Lot 308488	1.74E+00	$TCID_{50}$/mL
B/R75	ATCC VR-789	6.97E+00	$CEID_{50}$/mL
B/Russia/69	ATCC VR-790	3.63E+00	Copies/mL
B/R5	ATCC VR-787	1.74E+00	$CEID_{50}$/mL

Table 28 – NxTAG RPP Results on RSV strains

Type	Strain	Source	Concentration	
			Detected	Unit
A	A2	ATCC VR-1540	2.15E+00	$TCID_{50}$/mL
	A	ZeptoMetrix 0810040ACF	4.12E+02	$TCID_{50}$/mL
	Long	ATCC VR-26	1.65E+03	$TCID_{50}$/mL
B	18537	ATCC VR-1580	1.36E+00	$TCID_{50}$/mL
	CH93-18(18)	ZeptoMetrix 0810040CF	6.51E+01	$TCID_{50}$/mL
	B1	BEI NR-4052	6.51E+01	$TCID_{50}$/mL

Type	Strain	Source	Concentration	
			Detected	Unit
	B WV/14617/85	ATCC VR-1400	4.07E+00	$TCID_{50}$/mL
	9320	ATCC VR-955	4.07E+00	$TCID_{50}$/mL

Table 29 – NxTAG RPP Results on Parainfluenza virus strains

Type		Strain	Source	Concentration	
				Detected	Unit
PIV1		C35	ATCC VR-94	2.82E+01	$TCID_{50}$/mL
		Type 1	ZeptoMetrix 0810014CF	8.46E+01	$TCID_{50}$/mL
PIV2		Greer	ATCC VR-92	5.36E-01	$TCID_{50}$/mL
		Type 2	ZeptoMetrix 0810015CF	1.03E+02	$TCID_{50}$/mL
PIV3		C 243	ATCC VR-93	1.61E+01	$TCID_{50}$/mL
		Type 3	ZeptoMetrix 0810016CF	4.83E+01	$TCID_{50}$/mL
		NIH47885	BEI NR-3233	4.83E+01	$TCID_{50}$/mL
		ATCC-2011-5	ATCC VR-1782	4.83E+01	$TCID_{50}$/mL
PIV4	4A	Type 4A	ZeptoMetrix 0810060CF	2.54E+00	$TCID_{50}$/mL
		M-25	ATCC VR-1378	7.63E+00	$TCID_{50}$/mL
	4B	CH 19503	ATCC VR-1377	6.09E-01	$TCID_{50}$/mL
		Type 4B	ZeptoMetrix 0810060BCF	7.31E+00	$TCID_{50}$/mL
		19503	BEI NR-3238	4.68E+02	$TCID_{50}$/mL

Table 30 – NxTAG RPP Results on Coronavirus strains

Type	Source	Concentration	
		Detected	Unit
229E	ATCC VR-740	1.07E-02	$TCID_{50}$/mL
	ZeptoMetrix 0810229CF	5.15E-01	$TCID_{50}$/mL
NL63	ZeptoMetrix 0810228CF	3.37E-03	$TCID_{50}$/mL
	SJH (50608)	1.01E-02	$TCID_{50}$/mL
OC43	ATCC VR-1558	7.15E-02	$TCID_{50}$/mL
	ZeptoMetrix 0810024CF	2.15E-01	$TCID_{50}$/mL
HKU1	LMD-02, USA/HKU1-12/2009-2010	1.57E+04	Copies/mL
	LMD-100, Genotype A	9.45E+04	Copies/mL
	ZeptoMetrix 0810067CF, Recombinant	4.72E+04	Copies/mL

Table 31 – NxTAG RPP Results on Human Metapneumovirus strains

Subtype	Strain	Source	Concentration	
			Detected	Unit
A1	IA10-2003, hMPV-16	ZeptoMetrix VPL-030 Lot 305089	1.38E-01	$TCID_{50}$/mL
	A1	SJH 031709	4.15E-01	$TCID_{50}$/mL
	IA3-2002, hMPV-9	ZeptoMetrix 0810160CF Lot 310049	4.15E-01	$TCID_{50}$/mL
A2	IA14-2003, hMPV-20	ZeptoMetrix 0810163CF Lot 308415	8.85E+00	$TCID_{50}$/mL
	DHI 26583	SJH 030209	4.15E-01	$TCID_{50}$/mL
B1	Peru2-2002, hMPV-3	ZeptoMetrix 0810156CF Lot 308423	1.77E+01	$TCID_{50}$/mL
	Peru3-2003, hMPV-5	ZeptoMetrix VPL-030 Lot 305225	1.66E+00	$TCID_{50}$/mL
B2	Peru1-2002, hMPV-4	ZeptoMetrix VPL-030 Lot 305227	4.15E-01	$TCID_{50}$/mL
	Peru6-2003, hMPV-8	ZeptoMetrix 0810159CF Lot 308419	1.11E+00	$TCID_{50}$/mL
	IA18-2003, hMPV-18	ZeptoMetrix 0810162CF Lot 308411	2.65E+01	$TCID_{50}$/mL

Table 32 – NxTAG RPP Results on Rhinovirus strains

Species	Strain	Source	Concentration	
			Detected	Unit
A	1A	ZeptoMetrix 0810012CFN	5.18E-01	$TCID_{50}$/mL
			1.14E+02	Copies/mL
	Type 2, strain HGP	ATCC VR-482	1.55E+00	$TCID_{50}$/mL
	Type 7, strain 68-CV11	ATCC VR-1601	1.55E+00	$TCID_{50}$/mL
	Type 39, Strain 209	ATCC VR-340	1.55E+00	$TCID_{50}$/mL
	Type 54, strain FO 1-3774	ATCC VR-1661	1.55E+00	$TCID_{50}$/mL
	Type 60, Strain 2268-CV37	SJH 08/18/10 ATCC VR-1170	2.49E+01	$TCID_{50}$/mL
B	Type 3, strain FEB	ATCC VR-483	2.49E+01	$TCID_{50}$/mL
	Type 14, strain 1059	ATCC VR-284	9.95E+01	$TCID_{50}$/mL
	Type 17, strain 33342	ATCC VR-1663	2.49E+01	$TCID_{50}$/mL
	Type 27, strain 5870 [5870-CV28] (NIAID V-144-001-021)	ATCC VR-1137	3.43E+02	Copies/mL
	Type 42, strain 56822	ATCC VR-338	1.55E+00	$TCID_{50}$/mL
	Type 83, strain Baylor 7 (NIAID V-190-001-021)	ATCC VR-1193	1.55E+00	$TCID_{50}$/mL

Table 33 – NxTAG RPP Results on Enterovirus strains

Species	Strain	Source	Concentration	
			Detected	Unit
A	Type 71, strain H	ATCC VR-1432	1.00E+01	$TCID_{50}$/mL
	Human Coxsackievirus A10, strain M.K. (Kowalik)	ATCC VR-168	1.60E+02	$TCID_{50}$/mL
B	Coxsackievirus B DHI 20-4420010	DHI /SJH 20-4420010	1.00E+01	$TCID_{50}$/mL
	Coxsackievirus B1, strain Conn-5	ATCC VR-28	4.01E+01	$TCID_{50}$/mL
	Human Echovirus 11, strain Gregory	ATCC VR-41	1.00E+04	$TCID_{50}$/mL
	Human Echovirus 13, strain Del Carmen NIAID V-046-001-010	ATCC VR-1054	1.00E+01	$TCID_{50}$/mL
	Type 69, strain Toluca-1 [V-068-001-021]	ATCC VR-1077	1.00E+01	$TCID_{50}$/mL
C	Human coxsackievirus A21 , strain Kuykendall	ATCC VR-850	1.00E+01	$TCID_{50}$/mL
	Human coxsackievirus A24, strain DN-19	ATCC VR-1662	1.00E+01	$TCID_{50}$/mL
D	Type 68, strain 2007 isolate	ZeptoMetrix 0810237CF	3.34E+00	$TCID_{50}$/mL
	Type 68, strain Fermon	ATCC VR-1076	1.00E+01	$TCID_{50}$/mL
	Type 68, strain US/MO/14-18947	ATCC VR-1823	1.60E+02	$TCID_{50}$/mL
	Type 68, strain US/IL/14-18952	ATCC VR-1824	1.60E+02	$TCID_{50}$/mL
	Type 68, strain US/KY/14-18953	ATCC VR-1825	6.41E+02	$TCID_{50}$/mL
	Type 70, strain J670/71	ATCC VR-836	1.00E+01	$TCID_{50}$/mL

Table 34 – NxTAG RPP Results on Adenovirus strains

Species	Type	Strain	Source	Concentration	
				Detected	Unit
A	18	D.C.	ATCC VR-1095 NIAID V-218-003-014	4.00E+04	$TCID_{50}$/mL
B	3	Type 3	ZeptoMetrix 0810062CF	4.57E-01	$TCID_{50}$/mL
	7	Gomen	ATCC VR-7	9.76E+00	$TCID_{50}$/mL
	7A	Type 7A	ZeptoMetrix 0810021CF	7.32E+00	$TCID_{50}$/mL
	14	Type 14	ZeptoMetrix 0810108CF	1.52E-01	$TCID_{50}$/mL
	16	Ch.79 [V-216-003-014]	ATCC VR-1093 (NIAID V-216-003-014)	4.57E-01	$TCID_{50}$/mL
	21	AV-1645 [128]	ATCC VR-1098 NIAID V-221-011-014	4.57E-01	$TCID_{50}$/mL
C	1	Type 1	ZeptoMetrix 0810050CF	3.25E+00	$TCID_{50}$/mL
	1	Type 1, strain Adenoid 71	ATCC VR-1	9.76E+00	$TCID_{50}$/mL
	5	Type 5	ZeptoMetrix 0810020CF	9.76E+00	$TCID_{50}$/mL
D	8	Type 8	ZeptoMetrix 0810069CF	2.07E-01	$TCID_{50}$/mL
	10	J.J	ATCC VR-1087 NIAID V-210-003-014	2.07E-01	$TCID_{50}$/mL

Species	Type	Strain	Source	Concentration	
				Detected	Unit
	13	A.A [V-213-502-565]	ATCC VR-1090 (NIAID V-213-003-014)	2.07E-01	$TCID_{50}$/mL
	30	BP-7	ATCC VR-273	2.07E-01	$TCID_{50}$/mL
	37	GW [76-19026]	ATCC VR-929	2.07E-01	$TCID_{50}$/mL
E	4	Type 4	ZeptoMetrix 0810070CF	6.91E-02	$TCID_{50}$/mL
F	40	Dugan [79-18025]	ATCC VR-931	2.07E-01	$TCID_{50}$/mL
	41	Type 41 (Tak)	ZeptoMetrix 0810085CF Lot 306184	2.50E+03	$TCID_{50}$/mL

