510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K103776

B. Purpose for Submission:
An updated version of the Luminex RVP with a different Intended Use and an abbreviated testing process.

C. Measurand:
Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus, Human Metapneumovirus, Rhinovirus, and Adenovirus nucleic acids target sequences.

D. Type of Test:
A multiplexed nucleic acid test followed by Universal Tag sorting on the Luminex® 100/200 platform for the qualitative *in vitro* detection and identification of multiple respiratory pathogen nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections.

E. Applicant:
Luminex Molecular Diagnostics, Inc

F. Proprietary and Established Names:
xTAG® Respiratory Viral Panel FAST
Common Name: RVP FAST

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay

2. Classification:
   Class II

3. Product code:
   OCC, OEM, OEP

4. Panel:
   Microbiology (83)

H. Intended Use:

1. Intended use:

The xTAG® Respiratory Viral Panel Fast (RVP FAST) is a qualitative nucleic acid multiplex test intended for the simultaneous detection and identification of multiple respiratory virus nucleic acids in nasopharyngeal swabs from individuals suspected of respiratory tract infections. The following virus types and subtypes are identified using RVP FAST: Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus, Human Metapneumovirus, Rhinovirus, and Adenovirus. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory viral infection if used in conjunction with other clinical and epidemiological information.

Negative results do not preclude respiratory viral infection and should not be used as the sole basis for diagnosis, treatment or other management decisions. Positive results do not rule out bacterial infection or co-infection with other organisms. 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 infection.

Due to the genetic similarity between human rhinovirus and enterovirus, the RVP FAST primers for detection of rhinovirus cross-react with enterovirus. A rhinovirus reactive result should be confirmed by an alternate method (e.g., cell culture).

Performance characteristics for Influenza A Virus were established 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 infections 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 a state or local health department 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 for Use:
   Same as Intended Use

3. Special conditions for use statement(s):
   For prescription use only

4. Special instrument requirements:
   Luminex 100/200 instrument

I. Device Description:

RVP FAST is a PCR-based system for detecting viral nucleic acids in clinical specimens. The primers and probes of the RVP FAST have been designed to specifically target unique regions in the RNA / DNA of each virus listed in the intended use. Amplified products are sorted and analyzed on the Luminex 100 or 200 instrument, which generates

signals based on the acquisition of spectrofluorometric data. The raw signals are median fluorescence intensities (MFI) which are captured in a Luminex Output.csv file that is subsequently analyzed by the xTAG Data Analysis Software (TDAS RVP FAST) to establish the presence or absence of the viral nucleic acids hybridized to Luminex microsphere population. A summary of the RVP FAST assays and targets is presented in Table 1:

Table 1. Gene Targets Used for RVP FAST Analytes

RVP FAST Analytes	Gene Targets
RSV	RNA-dependent RNA polymerase (L) gene
Influenza A	matrix protein 1 (M1)
H1	hemagglutinin (HA) gene
H3	hemagglutinin (HA) gene
Influenza B	hemagglutinin (HA) gene
Rhinovirus	5' untranslated region
Human Metapneumovirus	nucleoprotein (N) gene
Adenovirus	hexon protein

Table 2. Reagents Supplied in the Kit

Reagent	Volume for 96 Tests	Storage Conditions
xTAG® RVP FAST Primer Mix	192 µL x 1 vial	Store at -25°C to -15°C upon receipt.
xTAG® OneStep Enzyme Mix	57 µL x 3 vials	
xTAG® OneStep Buffer, 5X	1.0 mL x 1 vial	
xTAG® dNTP Mix	200 µL x 1 vial	
xTAG® RNase-Free Water	1.9 mL x 1 vial	
xTAG® RVP FAST Bead Mix	1.92 mL x 1 vial	Store at -25°C to -15°C protected from light upon receipt. Store at 2°C to 8°C protected from light after first use.
xTAG® Reporter Buffer	12.0 mL x 1 vial	Store at -25°C to -15°C upon receipt. Store at 2°C to 8°C after first use.
xTAG® Streptavidin, R-Phycoerythrin G15 (SA-PE)	120 µL x 1 vial	Store at -25°C to -15°C protected from light upon receipt.
xTAG® MS2	1.5 mL x 2 vials	Store at -25°C to -15°C upon receipt.
xTAG® Bacteriophage Lambda DNA	200 µL x 1 vial	Store at -25°C to -15°C upon receipt.

Materials Required But Not Provided

Equipment

- Luminex® 100/200™ instrument (including IS or xPONENT software, calibrators and controls)
- Mini centrifuge (InterScience, C-1301) or equivalent
- Multichannel pipette (1-10 µL or 5-50 µL, 50-200 µL)
- Pipettes (P10, P100, P200, P1000)
- Pipetting aid
- Racks for 1.5 mL and 0.5 mL microcentrifuge tubes
- Racks for 0.2 mL thin wall tubes for PCR
- Sonicator bath (Ultrasonic Cleaner, Cole-Parmer®, A-08849-00) or equivalent
- Thermal Cycler capable of using 0.2 ml reaction tubes and 96-well reaction plates
- PCR cooler rack (Eppendorf, 022510509) or equivalent
- Vortex
- Biomérieux NucliSENS® easyMAG instrument

Consumables

- 0.2 mL thin wall polypropylene tubes for PCR (appropriate for thermal cycler)
- 0.5 mL or 1.5 mL polypropylene microcentrifuge tubes
- 25 mL pipettes
- 15 mL polypropylene tubes (Falcon® Tubes) or borosilicate glass tubes (5 or 10 mL)
- 50 mL Falcon tubes
- Costar® Thermowell® thin-wall polycarbonate 96-well plates (Corning) or equivalent
- Microseal® to cover 96-well plate
- Parafilm®
- Aerosol resistant tips for pipettes
- Reservoir basins
- BioMérieux NucliSENS® easyMAG lysis buffer
- BioMérieux NucliSENS® easyMAG extraction buffer 1
- BioMérieux NucliSENS® easyMAG extraction buffer 2
- BioMérieux NucliSENS® easyMAG extraction buffer 3
- BioMérieux NucliSENS® easyMAG magnetic silica

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Luminex® xTAG™ Respiratory Viral Panel (RVP).

2. Predicate K numbers:

   K063765, K081483, K091677

3. Comparison with predicate(s):

Table 3: Similarities between New Device and Predicate

Item	**New Device (Ref. No. to be determined) xTAG RVP FAST**	**Predicate (k063765, k081483, k091667) xTAG RVP**
Manufacturer	Luminex Molecular Diagnostics	Luminex Molecular Diagnostics
Specimen Types	Nasopharyngeal swabs	Nasopharyngeal swabs
Amplification Method	Multiplex end point RT-PCR	Multiplex end point RT-PCR
Test Format	Multiplex bead-based universal array sorting on Luminex 100/200 instrument	Multiplex bead-based universal array sorting on Luminex 100/200 instrument
Detection Method	Fluorescence based	Fluorescence based
Quality Control	Internal Control (E. coli phage MS2), Run Control (bacteriophage Lambda DNA), rotating analyte control and negative controls	Internal Control (E. coli phage MS2) and Run Control (bacteriophage Lambda DNA), rotating analyte control and negative controls
Results	Qualitative	Qualitative
Instrument	LX100 or LX200	LX100 or LX200

Table 4: Differences between New Device and Predicate

Item	**New Device (k103776) xTAG RVP FAST**	**Predicate (k063765, k081483, k091667) xTAG RVP**
Intended Use	The xTAG® Respiratory Viral Panel Fast (RVP FAST) is a qualitative nucleic acid multiplex test intended for the simultaneous detection and identification of multiple respiratory virus nucleic acids in nasopharyngeal swabs from individuals suspected of respiratory tract infections. The following virus types and subtypes are identified using RVP FAST: Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus, Human Metapneumovirus, Rhinovirus, and Adenovirus. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory viral infection if used in conjunction with other clinical and epidemiological information. Negative results do not preclude respiratory viral infection and should not be used as the sole basis for diagnosis, treatment or other management decisions. Positive results do not rule out bacterial infection or co-infection with other organisms. The agent detected may not be the definite cause of disease.	The xTAG® Respiratory Viral Panel (RVP) is a qualitative nucleic acid multiplex test intended for the simultaneous detection and identification of multiple respiratory virus nucleic acids in nasopharyngeal swabs from individuals suspected of respiratory tract infections. The following virus types and subtypes are identified using RVP: Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus subtype A, Respiratory Syncytial Virus subtype B, Parainfluenza 1, Parainfluenza 2, and Parainfluenza 3 virus, Human Metapneumovirus, Rhinovirus, and Adenovirus. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory viral infection if used in conjunction with other clinical and laboratory findings. It is recommended that specimens found to be negative for Influenza B, Respiratory Syncytial Virus subtype A and B, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3 and Adenovirus, after examination using RVP be confirmed by cell culture. Negative results do not preclude

	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 infection. Due to the genetic similarity between human Rhinovirus and Enterovirus, the RVP FAST primers for the detection of rhinovirus cross react with enterovirus. A rhinovirus reactive result should be confirmed by an alternate method (e.g. cell culture). Performance characteristics for Influenza A Virus were established 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 a 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.	respiratory virus infection and should not be used as the sole basis for diagnosis, treatment or other management decisions. Positive results do not rule out bacterial infection, or co-infection with other viruses. The agent detected may not be the definite cause of disease. The use of additional laboratory testing (e.g. bacterial culture, immunofluorescence, radiography) and clinical presentation must be taken into consideration in order to obtain the final diagnosis of respiratory viral infection. Due to seasonal prevalence, performance characteristics for Influenza A/H1 were established primarily with retrospective specimens. The RVP assay cannot adequately detect Adenovirus species C, or serotypes 7a and 41. The RVP primers for detection of rhinovirus cross-react with enterovirus. A rhinovirus reactive result should be confirmed by an alternate method (e.g. cell culture). Performance characteristics for Influenza A Virus were established 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 infections 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 a state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
Targets Reported	Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus, Human Metapneumovirus, Rhinovirus, and Adenovirus	Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus, Human Metapneumovirus, Rhinovirus, Adenovirus, Parainfluenza 1, Parainfluenza 2 and Parainfluenza 3
Sample Preparation	Biomérieux NucliSENS® EasyMag®	QIAGEN QIAamp MiniElute, Biomérieux NucliSENS® EasyMag®, and Biomérieux MiniMag™
Amplification Enzyme	xTAG® OneStep Enzyme Mix	xTAG® OneStep Enzyme Mix and ancillary reagent TaKaRa Taq™ Hot

		Start
Primer Mixes	One primer mix (PCR and TSPE combined)	Two primer mixes (1 for PCR and 1 for TSPE)
Software	xTAG Data Analysis Software RVP FAST (US)	xTAG Data Analysis Software RVP (US)

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

Table 5: Guidance Documents

	Title	Date
1	Class II Special Controls Guidance: Respiratory Viral Panel Multiplex Nucleic Acid Assay	Oct. 9, 2009
2	Class II Special Control Guidance Document: Testing for Detection and Differentiation of Influenza A Virus Subtypes Using Multiplex Assays	Oct. 9, 2009
3	Guidance (Draft) for Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses	Feb. 15, 2008
4	Guidance for In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path	May 1, 2007
5	Class II Special Controls Guidance: Reagents for Detection of Specific Novel Influenza A Viruses	Mar. 22, 2006
6	Class II Special Control Guidance Document: "Testing for Human Metapneumovirus (hMPV) Using Nucleic Acid Assays"	Oct. 9, 2009
7	Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices	May 11, 2005
8	Guidance document for Format for Traditional and Abbreviated 510(k)s	Aug. 12, 2005

L. Test Principle:

RVP FAST incorporates multiplex Reverse-Transcriptase Polymerase Chain Reaction (RT-PCR) with Luminex's Universal Tag sorting system on the Luminex® platform. The assay also detects an internal control (*E. coli* phage MS2) which should be added to each sample prior to extraction, and a run control (bacteriophage Lambda DNA) which should be added as a separate RT-PCR reaction in each run performed.