Table 35 – NxTAG RPP Results on *Chlamydophila pneumoniae* strains

Strain	Source	Concentration	
		Detected	Unit
TW-183	ATCC VR-2282	6.43E-02	$TCID_{50}$/mL
AR-39	ATCC 53592	3.09E+00	$TCID_{50}$/mL
TWAR (CDC/CWL-029)	ATCC VR-1310	1.24E+01	$TCID_{50}$/mL
TWAR strain 2043	ATCC VR-1355	7.72E-01	$TCID_{50}$/mL
TWAR strain 2023	ATCC VR-1356	1.93E-01	$TCID_{50}$/mL
CM-1	ATCC VR-1360	1.93E-01	$TCID_{50}$/mL
J-21	ATCC VR-1435	7.72E-01	$TCID_{50}$/mL
AO3	ATCC VR-1452	1.93E-01	$TCID_{50}$/mL

Table 36 – NxTAG RPP Results on *Mycoplasma pneumoniae* strains

Strain	Source	Concentration	
		Detected	Unit
M129	ZeptoMetrix 0801579	7.04E+02	Copies/mL
[M52]	ATCC 15293	2.11E+03	Copies/mL
[Bru]	ATCC 15377	2.11E+03	Copies/mL
[Mac]	ATCC 15492	2.11E+03	Copies/mL
PI 1428	ATCC 29085	2.11E+03	Copies/mL
M129-B7	ATCC 29342	2.11E+03	Copies/mL
M129-B170	ATCC 29343	2.11E+03	Copies/mL
Mutant 22	ATCC 39505	2.11E+03	Copies/mL
UTMB-10P	ATCC 49894	2.11E+03	Copies/mL
FH strain of Eaton Agent [NCTC 10119], type strain	ATCC 15531-TTR	2.11E+03	Copies/mL

Laboratory testing was supplemented with *in silico* data where prediction rules were used to predict reactivity and cross-reactivity of specific Influenza A strains. GenBank sequences were aligned with all primer sequences in the NxTAG Respiratory Pathogen Panel assay. Reactivity and cross-reactivity was predicted

based on thermodynamic analysis of mismatches between the primers and Influenza A sequences. With the exception of an H5N1 swine strain (A/swine/East Java/UT6010/2007(H5N1), the strains analyzed were predicted to react to the Influenza A primers and showed no cross-reactivity with the other analyte primers in the NxTAG Respiratory Pathogen Panel assay. The H5N1 swine strain is not expected to react.

f. Analytical Specificity:

Potential cross-reactivity of the assay was assessed with pathogens that cause respiratory infections that are not probed by the assay, pathogens that may be found in respiratory specimens, as well as pathogens that the assay is designed to detect. Cross reactivity was evaluated by preparing simulated specimens by spiking cultured organisms into UTM. Viral and bacterial targets were prepared at 1 x 10^5 $TCID_{50}$/mL or 1 x 10^6 CFU/mL, respectively, or at the highest concentration possible based on the organism stock concentration. Three replicates of each pathogen were extracted and tested according to the package insert.

One hundred and seven pathogens were tested during the course of the study. Of these pathogens, 80 are not probed by the NxTAG RPP assay. The remaining 27 pathogens are probed by the assay (21 targets plus additional strains of Influenza A H1). None of the pathogenic agents included in this study cross-react with the targets probed by NxTAG RPP, with the exception of three strains of non-pandemic Influenza A H1 (A/Brisbane/59/07, A/Solomon Islands/3/2006 and A/Singapore/63/04). These strains cross-reacted with Coronavirus 229E, when the titer of these Influenza A H1 strains was above 1 x 10^4 $TCID_{50}$/mL. Based on both laboratory testing and *in silico* prediction analysis, high titers of these 3 non-pandemic Influenza A H1 may result in a false positive call for Coronavirus 229E. Based on *in silico* analysis, there is potential that the presence of Coronavirus 229E may cause a false positive Influenza H1 call and the presence of Parainfluenza 2 may cause a false positive Influenza H3 call, although no false positive calls were observed with these two targets in the analytical study. A limitation was added to the labeling to inform the users of this risk. Results are summarized in Table 37.

Table 37 – Potential cross-reactivity with pathogens not probed by the NxTAG RPP

Organism	Reference	Titer Tested	Cross-Reactive Yes / No
Acholeplasma laidlawii (PG8 [NCTC 10116, PG8; A])	ATCC 23206-TTR	1.00E+06 CFU/mL	N
Acinetobacter baumanii (strain 307-0294)	ZeptoMetrix 0801597	1.00E+06 CFU/mL	N
Bordetella bronchiseptica	ZeptoMetrix 0801649	1.00E+06 CFU/mL	N
Bordetella holmesii (strain F061)	ZeptoMetrix 0801464	1.00E+06 CFU/mL	N
Bordetella parapertussis (strain	ZeptoMetrix	1.00E+06 CFU/mL	N

Organism	Reference	Titer Tested	Cross-Reactive Yes / No
A747)	0801461		
Bordetella pertussis (strain A639)	ZeptoMetrix 0801459	1.00E+06 CFU/mL	N
Burkholderia cepacia (strain Z066)	ZeptoMetrix 0801584	1.00E+06 CFU/mL	N
Candida albicans (strain 3147)	ATCC 10231	1.00E+06 CFU/mL	N
Candida glabrata (strain Z007)	ZeptoMetrix 0801535	1.00E+06 CFU/mL	N
Chlamydia trachomatis (strain IC-Cal-3)	ATCC VR-346	1.00E+06 CFU/mL	N
Corynebacterium diphtheriae	ZeptoMetrix 0801882	1.00E+06 CFU/mL	N
Corynebacterium genitalium (strain 392-1)	ATCC 33030	1.00E+06 CFU/mL	N
Corynebacterium glutamicum (Type strain 534 [NCIB 10025])	ATCC 13032	1.00E+06 CFU/mL	N
Cytomegalovirus (strain AD-169)	ZeptoMetrix 0810003CF	1.00E+05 $TCID_{50}$/mL	N
Epstein-Barr virus (strain B95-8)	ZeptoMetrix 0810008CF	1.00E+06 cp/mL	N
Escherichia coli (strain Crooks type)	ATCC 8739	1.00E+06 CFU/mL	N
Escherichia coli ((Migula) Castellani and Chalmers; serotype O17:K52:H18; strain UMN 026)	ATCC BAA-1161	>5000 CFU/mL	N
Fluoribacter bozemanae (Brenner et al.) Garrity et al., strain WIGA)	ATCC 33217	9.98E+05 CFU/mL	N
Fluoribacter dumoffii (strain NY 23)	ATCC 33279	9.99E+05 CFU/mL	N
Fluoribacter gormanii (strain LS-13 [ALLO3])	ATCC 33297	1.00E+06 CFU/mL	N
Haemophilus influenza (strain Minn A)	ZeptoMetrix 0801680	1.00E+06 CFU/mL	N
Herpesvirus (Simplex Type 1) (strain Macintyre)	ZeptoMetrix 0810005CF	1.00E+05 $TCID_{50}$/mL	N
Herpesvirus 3 (VZV)	ATCC VR-7367	8.89E+04 $TCID_{50}$/mL	N
Klebsiella pneumoniae	ATCC 13383	1.00E+06 CFU/mL	N
Lactobacillus acidophilus (Strain Scav [IFO 13951, M. Rogosa 210X, NCIB 8690, P.A. Hansen L 917)	ATCC 4356	1.00E+06 CFU/mL	N
Lactobacillus casei (strain 03)	ATCC 393	1.00E+06 CFU/mL	N
Lactobacillus plantarum (strain 17-5 [BUCSAV 217, BUCSAV 449, Glaxo 664, ICPB 2080, NCDO 82, NCIB 6376, NCIB 8014, NCIB 8030])	ZeptoMetrix 0801507	9.96E+05 CFU/mL	N

Organism	Reference	Titer Tested	Cross-Reactive Yes / No
Lactobacillus reuteri (strain type F275)	ATCC 23272	1.00E+06 CFU/mL	N
Legionella anisa (Gorman et al., strain WA-316-C3 [NCTC 11974])	ATCC 35292	1.00E+06 CFU/mL	N
Legionella birminghamensis (strain 1407-AL-H)	ATCC 43702	1.00E+06 CFU/mL	N
Legionella cincinnatiensis (strain 72-OH-0)	ATCC 43753	9.99E+05 CFU/mL	N
Legionella feeleii (Herwaldt et al., strain WO-44C [NCTC 12022])	ATCC 35072	9.96E+05 CFU/mL	N
Legionella hackeliae (strain Lansing 2 [NCTC 11979])	ATCC 35250	1.00E+06 CFU/mL	N
Legionella hackeliae (strain 8-PA-H [NCTC 11980])	ATCC 35999	1.00E+06 CFU/mL	N
Legionella lansingensis (strain 1677-MI-H)	ATCC 49751	1.00E+06 CFU/mL	N
Legionella longbeachae (strain Long Beach 4 [NCTC 11477])	ATCC 33462	1.00E+06 CFU/mL	N
Legionella micdadei (strain Tatlock)	ZeptoMetrix 0801576	1.00E+06 CFU/mL	N
Legionella pneumophila (strain Philadelphia)	ZeptoMetrix 0801645	9.99E+05 CFU/mL	N
Measles virus (Rubeola) (strain Edmonston)	ATCC VR-24	1.00E+05 $TCID_{50}$/mL	N
Moraxella catarrhalis (strain Ne11)	ZeptoMetrix 0801509	9.98E+05 CFU/mL	N
Mycobacterium avium (Serotype 2)	ZeptoMetrix 0801663	9.99E+05 CFU/mL	N
Mycobacterium intracellulare (strain 3600 [TMC 1406])	ATCC 13950	Not available	N
Mycobacterium tuberculosis (strain H37Rv)	ATCC 25618D-5	6.26E-01 ng/µL	N
Mycoplasma arginini (G230 [NCTC 10129])	ATCC 23838-TTR	1.00E+06 CFU/mL	N
Mycoplasma fermentans (PG18 [G, NCTC 10117])	ATCC 19989-TTR	1.00E+06 CFU/mL	N
Mycoplasma gallisepticum [NCTC 10115, PG 31, X95]	ATCC 19610-TTR	1.00E+06 CFU/mL	N
Mycoplasma genitalium (Tully et al., [UMTB-10G])	ATCC 49899	Not available	N
Mycoplasma hominis [LBD-4]	ATCC 27545-TTR	1.00E+06 CFU/mL	N
Mycoplasma hyorhinis (BTS-7 [ATCC 23234, D.G. ff. Edward PG 42, NCTC 10130])	ATCC 17981-TTR	1.00E+06 CFU/mL	N
Mycoplasma orale (CH 19299 [NCTC 10112])	ATCC 23714-TTR	1.00E+06 CFU/mL	N
Mycoplasma pneumonia (strain	ZeptoMetrix	1.00E+06 CCU/mL	N

Organism	Reference	Titer Tested	Cross-Reactive Yes / No
M129)	0801579		
Mycoplasma salivarium ([H110, NCTC 10113, PG 20])	ATCC 23064-TTR	1.00E+06 CFU/mL	N
Mycoplasma synoviae (WVU 1853 [NCTC 10124])	ATCC 25204-TTR	1.00E+06 CFU/mL	N
Mumps virus	ZeptoMetrix 0810079CF	3.03E+05 $TCID_{50}$/mL	N
Neisseria elongata (strain Z071)	ZeptoMetrix 0801510	1.00E+06 CFU/mL	N
Neisseria gonorrhoeae (strain Z017)	ZeptoMetrix 0801482	1.00E+06 CFU/mL	N
Neisseria meningitides (Serotype A)	ZeptoMetrix 0801511	1.00E+06 CFU/mL	N
Neisseria sicca (strain Z043)	ZeptoMetrix 0801754	1.00E+06 CFU/mL	N
Porphyromonas gingivalis (strain 2561)	ATCC 33277	1.00E+06 CFU/mL	N
Proteus vulgaris	ATCC 6380	1.00E+06 CFU/mL	N
Pseudomonas aeruginosa (strain Boston 41501)	ATCC 27853	1.00E+06 CFU/mL	N
Pseudomonas pertucinogena	ATCC 190	1.00E+06 CFU/mL	N
*Pseudomonas pseudoalcaligenes (*Stanier U-188 [FERM-P 2922])	ATCC 31200	Not available	N
Serratia proteamaculans (subsp. quinovora Grimont et al; strain 4364 [CIP 8195])	ATCC 33765	Not available	N
Serratia proteamaculans (subsp. proteamaculans (Paine and Stansfield) Grimont et al, strain NCPPB 245 [D. Dye ZL1, ICPB XP176, NCTC 394])	ATCC 19323	>5000 CFU/mL	N
Staphylococcus aureus subsp. aureus (protein A producer) (strain NCTC 8530)	ATCC 12598	1.00E+06 CFU/mL	N
Staphylococcus aureus (MSSA, delta mecA)	ZeptoMetrix 0801675	1.00E+06 CFU/mL	N
Staphylococcus epidermidis (MRSE, RP62A)	ZeptoMetrix 0801651	1.00E+06 CFU/mL	N
Staphylococcus epidermidis (strain MSSE, HER 1292)	ZeptoMetrix 0801689	1.00E+06 CFU/mL	N
Staphylococcus epidermidis (strain PCI 1200)	ATCC 12228	1.00E+06 CFU/mL	N
Staphylococcus haemolyticus (strain Z067)	ZeptoMetrix 0801591	9.98E+05 CFU/mL	N
Streptococcus dysgalactiae	ATCC 43078	1.00E+06 CFU/mL	N
Streptococcus mitis	ZeptoMetrix 0801695	1.00E+06 CFU/mL	N

Organism	Reference	Titer Tested	Cross-Reactive Yes / No
Streptococcus pneumoniae (strain Z022, 19F)	ZeptoMetrix 0801439	1.00E+06 CFU/mL	N
Streptococcus pyogenes (strain M-3 [DLS 88002, Weller])	ATCC 51500	1.00E+06 CFU/mL	N
Streptococcus salivarius (strain 7073)	ATCC 7073	1.00E+06 CFU/mL	N
Tatlockia micdadei (strain TATLOCK [CIP 103882, NCTC 11371])	ATCC 33218	1.00E+06 CFU/mL	N
Thermanaerovibrio acidaminovorans (Guangsheng et al.; Baena et al, strain Su883 [DSM 6589])	ATCC 49978	Not available	N
Thielavia terrestris ((Apinis) Malloch et Cain, teleomorph, NRRL 8126)	ATCC 38088	Not available	N
Ureaplasma urealyticum (T-strain 960)	ATCC 27618	5.00E+06CCU/mL	N

Table 38 – Potential cross-reactivity with pathogens probed by the NxTAG RPP

Organism	Reference	Titre Tested	Cross-Reactive Yes / No
Adenovirus (Type 01 Species C)	ZeptoMetrix 0810050CF	1.00E+05 $TCID_{50}$/mL	N
Bocavirus (clinical specimen)	Saint Joseph's Hospital, Ontario (SJH), 04-0178	Not Available	N
Chlamydophila pneumoniae (strain CM-1)	ATCC VR-1360	1.00E+06 CFU/mL	N
Human coronavirus HKU1 (recombinant, in Sendai virus)	ZeptoMetrix 0810067CF	1.00E+05 $TCID_{50}$/mL	N
Human coronavirus OC43 (Betacoronavirus 1)	ATCC VR-1558	1.00E+05 $TCID_{50}$/mL	N
Human coronavirus NL63	ZeptoMetrix 0810228CF	1.00E+05 $TCID_{50}$/mL	N
Human coronavirus NL63	Saint Joseph's Hospital, Ontario (SJH)	1.00E+05 $TCID_{50}$/mL	N
Human coronavirus 229E	ATCC VR-740	2.81E+04 $TCID_{50}$/mL	N
Human coronavirus 229E	ZeptoMetrix 0810229CF	1.00E+05 $TCID_{50}$/mL	N
Human metapneumovirus (strain IA10-2003)	ZeptoMetrix VPL-030	1.00E+05 $TCID_{50}$/mL	N
Human parainfluenza virus (Type 1)	ZeptroMetrix 0810014CF	1.00E+05 $TCID_{50}$/mL	N
Human parainfluenza virus (Type 2)	ZeptoMetrix 0810015CF	1.00E+05 $TCID_{50}$/mL	N
Human parainfluenza virus (Type 3)	ZeptoMetrix 0810016CF	1.00E+05 $TCID_{50}$/mL	N
Human parainfluenza virus (Type 4A)	ZeptoMetrix 0810060CF	1.00E+05 $TCID_{50}$/mL	N
Human respiratory syncytial virus (Type A)	ZeptoMetrix 0810040ACF	1.00E+05 $TCID_{50}$/mL	N
Human respiratory syncytial virus (CH93-18(18), Type B)	ZeptoMetrix 0810040CF	1.00E+05 $TCID_{50}$/mL	N
Human rhinovirus (strain 1A)	ZeptoMetrix 0810012CFN	1.00E+05 $TCID_{50}$/mL	N
Influenza A H1N1 (A/Brisbane/59/07)	ZeptoMetrix 0810036CF	1.00E+05 $TCID_{50}$/mL	Y
Influenza A H1N1 (strain A/NWS/33)	ATCC VR-219	1.51E+05 $TCID_{50}$/mL	N
Influenza A H1N1 (strain A/WS/33)	ATCC VR-1520	1.51E+05 $TCID_{50}$/mL	N
Influenza A H1N1 (strain A1/Mal/302/54)	ATCC VR-98	1.51E+05 $TCID_{50}$/mL	N
Influenza A H1N1 (strain A/New Caledonia/20/99)	ZeptoMetrix 0810036CF	1.51E+05 $TCID_{50}$/mL	N
Influenza A H1N1 (strain A/Singapore/63/04)	ZeptoMetrix 0810246CF	1.00E+05 $TCID_{50}$/mL	Y
Influenza A H1N1 (strain A/Solomon Islands/3/2006)	ZeptoMetrix 0810036CFN	1.00E+05 $TCID_{50}$/mL	Y
Influenza A H1N1 pandemic 2009 (A/SwineNY/03/2009)	ZeptoMetrix 0810109CFN	1.00E+05 $TCID_{50}$/mL	N
Influenza A H3N2 (strain A/Victoria/3/75)	ATCC VR-822	1.00E+05 $TCID_{50}$/mL	N
Influenza B virus (B/Florida/04/2006)	ZeptoMetrix 0810037CF	1.00E+05 $TCID_{50}$/mL	N

g. Potentially Interfering Substances:

In a non-clinical study, potentially interfering substances that may be present in the nasopharynx were evaluated relative to the performance of the NxTAG RPP Assay. Single analyte and/or multiple analytes (2 – 4 different analytes probed by the NxTAG RPP assay) were spiked into UTM at 3x their respective LoDs with and without the potential interfering substance or non-panel organism. The potential interfering substances (25 total microbial and chemical) were also spiked into individual negative specimens to evaluate the effect on the device when elevated levels of potential interfering substances are present. Negative extraction controls were included in every nucleic acid extraction run. Either RNase/Dnase free water or UTM were used as negative controls. Each specimen was assayed in triplicate. The evaluated substances are listed in Table 39 with active ingredients and concentrations tested shown.

Out of 777 total positive samples analyzed during the interference study, 5 (0.64%) resulted in false negative results; 8 (1.03%) resulted in false positive results; and 764 (98.32%) resulted in correct positive calls. The five false negative samples were re-extracted and re-analyzed and all five provided correct results. Six of the eight false positive results were re-analyzed from the extracted sample and provided correct results. One false positive was confirmed as dual positive when retested from the extracted material suggesting contamination at the extraction step. Re-extraction and retest of the material in triplicate provided the correct result. One false positive was determined to be invalid due to an instrument error. The sample was re-extracted and retested in triplicate and provided the correct result.

Although FluMist did not interfere with the assay's ability to identify other analytes, as expected NxTAG RPP was able to recognize and make a positive call for the attenuated viruses present in the FluMist vaccine (Influenza A, Influenza A 2009 H1N1, Influenza A H3, and Influenza B). Positive influenza results obtained in a patient who received FluMist prior to sample collection may be due to detection of vaccine virus and may mask a true positive result due to infection by one or more of these analytes.

Table 39 – Potential interferents tested for the NxTAG RPP

Interferent	Concentration Tested	Interferent	Concentration Tested
Blood (human)	5% v/v	Cytomegalovirus	1.67E+04 $TCID_{50}$/mL
Mucin: bovine submaxillary gland, type I-S	100 µg/mL	Human Rhinovirus	1.10E+04 $TCID_{50}$/mL
Vicks DayQuil	15% v/v	Influenza A H1N1 (2009)	1.00E+05 $TCID_{50}$/mL
Drixoral	15% v/v	Measles Virus	4.20E+04 $TCID_{50}$/mL
Salinex	15% v/v	Mumps Virus	5.03E+04 $TCID_{50}$/mL
Pulmicort	25 µg/mL	RSV	2.80E+04 $TCID_{50}$/mL
Apo-Mometasone	16.7 µg/mL	*Bordetella pertussis*	1.00E+06 CFU/mL
Advair MDI 25/250 mcg	4.17 µg/mL / 41.7 µg/mL	*Corynebacterium diphtheriae*	1.00E+06 CFU/mL
Zicam Allergy Relief	5% v/v	*Neisseria meningitides*	1.00E+06 CFU/mL
Cepacol	1.25 mg/mL benzocaine / 0.17 mg/mL cetylpyridinium chloride	*Staphylococcus aureus*	1.00E+06 CFU/mL
Relenza	5 mg/mL	FluMist	0.5% v/v
Taro-Mupirocin ointment 2%	0.02%	FluMist	0.05% v/v
Tobramycin USP	0.6 mg/mL		

Competitive interference due to pathogens probed by NxTAG Respiratory Pathogen Panel was also evaluated to assess the effects of clinically relevant co-infections with pathogens probed by the assay. No interference was seen when analytes that are a part of the NxTAG RPP assay were evaluated for competitive interference with one pathogen present at a high titer (1.00E+05 $TCID_{50}$/mL), and a second pathogen at a low titer (3x LoD) as shown in table 40 below.