For each sample, viral extract (RNA or DNA) is amplified in a single multiplex RT-PCR reaction. For each of the viruses/subtypes or internal control present in the sample, PCR amplimers are produced. The RT-PCR product is then added to a hybridization/detection reaction containing the universal bead array and the Streptavidin-R-Phycoerythrin reporter. Each Luminex bead population detects a specific viral target or assay control through a highly specific anti-tag/tag hybridization. Following the incubation of the RT-PCR products with the bead mix and reporter, the hybridization/detection reactions are sorted and fluorescent signal is captured on the Luminex instrument. These fluorescence values (median fluorescence intensity, MFI) generated for each bead population are analyzed to establish the presence or absence of viral targets and/or controls in each sample tested. The data generated by the xMAP instrument is analyzed by the xTAG Data Analysis Software RVP Fast (TDAS RVP FAST) to provide a qualitative summary report on which viruses are present in the sample, if any.

Interpretation of Results

Each sample includes an individual column for each viral target, the internal control (bacteriophage MS2) and the run control (bacteriophage Lambda). Additionally, characterized positive controls and a negative control (RNase-free water) should be included with each run.

Control Calls (Internal and Run Control)

- **PRES** - the recommended Internal / Run Control is detected (MFI ≥ 800)
- **ABS** - the recommended Internal / Run Control is not detected (MFI < 800)
- **No Call** - unable to determine presence or absence of the Internal / Run Control due to an assay-specific criterion not being met (see Tables 6-7 for re-test recommendations).

  Note: TDAS RVP FAST (US) uses notes in the **Notes** column and the **Warnings/Errors** section of the data file to determine whether there was a problem during reading. TDAS RVP FAST (US) does not interpret the data if the system gives a "Sample Empty" or "User cancel" in the **Notes** column, or reports an error status for a sample. DO NOT EDIT the Luminex data file at any time or TDAS RVP FAST (US) cannot correctly interpret the data.

External Controls (Negative and Positive Controls)

At least one negative control should be included in the run. The software considers the last sample on the plate to be the external negative control. If the external negative control fails, then a message appears and the plate fails (see Table 7 for Re-Run recommendations).

Characterized external positive controls should be included in the run. If a given analyte positive control does not perform as expected, all results for that analyte in the batch of samples are invalid and samples should be re-run (see Table 7 for Re-Run recommendations).

Viral Target (Sample) Calls (except Influenza A)

- **POS** - the viral target is detected. The positive thresholds for each target are provided below:
  - Influenza B (MFI ≥ 400)
  - RSV-probe 1 (MFI ≥ 120) or RSV-probe 2 (MFI ≥ 150)
  - Rhinovirus (MFI ≥ 300)
  - Human Metapneumovirus (MFI ≥ 200)
  - Adenovirus (MFI ≥ 150)
- **NEG** - the viral target is not detected. The negative thresholds for each target is provided below:
  - Influenza B (MFI < 400)
  - RSV-probe 1 (MFI < 120) and RSV-probe 2 (MFI < 150)
  - Rhinovirus (MFI < 300)

- Human Metapneumovirus (MFI < 200)
- Adenovirus (MFI < 150)

- **No Call** - invalid result due to a failure in one or more assay parameters / controls (refer to Tables 6-7 for re-test recommendations)

Influenza A (Sample) Calls

- The viral target is detected. The positive thresholds are provided below:
  - **H1 D** - the Influenza A H1 subtype (seasonal H1) (MFI ≥ 450) and the Influenza A matrix gene are detected
    (MFI ≥ 300)
  - **H3 D** - the Influenza A H3 subtype (MFI ≥ 200) and the Influenza A matrix gene are detected (MFI ≥ 300)
  - **H1, H3 D** - the Influenza A H1 (seasonal H1), the H3 (seasonal H3) subtypes and the Influenza A matrix gene are detected
  - **Ut D** - the Influenza A matrix gene is detected and both the H1 and H3 subtypes are not detected. NOTE: please refer to. Influenza A precautions listed below).
- **NEG** - the viral target is not detected and all thresholds must be negative. The negative thresholds are provided below:
  - Flu A matrix (MFI < 300)
  - H1 (MFI < 450)
  - H3 (MFI < 200)
- **No Call** - an invalid result due to a failure in one or more assay parameters / controls (see re-test recommendations below Tables 6-7)

  It is recommended that the sample be re-tested once according to the instructions provided in Table 6 and 7. If a re-test needs to be carried out due to an invalid result producing a "No Call" for a sample or target, the re-test results should be considered the final result for that sample or target. For analytes other than influenza, if the final RVP FAST result is a "No Call" then follow-up testing is recommended. For detection of Influenza A H1 and H3 subtypes, specific precautions listed below must be followed:

- **Unsubtypeable (i.e., Influenza A matrix gene is detected), but not for H1 and H3 Subtype**: If RVP FAST positively identifies the Flu A matrix gene target but fails to identify a hemagglutinin gene target (H1 or H3), retest the sample with RVP FAST from the extraction step with external controls for these two analytes. Run sample extract in duplicate. If the re-test on both replicates does not yield a positive result for H1 or H3 and external controls are properly typed, follow up with appropriate public health authorities to determine whether the unsubtypeable Flu A specimen represents a novel strain of Influenza A.

- If infections 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 a state or local health department for testing. Viral culture

should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

- The performance of influenza B may vary depending on whether sequence variants that could potentially affect primer binding are present among the circulating influenza B viruses (Pabbaraju et al. 2011).

- Scientific literature reports that some *E. coli* strains may be infected with lambdoid phages in the wild resulting in a risk of a 'present' call for the lambda bead in a patient sample containing a strain of *E. coli* infected with lambda phage. This does not alter the ability of RVP FAST to detect the other viral targets.

Table 6. RVP FAST Retest Recommendations: Sample Related Issues

TDAS Warning Message (For messages in the detailed views, refer to the User Manual)	Problem	Possible Cause(s)	Recommendation(s)
"Warning: Influenza A detected but subtype could not be determined. Refer to Kit Package insert for further instructions"	Influenza A matrix gene detected but seasonal H1 and H3 subtypes not identified (FluA Ut D)	Novel strain of influenza A or low titer specimen	Refer to section 7
"Target failed: incompatible signals between targets"	Ambiguous calls between Influenza A matrix gene and one of its subtypes (FluA No Call), i.e. POS call for H1 or H3 hemagglutinin gene in the absence of a POS call for the matrix gene	Sample titer failure or contamination	Re-run from RNA step (or re-extract, or obtain new specimen at laboratory's discretion)
"Sample failed: unexpected control call(s)"	All viral target signals are not detected and internal control ABSENT (No Call for entire sample)	Sample extraction failure or did not spike in MS-2 to the sample (and no viral targets were detected)	Re-extract (or obtain new specimen at laboratory's discretion)

Table 7. RVP FAST Re-Test Recommendations: Instrument and Run-Related Issues

TDAS Warning Message (For messages in the detailed views, refer to the User Manual)	Problem	Possible Cause(s)	Recommendation(s)

Instrument-Related Issues			
"Assay failed: low bead count(s) for the primary negative control sample"	Plate failure (No Call for whole plate)	No beads collected Too few beads collected Incorrect probe height Incorrect template/protocol chosen Beads shifting	Select a different primary negative control or Re-run from bead hybridization step
"Sample failed: low bead count(s) for control(s)" or "Target(s) failed: low bead count(s)"	No Call for all targets and No Call for internal control of the corresponding sample or No Call for targets that have a low bead count	No beads collected Too few beads collected Incorrect probe height Incorrect template/protocol chosen Beads shifting	Select a different primary negative control or Re-run from bead hybridization step
"Assay failed: '<instrument error message>'. Check the Luminex instrument for details" or "Sample failed: '<instrument error message>'. Check the Luminex instrument for details"	Plate failure (No Call for whole plate) or No Call for all targets of a sample that has a Luminex error status during data acquisition stage	No beads collected Time out during acquiring data Luminex instrument error	Select a different primary negative control or Re-run from bead hybridization step
Run-Related Issues			
"Assay failed: unexpected value(s) encountered for the primary negative control sample"	Plate failure (No Call for whole plate)	Omitting enzyme Omitting primer mix Omitting reporter solution	Select a different primary negative control or Re-run from RNA step (re-extract at laboratory's discretion)
"Assay failed: a primary negative control signal exceeds acceptable value"	Plate failure (No Call for whole plate)	Contamination	Re-run from RNA step (re-extract at laboratory's discretion)

"Assay failed: 'Sample Empty' message from the Luminex machine for the primary negative control sample" or "Assay failed: 'User cancel' message from the Luminex machine for the primary negative control sample"	Plate failure (No Call for whole plate)	Sample is empty for the primary negative control sample User cancelled the primary negative control sample during data acquisition stage	Select a different primary negative control or Re-run from bead hybridization step
"Sample failed: 'Sample Empty' message from the Luminex machine for this well" or "Sample failed: 'User Cancel' message from the Luminex machine for this well"	No Call for all viral targets and all control targets	Sample is empty User cancelled the plate during data acquisition stage	Re-run from bead hybridization step
"Sample failed: unexpected value(s) encountered for control(s)"	No Call for all viral targets and all control targets due to unexpected signals in the control target(s) without detecting any viral targets	Omitting enzyme Omitting primer mix Omitting reporter solution	Re-run from RNA step (re-extract at laboratory's discretion)
"Target(s) failed: unexpected value(s) encountered"	No Call for targets that have unexpected signals	Time out during acquiring data for those targets	Re-run from RNA step (re-extract at laboratory's discretion)

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility was assessed by using simulated samples with viral loads generating MFI signals bracketing the cut-off for positive calls (from both below and above). Simulated samples were prepared by spiking cultured viruses into Universal Transport Medium (UTM). The study was conducted at three independent sites by two operators at each site. Each operator conducted five separate runs with each sample in triplicates (starting from EasyMag nucleic acids extraction) on non-consecutive days. Thus, there were a total of 90 replicates for each sample: (3 sites) x (2 operators/site) x (5 xTAG runs/operator) x 3 replicates.

Each of the following viral analyte targets were examined in the form of simulated sample arranged in a dilution series: Flu A (matrix gene), Flu A (hemagglutinin H1), Flu A (hemagglutinin H3), Flu B, RSV A, RSV B, hMPV, Adenovirus, and Rhinovirus. Each target was assessed at three dilution levels: High Negative (HN), Low Positive (LP), and Moderate Positive (MP). RVP FAST calls (Positive and

Negative) generated for each viral analyte for this reproducibility study are summarized in Tables 8 through 15 below.

Table 8. Summary of Flu A and H1 calls in simulated Influenza A-H1 samples

Virus / Level			**All Days (5 extraction days x 6 extraction runs per day)**					
Flu A-H1 (Strain: A/Solomon Islands/3/2006)	**Site**	**Operator**	**# Pos Call[1]**	**# Neg Call[1]**	**25th Percentile MFI**	**Median MFI**	**75th Percentile MFI**	**% CV[2]**
Flu A – Moderate Positive (MP) / (6.1 TCID50/mL)	Site 1	Operator	15	0	3,931.5	4278.5	4514.0	14.88
		Operator	15	0	4,040.0	4519.0	4603.0	7.81
		Site 1	30	0	4,033.5	4362.5	4594.0	11.66
	Site 2	Operator	15	0	4,234.0	4606.0	4967.5	10.17
		Operator	15	0	4,372.0	4749.0	5145.5	8.34
		Site 2	30	0	4,318.0	4618.3	4967.5	9.15
	Site 3	Operator	15	0	1,706.0	2024.0	2657.0	41.17
		Operator	14	1	2,022.0	2192.0	2620.5	36.53
		Site 3	29	1	1,820.0	2117.0	2620.5	38.22
	Overall Total		**89**	**1**	**2,620.5**	**4212.5**	**4606.0**	**34.09**
H1 – Moderate Positive (MP) / (6.1 TCID50/mL)	Site 1	Operator	15	0	4,030.0	4522.0	4758.0	12.72
		Operator	15	0	4,173.0	4448.0	4734.5	10.43
		Site 1	30	0	4,131.0	4455.5	4734.5	11.45
	Site 2	Operator	15	0	4,908.0	4996.0	5493.0	7.93
		Operator	15	0	5,093.0	5420.0	5633.0	6.87
		Site 2	30	0	4,968.0	5174.5	5615.5	7.70
	Site 3	Operator	15	0	2,253.5	2730.0	3567.0	37.00
		Operator	14	1	2,572.0	3098.5	3802.0	39.81
		Site 3	29	1	2,379.0	2886.0	3567.0	37.87
	Overall Total		**89**	**1**	**3,425.0**	**4558.0**	**5093.0**	**28.19**
Flu A – Low Positive (LP) / (1.6 TCID50/mL)	Site 1	Operator	15	0	1,489.0	1713.0	2191.5	24.94
		Operator	15	0	1,307.5	1743.0	1947.0	26.64
		Site 1	30	0	1,378.0	1728.0	1953.0	25.60