Table 40 – Potential interferents tested for the NxTAG RPP

High Titer Pathogen	Low Titer Pathogen
Influenza A H1	Rhinovirus
Influenza A H3	Rhinovirus
Influenza A H1	RSV A
Influenza A H3	RSV A
Influenza A H1	RSV B
Influenza A H3	RSV B
RSV B	Rhinovirus
RSV A	Adenovirus
RSV B	Adenovirus
Adenovirus	Enterovirus
Rhinovirus	Influenza A H1

High Titer Pathogen	Low Titer Pathogen
Rhinovirus	Influenza A H3
RSV A	Influenza A H1
RSV A	Influenza A H3
RSV B	Influenza A H1
RSV B	Influenza A H3
Rhinovirus	RSV B
Adenovirus	RSV A
Adenovirus	RSV B
Enterovirus	Adenovirus

h. Assay cut-off:

The NxTAG RPP data analysis algorithm uses the MAGPIX instrument outputs – median fluorescence intensity (MFI) measure – to determine the validity of a sample, followed by the multi-dimension detection (MDD) measure to make a target call of positive or negative for a valid sample. MDD is a measure resulting from the subtraction of the median MFI signal of all analytes within the sample from the signal of that particular analyte. The result is a measure that has been adjusted for the noise within the sample. During cutoff determination, both MFI and MDD thresholds were set for each target; however, only MDD cutoffs are used to determine the presence of a target.

Assay cut-off determination was performed via a three step process for each analyte: 1) setting an initial cut-off range using a validated algorithm, 2) performing Receiver Operating Characteristic (ROC) analysis of empirical data based on cut-offs within this range, and 3) establishing a cut-off value through a Design Review Committee (DRC) assessment of ROC curves. Distinct sample sets were used for setting initial cut-off ranges (step 1 above) and for finding the performance of the assay within the cut-off ranges (step 2 above). The sample sets consisted of clinical specimens, cultured isolates with confirmed viral or bacterial identity which were serially diluted into negative matrix (UTM), and multi-analyte contrived samples consisting of cultured isolates of multiple targets to simulate co-infection samples. Clinical samples were assigned a “positive” or “negative” call for the analyte in question based on results obtained at the clinical site. These results were based on the routine diagnostic algorithm at the collection site (e.g. real-time PCR, nucleic acid amplification tests followed by bi-directional sequencing). Both sample sets were supplemented with negative extraction controls (negative matrix) that were coded as negative for all targets.

The initial cut-off range for both MFI and MDD for each analyte was determined using an 830 sample training set. The signal range (from minimum to maximum signal) was divided into 100 equally spaced parameters. These 100 parameters, independently determined for each analyte and each measure (i.e. MFI, MDD),

were used to calculate the input values required by the validated threshold-setting algorithm to produce an initial range of cut-off values. A separate data set was used to determine the performance of cut-off values within the initial MFI and MDD ranges for each analyte. This performance assessment step included 189 clinical samples. For each analyte, the initial cut-off range was divided into 15 equally-spaced levels, which were used in ROC analysis of the Test Set. The objective of this step was to evaluate the cut-off recommendations for each analyte using a ROC curve. These cut-off recommendations and their performances were generated for the purpose of a formal DRC review. The DRC took into consideration the data set and performance of the MFI/MDD cut-off ranges generated in Step 2 together with technological factors such as the NxTAG RPP chemistry and the observed background signals. The established (final) cut-off values for each analyte are listed in Table 41 below.

Table 41 – Assay cut-off for the NxTAG RPP

Analyte	Final MFI Cutoff	Final MDD Cutoff
Influenza A	45	35
Influenza A H1 (H1-A)	90	75
Influenza A H1 (H1-B)	55	45
Influenza A H3	80	50
Influenza B	60	40
RSV A	50	45
RSV B	45	35
Parainfluenza virus 1	75	60
Parainfluenza virus 2	70	55
Parainfluenza virus 3	60	50
Parainfluenza virus 4 (PIV4-A)	80	60
Parainfluenza virus 4 (PIV4-B)	55	35
Coronavirus 229E	60	50
Coronavirus NL63	75	60
Coronavirus OC43	50	40
Coronavirus HKU1	65	55
Human Metapneumovirus	100	90
Rhinovirus/Enterovirus	50	40
Adenovirus	75	65
Human Bocavirus	75	65
Chlamydophila pneumoniae	45	40
Mycoplasma pneumoniae	40	30
Internal Control	100	80

i. Carryover:

A study was conducted using negative samples (UTM) alternating with replicates of a high titer purified nucleic acid sample in a checkerboard pattern. Two representative NxTAG RPP analytes were examined in separate runs, one viral (Parainfluenza 3) and one bacterial (*Mycoplasma pneumoniae*). High titer purified

viral nucleic acid samples (1.98 x 10^5 $TCID_{50}$/mL for Parainfluenza 3 and 1.0 x 10^6 CCU/mL for *Mycoplasma pneumonia*) were prepared in UTM, in order to obtain positive calls 100 percent of the time and maximize the potential for cross contamination. No carryover contamination was observed.

2. Comparison studies:

a. Method comparison with predicate device:

N/A

b. Matrix comparison:

This study was designed to compare the performance of the NxTAG RPP in an artificial matrix (UTM) with its performance in a negative clinical matrix (NCM, pooled negative clinical specimens). The performance of NxTAG RPP in these two matrices was assessed by comparing serial dilution curves of five representative NxTAG RPP targets: one DNA viral target (Human Bocavirus), one RNA viral target (Influenza A H1), and three bacterial targets (*Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, and one masked bacterial target). Ten 4-fold serial dilutions were prepared for each target from the stock material and diluted into each matrix (UTM or NCM) at concentrations ranging from assay response plateau to baseline. Samples were extracted using NucliSENS easyMag extraction method. Serial dilution curves for each target were generated by assessing three replicates at each dilution level, and the entire dilution series for both matrices was assayed in a single NxTAG RPP run. Following data analysis, Multi-Dimension Detection (MDD) values for each target's serial dilutions were used to generate system response curves and 95% confidence intervals (CI) for both matrices at the low point of $logEC_{10}$ (effective concentration 10%; i.e. the concentration that gives 10% response in the dilution curve). The data showed that the dilution curves of all five targets covered the response plateau at the highest concentration tested and reached the base line at the lowest concentration tested. There was an overlap in the 95% confidence intervals between both matrices at the low point $logEC_{10}$ for all analytes tested. Based on these results, the performance of the two matrices is considered equivalent in the NxTAG RPP assay.

c. Fresh versus frozen equivalency

A study was performed with each analyte target probed by the assay to assess storage at 2 to 8°C. Simulated specimens were prepared as multi-analyte combinations by spiking cultured organisms into Universal Transport Media (UTM) at three different concentration levels and extracted using bioMérieux NucliSENS easyMAG extraction method followed by analysis with NxTAG Respiratory Pathogen Panel. Analyte concentrations tested included low positive (1-3x LoD), moderate positive (6x LoD), and strongly positive (12x LoD). A total of 60 replicates were tested fresh (day 0), day 4, and day 7 after storage at 2 to 8°C.

Storage of un-extracted specimens at 2 to 8°C for 1 week did not alter the performance of the NxTAG Respiratory Pathogen Panel assay in comparison to testing of fresh specimens.

In addition, nucleic acid stability was evaluated after storage at -70°C to -80°C. Identical panels of specimens used in the 2 to 8°C study were extracted and the nucleic acid was either tested fresh or stored at -70°C to -80°C for 1 month, 6 months, or 12 months. Data for the one month time-point was provided while additional time-points await testing. No affect was seen in the performance when extracted nucleic acid was stored at -70°C to -80°C for 1 month. Specimens used in the clinical study support the storage of un-extracted specimens at -70°C to -80°C for at least 12 months.

3. Clinical studies:

a. Clinical Sensitivity and Specificity

Prospective clinical study

The clinical performance of the NxTAG RPP assay was established in two prospective phases using nasopharyngeal swabs (NPS) prospectively collected from pediatric or adult patients suspected of having respiratory tract infection during the 2013/2014 and 2014/2015 flu seasons. In addition to the prospective specimens collected during the two influenza seasons, performance was also examined using a pre-selected (banked) set and a contrived specimen set.

In the first phase of the prospective study (2013/2014 flu season), specimens were collected between January 29 and April 09, 2014 and tested at 2 clinical sites located in the United States. Clinical specimens accrued during the second phase of the prospective study (2014/2015 flu season) were collected between January 18 and March 20, 2015 and tested at 3 clinical sites located in the United States and Canada. The clinical specimen collection sites were chosen based on the types of patients usually referred to them, and the prevalence of respiratory pathogens. A total of 2209 specimens were collected of which 77 did not meet the inclusion criteria. The remaining 2132 clinical specimens were used for the prospective data set. Of these, 934 were collected during the 2013/2014 Flu season and the remaining 1198 specimens were enrolled during the 2014/2015 Flu season. There were 101 initial invalid results (4.7%); all invalids were retested as per the clinical protocol and generated valid results on retest. Demographic information for the combined prospective dataset is provided in Table 42 below.