	Site 2	Operator	15	0	1,840.0	2221.5	2862.0	39.77
		Operator	15	0	1,528.0	1874.0	2172.5	25.62
		Site 2	30	0	1,549.0	1982.3	2297.5	38.17
	Site 3	Operator	14	1	756.5	923.0	1174.0	40.83
		Operator	15	0	718.0	875.0	952.5	34.70
		Site 3	29	1	733.0	878.5	1025.0	37.68
	Overall Total		**89**	**1**	**952.5**	**1524.0**	**1953.0**	**48.57**
H1 – Low Positive (LP) / (1.6 TCID50/mL)	Site 1	Operator	15	0	1,621.0	1698.0	1869.0	12.65
		Operator	15	0	1,256.5	1464.0	1953.0	29.97
		Site 1	30	0	1,335.0	1685.8	1888.5	22.36
	Site 2	Operator	15	0	2,305.0	2680.5	3249.0	23.23
		Operator	15	0	1,991.0	2362.5	2710.0	20.49
		Site 2	30	0	2,139.0	2528.5	3002.0	22.69
	Site 3	Operator	14	1	1,261.5	1481.0	2346.0	45.28
		Operator	15	0	957.5	1168.0	1408.0	24.47
		Site 3	29	1	1,155.0	1326.0	1698.0	42.89
	Overall Total		**89**	**1**	**1,328.0**	**1793.0**	**2346.0**	**39.25**
Flu A – High Negative (HN) / (4.0×10^{-1} TCID50/mL)	Site 1	Operator	6	9	206.5	253.5	351.0	N/A
		Operator	8	7	214.0	326.0	401.0	N/A
		Site 1	14	16	214.0	273.3	382.0	N/A
	Site 2	Operator	13	2	347.0	463.0	774.0	N/A
		Operator	13	2	504.0	605.0	936.5	N/A
		Site 2	26	4	385.0	549.3	914.5	N/A
	Site 3	Operator	0	15	100.5	149.0	198.0	N/A
		Operator	4	11	132.0	161.0	455.5	N/A
		Site 3	4	26	114.0	155.0	230.0	N/A
	Overall Total		**44**	**46**	**168.0**	**273.3**	**512.0**	**N/A**
H1 – High Negative (HN) / (4.0×10^{-1}	Site 1	Operator	6	9	258.0	358.0	455.0	N/A
		Operator	4	11	262.0	345.0	492.0	N/A

		Site 1	10	20	262.0	353.3	455.0	N/A
	Site 2	Operator	13	2	562.5	711.5	1005.0	N/A
		Operator	13	2	515.0	605.0	1293.0	N/A
		Site 2	26	4	527.0	643.8	1005.0	N/A
	Site 3	Operator	0	15	148.0	216.0	289.0	N/A
		Operator	4	11	225.0	258.0	501.0	N/A
		Site 3	4	26	189.0	235.5	324.0	N/A
	Overall Total		**40**	**50**	**242.0**	**376.5**	**592.0**	**N/A**

[1]The total number of calls per operator was 15, per site was 30, and across all sites and operators, 90.
[2]For Table 12 – 19, %CV = Standard Deviation / Mean*100; N/A = not applicable.

Table 9. Summary of flu A and H3 calls in simulated Influenza A-H3 samples

Virus / Level			**All Days (5 extraction days x 6 extraction runs per day)**					
Flu A-H3 (Strain: A/Victoria/ 3/75)	**Site**	**Operator**	**# Pos Call**	**# Neg Call**	**25th Percentile MFI**	**Median MFI**	**75th Percentile MFI**	**% CV**
Flu A – Moderate Positive (MP) / (1.5 x 10^1 TCID50/mL)	Site 1	Operator 1	15	0	3,680.0	3956.0	4119.0	7.25
		Operator 2	15	0	3,596.0	3870.5	4084.0	8.60
		Site 1	30	0	3,632.0	3888.3	4084.0	7.82
	Site 2	Operator 1	15	0	4,062.5	4406.0	4702.0	9.63
		Operator 2	15	0	4,031.0	4174.0	4488.0	7.61
		Site 2	30	0	4,062.5	4354.0	4642.0	8.71
	Site 3	Operator 1	15	0	2,501.0	2891.0	2999.0	17.40
		Operator 2	15	0	2,512.0	2626.0	3074.0	20.25
		Site 3	30	0	2,512.0	2706.8	3070.0	18.57
	Overall Total		**90**	**0**	**3,070.0**	**3833.0**	**4179.5**	**20.86**
H3 – Moderate Positive (MP) / (1.5 x 10^1 TCID50/mL)	Site 1	Operator 1	15	0	1,709.0	2008.0	2382.0	18.88
		Operator 2	15	0	1,846.5	1935.0	2353.0	16.72
		Site 1	30	0	1,738.5	1971.5	2353.0	17.65
	Site 2	Operator 1	15	0	2,813.0	3164.0	3254.5	8.71
		Operator 2	15	0	2,809.0	3031.0	3171.5	9.88
		Site 2	30	0	2,813.0	3051.3	3196.0	9.15
	Site 3	Operator 1	15	0	951.5	1265.5	1491.5	38.73

		Operator 2	15	0	1,124.0	1251.5	1682.0	27.11
		Site 3	30	0	1,080.0	1259.3	1505.5	32.99
	Overall Total		**90**	**0**	**1,492.0**	**2088.8**	**2845.5**	**36.53**
Flu A – Low Positive (LP) / (5.5 TCID50/mL)	Site 1	Operator 1	15	0	1,839.0	2054.0	2281.0	17.36
		Operator 2	15	0	1,732.0	1903.0	2175.0	17.35
		Site 1	30	0	1,780.5	1936.5	2215.0	17.14
	Site 2	Operator 1	15	0	2,202.5	2637.0	2732.0	18.99
		Operator 2	15	0	2,267.0	2485.5	2638.0	15.74
		Site 2	30	0	2,267.0	2533.0	2685.0	17.28
	Site 3	Operator 1	14	1	1,050.5	1256.0	1439.0	34.16
		Operator 2	15	0	1,191.5	1433.5	1704.0	22.87
		Site 3	29	1	1,152.5	1373.0	1503.0	28.88
	Overall Total		**89**	**1**	**1,460.0**	**1905.0**	**2446.0**	**31.44**
H3 – Low Positive (LP) / (5.5 TCID50/mL)	Site 1	Operator 1	15	0	601.5	776.5	930.0	24.31
		Operator 2	15	0	610.0	771.0	888.5	18.76
		Site 1	30	0	610.0	773.8	889.0	21.56
	Site 2	Operator 1	15	0	1,106.0	1219.0	1489.0	20.43
		Operator 2	15	0	1,129.0	1354.0	1580.0	25.51
		Site 2	30	0	1,125.0	1305.5	1509.5	23.17
	Site 3	Operator 1	14	1	291.0	401.0	512.0	50.52
		Operator 2	15	0	383.0	543.0	567.5	24.92
		Site 3	29	1	328.0	473.0	547.5	38.36
	Overall Total		**89**	**1**	**535.0**	**773.8**	**1125.0**	**50.38**
Flu A – High Negative (HN) / (1.8 TCID50/mL)	Site 1	Operator 1	14	1	591.5	675.0	771.5	N/A
		Operator 2	15	0	459.5	647.5	857.5	N/A
		Site 1	29	1	483.0	652.3	801.0	N/A
	Site 2	Operator 1	15	0	822.0	1059.0	1247.0	N/A
		Operator 2	15	0	706.5	786.5	1045.0	N/A
		Site 2	30	0	735.0	914.3	1138.0	N/A
	Site 3	Operator 1	4	11	204.5	259.0	314.0	N/A

		Operator 2	11	4	292.0	443.5	500.0	N/A
		Site 3	15	15	230.0	303.8	447.5	N/A
	Overall Total		**74**	**16**	**427.0**	**639.0**	**891.0**	**N/A**
H3 – High Negative (HN) / (1.8 TCID50/mL)	Site 1	Operator 1	11	4	191.0	223.0	274.0	N/A
		Operator 2	11	4	175.0	223.0	270.0	N/A
		Site 1	22	8	191.0	223.0	271.5	N/A
	Site 2	Operator 1	14	1	304.5	341.0	440.0	N/A
		Operator 2	15	0	322.0	383.5	515.0	N/A
		Site 2	29	1	311.0	358.5	440.0	N/A
	Site 3	Operator 1	1	14	116.0	134.5	163.5	N/A
		Operator 2	7	8	132.0	183.5	227.0	N/A
		Site 3	8	22	123.0	147.0	200.5	N/A
	Overall Total		**59**	**31**	**163.5**	**228.3**	**334.0**	**N/A**

Table 10. Summary of Flu B calls in simulated Influenza B samples

Virus / Level			**All Days (5 extraction days x 6 extraction runs per day)**					
Flu B (Strain: B/Malaysia2506/04)	**Site**	**Operator**	**# Pos Call**	**# Neg Call**	**25th Percentile MFI**	**Median MFI**	**75th Percentile MFI**	**%CV**
Flu B - Moderate Positive (MP) / (1.1 x 10^{-1} TCID50/mL)	Site 1	Operator 1	15	0	3,690.0	4155.0	4855.0	14.30
		Operator 2	15	0	3,966.0	4189.0	4578.0	11.17
		Site 1 Total	30	0	3,923.0	4184.5	4578.0	12.64
	Site 2	Operator 1	15	0	3,804.0	4111.0	4758.0	20.85
		Operator 2	15	0	3,567.0	3769.0	4167.5	9.26
		Site 2 Total	30	0	3,715.5	4007.5	4428.0	16.15
	Site 3	Operator 1	15	0	2,435.0	2803.5	3191.0	17.94
		Operator 2	15	0	2,138.0	2930.0	3403.0	23.73
		Site 3 Total	30	0	2,435.0	2895.8	3341.0	20.89
	Overall Total		**90**	**0**	**3,275.0**	**3780.0**	**4196.0**	**22.72**
Flu B - Low Positive (LP) / (2.9 x 10^{-2} TCID50/mL)	Site 1	Operator 1	15	0	1,663.0	1770.5	2434.0	22.28
		Operator 2	15	0	1,657.0	1886.5	2191.0	18.25

		Site 1 Total	30	0	1,663.0	1878.3	2199.0	20.01
	Site 2	Operator 1	15	0	1,896.0	2187.0	2303.5	12.82
		Operator 2	15	0	1,618.0	1849.5	2026.0	16.21
		Site 2 Total	30	0	1,732.0	1965.0	2191.0	16.01
	Site 3	Operator 1	15	0	868.0	1222.0	1627.0	34.89
		Operator 2	15	0	1,124.5	1212.0	1650.5	24.87
		Site 3 Total	30	0	1,083.5	1217.0	1627.0	29.70
	Overall Total		**90**	**0**	**1,456.0**	**1747.5**	**2025.5**	**27.42**
Flu B – High Negative (HN) / (7.2×10^{-3} TCID50/mL)	Site 1	Operator 1	9	6	373.5	465.5	667.0	N/A
		Operator 2	9	6	344.0	490.0	630.0	N/A
		Site 1 Total	18	12	373.0	480.0	630.0	N/A
	Site 2	Operator 1	14	1	456.0	594.0	670.0	N/A
		Operator 2	7	8	284.5	369.5	506.5	N/A
		Site 2 Total	21	9	369.5	472.0	630.0	N/A
	Site 3	Operator 1	6	9	289.5	361.0	426.5	N/A
		Operator 2	7	8	215.0	383.5	459.0	N/A
		Site 3 Total	13	17	260.0	366.5	433.0	N/A
	Overall Total		**52**	**38**	**344.0**	**421.5**	**565.0**	**N/A**

Table 11. Summary of RSV calls in simulated RSV A samples

Virus / Level			**All Days (5 extraction days x 6 extraction runs per day)**					
RSV A (Strain: Long strain ATCC VR-26	**Site**	**Operator**	**# Pos Call**	**# Neg Call**	**25th Percentile MFI**	**Median MFI**	**75th Percentile MFI**	**% CV**
RSV A – Moderate Positive (MP) / (3.5 TCID50/mL)	Site 1	Operator 1	15	0	1,141.5	1237.0	1774.0	60.64
		Operator 2	15	0	1,285.0	1818.0	2565.0	44.50
		Site 1 Total	30	0	1,145.5	1406.3	2042.0	52.24
	Site 2	Operator 1	15	0	1,891.0	2164.5	2359.0	60.54
		Operator 2	15	0	2,199.0	2629.5	3165.5	51.48
		Site 2 Total	30	0	1,964.0	2264.8	2932.0	55.37

	Site 3	Operator 1	15	0	1,186.5	1556.0	2793.5	65.26
		Operator 2	15	0	1,214.0	1726.0	5845.0	77.18
		Site 3 Total	30	0	1,214.0	1702.0	2987.5	75.19
	Overall Total		**90**	**0**	**1,309.5**	**1869.0**	**2667.0**	**66.04**
RSV A - – Low Positive (LP) / (1.8 TCID50/mL)	Site 1	Operator 1	15	0	585.0	690.0	1198.0	126.13
		Operator 2	15	0	513.0	680.5	1062.0	83.15
		Site 1 Total	30	0	536.0	685.3	1152.5	116.70
	Site 2	Operator 1	15	0	714.0	1007.0	1321.0	52.55
		Operator 2	15	0	947.5	1171.0	1535.0	30.00
		Site 2 Total	30	0	936.5	1098.3	1526.0	41.34
	Site 3	Operator 1	15	0	341.5	417.0	1204.0	118.97
		Operator 2	15	0	527.0	689.0	1265.0	49.92
		Site 3 Total	30	0	402.5	636.0	1208.0	96.44
	Overall Total		**90**	**0**	**543.0**	**904.8**	**1279.0**	**89.11**
RSV A - High Negative (HN) / (.2 x 10^{-1} TCID50/mL)	Site 1	Operator 1	5	10	57.0	69.0	131.0	N/A
		Operator 2	3	12	80.0	88.0	110.5	N/A
		Site 1 Total	8	22	67.0	86.3	127.5	N/A
	Site 2	Operator 1	2	13	78.5	98.0	113.0	N/A
		Operator 2	5	10	87.0	107.0	122.0	N/A
		Site 2 Total	7	23	86.0	102.8	116.5	N/A
	Site 3	Operator 1	0	15	51.0	67.0	77.0	N/A
		Operator 2	1	14	68.0	86.5	107.5	N/A
		Site 3 Total	1	29	64.0	70.5	96.0	N/A
	Overall Total		**16**	**74**	**68.0**	**86.5**	**109.5**	**N/A**

Table 12. Summary of RSV calls in simulated RSV B samples

Virus / Level			All Days (5 extraction days x 6 extraction runs per day)					
RSV B (Strain: Wash/ 18537/62)	**Site**	**Operator**	**# Pos Call**	**# Neg Call**	**25th Percentile MFI**	**Median MFI**	**75th Percentile MFI**	**% CV**
RSV B – Moderate Positive (MP) / (3.1 x 10^{-2} TCID50/mL)	Site 1	Operator 1	15	0	1,212.0	1545.0	1930.5	26.81
		Operator 2	15	0	1,119.0	1419.0	2114.0	94.33
		Site 1 Total	30	0	1,197.5	1485.5	1947.0	79.94
	Site 2	Operator 1	15	0	1,877.5	2528.0	3120.0	54.82
		Operator 2	15	0	2,358.0	3419.0	6593.0	52.24
		Site 2 Total	30	0	2,239.0	2763.8	4486.0	55.63
	Site 3	Operator 1	15	0	800.5	1050.0	1551.0	78.41
		Operator 2	15	0	931.0	989.0	1978.0	74.10
		Site 3 Total	30	0	900.5	991.8	1551.0	74.94
	Overall Total		**90**	**0**	**1,085.5**	**1800.5**	**2678.0**	**77.60**
RSV B - – Low Positive (LP) / (1.1 x 10^{-2} TCID50/mL)	Site 1	Operator 1	15	0	419.0	717.0	1730.5	111.46
		Operator 2	15	0	375.0	542.0	1116.0	158.61
		Site 1 Total	30	0	407.0	618.8	1335.5	131.83
	Site 2	Operator 1	15	0	735.0	1153.5	1465.0	52.08
		Operator 2	15	0	678.5	887.0	1113.5	53.25
		Site 2 Total	30	0	735.0	984.8	1323.5	53.00
	Site 3	Operator 1	11	4	142.0	293.5	779.0	98.63
		Operator 2	15	0	352.5	655.0	1285.0	97.58
		Site 3 Total	26	4	252.0	517.0	990.5	107.76
	Overall Total		**86**	**4**	**407.0**	**720.5**	**1281.5**	**105.94**
RSV B - High Negative (HN) / (2.9 x 10^{-3} TCID50/mL)	Site 1	Operator 1	5	10	70.0	89.0	165.5	N/A
		Operator 2	4	11	82.0	106.5	173.0	N/A
		Site 1 Total	9	21	82.0	103.0	165.5	N/A
	Site 2	Operator 1	10	5	121.0	203.0	299.0	N/A
		Operator 2	8	7	82.0	152.0	218.0	N/A
		Site 2 Total	18	12	110.0	159.8	233.0	N/A

	Site 3	Operator 1	3	12	70.0	97.0	120.0	N/A
		Operator 2	3	12	58.0	109.0	143.0	N/A
		Site 3 Total	6	24	63.0	101.5	133.0	N/A
	Overall Total		**33**	**57**	**82.0**	**114.0**	**190.0**	**N/A**

Table 13. Summary of hMPV calls in simulated hMPV samples

Virus / Level			**All Days (5 extraction days x 6 extraction runs per day)**					
hMPV (Strain: Subtype A2 CDC Isolate 26583)	**Site**	**Operator**	**# Pos Call**	**# Neg Call**	**25th Percentile MFI**	**Median MFI**	**75th Percentile MFI**	**% CV**
hMPV - Moderate Positive (MP) / (3.8 x 10^1 TCID50/mL)	Site 1	Operator 1	15	0	1,976.0	2192.0	2599.0	35.57
		Operator 2	15	0	2,012.5	2766.0	2853.0	17.70
		Site 1 Total	30	0	2,012.5	2362.5	2785.5	27.33
	Site 2	Operator 1	15	0	2,817.0	3064.0	3575.0	31.04
		Operator 2	15	0	2,897.0	3055.5	3293.0	11.53
		Site 2 Total	30	0	2,855.0	3059.8	3326.0	24.14
	Site 3	Operator 1	15	0	1,151.5	1440.0	1757.0	23.82
		Operator 2	15	0	1,361.0	1513.0	2016.0	19.47
		Site 3 Total	30	0	1,316.5	1493.3	1769.0	21.73
	Overall Total		**90**	**0**	**1,757.0**	**2307.3**	**2909.0**	**38.16**
hMPV - Low Positive (LP) / (1.9 x 10^1 TCID50/mL	Site 1	Operator 1	15	0	889.5	1129.0	1396.5	22.91
		Operator 2	15	0	870.5	1136.0	1385.0	27.94
		Site 1 Total	30	0	880.0	1132.5	1385.0	25.10
	Site 2	Operator 1	15	0	1,474.5	1613.0	1911.0	19.63
		Operator 2	15	0	1,499.0	1716.0	2323.0	24.57
		Site 2 Total	30	0	1,476.0	1708.3	2128.0	23.06
	Site 3	Operator 1	15	0	444.0	650.5	800.0	31.79
		Operator 2	15	0	665.0	965.0	1193.0	26.54
		Site 3 Total	30	0	650.0	757.5	969.0	34.28

	Overall Total		**90**	**0**	**830.0**	**1216.8**	**1502.0**	**43.10**
hMPV – High Negative (HN) / 6.4 TCID50/mL)	Site 1	Operator 1	13	2	233.0	330.0	459.5	N/A
		Operator 2	13	2	223.0	302.5	559.0	N/A
		Site 1 Total	26	4	233.0	326.5	459.5	N/A
	Site 2	Operator 1	15	0	391.0	486.0	709.0	N/A
		Operator 2	15	0	395.0	502.0	1101.5	N/A
		Site 2 Total	30	0	395.0	494.0	709.0	N/A
	Site 3	Operator 1	9	6	139.0	243.0	413.5	N/A
		Operator 2	13	2	234.0	263.0	308.0	N/A
		Site 3 Total	22	8	151.0	256.0	373.5	N/A
	Overall Total		**78**	**12**	**243.0**	**371.8**	**533.0**	**N/A**

Table 14. Summary of Adenovirus calls in simulated Adenovirus samples

Virus / Level			**All Days (5 extraction days x 6 extraction runs per day)**					
Adeno (Strain: Type 1, Adenoid 71)	**Site**	**Operator**	**# Pos Call**	**# Neg Call**	**25th Percentile MFI**	**Median MFI**	**75th Percentile MFI**	**% CV**
Adeno - Moderate Positive (MP) / (1.1 x 10^3 TCID50/mL)	Site 1	Operator 1	15	0	1,318.0	1695.0	1964.5	24.65
		Operator 2	15	0	1,729.5	1845.5	1975.5	16.33
		Site 1	30	0	1,626.0	1770.0	1964.5	21.09
	Site 2	Operator 1	15	0	2,824.0	3026.0	3575.0	14.53
		Operator 2	15	0	2,886.0	3009.0	3157.0	6.90
		Site 2	30	0	2,833.0	3013.3	3166.0	11.37
	Site 3	Operator 1	15	0	587.0	807.5	931.5	27.84
		Operator 2	15	0	693.0	766.0	834.0	38.43
		Site 3	30	0	650.0	772.8	920.0	34.20
	Overall Total		**90**	**0**	**897.0**	**1805.5**	**2833.0**	**52.08**
Adeno - Low Positive (LP) / (3.8 x 10^2 TCID50/mL)	Site 1	Operator 1	15	0	448.0	528.0	601.0	22.67
		Operator 2	15	0	534.0	596.5	749.0	20.71

		Site 1	30	0	522.0	553.5	649.0	24.25
	Site 2	Operator 1	15	0	1,355.0	1427.5	1530.0	8.28
		Operator 2	15	0	1,161.0	1343.5	1461.0	15.32
		Site 2	30	0	1,299.0	1358.5	1499.0	12.84
	Site 3	Operator 1	13	2	159.5	220.0	294.0	37.76
		Operator 2	15	0	200.0	227.5	272.5	16.60
		Site 3	28	2	188.5	226.0	273.0	28.59
	Overall Total		**88**	**2**	**273.0**	**553.5**	**1299.0**	**67.74**
Adeno - High Negative (HN) / (9.5 x 10^{1} TCID50/mL)	Site 1	Operator 1	5	10	115.0	134.0	167.0	N/A
		Operator 2	6	9	124.5	145.0	198.5	N/A
		Site 1	11	19	122.0	137.5	167.0	N/A
	Site 2	Operator 1	15	0	248.0	291.0	339.5	N/A
		Operator 2	15	0	290.0	321.0	347.0	N/A
		Site 2	30	0	260.0	304.3	339.5	N/A
	Site 3	Operator 1	0	15	49.0	69.5	90.0	N/A
		Operator 2	0	15	67.5	84.0	108.0	N/A
		Site 3	0	30	56.0	73.8	90.0	N/A
	Overall Total		**41**	**49**	**88.5**	**139.3**	**260.0**	**N/A**

Table 15. Summary of Rhinovirus calls in simulated Rhinovirus samples

Virus / Level			**All Days (5 extraction days x 6 extraction runs per day)**					
Rhino (Strain: FO 1-3774, Type 54)	**Site**	**Operator**	**# Pos Call**	**# Neg Call**	**25th Percentile MFI**	**Median MFI**	**75th Percentile MFI**	**% CV**
Rhino - Moderate Positive (MP) / (5.6 x 10^{-2} TCID50/mL)	Site 1	Operator 1	15	0	2,422.0	3067.5	3892.5	32.64
		Operator 2	15	0	2,774.0	3383.5	3547.0	22.17
		Site 1 Total	30	0	2,516.0	3180.3	3596.0	27.50
	Site 2	Operator 1	15	0	3,263.0	3503.0	4030.0	13.31
		Operator 2	15	0	2,934.0	3428.0	4082.5	18.58
		Site 2 Total	30	0	3,248.0	3470.3	4030.0	15.87