Table 42 – Demographic details for the combined prospective dataset

Gender	Site 1	Site 2	Site 3	Site 4	All Sites
Male	322 (47.7%)	155 (41.3%)	264 (53.0%)	281 (48.1%)	1022 (47.9%)
Female	353 (52.3%)	220 (58.7%)	234 (47.0%)	303 (51.9%)	1110 (52.1%)
Total	**675**	**375**	**498**	**584**	**2132**
AGE (yrs)					
0 – 1	105 (15.6%)	25 (6.7%)	155 (31.1%)	168 (28.8%)	453 (21.2%)
>1 – 5	66 (9.8%)	22 (5.9%)	92 (18.5%)	70 (12.0%)	250 (11.7%)
>5 – 21	87 (12.9%)	73 (19.5%)	101 (20.3%)	92 (15.8%)	353 (16.6%)
>21 – 65	174 (25.8%)	152 (40.5%)	111 (22.3%)	147 (25.2%)	584 (27.4%)
>65	243 (36.0%)	103 (27.5%)	39 (7.8%)	107 (18.3%)	492 (23.1%)
Total	**675**	**375**	**498**	**584**	**2132**
SUBJECT STATUS					
Outpatients	309 (45.8%)	157 (41.9%)	49 (9.8%)	39 (6.7%)	554 (26.0%)
Hospitalized	255 (37.8%)	144 (38.4%)	332 (66.7%)	329 (56.3%)	1060 (49.7%)
Emergency Department	111 (16.4%)	74 (19.7%)	117 (23.5%)	216 (37.0%)	518 (24.3%)
Total	**675**	**375**	**498**	**584**	**2132**
IMMUNE STATUS					
Immuno-compromised	116 (17.2%)	89 (23.7%)	0 (0.0%)	59 (10.1%)	264 (12.4%)
Immuno-competent	506 (75.0%)	285 (76.0%)	0 (0.0%)	525 (89.9%)	1316 (61.7%)
Not Determined	53 (7.9%)	1 (0.3%)	498 (100%)	0 (0.0%)	552 (25.9%)
Total	**675**	**375**	**498**	**584**	**2132**

All prospective clinical specimens were analyzed by comparator methods for each analyte target at a centralized testing facility (Luminex Molecular Diagnostic, Toronto, ON). An FDA-cleared molecular assay was used as the comparator method for the following targets: Influenza A H3, Influenza B, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3 and Human Metapneumovirus. Amplification followed by bi-directional sequencing (using validated primers) directly from extracted clinical specimens using two nucleic acid amplification tests (NAATs) was used as the comparator method for the following targets: Adenovirus, Influenza A (matrix), Influenza A H1, Parainfluenza 4, Coronavirus 229E, Coronavirus OC43, Coronavirus NL63, Coronavirus HKU1, Rhinovirus/Enterovirus, Human Bocavirus, *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*.

The clinical performance of the NxTAG Respiratory Pathogen Panel assay in the

prospective study (N=2132) is summarized for each individual target in Table 43 below.

Table 43 – NxTAG RPP Assay Clinical Performance (Prospective dataset)

Target	PPA		95% CI	NPA		95% CI	# "No Call"
Influenza A	259/273[1]	94.9%	91.5% - 97.2%	1822/1859[2]	98.0%	97.3% - 98.6%	0
Influenza A H1	21/21	100%	83.9% - 100%	2091/2111	99.1%	98.5% - 99.4%	0
Influenza A H3	203/206[3]	98.5%	95.8% - 99.7%	1872/1917[4]	97.7%	96.9% - 98.3%	9
Influenza B	87/91[5]	95.6%	89.1% - 98.8%	2019/2033[6]	99.3%	98.8% - 99.6%	8
RSV A	73/73	100%	95.1% - 100%	2037/2052[7]	99.3%	98.8% - 99.6%	7
RSV B	131/133	98.5%	94.7% - 99.8%	1978/1990[8]	99.4%	98.9% - 99.7%	9
Coronavirus 229E	21/21	100%	83.9% - 100%	2098/2111	99.4%	98.9% - 99.7%	0
Coronavirus OC43	30/31	96.8%	83.3% - 99.9%	2092/2101	99.6%	99.2% - 99.8%	0
Coronavirus NL63	62/65	95.4%	87.1% - 99.0%	2053/2065	99.4%	99.0% - 99.7%	2[9]
Coronavirus HKU1	13/14	92.9%	66.1% - 99.8%	2113/2118	99.8%	99.4% - 99.9%	0
Human Metapneumovirus	135/144[10]	93.8%	88.5% - 97.1%	1958/1976[11]	99.1%	98.6% - 99.5%	12
Rhinovirus /Enterovirus	286/300[12]	95.3%	92.3% - 97.4%	1764/1832[13]	96.3%	95.3% - 97.1%	0
Adenovirus	20/20	100%	83.2% - 100%	2078/2112[14]	98.4%	97.8% - 98.9%	0
Parainfluenza 1	5/5	100%	47.8% - 100%	2115/2116	99.9%	99.7% - 100%	11
Parainfluenza 2	1/2[15]	50.0%	1.3% - 98.7%	2121/2122	99.9%	99.7% - 100%	8
Parainfluenza 3	20/21[16]	95.2%	76.2% - 99.9%	2086/2103[17]	99.2%	98.7% - 99.5%	8
Parainfluenza 4	3/5	60.0%	14.7% - 94.7%	2116/2127	99.5%	99.1% - 99.7%	0
Human Bocavirus[18]	27/28	96.4%	81.7% - 99.9%	2081/2104	98.9%	98.4% - 99.3%	0
Chlamydophila pneumoniae[18]	0/1	0.0%	0.0% - 97.5%	2131/2131	100%	99.8% - 100%	0
Mycoplasma pneumoniae[18]	7/9	77.8%	40.0% - 97.2%	2121/2123	99.9%	99.7% - 100%	0

[1] All fourteen (14) NxTAG Respiratory Pathogen Panel Flu A negative specimens that were positive by the reference method (i.e. False Negative) were negative by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).

[2] Twelve (12) NxTAG Respiratory Pathogen Panel Flu A positive specimens that were negative by the reference method (i.e. False Positive) were positive by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).

[3] Two (2) NxTAG Respiratory Pathogen Panel Flu A H3 negative specimens that were positive by the reference method (i.e. False Negative) were confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.

[4] Thirty-four (34) NxTAG Respiratory Pathogen Panel Flu A H3 positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis

using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.
[5] Three (3) NxTAG Respiratory Pathogen Panel Flu B negative specimens that were positive by the reference method (i.e. False Negative) were confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.
[6] Four (4) NxTAG Respiratory Pathogen Panel Flu B positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.
[7] Eight (8) NxTAG Respiratory Pathogen Panel Respiratory Syncytial Virus A positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.
[8] Three (3) NxTAG Respiratory Pathogen Panel Respiratory Syncytial Virus B positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.
[9] Two (2) clinical specimens did not have sufficient volume for confirmatory sequencing (QNS).
[10] Six (6) NxTAG Respiratory Pathogen Panel Human Metapneumovirus negative specimens that were positive by the reference method (i.e. False Negative) were confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.
[11] Two (2) NxTAG Respriatory Pathogen Panel Human Metapneumovirus positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.
[12] Eight (8) NxTAG Respiratory Pathogen Panel Rhinovirus/Enterovirus negative specimens that were positive by the reference method (i.e. False Negative) were negative by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).
[13] Eighteen (18) NxTAG Respiratory Pathogen Panel Rhinovirus/Enterovirus positive specimens that were negative by the reference method (i.e. False Positive) were positive by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).
[14] Two (2) NxTAG Respiratory Pathogen Panel Adenovirus positive specimens that were negative by the reference method (i.e. False Positive) were positive by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).
[15] The one (1) NxTAG Respiratory Pathogen Panel Parainfluenza 2 negative specimen that was positive by the reference method (i.e. False Negative) was confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.
[16] The one (1) NxTAG Respiratory Pathogen Panel Parainfluenza 3 negative specimen that was positive by the reference method (i.e. False Negative) was confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.
[17] Two (2) NxTAG Respiratory Pathogen Panel Parainfluenza 3 positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.
[18] Site testing results on discrepant specimens were unavailable for Human Bocavirus, *Chlamydophila pneumonia*, and *Mycoplasma pneumoniae.*

Table 44 - 3x3 Table for Influenza A (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	259	36[2]	0	295
Negative	14[1]	1822	0	1836
No Call	0	1	0	1
TOTAL	273	1859	0	2132
		95% CI		
Positive Percent Agreement	94.9%	91.5% - 97.2%		
Negative Percent Agreement	98.0%	97.3% - 98.6%		

[1] All fourteen (14) NxTAG Respiratory Pathogen Panel negative specimens that were positive by the reference method (i.e. False Negative) were negative by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).
[2] Twelve (12) NxTAG Respiratory Pathogen Panel positive specimens that were negative by the reference method (i.e. False Positive) were positive by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).

Table 45 - 3x3 Table for Influenza A H1 (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	21	20	0	41
Negative	0	2091	0	2091
No Call	0	0	0	0
TOTAL	21	2111	0	2132
		95% CI		
Positive Percent Agreement	100%	83.9% - 100%		
Negative Percent Agreement	99.1%	98.5% - 99.4%		

Table 46 - 3x3 Table for Influenza A H3 (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	203	45[2]	1	249
Negative	3[1]	1872	8	1883
No Call	0	0	0	0
TOTAL	206	1917	9	2132
		95% CI		
Positive Percent Agreement	98.5%	95.8% - 99.7%		
Negative Percent Agreement	97.7%	96.9% - 98.3%		

[1] Two (2) NxTAG Respiratory Pathogen Panel negative specimens that were positive by the reference method (i.e. False Negative) were confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.

[2] Thirty-four (34) NxTAG Respiratory Pathogen Panel positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.

Table 47 - 3x3 Table for Influenza B (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	87	14[2]	0	101
Negative	4[1]	2019	8	2031
No Call	0	0	0	0
TOTAL	91	2033	8	2132
		95% CI		
Positive Percent Agreement	95.6%	89.1% - 98.8%		
Negative Percent Agreement	99.3%	98.8% - 99.6%		

[1] Three (3) NxTAG Respiratory Pathogen Panel negative specimen that was positive by the reference method (i.e. False Negative) was confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.

[2] Four (4) NxTAG Respiratory Pathogen Panel positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.

Table 48 - 3x3 Table for RSV A (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	73	15[1]	0	88
Negative	0	2037	7	2044
No Call	0	0	0	0
TOTAL	73	2052	7	2132
		95% CI		
Positive Percent Agreement	100%	95.1% - 100%		
Negative Percent Agreement	99.3%	98.8% - 99.6%		

[1] Eight (8) NxTAG Respiratory Pathogen Panel positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.

Table 49 - 3x3 Table for RSV B (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	131	12[1]	1	144
Negative	2	1978	8	1988
No Call	0	0	0	0
TOTAL	133	1990	9	2132
		95% CI		
Positive Percent Agreement	98.5%	94.7% - 99.8%		
Negative Percent Agreement	99.4%	98.9% - 99.7%		

[1] Three (3) NxTAG Respiratory Pathogen Panek positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.

Table 50 - 3x3 Table for Coronavirus 229E (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	21	13	0	34
Negative	0	2098	0	2098
No Call	0	0	0	0
TOTAL	21	2111	0	2132
		95% CI		
Positive Percent Agreement	100%	83.9% - 100%		
Negative Percent Agreement	99.4%	98.9% - 99.7%		

Table 51 - 3x3 Table for Coronavirus OC43 (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	30	9	0	39
Negative	1	2092	0	2093
No Call	0	0	0	0
TOTAL	31	2101	0	2132
		95% CI		
Positive Percent Agreement	96.8%	83.3% - 99.9%		
Negative Percent Agreement	99.6%	99.2% - 99.8%		

Table 52 - 3x3 Table for Coronavirus NL63 (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	62	12	0	74
Negative	3	2053	2[1]	2058
No Call	0	0	0	0
TOTAL	65	2065	2	2132
		95% CI		
Positive Percent Agreement	95.4%	87.1% - 99.0%		
Negative Percent Agreement	99.4%	99.0% - 99.7%		

[1] Two (2) clinical specimens did not have sufficient volume for confirmatory sequencing

Table 53 - 3x3 Table for Coronavirus HKU1 (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	13	5	0	18
Negative	1	2113	0	2114
No Call	0	0	0	0
TOTAL	14	2118	0	2132
		95% CI		
Positive Percent Agreement	92.9%	66.1% - 99.8%		
Negative Percent Agreement	99.8%	99.4% - 99.9%		

Table 54 - 3x3 Table for Human Metapneumovirus (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	135	18[2]	1	154
Negative	9[1]	1958	11	1978
No Call	0	0	0	0
TOTAL	144	1976	12	2132
		95% CI		
Positive Percent Agreement	93.8%	88.5% - 97.1%		
Negative Percent Agreement	99.1%	98.6% - 99.5%		

[1] Six (6) NxTAG Respiratory Pathogen Panel negative specimens that were positive by the reference method (i.e. False Negative) were confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.

[2] Two (2) NxTAG Respiratory Pathogen Panel positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.

Table 55 - 3x3 Table for Parainfluenza virus 1 (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	5	1	0	6
Negative	0	2115	11	2226
No Call	0	0	0	0
TOTAL	5	2116	11	2132
		95% CI		
Positive Percent Agreement	100%	47.8% - 100%		
Negative Percent Agreement	99.9%	99.7% - 100%		

Table 56 - 3x3 Table for Parainfluenza virus 2 (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	1	1	0	2
Negative	1[1]	2121	8	2130
No Call	0	0	0	0
TOTAL	2	2122	8	2132
		95% CI		
Positive Percent Agreement	50.0%	1.3% - 98.7%		
Negative Percent Agreement	99.9%	99.7% - 100%		

[1] The one (1) NxTAG Respiratory Pathogen Panel negative specimen that was positive by the reference method (i.e. False Negative) was confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.

Table 57 - 3x3 Table for Parainfluenza virus 3 (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	20	17[2]	1	38
Negative	1[1]	2086	7	2094
No Call	0	0	0	0
TOTAL	21	2103	8	2132
		95% CI		
Positive Percent Agreement	95.2%	76.2% - 99.9%		
Negative Percent Agreement	99.2%	98.7% - 99.5%		

[1] The one (1) NxTAG Respiratory Pathogen Panel negative specimen that was positive by the reference method (i.e False Negative) was confirmed as negative by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.

[2] Two (2) NxTAG Respiratory Pathogen Panel positive specimens that were negative by the reference method (i.e. False Positive) were confirmed as positive by bi-directional sequencing analysis using analytically validated primers that targeted genomic regions distinct from the NxTAG Respiratory Pathogen Panel Assay.

Table 58 - 3x3 Table for Parainfluenza virus 4 (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	3	11	0	14
Negative	2	2116	0	2118
No Call	0	0	0	0
TOTAL	5	2127	0	2132
		95% CI		
Positive Percent Agreement	60.0%	14.7% - 94.7%		
Negative Percent Agreement	99.5%	99.1% - 99.7%		

Table 59 - 3x3 Table for Rhinovirus/Enterovirus (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	286	68[2]	0	354
Negative	14[1]	1764	0	1778
No Call	0	0	0	0
TOTAL	300	1832	0	2132
		95% CI		
Positive Percent Agreement	95.3%	92.3% - 97.4%		
Negative Percent Agreement	96.3%	95.3% - 97.1%		

[1] Eight (8) NxTAG Respiratory Pathogen Panel negative specimens that were positive by the reference method (i.e. False Negative) were negative by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).

[2] Eighteen (18) NxTAG Respiratory Pathogen Panel positive specimens that were negative by the reference method (i.e. False Positive) were positive by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).

Table 60 - 3x3 Table for Adenovirus (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	20	34[1]	0	54
Negative	0	2078	0	2078
No Call	0	0	0	0
TOTAL	20	2112	0	2132
		95% CI		
Positive Percent Agreement	100%	83.2% - 100%		
Negative Percent Agreement	98.4%	97.8% - 98.9%		

[1] Two (2) NxTAG Respiratory Pathogen Panel positive specimens that were negative by the reference method (i.e. False Positive) were positive by the method routinely used at the clinical sites (FDA-cleared RT-PCR assay or site-validated real-time RT PCR).

Table 61 - 3x3 Table for Human Bocavirus (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	27	23	0	50
Negative	1	2081	0	2082
No Call	0	0	0	0
TOTAL	28	2104	0	2132
		95% CI		
Positive Percent Agreement	96.4%	81.7% - 99.9%		
Negative Percent Agreement	98.9%	98.4% - 99.3%		

Table 62 - 3x3 Table for *Chlamydophila pneumoniae* (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	0	0	0	0
Negative	1	2131	0	2132
No Call	0	0	0	0
TOTAL	1	2131	0	2132
		95% CI		
Positive Percent Agreement	0.0%	0.0% - 97.5%		
Negative Percent Agreement	100%	99.8% - 100%		

Table 63 - 3x3 Table for *Mycoplasma pneumoniae* (Prospective Sample Set)

NxTAG RPP	Reference			
	Positive	Negative	No Call	TOTAL
Positive	7	2	0	9
Negative	2	2121	0	2123
No Call	0	0	0	0
TOTAL	9	2123	0	2132
		95% CI		
Positive Percent Agreement	77.8%	40.0% - 97.2%		
Negative Percent Agreement	99.9%	99.7% - 100%		

NxTAG RPP detected a total of 96 mixed infections in the first phase of the prospective clinical evaluation (2013/2014 flu season). This represents 18.6% of the total number of NxTAG RPP positive specimens (96/517) during that study period. Eighty-four (84/96; 87.5%) were double infections, 8 (8/96; 8.3%) were triple infections, 3 (3/96; 3.1%) were quadruple infections and 1 (1/96; 1.0%) was quintuple infection. The single most common co-infection (9/96; 9.4%) was Human Metapneumovirus with Rhinovirus/Enterovirus. Out of the 96 co-infections, 47 contained one or more analytes that had not been detected with the reference/comparator methods, i.e. discrepant co-infections. Distinct co-infection combinations detected by NxTAG RPP during the first phase of the prospective study (2013/2014 flu season) are summarized in Table 64**.**

Table 64 – Distinct co-infections detected by the NxTAG RPP in the prospective clinical study (January 2014 to April 2014)

Distinct Co-infection Combination Detected by NxTAG® RPP					Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	**Target 2**	**Target 3**	**Target 4**	**Target 5**			
CoV 229E	Rhino/Entero				1	1	CoV 229E (x1);
CoV 229E	HMPV				3	3	CoV 229E (x3);
CoV HKU1	Adenovirus				1	0	
CoV HKU1	Rhino/Entero				1	0	
CoV HKU1	HMPV				2	0	
CoV NL63	Rhino/Entero				3	2	CoV NL63 (x2); Rhino/Entero (x1);
CoV NL63	HMPV				1	0	
Rhino/Entero	Adenovirus				4	1	Adenovirus (x1);
Rhino/Entero	HBoV				9	5	Rhino/Entero (x1); HBoV (x5);
Rhino/Entero	*M. Pneumoniae*				1	0	
HMPV	Adenovirus				3	3	HMPV (x2); Adenovirus (x3);
HMPV	Rhino/Entero				9	4	HMPV (x1); Rhino/Entero (x3);
HMPV	HBoV				4	3	HBoV (x3);
Flu A H1	CoV 229E				2	2	Flu A H1 (x2);
Flu A H1	CoV HKU1				2	1	Flu A H1 (x1);
Flu A H1	Rhino/Entero				1	1	Flu A H1; Rhino/Entero (x1);
Flu A H1	HMPV				1	0	
Flu A H1	Flu A H3				1	1	Flu A H3 (x1);

Distinct Co-infection Combination Detected by NxTAG® RPP					Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	**Target 2**	**Target 3**	**Target 4**	**Target 5**			
Flu A H3	CoV 229E				1	0	
Flu A H3	CoV NL63				2	0	
Flu A H3	Rhino/Entero				4	2	Flu A H3 (x1); Rhino/Entero (x1);
Flu A H3	RSV A				1	1	Flu A H3 (x1); RSV A (x1);
Flu B	Adenovirus				1	1	Adenovirus (x1);
Flu B	CoV 229E				1	1	CoV 229E (x1);
Flu B	Rhino/Entero				1	1	Rhino/Entero (x1);
Flu B	RSV B				1	0	
RSV A	Rhino/Entero				3	1	Rhino/Entero (x1);
RSV A	HBoV				1	1	HBoV (x1);
RSV A	PIV 1				1	0	
RSV A	PIV 4				1	1	PIV 4 (x1);
RSV A	RSV B				2	1	RSV A (x1); RSV B (x1);
RSV B	Adenovirus				1	1	Adenovirus (x1);
RSV B	CoV 229E				1	1	CoV 229E (x1);
RSV B	CoV NL63				1	0	
RSV B	CoV OC43				1	0	
RSV B	Rhino/Entero				8	1	Rhino/Entero (x1);
RSV B	HBoV				3	1	HBoV (x1);
Adenovirus	PIV 3	PIV 4			1	1	Adenovirus (x1); PIV 4 (x1);
CoV NL63	Rhino/Entero	HBoV			2	0	
Flu A H1	CoV 229E	CoV NL63			1	1	Flu A H1 (x1); CoV NL63 (x1);
Flu A H3	CoV NL63	Rhino/Entero			1	0	
RSV B	CoV NL63	Rhino/Entero			1	0	
RSV B	CoV NL63	HMPV			2	2	RSV B (x1); CoV NL63 (x1); HMPV (x1);
Flu A H1	PIV 3	PIV 4	*M. Pneumoniae*		1	1	*M. Pneumoniae* (x1);
Flu B	HMPV	PIV 3	PIV 4		1	1	HMPV (x1); PIV 4 (x1);
RSV B	CoV HKU1	Rhino/Entero	Adenovirus		1	0	

Distinct Co-infection Combination Detected by NxTAG® RPP					Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	**Target 2**	**Target 3**	**Target 4**	**Target 5**			
RSV B	CoV NL63	HMPV	Rhino/Entero	HBoV	1	0	
Total Co-infections					**96**	**47**	
Double Infections					**84**	**41**	
Triple Infections					**8**	**4**	
Quadruple Infections					**3**	**2**	
Quintuple Infections					**1**	**0**	

* A discrepant co-infection or discrepant analyte was defined as one that was detected by NxTAG RPP but not by the reference/comparator methods.
Note: the following abbreviations are used for Table 64: Flu A=Influenza A; CoV=Coronavirus; Rhino/Entero = Rhinovirus/Enterovirus; HMPV= Human Metapneumovirus; HBoV=Human Bocavirus; *M. pneumonia= Mycoplasma pneumoniae*; RSV= Respiratory Syncytial Virus; PIV=Parainfluenza

During the second phase of the prospective clinical evaluation (2014/2015 flu season), NxTAG RPP detected a total of 120 mixed infections. This represents 17.3% of the total number of NxTAG RPP positive specimens (120/694) during that study period. Ninety seven (97/120; 80.8%) were double infections, 15 (15/120; 12.5%) were triple infections and 8 (8/120; 6.7%) were quadruple infections. The single most common co-infection (7/120; 5.8%) was Adenovirus with Rhinovirus/Enterovirus. Out of the 120 co-infections, 75 contained one or more analytes that had not been detected with the reference/comparator methods, i.e. discrepant co-infections. Distinct co-infection combinations detected by NxTAG RPP during the second phase of the prospective study (2014/2015 flu season) are summarized in Table 65.