	Site 3	Operator 1	15	0	2,060.0	2383.0	2535.0	26.62
		Operator 2	15	0	2,037.0	2308.0	2885.0	31.48
		Site 3 Total	30	0	2,060.0	2364.5	2797.5	28.86
	Overall Total		**90**	**0**	**2,434.0**	**3060.3**	**3635.0**	**27.26**
Rhino - Low Positive (LP) / (1.4×10^{-2} TCID50/mL)	Site 1	Operator 1	15	0	639.0	931.0	1502.0	47.22
		Operator 2	15	0	526.0	655.0	796.5	40.93
		Site 1 Total	30	0	630.0	763.8	1257.0	50.11
	Site 2	Operator 1	15	0	1,018.0	1349.0	2080.0	50.94
		Operator 2	15	0	991.0	1369.0	1778.0	40.84
		Site 2 Total	30	0	1,018.0	1354.3	1778.0	46.17
	Site 3	Operator 1	11	4	258.0	521.0	731.0	63.84
		Operator 2	14	1	490.0	811.5	1231.0	48.43
		Site 3 Total	25	5	392.0	632.5	950.0	56.35
	Overall Total		**85**	**5**	**630.0**	**944.0**	**1359.5**	**61.25**
Rhino – High Negative (HN) / (5.2×10^{-3} TCID50/mL)	Site 1	Operator 1	6	9	203.0	253.0	364.0	N/A
		Operator 2	9	6	220.0	334.0	448.0	N/A
		Site 1 Total	15	15	204.5	289.5	424.0	N/A
	Site 2	Operator 1	9	6	218.0	365.5	415.5	N/A
		Operator 2	12	3	302.0	433.0	740.0	N/A
		Site 2 Total	21	9	238.0	397.0	551.0	N/A
	Site 3	Operator 1	4	11	107.5	155.0	317.0	N/A
		Operator 2	6	9	178.0	248.0	440.0	N/A
		Site 3 Total	10	20	148.0	243.0	423.5	N/A
	Overall		**46**	**44**	**201.5**	**302.0**	**440.0**	**N/A**

The reproducibility study was designed to examine maximum variability of the entire assay system – the xTAG® RVP FAST assay as well as the bioMerieux NucliSENS® EasyMag extraction method. As with any nucleic acid extraction method, the bioMerieux NucliSENS® EasyMag exhibits within run and between runs variability and contributes to the total variability of the assay. Therefore, the observed variability in this reproducibility study is partially attributable to nucleic acid extraction.

Site-to-site reproducibility for dual-analyte targets was investigated at three independent sites. Each site employed two operators and one or two Luminex instruments. Each operator conducted 5 separate extractions using Biomerieux NucliSENS® EasyMag

extraction Kit and 5 runs on non-consecutive days. Analyte levels ($TCID_{50}$/mL) were established on the high-titer viral stocks (Table 16). Each concentrated viral stock was subjected to an initial dilution which was then processed by a series of 12 dilution steps.

Table 16. Viruses used for Dual-Analyte Samples (Formulated in UTM)

Sample Type	Strain ID	Titer for Dilution 1 ($TCID_{50}$/mL)
Flu A H3	A/Victoria/3/75 DHI 20-4710010, (original ATCC VR-822)	5.9E+04
Flu B	Influenza B/Malaysia/2506/04 (PHL)	9.6E+02
Adenovirus	Type 1 Strain Adenoid 71 DHI 20-4740010, (original ATCC VR-1)	1.3E+07
RSV A	Long strain ATCC VR-26	7.3E+03
hMPV, subtype A2	CAN97-83 (CDC isolate 26583)	1.0E+05

Four different clinically relevant co-infections were represented by the dual-analyte samples. The dual-analyte samples were prepared using a "Low" concentration of one virus expected in a clinically relevant co-infection, and a "High" concentration of the other virus, and vice-versa, to create eight dual-analyte samples in UTM (Universal Transport Medium) matrix. "Low" concentration sample - a titer that generates an MFI value above the cutoff in an estimated 95% of replicates, and an MFI value below the cutoff in an estimated 5% of replicates.
"High" concentration sample - a titer that is expected to yield MFI values approaching the plateau. Results of the dual analyte testing are summarized in Tables 17 – 20.

Table 17. Summary of Calls in Adenovirus and RSV A Dual Analyte Samples

Dual Analyte Combination	Site	Adenovirus Calls		RSV Calls	
		# Positive	# Negative	# Positive	# Negative
Adeno **High** / RSV A **Low** ($4.9E+04$ $TCID_{50}$/mL / $1.1E+02$ $TCID_{50}$/mL)	Site 1	10/10	0/10	9/10	1/10
	Site 2	10/10	0/10	10/10	0/10
	Site 3	10/10	0/10	10/10	0/10
	Total	**30/30**	**0/30**	**29/30**	**1/30**
Adeno **Low** / RSV A **High** ($3.1E+03$ $TCID_{50}$/mL / $3.7E+03$ $TCID_{50}$/mL)	Site 1	10/10	0/10	10/10	0/10
	Site 2	10/10	0/10	10/10	0/10
	Site 3	10/10	0/10	10/10	0/10
	Total	**30/30**	**0/30**	**30/30**	**0/30**

Table18. Summary of Calls in Flu A H3 and RSV A Dual Analyte Samples

Dual Analyte Combination	Site	Flu A H3 Calls		RSV Calls	
		# Positive	# Negative	# Positive	# Negative
FluA.H3 **High** / RSV A **Low** (5.9E+04 $TCID_{50}$/mL / 1.1E+02 $TCID_{50}$/mL)	Site 1	10/10	0/10	8/10	2/10
	Site 2	10/10	0/10	9/10	1/10
	Site 3	10/10	0/10	9/10	1/10
	Total	**30/30**	**0/30**	**26/30**	**4/30**
FluA.H3 **Low** / RSV A **High** (3.7E+03 $TCID_{50}$/mL / 3.7E+03 $TCID_{50}$/mL)	Site 1	10/10	0/10	10/10	0/10
	Site 2	10/10	0/10	10/10	0/10
	Site 3	10/10	0/10	10/10	0/10
	Total	**30/30**	**0/30**	**30/30**	**0/30**

Table 19. Summary of Calls in Flu B and RSV A Dual Analyte Samples

Dual Analyte Combination	Site	Flu B Calls		RSV Calls	
		# Positive	# Negative	# Positive	# Negative
Flu B **High** / RSV A **Low** (9.6E+02 $TCID_{50}$/mL / 1.1E+02 $TCID_{50}$/mL)	Site 1	10/10	0/10	8/10	2/10
	Site 2	10/10	0/10	9/10	1/10
	Site 3	10/10	0/10	10/10	0/10
	Total	**30/30**	**0/30**	**27/30**	**3/30**
Flu B **Low** / RSV A **High** (6.0E+01$TCID_{50}$/mL / 3.7E+03$TCID_{50}$/mL)	Site 1	10/10	0/10	10/10	0/10
	Site 2	10/10	0/10	10/10	0/10
	Site 3	10/10	0/10	10/10	0/10
	Total	**30/30**	**0/30**	**30/30**	**0/30**

Table 20. Summary of Calls in hMPV and RSV A Dual Analyte Samples

Dual Analyte Combination	Site	hMPV Calls		RSV Calls	
		# Positive	# Negative	# Positive	# Negative
hMPV **High** / RSV A **Low** (2.6E+04 $TCID_{50}$/mL / 1.1E+02 $TCID_{50}$/mL)	Site 1	10/10	0/10	8/10	2/10
	Site 2	10/10	0/10	10/10	0/10
	Site 3	10/10	0/10	9/10	1/10

	Total	**30/30**	**0/30**	**27/30**	**3/30**
hMPV **Low** / RSV A **High** (1.6E+03 $TCID_{50}$/mL / 3.7E+03$TCID_{50}$/mL)	Site 1	10/10	0/10	10/10	0/10
	Site 2	10/10	0/10	10/10	0/10
	Site 3	10/10	0/10	10/10	0/10
	Total	**30/30**	**0/30**	**30/30**	**0/30**

Data generated form the reproducibility studies suggest that there are no significant differences in calls generated from dual-analyte samples between operators, instruments, sites or days. All High concentration dilutions in the dual-analyte combinations made a POS call by RVP FAST. False negative results for RSV low-titer infections may be observed in samples when in the presence of high concentrations of other analytes (co-infected samples). Clinically, when a dual infection is encountered, the low titer virus may be from an infection that has already passed or from a new infection.

Precision (Repeatability)

The repeatability of the RSV FAST Assay was evaluated by repeated testing of twenty (20) replicates of each analyte at LoD concentration after extraction with the Biomérieux NucliSENS® EasyMag system in a single run. The viral strains and the concentrated viral stock solution (termed as the initial stock) used in the study are shown in Table 21. Since there are two separate probes for the analyte RSV, two samples (RSV A for probe 1 and RSV B for probe 2) were used to assess repeatability for RSV. Repeatability of the Flu A analyte was examined twice since both the Flu A H1 and Flu A H3 viral samples also contain the Flu A matrix target.

Table 21 – Cultured Isolates in Universal Transport Medium (UTM)

Sample Type	**Strain ID**	**Titer of Initial Stock ($TCID_{50}$/mL)**
Flu A H1	A/Solomon Islands/3/2006 (NML)	3.1×10^{3}
Flu A H3	A/Victoria/3/75 DHI 20-4710010 (original ATCC VR-822)	5.9×10^{4}
Flu B	Influenza B/Malaysia/2506/04 (PHL)	3.1×10^{2}
Adenovirus	Type 1 Strain Adenoid 71 DHI 20-4740010 (original ATCC VR-1)	1.3×10^{7}
Rhinovirus	FO 1-3774, Type 54 ATCC 1164	2.3×10^{2}
hMPV sublineage A1	U of Iowa, Dept. Public Health. (Isolate # 16, Iowa, January 2003) hMPV-16 (IA10-2003)	4.5×10^{4}
RSV-A	Long strain ATCC VR-26	7.3×10^{3}
RSV-B	Wash/18537/62 DHI 20-4730010 (ATCC VR-1401)	1.3×10^{2}

Repeatability was assessed by confirming that the sample was consistently called across all replicates. The coefficient of variation (Standard Deviation / Mean) of the MFI values was also calculated for the 20 replicates. xTAG RVP FAST is a qualitative assay that has an underlying numeric output. As a qualitative assay based on end-point PCR, the distribution of the MFI values for each sample used in this repeatability study may show considerable variability. The repeatability study results are presented in Tables 22-23

Table 22: Assay Repeatability Assessed by Confirmation of Calls

Sample	Viral Titer ($TCID_{50}$/mL)*	xTAG RVP FAST Result
Flu A H1	7.6×10^{-1}	19 of 20 POS for both matrix FluA & hemagglutinin H1
Flu A H3	3.6	20 of 20 POS for both matrix FluA & hemagglutinin H3
Flu B	2.9×10^{-2}	20 of 20 POS
Adenovirus	3.9×10^{2}	20 of 20 POS
RSV-A	1.8	20 of 20 POS
RSV-B	1.6×10^{-2}	19 of 20 POS
Rhinovirus	1.4×10^{-2}	19 of 20 POS
hMPV sublineage A1	3.4×10^{-1}	19 of 20 POS

*These titers are at the Limit of Detection for each analyte tested.

Table 23: Assay Repeatability Assessed by Coefficient of Variation

Sample tested	Viral Titer ($TCID_{50}$/mL) Tested^	Analyte assessed	Mean MFI	Standard Deviation (SD) of MFI	CV% (SD / Mean)
FluA H1	7.6×10^{-1}	hemagglutinin H1	1076.6	354.1	32.9%
		FluA matrix	979.2	404.9	41.3%
FluA H3	3.6	hemagglutinin H3	467.2	167.8	35.9%
		FluA matrix	1425.7	322.6	22.6%
Flu B	2.9×10^{-2}	Flu B	1425.1	324.5	22.8%
Adenovirus	3.9×10^{2}	Adeno	470.6	112.9	24.0%
RSV A	1.8	RSV probe 1	1248.9	1134.8	90.9%
RSV B	1.6×10^{-2}	RSV probe 2	537.3	869.6	161.8%
Rhinovirus	1.4×10^{-2}	Rhino	731.4	503.8	68.9%
hMPV, subtype A1	3.4×10^{-1}	hMPV	514.1	246.9	48.0%

b. Linearity/assay reportable range:

Not applicable, qualitative assay

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

Before using the Luminex system to read samples prepared by the xTAG assay, prepare and calibrate the Luminex instrument system following the procedures in the appropriate system user manual

Assay Controls

An **internal control** Bacteriophage MS2 is included in the RVP FAST assay. This internal positive control is added to each patient specimen and each external control prior to extraction and serves to monitor the extraction and reverse-transcription steps. Failure to generate a PRES (present) call for the MS2 control indicates a failure at either the extraction step or reverse-transcription step, and may be indicative of the presence of amplification inhibitors which could lead to false negative results. A positive result (PRES) indicates that the extraction and amplification steps are functioning properly.