Table 65 – Distinct co-infections detected by the NxTAG RPP in the prospective clinical study (January 2015 to March 2015)

Distinct Co-infection Combination Detected by NxTAG® RPP					Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	Target 2	Target 3	Target 4	Target 5			
Adenovirus	HBoV			1	0		Adenovirus
Adenovirus	*M. Pneumoniae*			1	1	Adenovirus (x1); *M. Pneumoniae* (x1);	Adenovirus
Adenovirus	PIV 3			2	1	PIV 3 (x1);	Adenovirus
CoV 229E	CoV NL63			1	0		CoV 229E
CoV 229E	HBoV			1	0		CoV 229E
CoV 229E	HMPV			1	0		CoV 229E
CoV 229E	PIV 3			1	0		CoV 229E
CoV HKU1	PIV 1			1	1	CoV HKU1 (x1); PIV 1 (x1);	CoV HKU1
CoV NL63	Adenovirus			1	1	Adenovirus (x1);	CoV NL63
CoV NL63	Rhino/Entero			2	1	Rhino/Entero (x1);	CoV NL63
CoV NL63	HMPV			3	1	HMPV (x1);	CoV NL63
CoV NL63	PIV 4			1	1	CoV NL63 (x1); PIV 4 (x1);	CoV NL63
CoV OC43	Adenovirus			1	1	Adenovirus (x1);	CoV OC43
CoV OC43	CoV NL63			1	0		CoV OC43
CoV OC43	Rhino/Entero			1	0		CoV OC43
CoV OC43	HBoV			1	1	HBoV (x1);	CoV OC43
CoV OC43	HMPV			1	1	HMPV (x1);	CoV OC43
Rhino/Entero	Adenovirus			7	7	Rhino/Entero (x7); Adenovirus (x7);	Rhino/Entero
Rhino/Entero	HBoV			3	3	HBoV (x3);	Rhino/Entero
Rhino/Entero	PIV 3			2	2	Rhino/Entero (x1); PIV 3 (x1);	Rhino/Entero
HMPV	Rhino/Entero			4	1	HMPV (x1);	HMPV
HMPV	PIV 3			1	0		HMPV
Flu A (unsubtypeable)	Rhino/Entero			1	1	Flu A (matrix gene) (x1);	Flu A (unsubtypeable)

Distinct Co-infection Combination Detected by NxTAG® RPP					Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	**Target 2**	**Target 3**	**Target 4**	**Target 5**			
Flu A (unsubtypeable)	RSV B			1	1	Flu A (matrix gene) (x1);	Flu A (unsubtypeable)
Flu A H1	CoV NL63			1	1	CoV NL63 (x1);	Flu A H1
Flu A H3	CoV 229E			1	1	Flu A H3(x1);	Flu A H3
Flu A H3	CoV HKU1			1	1	CoV HKU1 (x1);	Flu A H3
Flu A H3	CoV NL63			2	0		Flu A H3
Flu A H3	CoV OC43			2	0		Flu A H3
Flu A H3	Rhino/Entero			6	3	Rhino/Entero (x3);	Flu A H3
Flu A H3	HBoV			2	0		Flu A H3
Flu A H3	HMPV			4	3	HMPV (x3);	Flu A H3
Flu A H3	Flu B			1	1	Flu B (x1);	Flu A H3
Flu A H3	PIV 1			1	1	Flu A H3(x1);	Flu A H3
Flu A H3	PIV 2			1	1	PIV 2 (x1);	Flu A H3
Flu A H3	PIV 3			1	1	PIV 3 (x1);	Flu A H3
Flu A H3	RSV A			4	3	Flu A H3 (x1); RSV A (x3);	Flu A H3
Flu B	Adenovirus			1	1	Adenovirus (x1);	Flu B
Flu B	Rhino/Entero			4	4	Flu B (x2); Rhino/Entero (x2);	Flu B
Flu B	HBoV			1	1	Flu B (x1);	Flu B
Flu B	RSV A			2	2	Flu B (x1); RSV A (x1);	Flu B
Flu B	RSV B			1	1	RSV B (x1);	Flu B
PIV 3	HBoV			1	0		PIV 3
RSV A	CoV OC43			1	1	CoV OC43 (x1);	RSV A
RSV A	Rhino/Entero			4	1	Rhino/Entero (x1);	RSV A
RSV A	HBoV			1	0		RSV A
RSV A	HMPV			1	1	RSV A (x1);	RSV A
RSV A	PIV 3			1	0		RSV A
RSV B	CoV NL63			1	0		RSV B
RSV B	CoV OC43			1	0		RSV B
RSV B	Rhino/Entero			5	2	RSV B (x1); Rhino/Entero	RSV B

Distinct Co-infection Combination Detected by NxTAG® RPP					Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	Target 2	Target 3	Target 4	Target 5			
						(x1);	
RSV B	HBoV			2	1	HBoV (x1);	RSV B
RSV B	HMPV			2	0		RSV B
RSV B	PIV 4			1	0		RSV B
CoV NL63	Rhino/Entero	Adenovirus		1	0		CoV NL63
Rhino/Entero	Adenovirus	PIV 3		1	1	Adenovirus (x1);	Rhino/Entero
HMPV	Adenovirus	HBoV		1	1	Adenovirus (x1); HBoV (x1);	HMPV
HMPV	Adenovirus	PIV 4		1	1	Adenovirus (x1); PIV 4 (x1);	HMPV
Flu A (unsubtypeable)	Rhino/Entero	Adenovirus		1	1	Flu A (matrix gene) (x1); Rhino/Entero (x1); Adenovirus (x1);	Flu A (unsubtypeable)
Flu A H1	RSV B	CoV 229E		1	1	Flu A H1 (x1);	Flu A H1
Flu A H3	CoV HKU1	PIV 3		1	1	CoV HKU1 (x1); PIV 3 (x1);	Flu A H3
Flu A H3	CoV OC43	HMPV		1	1	HMPV (x1);	Flu A H3
Flu A H3	Rhino/Entero	PIV 3		1	1	Flu A H3 (x1); PIV 3 (x1);	Flu A H3
Flu A H3	RSV A	Rhino/Entero		1	1	Flu A H3 (x1); RSV A (x1); Rhino/Entero (x1);	Flu A H3
Flu B	Adenovirus	PIV 3		1	1	Flu B (x1); Adenovirus (x1); PIV 3 (x1);	Flu B
Flu B	Rhino/Entero	Adenovirus		1	1	Rhino/Entero (x1); Adenovirus (x1);	Flu B
RSV A	RSV B	CoV NL63		1	0		RSV A
RSV A	RSV B	Rhino/Entero		1	1	RSV A (x1);	RSV A
RSV B	Rhino/Entero	HBoV		1	0		RSV B
CoV 229E	Adenovirus	PIV 3	PIV 4	1	1	CoV 229E (x1); Adenovirus (x1); PIV 4 (x1);	CoV 229E
CoV 229E	CoV NL63	Rhino/Entero	Adenovirus	1	1	Rhino/Entero (x1);	CoV 229E

Distinct Co-infection Combination Detected by NxTAG® RPP					Total # co-infections	# discrepant results*	Discrepant Analytes*
Target 1	**Target 2**	**Target 3**	**Target 4**	**Target 5**			
						Adenovirus (x1);	
CoV NL63	Rhino/Entero	PIV 3	PIV 4	1	1	CoV NL63 (x1); Rhino/Entero (x1); PIV 4 (x1);	CoV NL63
HMPV	Adenovirus	PIV 3	HBoV	1	0		HMPV
Flu A H3	CoV NL63	PIV 3	PIV 4	1	1	PIV 4 (x1);	Flu A H3
Flu B	CoV 229E	CoV HKU1	Rhino/Entero	1	1	Flu B (x1); CoV 229E (x1); CoV HKU1 (x1); Rhino/Entero (x1);	Flu B
Flu B	CoV NL63	HMPV	Rhino/Entero	1	1	HMPV (x1);	Flu B
RSV B	CoV 229E	PIV 3	PIV 4	1	1	CoV 229E (x1); PIV 4 (x1);	RSV B
Total Co-infections					**120**	**75**	
Double Infections					**97**	**56**	
Triple Infections					**15**	**12**	
Quadruple Infections					**8**	**7**	

* A discrepant co-infection or discrepant analyte was defined as one that was detected by NxTAG RPP but not by the reference/comparator methods.

Note: the following abbreviations are used for Table 64: Flu A=Influenza A; CoV=Coronavirus; Rhino/Entero = Rhinovirus/Enterovirus; HMPV= Human Metapneumovirus; HBoV=Human Bocavirus; *M. pneumonia= Mycoplasma pneumoniae*; RSV= Respiratory Syncytial Virus; PIV=Parainfluenza

Pre-selected Clinical Specimens

Due to low prevalence rates of some of the pathogens in the NxTAG Respiratory Pathogen Panel, an additional study was conducted and the prospective sample set was supplemented with banked (pre-selected) positive specimens collected at selected sites. In order to minimize bias, pre-selected positive specimens were tested along with negative clinical specimens in a randomized, blinded fashion at 4 testing sites (3 of which were external to Luminex). The results from pre-selected specimens were analyzed separately from those of the prospective data set and performance of the assay was calculated as Positive Percent Agreement (PPA). Table 66 provides a summary of the subject demographic information from the 326 nasopharyngeal swabs that were included in the data analysis of the pre-selected study.

Table 66 – Demographic information for the pre-selected dataset

SEX	NUMBER OF SUBJECTS
Male	179 (54.9%)
Female	147 (45.1%)
Not known	0 (0.0%)
Total	**326**
AGE (yrs)	
0 - 1	75 (23.0%)
>1 - 5	76 (23.3%)
>5 - 21	40 (12.3%)
>21 - 65	83 (25.4%)
>65	52 (16.0%)
Not known	0 (0.0%)
Total	**326**

Table 67 – Positive percent agreement for the NxTAG RPP in the pre-selected dataset

Target	Positive Agreement		95%CI for Positive Agreement
	TP / (TP+FN)	Percent	
Adenovirus	30/30	100%	88.6% - 100%
Influenza A H1	35/35	100%	90.1% - 100%
Parainfluenza 1	38/38	100%	90.8% - 100%
Parainfluenza 2	33/33	100%	89.6% - 100%
Parainfluenza 3	34/34	100%	89.8% - 100%
Parainfluenza 4	41/42	97.6%	87.7% - 99.6%
Coronavirus 229E	17/17	100%	81.6% - 100%
Coronavirus OC43	16/16	100%	80.6% - 100%
Coronavirus NL63	15/15	100%	79.6% - 100%
Coronavirus HKU1	44/49	89.8%	78.2% - 95.6%
Enterovirus D68	14/14	100%	78.5% - 100%
Chlamydophila pneumoniae	2/2	100%	34.2% - 100%
Mycoplasma pneumoniae	4/4	100%	51.0% - 100%

Contrived Samples

Due to the limited number of samples positive for atypical bacteria, an additional study was performed using contrived *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* specimens. Contrived samples were prepared by spiking varying

concentrations of pathogen into negative clinical specimens. Fifty (50) contrived specimens for each of the two atypical bacteria were prepared for testing based on clinically relevant titers as reported in published scientific literature. A summary of the contrived sample set is provided in Table 68 below. Contrived specimens were tested along with 50 distinct negative clinical specimens in a randomized, blinded fashion at 3 testing sites as per pre-selected specimens. A limitation was added to the labeling to inform the users of the use of contrived specimens for *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* for performance evaluation.

Table 68 – Contrived Sample Set

Analyte	Strain Information	Source	Spiking Level (copies/mL)	Number of Samples
Negative	N/A	N/A	0	50
Chlamydophila pneumoniae	AR39	ATCC 53592	10^2	5
Chlamydophila pneumoniae	TWAR strain 2023	ATCC VR-1356	10^3	15
Chlamydophila pneumoniae	J-21	ATCC VR-1435	10^3	15
Chlamydophila pneumoniae	AO3	ATCC VR-1452	10^4	5
Chlamydophila pneumoniae	CM-1	ATCC VR-1360	10^5	5
Chlamydophila pneumoniae	TW-183	ATCC VR-2282	10^6	5
Mycoplasma pneumoniae	UTMB-10P	ATCC 49894	10^2	5
Mycoplasma pneumoniae	[Mac] (Type 2)	ATCC 15492	10^3	15
Mycoplasma pneumoniae	M129-B7 (Type 1)	ATCC 29342	10^3	15
Mycoplasma pneumoniae	PI 1428 (Type 1)	ATCC 29085	10^4	5
Mycoplasma pneumoniae	[Bru]	ATCC 15377	10^6	5
Mycoplasma pneumoniae	FH strain of Eaton Agent [NCTC 10119]; (Type 2)	ATCC 15531-TTR	10^8	5

Table 69 – Positive and Negative Agreement of NxTAG RPP for the *Chlamydophila pneumoniae* contrived sample set

NxTAG RPP Result	Contrived sample		
	Positive	Negative	Total
Positive	50	0	50
Negative	0	50	50
Total	50	50	100
Positive Percent Agreement: 50/50 100% (95%CI: 92.9% - 100%)			
Negative Percent Agreement: 50/50 100% (95%CI: 92.9% - 100%)			

Table 70 – Positive and Negative Agreement of NxTAG RPP for the *Mycoplasma pneumoniae* contrived sample set

NxTAG RPP Result	Contrived sample		
	Positive	Negative	Total
Positive	50	0	50
Negative	0	50	50
Total	50	50	100
Positive Percent Agreement: 50/50 100% (95%CI: 92.9% - 100%)			
Negative Percent Agreement: 50/50 100% (95%CI: 92.9% - 100%)			

4. Clinical cut-off**:**

   See: *Assay cut-off*; section *M-1-h*

5. Expected values/Reference range:

   The NxTAG RPP clinical study included a total of 2132 prospectively collected specimens collected and tested during two phases of the prospective study. The number and percentage of cases positive for one or more targets, as determined by the NxTAG RPP Assay are shown by age category in Tables 71 and 72:

Table 71 - Expected Values for NxTAG RPP Clinical Samples (Jan 2014 – Apr 2014)

Target (Analyte)	Overall (n=934)		0-1 year (n=248)		>1-5 years (n=151)		>5-21 years (n=180)		>21-65 years (n=212)		>65 years (n=143)	
	No.	EV	No.	EV	No.	EV	No.	EV	No.	EV	No.	EV
Adenovirus	18	1.9%	11	4.4%	4	2.6%	2	1.1%	1	0.5%	0	0.0%
Influenza A	70[1]	7.5%	13	5.2%	13	8.6%	11	6.1%	23	10.8%	10	7.0%
Influenza A H1	38	4.1%	7	2.8%	7	4.6%	3	1.7%	17	8.0%	4	2.8%
Influenza A H3	32	3.4%	5	2.0%	6	4.0%	9	5.0%	6	2.8%	6	4.2%

Influenza B	52	5.6%	2	0.8%	4	2.6%	13	7.2%	14	6.6%	19	13.3%
Respiratory Syncytial Virus A	19	2.0%	13	5.2%	2	1.3%	1	0.6%	2	0.9%	1	0.7%
Respiratory Syncytial Virus B	80	8.6%	42	16.9 %	18	11.9%	3	1.7%	11	5.2%	6	4.2%
Parainfluenza 1	2	0.2%	1	0.4%	1	0.7%	0	0.0%	0	0.0%	0	0.0%
Parainfluenza 2	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
Parainfluenza 3	3	0.3%	1	0.4%	1	0.7%	1	0.6%	0	0.0%	0	0.0%
Parainfluenza 4	4	0.4%	2	0.8%	1	0.7%	1	0.6%	0	0.0%	0	0.0%
Coronavirus 229E	17	1.8%	4	1.6%	5	3.3%	3	1.7%	4	1.9%	4	4
Coronavirus OC43	6	0.6%	1	0.4%	0	0.0%	1	0.6%	2	0.9%	2	1.4%
Coronavirus NL63	33	3.5%	7	2.8%	14	9.3%	4	2.2%	1	0.5%	7	4.9%
Coronavirus HKU1	11	1.2%	5	2.0%	3	2.0%	0	0.0%	2	0.9%	1	0.7%
Rhinovirus/ Enterovirus	195	20.9 %	76	30.6 %	57	37.7%	45	25.0 %	11	5.2%	6	4.2%
Human Metapneumovirus	88	9.4%	34	13.7 %	21	13.9%	14	7.8%	9	4.2%	10	7.0%
Human Bocavirus	28	3.0%	16	6.5%	10	6.6%	2	1.1%	0	0.0%	0	0.0%
C. pneumoniae	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
M. pneumoniae	3	0.3%	0	0.0%	2	1.3%	1	0.6%	0	0.0%	0	0.0%

[1]One (1) specimen generated Influenza A un-subtypeable result by NxTAG RPP (i.e. Influenza A matrix positive but H1 and H3 subtype negative). This specimen was negative for Influenza A H1 and H3 by comparator. Two (2) Influenza A positive specimens generated both H1 and H3 positive calls by NxTAG RPP.

Table 72 - Expected Values for NxTAG RPP Clinical Samples (Jan 2015 – Mar 2015)

Target (Analyte)	Overall (n=1198)		0-1 year (n=205)		>1-5 years (n=99)		>5-21 years (n=173)		>21-65 years (n=372)		>65 years (n=349)	
	No.	EV	No.	EV	No.	EV	No.	EV	No.	EV	No.	EV
Adenovirus	36	3.0%	7	3.4%	8	8.1%	6	3.5%	6	1.6%	9	2.6%
Influenza A	225[1]	18.8%	9	4.4%	16	16.2%	40	23.1%	80	21.5%	80	22.9%
Influenza A H1	3	0.3%	1	0.5%	0	0.0%	0	0.0%	2	0.5%	0	0.0%
Influenza A H3	217	18.1%	8	3.9%	15	15.2%	39	22.5%	75	20.2%	80	22.9%
Influenza B	49	4.1%	2	1.0%	3	3.0%	21	12.1%	13	3.5%	10	2.9%
Respiratory Syncytial Virus A	69	5.8%	28	13.7%	8	8.1%	5	2.9%	12	3.2%	16	4.6%
Respiratory Syncytial Virus B	64	5.3%	31	15.1%	8	8.1%	4	2.3%	9	2.4%	12	3.4%
Parainfluenza 1	4	0.3%	1	0.5%	1	1.0%	0	0.0%	0	0.0%	2	0.6%
Parainfluenza 2	2	0.2%	0	0.0%	0	0.0%	0	0.0%	1	0.3%	1	0.3%
Parainfluenza 3	35	2.9%	17	8.3%	2	2.0%	1	0.6%	7	1.9%	8	2.3%
Parainfluenza 4	10	0.8%	1	0.5%	2	2.0%	0	0.0%	3	0.8%	4	1.1%
Coronavirus 229E	17	1.4%	2	1.0%	2	2.0%	2	1.2%	6	1.6%	5	1.4%
Coronavirus OC43	33	2.8%	9	4.4%	2	2.0%	5	2.9%	10	2.7%	7	2.0%
Coronavirus NL63	41	3.4%	10	4.9%	10	10.1%	10	5.8%	10	2.7%	1	0.3%
Coronavirus HKU1	7	0.6%	1	0.5%	0	0.0%	1	0.6%	3	0.8%	2	0.6%
Rhinovirus/ Enterovirus	159	13.3%	39	19.0%	23	23.2%	24	13.9%	45	12.1%	28	8.0%
Human Metapneumovirus	66	5.5%	19	9.3%	13	13.1%	11	6.4%	15	4.0%	8	2.3%
Human Bocavirus	22	1.8%	13	6.3%	5	5.1%	1	0.6%	1	0.3%	2	0.6%
C. pneumoniae	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%	0	0.0%
M. pneumoniae	6	0.5%	0	0.0%	0	0.0%	2	1.2%	4	1.1%	0	0.0%

[1] Five (5) specimens generated Influenza A un-subtypeable result by NxTAG RPP (i.e. Influenza A matrix positive but H1 and H3 subtype negative). All 5 specimens were negative for Influenza A H1 and H3 by comparator.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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