Bacteriophage lambda DNA included in RVP FAST assay kit is the **run control** is used to monitor the entire assay. For each run, the control is included as a separate sample during the RT-PCR set-up. The run control is an analyte recognized by the TDAS RVP FAST (US) that is distinct from the viral analytes the assay is intended to detect.

These **external positive controls** for target analytes are not provided with the kit but should be included with each batch of patient specimens. External controls should be prepared, extracted and tested in the same manner as patient samples. If a given analyte positive control does not perform as expected, all results for that analyte in the batch of samples are invalid and samples should be re-run.

The sponsor is also recommending including at least one negative control with each run of RVP FAST. By default, TDAS RVP FAST (US) considers the last sample on the plate to be the primary negative control. Multiple negative controls should be interspersed throughout the plate (e.g., the beginning, middle, and end).

Specimen Stability

An analytical study was performed to establish the storage conditions for nasopharyngeal swab (NPS) specimens tested using the RVP FAST assay. The performance of RVP FAST was evaluated using a set of clinical specimens that were tested following their extraction from the fresh state or after freezing at -70°C for a prolonged period of time. A total of 142 nasopharyngeal swabs, after initial testing, were stored frozen at -70°C for up to 20 months then thawed and extracted using the Biomerieux EasyMag extraction method. The nucleic acid was stored frozen at -70°C prior to testing with RVP FAST. Testing was performed in the presence of external positive pooled controls (representing analytes probed by RVP FAST). Results from all 142 specimens that were tested in these experiments are summarized in Tables 24-25

Table 24. Overall positive agreement between fresh and frozen specimens (long-term stability):

	Fresh Positive Calls	Expected Positive Calls from Frozen	Unexpected Negative Calls from Frozen	Positive Agreement (# expected positive calls / # positive calls included x 100%)	
Analyte (Virus)	**# Calls Included**	**# Expected Positive Calls**	**# Unexpected Negative Calls**	**Proportion**	**95% Lower Confidence Interval (CI)**
All Analytes	**74**	**73**	**1**	**98.64%**	**92.70%**
Flu A	11	11	0	100%	71.51%
H1	5	5	0	100%	47.82%
H3	5	5	0	100%	47.82%
Flu B	9	8	1	88.89%	51.75%
RSV	21	21	0	100%	83.89%
Adenovirus	3	3	0	100%	29.24%
hMPV	12	12	0	100%	73.53%
Rhino	8	8	0	100%	63.06%

Table 25. Overall negative agreement between fresh and frozen specimens (long-term stability):

	Fresh Negative Calls	Expected Negative Calls from Frozen	Unexpected Positive Calls from Frozen	Negative Agreement (# expected negative calls / # negative calls included x 100%)	
Analyte (Virus)	**# Calls Included**	**# Expected Negative Calls**	**# Unexpected Positive Calls**	**Proportion**	**95% Lower Confidence Interval (CI)**
All Analytes	**1062**	**1048**	**14**	**98.68%**	**97.80%**
Flu A	131	129	2	98.47%	94.59%
H1	137	136	1	99.27%	96.00%
H3	137	135	2	98.54%	94.83%
Flu B	133	131	2	98.49%	94.67%
RSV	121	121	0	100%	97.00%
Adenovirus	139	138	1	99.28%	96.06%
hMPV	130	128	2	98.46%	94.55%
Rhino	134	130	4	97.01%	92.53%

All targets included in RVP FAST were represented in the sample set. Positive agreement between fresh and frozen specimens was 100% for each target, with the exception of Flu B (88.89% with a95% lower bound CI 51.75%).

Fresh vs. Frozen Study

In order to utilize frozen banked clinical samples in the evaluation of RVP FAST assay, a study was conducted to demonstrate that preservation of samples (by freezing at ≤-

70°C does not affect the accuracy of test results compared to freshly collected or freshly prepared samples. The "fresh vs. frozen" study evaluated the performance of xTAG® RVP FAST on a set of clinical specimens (nasopharyngeal swabs) that were tested following their extraction from the fresh state or thawed state (i.e. after freezing at -70°C for a short period of time). A total of 202 nasopharyngeal swabs (NP swabs) were prospectively collected from symptomatic pediatric and adult subjects. Upon receipt at the virology laboratory, each specimen was split into 2 aliquots: (1) the first aliquot was extracted from the fresh state using the Biomerieux EasyMag extraction method and the nucleic acid stored frozen at -70°C; (2) the second aliquot was stored frozen at -70°C in its un-extracted state. Upon accrual of approximately 40 prospective clinical specimens, frozen aliquots of the original specimens were thawed and extracted using the Biomerieux EasyMag extraction method. The nucleic acid was stored frozen at -70°C. Extracts from both fresh and frozen specimens (batches of approx 40 specimens, both fresh and frozen) were then thawed and tested in the same run by RVP FAST in the presence of external positive pooled controls (representing analytes probed by RVP FAST). Twenty three (23) specimens and their corresponding calls were excluded from the analysis due to failure of the internal control when tested from the fresh state. One (1) additional specimen was excluded from the analysis due to failure of the internal control when tested from the frozen state. Results from the remaining 178 specimens that were analyzed (fresh and frozen) are summarized in Tables 26 and 27 below.

Table 26. Overall positive agreement between fresh and frozen specimens (short-term stability):

	Fresh Positive Calls	**Expected Positive Calls from Frozen**	**Unexpected Negative Calls from Frozen**	**Positive Agreement (# expected positive calls / # positive calls included x 100%)**	
Analyte (Virus)	**# Calls Included**	**# Expected Positive Calls**	**# Unexpected Negative Calls**	**Proportion**	**95% Lower Confidence Interval (CI)**
All Analytes	**89**	**88**	**1**	**98.88%**	**93.90%**
Flu A	11	11	0	100%	71,51%
H1	6	6	0	100%	54.07%
H3	5	5	0	100%	47.82%
Flu B	10	10	0	100%	69.15%
RSV	29	28	1	96.55%	82.24%
Adenovirus	4	4	0	100%	39.76%
hMPV	14	14	0	100%	76.84%
Rhino	10	10	0	100%	69.15%

Table 27. Overall negative agreement between fresh and frozen specimens (short-term stability):

	Fresh Negative Calls	Expected Negative Calls from Frozen	Unexpected Positive Calls from Frozen	Negative Agreement (# expected negative calls / # negative calls included x 100%)	
Analyte (Virus)	**# Calls Included**	**# Expected Negative Calls**	**# Unexpected Positive Calls**	**Proportion**	**95% Lower Confidence Interval (CI)**
All Analytes	**1335**	**1328**	**7**	**99.48%**	**98.92%**
Flu A	167	165	2	98.80%	95.74%
H1	172	171	1	99.42%	96.80%
H3	173	172	1	99.42%	96.82%
Flu B	168	167	1	99.40%	96.73%
RSV	149	149	0	100%	97.55%
Adenovirus	174	173	1	99.43%	96.84%
hMPV	164	164	0	100%	97.78%
Rhino	168	167	1	99.40%	96.73%

All targets included in RVP FAST were represented in the sample set. Positive agreement between fresh and frozen specimens was 100% for each target, with the exception of RSV (96.55% with a 95% lower bound CI 82.24%).

d. Detection limit:

Analytical studies were carried out to determine the LoD for each RVP FAST targeted organism. Samples were prepared from viral strains listed in column 2 of Table 28. Serial dilutions of the viral analyte targets were prepared from a high titer stock in Universal Transport Medium (UTM) for the following analytes: Flu A (hemagglutinin H1), Flu A (hemagglutinin H3), Flu B, Adenovirus, RSV A, RSV B, Rhinovirus and hMPV subtypes A1, A2, B1 and B2. Serial dilutions were prepared from concentrated viral stocks in UTM.

In the first part of this study, serial dilution curves for the Flu A H1, Flu A H3, Flu B, Adenovirus, RSV-A, RSV-B, Rhinovirus and hMPV subtypes A1, A2, B1 and B2 were obtained. These curves were generated by assessing 3 to 4 replicates of each dilution level starting from the sample extraction step with the xTAG RVP FAST assay. a dilution or dilutions for each target were selected for further confirmation testing. Confirmation of LoD for each target was achieved through testing of 20 replicates of the selected dilutions starting from sample extraction. In general, the dilution level corresponding to the lowest concentration of the analyte for which 3/3 (or 4/4) replicates generated positive calls by xTAG RVP assay was selected for LoD confirmation testing. LoD was considered as confirmed if the selected dilution level gave positive calls for ≥19/20 of the replicates.

Table 28. Summary results for the LoD confirmation studies

Analyte	Strain ID	$TCID_{50}$/mL (corresponding to the estimated LoD)	Mean MFI values (n=20)
Adenovirus	Type 1 Strain Adenoid 71 DHI 20-4740010 (original ATCC VR-1)	3.9×10^{2}	471
Flu A H1	A/Solomon Islands/3/2006 (NML)	7.6×10^{-1} (matrix)	979
		7.6×10^{-1} (H1)	1077
Flu A H3	A/Victoria/3/75 DHI 20-4710010 (original ATCC VR-822)	1.8 (matrix)	628
		3.6 (H3)	467
Flu B	Influenza B/Malaysia/2506/04 (PHL)	2.9×10^{-2}	1425
Rhinovirus	FO 1-3774, Type 54 ATCC 1164	1.4×10^{-2}	731
hMPV	University of Iowa, Dept. Public Health. (hMPV sublineage A1, Isolate # 16, Iowa, January 2003)	3.4×10^{-1}	514
	CAN97-83 (hMPV sublineage A2, CDC isolate 26583)	1.3×10^{1}	928
	University of Iowa, Dept. Public Health. (hMPV sublineage B1, Isolate #5, Iowa)	1.1	1091
	University of Iowa, Dept. Public Health. (hMPV sublineage B2, Isolated October, 2003, Cusco, Peru)	1.2	638
RSV	RSV A Long strain ATCC VR-26	1.8	1249
	RSV B Wash/18537/62 DHI 20-4730010 (original ATCC VR-1401)	1.6×10^{-2}	537

e. Analytical Reactivity:

The analytical **inclusivity** study was performed to determine whether the RVP FAST test is able to detect a variety of strains that represent the temporal and geographic diversity of each of RVP target organism. The dilution series for each

viral stock was intended to cover the detection range of the assay including viral loads at or near the limit of detection (LoD). All 10-fold dilution levels of a given viral strain were tested in a single run with the RVP FAST assay, together with the controls (DNAse-free and RNAse free water, lambda DNA, and MS2 bacteriophage) as recommended in the package insert. In the event that the MFI generated for a particular organism in the initial 10-fold dilution series fell well above or below the assay cut-off, then additional dilutions were generated and tested with RVP FAST. The starting concentration and dilution factor of each successive dilution was determined upon review of the initial 10-fold dilution series

To supplement the known cultured isolate strains tested, sequencing data was generated from clinical specimens that tested positive by RVP FAST for Flu A H1, Flu A H3, RSV, Rhinovirus or hMPV, to verify that the 'positive' call by the assay concurred with a 'positive' sequence in the sample.

Summary tables of Analytical Reactivity (Inclusivity) Testing with the RVP FAST Assay are presented below in Tables 29 – 36.

RVP FAST inclusivity testing showed broad coverage of influenza A subtypes. In addition, phylogenetic analysis of sequencing data from positive clinical samples show that all of the Flu A H1 sequences are closely related to A/Brisbane/59/2007, the reference strain recommended by WHO for inclusion in the 2009-2010 trivalent vaccine for the Northern Hemisphere. The A/Brisbane/59/2007 was the predominant Flu A H1N1 strain in human circulation, just before the outbreak of the 2009 pandemic. Most of the Flu A H3 sequences obtained from positive clinical samples were closely related to either A/Brisbane/10/2007 (H3N2) or A/Perth/16/2009 (H3N2) reference strains which were the strains recommended by WHO for inclusion in the 2009-2010 influenza virus trivalent vaccine for the Northern and Southern hemispheres, respectively.

Table 29. RVP FAST results on Influenza A strains, subtype H1

Subtype	Strain ID	Concentration	Flu A matrix call	H1 call	H3 call
H1N1	A/ Caledonia/20/99	Unknown	POS	POS	NEG
	A/ Solomon Islands/03/06	2.8 $TCID_{50}$/mL	POS	POS	NEG
	A/Brisbane/59/2007	Flu A Ct: 33.48 H1 Ct: 34.75	POS	POS	NEG
	A/Swine/Iowa/1976/31	Ct: 28.59	POS	POS	NEG
	A/Swine/Ontario/52156/03	Ct: 33.24	POS	POS	NEG

Table 30. RVP FAST results on Influenza A strains, subtype H3

Subtype	Strain ID	Concentration (Ct value)	Flu A matrix call	H1 call	H3 call
H3N2	A/ Wyoming/03/2003	36.73	POS	NEG	POS
	A/Perth/16/2009	Flu A: 33.77 H3: 32.02	POS	NEG	POS
	A/ Panama/2007/99	33.64	POS	NEG	POS
	A/ Victoria/3/75	36.46	POS	NEG	POS
	A/ Christchurch/90/2004	33.43	POS	NEG	POS
	A/ Zhejiang/209/2005	36.20	POS	NEG	POS
	A/ Aichi/174/2005	34.06	POS	NEG	POS
	A/ New York/206/2005	37.34	POS	NEG	POS
	A/ Nairobi/5842/2006	26.86	POS	NEG	POS
	A/ New York/376/2005	36.99	POS	NEG	POS
	A/ Italy/384/2005	36.69	POS	NEG	POS
	A/ Minnesota/04/2008	unknown	POS	NEG	POS

Table 31. RVP FAST results on Influenza A non-H1N1 (seasonal) or non-H3N2 strains

Subtype	Strain ID	Concentration (Ct value)	Flu A matrix call	H1 call	H3 call
2009 H1N1 (swine)	A/England/195/2009	FluA 36.10 H1v 30.83 N1v 33.46	POS	NEG	NEG
	A/Aragon/3218/2008	FluA 30.96 H1v 33.82 N1v35.15	POS	NEG	NEG
	A/England/935240/2009	FluA 26.58 H1v 21.87 N1v 24.40	POS	NEG	NEG
	A/England/935240/2009 and A/Scotland/8/2009	FluA 28.04 H1v 22.84 N1v 25.69	POS	NEG	NEG
H5N1	NIB23A/Turkey/Turkey/1/2005	FluA 23.18 H5 20.37	POS	NEG	NEG
H6N1	A/Turkey/England/198/2009	FluA 32.84 H6 28.42	POS	NEG	NEG

H7N7	H7N7/Prague/56	Unknown	POS	NEG	NEG

Table 32. RVP FAST results on Influenza B strains

Strain ID	Concentration (Ct value)	Flu B call
B/ Sichuan/379/99	31.89	POS
B/ Hong Kong/330/01	31.13	POS
B/ Jiangsu/10/2003	31.48	POS
B/ Shanghai/361/2002	Unknown	POS
B/ Malaysia/2504/04	38.72	POS
B/ England/145/2008	27.84	POS
B/England/393/2008	32.48	POS

Table 33. RVP FAST results on human rhinovirus strains

Strain ID	Group	Serotype	Concentration	Rhinovirus call
Rhino 1a	HRV-A	1	Unknown	POS
Rhino 39	HRV-A	39	Unknown	POS
Rhino 54	HRV-A	54	Unknown	POS
Rhino 60	HRV-A	60	Unknown	POS

Three different sets of validated rhinovirus sequencing primers were used to sequence clinical samples from the RVP FAST multi-site study. Based on the empirical testing of reference strains using RVP FAST and sequence analysis of clinical samples that tested "positive" with RVP FAST, the data show wide coverage among all three rhinovirus species A, B and C.

Table 34. RVP FAST results on Adenovirus strains

Species	Serotype ID	Concentration	Adenovirus call
A	18	3.2×10^6 $TCID_{50}$/mL	POS
	31	Unknown	POS
B	7	Ct 33.21	POS
	14	Ct 38.63	POS

	16	Ct 31.08	POS
	21	Ct 30.11	POS
	35	Unknown	POS
C	2	Ct 27.51	POS
D	8	Ct 32.16	POS
	10	Ct 29.54	POS
	13	Ct 28.93	POS
	19	Ct 32.47	POS
	22	Unknown	POS
	25	Ct 39.53	POS
	30	1.6×10^{-1} $TCID_{50}$/mL	POS
	37	1.6 $TCID_{50}$/mL	POS
	45	Unknown	POS
E	4	1.6×10^{1} $TCID_{50}$/mL	POS
F	40	1.6×10^{1} $TCID_{50}$/mL	POS
	41	Ct 30.58	POS

A total of 20 adenovirus serotypes were empirically tested with RVP FAST assay. The RVP FAST assay was able to detect a broad coverage of Adenoviruses including representatives of all 6 species (A-F).

In addition to this empirical data, 4 additional hMPV strains representing groups A1, A2, B1 and B2 were tested as part of the LoD study. All four phylogenetic groups of human Metapneumovirus (hMPV) were detected by RVP FAST (A1, A2, B1, B2).

Table 35. RVP FAST results on hMPV results

Phylogenetic group	Concentration	hMPV call
A1	Unknown	POS
A2	Unknown	POS
B1	Unknown	POS
B2	Unknown	POS

Two different sets of validated hMPV primers were used, to sequence clinical samples from the RVP FAST multi-site study. A total of 38 RVP FAST hMPV positive

specimens generated positive sequences for hMPV. Of these, 15 generated positive sequences for the Nucleocapsid gene, and 28 generated positive sequences for the Phosphoprotein gene (5 samples generated positive sequences for both genes) Sequence analysis of positive clinical samples show coverage among three hMPV phylogenetic groups (A2, B1 and B2). hMPV group A1 was not represented in the clinical sample set.

Table 36. Respiratory Syncytial Virus Reference Strains

Strain ID	Source	Group	Concentration (Ct Value)	RSV call
Long	ATCC VR-26	RSV-A	36.32	POS
Wash/18537/62	ATCC VR-1401	RSV-B	27.07	POS
9320	ATCC VR-955	RSV-B	29.4	POS
A-2	ATCC VR-1540	RSV-A	36.52	POS
B WV/14617/85	ATCC VR-1400	RSV-B	27.85	POS
RSV A2 cpts-530	ATCC VR-2452	RSV-A	37.88	POS
Subgroup B cp23 Clone 1A2	ATCC VR2579	RSV-B	26.14	POS

f. Analytical Specificity/Cross-reactivity Evaluation:

An analytical exclusivity study was carried out to assess the potential for false positive results due to cross-reactivity between RVP FAST assays and other common respiratory tract pathogens that are not probed by the RVP FAST assay. A total of 30 potentially cross-reactive pathogens (Table 37) were assessed with RVP FAST. Each replicate underwent a single EasyMag (bioMerieux NucliSENS®) extraction prior to testing. Potentially cross-reactive viruses were prepared by growing each virus in the appropriate cell host, to a high titer of ~10^5 pfu/mL (≈10^5 $TCID_{50}$/mL). Potentially cross-reactive bacteria were prepared at a turbidity corresponding to a 1/100 dilution of a 0.5 McFarland Standard (approximately 1x10^6 cfu/mL). In all cases, UTM-RT medium (universal transport media; Copan, reference number 330C) was used as a matrix.

Table 37. Pathogens (Viral and Bacterial) Used in the Cross-Reactivity Study

Bacterial (n=20)	***Viral (n=10)***
Bordetella pertussis	*Herpes simplex virus Type 1*
Chlamydia pneumoniae	*Cytomegalovirus*
Haemophillus influenzae	*Varicella-zoster virus*
Pseudomonas aeruginosa	*Mumps*
Streptococcus pneumoniae	*Rubeola (Measles)*
Moraxella cartarrhalis	*Epstein Barr virus*

Mycobacterium intracellulare	*Parainfluenza 1*
Mycoplasma bovis	*Parainfluenza 2*
Mycoplasma pneumoniae	*Parainfluenza 3*
Klebsiella pneumoniae	*Bocavirus*
Legionella pneumophilia	
Neisseria meningitidis	
Staphylococcus aureus	
Staphylococcus epidermidis	
Streptococcus Group B	
Acinetobacter baumanii (calcoaceticus)	
Streptococcus pyogenes	
Mycobacterium avium	
Serratia marcescens	
Escherichia coli	

Analysis of the pathogens listed in Table 37 showed no cross-reactivity with any viral target probed by RVP FAST. The E. coli stock tested yielded a "present" call for the run control (bacteriophage lambda), as expected. Contamination of some E. coli strains with lambdoid phages is a known phenomenon in the natural ecology of E. coli in the wild thus yielding a "Present" call for the lambda run control (Krylog & Tsygankov 1976; Dhillon et al. 1980; Kameyama et al. 1999).

Cross-Reactivity with Enterovirus

Rhinoviruses and Enteroviruses are closely-related genera of the Picornaviridae family, small, non-enveloped ssRNA positive-strand viruses. Potential cross-reactivity with Entorovirus was assessed using cultured isolates. A list of the Enterovirus strains tested is presented in Table 38 below.

Table 38. Enterovirus Strains tested with RVP FAST

Enterovirus Sabin 1
Enterovirus Sabin 2
Enterovirus Sabin 3
Enterovirus 70
Enterovirus Coxsackie B1
Enterovirus Coxsackie B2
Enterovirus Coxsackie B4
Enterovirus Coxsackie B5
Enterovirus Coxsackie A9
Enterovirus Echo 6
Enterovirus Echo 7
Enterovirus Echo 11

Enterovirus Echo 13
Enterovirus Echo 14
Enterovirus Echo 30

All cultured isolates tested generated a positive call for Rhinovirus by RVP FAST suggesting that Enterovirus cross-reacts with Rhinovirus-specific primers in the RVP FAST assay.

Cross-Reactivity with Coronaviruses
Potential cross-reactivity with coronaviruses (HKU1, NL63 and OC43) was examined using clinical specimens that tested positive by a real-time PCR assay (N=9). No cross reactivity with viral targets probed by RVP FAST were reported in these samples.

g. Assay cut-off:

Assay cut-off determination (threshold-setting algorithm) consists of three steps for each analyte: 1) setting an initial cut-off range using a validated computer algorithm, 2) recommending optimized cut-offs within this range based on ROC analysis of empirical data, and 3) establishing an MFI cut-off value through a Design Review Committee (DRC) assessment of ROC curves. DRC assessments are carried out in accordance with internal procedures that are part of the Design Control sub-system of the LMD Quality System.

Distinct sample sets were used for setting initial cut-offs (step 1 above) and for finding the optimized cut-offs (step 2 above). Clinical specimens used in these two cut-off determination steps 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 and generated either by DSFA, culture followed by DFA, the 510k cleared xTAG RVP assay (k063765) or a lab-developed real-time RT-PCR assay. The sample set used in these 2 cut-off determination steps also included cultured isolates with confirmed viral identity which were serially diluted into universal transport media (UTM). Finally, the sample set was supplemented with extraction controls (UTM spiked with MS2) that were coded as negative for all viral targets. All samples were extracted using the Biomerieux EasyMag method prior to being tested with RVP FAST.

For each analyte, ten (10) alternative cut-off values within the initial cut-off range were assessed using ROC analysis of the optimization data set. The positive and negative agreements (with their corresponding lower bound 95% confidence interval) between xTAG® RVP FAST and the expected call were used to select optimal cut-offs for each analyte. Table 39 below lists the optimal MFI cut-off ranges generated for each analyte using the following acceptance criteria as a guide:
≥90% positive agreement with lower bound of the two-sided 95% CI ≥ 80%
≥90% negative agreement with lower bound of the two-sided 95% CI ≥ 90%

Table 39. Optimized threshold range for each analyte

Analytes	No. of negative samples	No. of positive samples	Optimal cutoffs	Positive agreement	95% CI - Lower bound	Negative agreement	95% CI - Lower bound
Adenovirus	236	22	98-135	92 - 95%	71 – 77%	95 - 99%	91 – 97%
Rhinovirus	218	40	273-441	95%	83%	94%	90%
H1	229	29	173-679	93%	77%	100%	98%
H3	240	18	151-410	94 - 100%	73 – 81%	99%	97%
hMPV	243	15	152-637	93%	68%	100%	98%
Influenza-A	208	50	179-836	92 - 96%	81 – 86%	100%	97%
Influenza-B	225	33	218-630	94 - 97%	80 – 84%	100%	98%
RSV-probe 1	211	47	98-798	94 - 100%	82 – 92%	94 – 100%	90 - 98%
RSV-probe 2	243	15	94-292	100%	78%	94 – 100%	90 - 98%

A design review committee (DRC) selected the final cut-offs. Final cut-offs fell within the optimized threshold range (Table 40) for all analytes with the exception of Adenovirus. For this target, a final cut-off value of 150 MFI was selected as most of the optimization samples that were not detected by the assay had high Ct values (>35) and were co-infected with multiple targets.

Table 40: Final cutoffs for xTAG RVP FAST

Analyte	Final Cut-off (MFI)
Adenovirus	150
Influenza A	300
H1	450
H3	200
Influenza B	400
Rhinovirus	300
HMPV	200
RSV Probe-1	120
RSV Probe-2	150

h. Interfering Substances:

An interference study was carried out to evaluate the influence of potential interfering substances on the accuracy of test results obtained with the RVP FAST. A total of 14 combinations of analyte and potential interferents (Table 41) were assessed with RVP FAST. Each replicate was extracted using the EasyMag method. Potentially interfering pathogens were assayed in the presence of RVP FAST targets. The RVP FAST targets were prepared at 4-10x the limit of detection for that analyte. Potential viral interferents were spiked in at titers of ~ 10^5 pfu/mL (≈ 10^5 $TCID_{50}$/mL) and potential bacterial interferents were spiked in at titers of ~ $1x10^6$ cfu/mL (by turbidity measurement, against the McFarland Standard).

Table 41. Potential Interferents tested with RVP FAST

Target analyte	***Potential interferent***
RSV	*Streptococcus pneumoniae*
	Bordetella pertussis
	Haemophilus influenzae
	CMV
Adenovirus	*Bordetella pertussis*
	CMV
	Chlamydia pneumoniae
Influenza A (H3)	*Streptococcus pneumoniae*
	Staphylococcus aureus
	Bordetella pertussis
	Chlamydia pneumoniae
Rhinovirus	*Streptococcus pneumoniae*
	Mycoplasma pneumoniae
	Haemophilus influenzae

Analysis of the pathogens listed in Table 41 showed no interference with the RVP FAST targets tested

Common Medications
Clinically significant interference by medications would result in lower RVP FAST detection rates in specimens obtained from medicated patients compared with non-medicated patients. Analysis of the clinical data set shows that no significant differences in the sensitivity of RVP FAST targets were observed between medicated patients and unmedicated patients. RVP FAST correctly identified 217/229 (94.75%) of the targets probed by the assay in the population receiving medications and 106/110 (96.36%) in the population not receiving medications. This suggests that commonly prescribed medications in the intended use population do not interfere with RVP FAST assay results. A complete list of medications recorded in patients charts extracted for the clinical dataset is presented in Table 42 below.

Table 42. Medications Administered to Subjects Included in the RVP Fast Prospective Dataset

Generic Medication List			
Acebutolol	Dalteparin	Insulin lispro	Piperacillin/Tazobactam
Acetaminophen	Dapsone	Ioperamide	Posaconazole
Acetylsystein	Darbepoetin	Ipratropium	Potassium chloride

Acne medications	Dexamethasone	Ipratropium bromide	Pramipexole
Acyclovir	Dextromethorphan	Irbesartan	Pravastatin
Albuterol	Diazepam	Iron supplements	Prednisone
Alendronate	Diclofenac	Itraconazole	Prochlorazine
Alendronate sodium/Cholecalciferol	Digoxin	Ketorolac	Prochlorperazine
Allopurinol	Diltiazem	Lactulose	Pseudoephedrine
Amcinonide	Dimenhydrinate	Lansoprazole	Psyllium
Amiloride	Diphenhydramine	Larazapam	Pyrazinamide
Amiloride hydrochloride/hctz	Docetaxel	Levalbuterol	Pyridostigmine
Amiodarone	Docusate	Levfloxacin	Quetiapine fumarate
Amitriptyline	Docusate sodium	Levodopa - carbidopa	Quinapril hydrochloride
Amlodipine	Domperidone	Levofloxacin	Quinine
Amlodipine besylate	Donepezil hydrochloride	Levomepromazine	Rabeprazole
Amoxicillin	Doxycycline	Levonorgestrel and Ethinyl estradiol	Rabeprazole sodium
Amoxicillin clavulanate	Doxylamine succinate	Levothyroxine	Raloxifene
Ampicillin	EC acetylsalicylic acid	Linezolid	Ramipril
Ampicillin - cefotaxime	Enalapril	Liothyronine	Ranitidine
Antihistamine	Enoxaparin	Lisinopril	Rifampin
Anti-malaria drugs	Erythromycin	Lorazepam	Risedronate sodium
Aspirin	Esomeprazole	Maalox	Risperidone
Atenolol	Etanercept	Magnesium	Rivastigmine
Atorvastatin	Ethinyl estradiol and norethindrone	Magnesium oxide	Rosiglitazone
Azathioprine	Ezetimibe	Magnesium sulphate	Rosuvastatin
Azithromycin	Famotidine	Megestrol acetate	Salbutamol
Aztreonam	Fentanyl	Meropenem	Salbutamol sulfate

Benzydamine	Ferrous fumarate	Metformin	Saline
Bevacizumab	Ferrous gluconate	Methadone	Salmeterol
Bicalutamide	Ferrous sulphate	Methotrexate	Scopolamine
Bisacodyl	Fexofenadine	Methylprednisolone	Senna
Bisoprolol	Filgrastim	Metoclopramide	Sennosides
Budesonide and formoterol	Finasteride	Metoprolol	Seroquil
Bupropion	Fish oil	Metoprolol tartrate	Sertraline hydrochloride
Calcitriol	Fluconazole	Metronidazole	Simvastatin
Calcium	Flunisolide nasal	Midazolam	Sitagliptin
Calcium carbonate	Fluticasone	Mineral oil	Sodium biphosphate
Calcium chloride	Fluticasone - salmeterol	Mirtazapine	Sodium phosphates
Calcium gluconate	Fluticasone - Salmeterol	Montelukast	Spironolactone
Candesartan cilexetil	Fluticasone propionate	Morphine	Spironolactone-hctz
Candesartan-hctz	Fluvoxamine maleate	Moxifloxacin	Sulfamethoxazole - trimethoprim
Carvedilol	Folic acid	Mucousitis mouth wash	Sulfonylurea
Caspofungin	Fosinopril	Multivatamins and minerals	Surfactant
Cefazolin	Furosemide	Mycophenolate mofetil	Tacrolimus
Cefepime	Gabapentin	Nadolol	Tamsulosin
Cefotaxime	Ganciclovir	Nicotine patch	Terazosin
Cefprozil	Gentamycin	Nifedipine	Testosterone undecanoate
Cefradine	Gliclazide	Nitrofurantoin	Theophylline, anhydrous
Ceftazidime	Glucosamine	Nitroglycerin	Thiamine (Vitamin B1)
Ceftriaxone	Glyburide	Nortriptyline	Thyroxine

Cefuroxime	Glycerin	Nystatin	Ticarcillin/Clavulanate
Celecoxib	Granisetron	Olanzapine	Tiotropium
Cephalexin	Guaifenesin - phenylephrine	Omeprazole	Tobramycin
Chaste tree	Haloperidol	Ondansetron	Tramadol
Chemotherapeutic drugs	Heparin	Oseltamivir	Tranexamic acid
Chlorpromazine	Hydrochlorothiazide	Oxazepam	Trazodone
Ciprofloxacin	Hydrochlorothiazide - losartan	Oxtriphylline	Triazolam
Citalopram	Hydrocodone	Oxycodone	Trimethoprim
Citalopram hydrobomide	Hydrocortisone	Packed red blood cells	Ursodeoxycholic acid
Clarithromycin	Hydromorphone	Pantoprazole	Ursodiol
Clindamycin	Hydroxychloroquine	Parachlorometaxylenol	Valganciclovir
Clonazepam	Hydroxychloroquine sulfate	paroxetine	Valproic acid
Clopidogrel	Hydroxyquine	Penicillamine	Vancomycin
Cloxacillin	Hypromellose	Penicillins and beta-lactamase inhibitors	Venlafaxine
Clozapine	Ibuprofen	Pentamidine	Vitamin A, Vitamin D, Vitamin C
Codeine	Imatinib	Pentostatin	Vitamin B12
Codeine phosphate	Imipenem	Pentoxifylline	Vitamin E
Conjugated estrogens	Indapamide	Perindopril	Voriconazole
Cyanocobalamin	Indomethacin	Phenylephrine	Wafarin
Cyclobenzaprine	Infliximab	Phenytoin	Zopiclone
Cyclophamide	Insulin aspart	Phosphate	
Cyclosporine	Insulin isophane and	Pioglitazon	

	insulin regular	hydrocholoride	

i. Carry-Over Contamination:

The carry-over study was conducted using water blanks (DNase and RNase free distilled water) alternating with replicates of a high titre purified viral nucleic acid sample in a checkerboard pattern. RSV B nucleic acid (Wash/18537/62DHI 20-4730010; original ATCC VR-1401) was prepared at a high titer far above the assay cut-off, in order to obtain positive calls 100% of the time and maximize the potential for cross contamination. RSV B was chosen as the analyte, since it is commonly observed in clinical specimens at high titre (Chidgey and Broadley, 2005). This study consisted of six identical runs tested over six different days each performed by one operator, using a single kit lot and equipment set. The mean MFI of the RSV B replicates was 10,860. No carryover contamination with RSV B was observed, as the MFI values obtained in the blank positions was not significantly greater than the LoB any blank position on the checkerboard plate layout.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable. Refer to the Clinical Studies Section of this document.

b. Matrix comparison:

Not applicable

3. Clinical studies:

Prospective Clinical Study

The clinical performance of the RVP FAST assay was evaluated during prospective studies at three clinical laboratories; two of the laboratories were in Southern Ontario (Canada) and one in New York (US). A total of 1191 nasopharyngeal swabs clinical specimens were collected from pediatric and adult patients. Of these 1191 specimens, 633 were prospectively collected during the 2007/2008 and 558 in the 2008/2009 influenza seasons (prospective data set). An additional 34 specimens were pre-selected for adenovirus (pre-selected data set). Of the 1191 prospectively collected clinical specimens, 178 were tested by RVP FAST from the "fresh" state and 1013 were tested from the "frozen" state. All 34 pre-selected adenovirus positive clinical specimens were tested by RVP FAST from the "frozen" state.

The study sites enrolled subjects from diverse demographic groups. Data summery of demographic information for the 1191 subjects that participated in the prospective study is presented in Table 43 below.

Table 43. Demographic Summary for RVP FAST Prospective Clinical Study

SEX	NUMBER OF SUBJECTS
Male	591 (49.6%)
Female	600 (50.4%)
AGE (yrs)	
0 - 1	187 (15.7%)
>1 - 5	82 (6.9%)
>5 - 21	112 (9.4%)
>21 - 65	471 (39.5%)
>65	339 (28.5%)
SUBJECT STATUS	
Outpatients	120 (10.1%)
Hospitalized	473 (39.7%)
Emergency Department	291 (24.4%)
Not Determined	307 (25.8%)

For Influenza A, Influenza B, RSV and Adenovirus, all specimens were assessed from the fresh state at each site by cleared Direct Specimen Fluorescent Antibody (DSFA) tests and/or culture followed by DFA. If DSFA or culture followed by DFA was positive, then the specimen was coded as positive by comparator. Comparator testing for these analytes was carried out at the clinical site processing the test requisition for that patient sample.

For subtypes of Influenza A, well-characterized RT-PCR amplification followed by bi-directional sequencing was performed on frozen extracts of all DSFA or culture followed by DFA positive specimens. Amplification primers used for Influenza A subtyping targeted different genomic regions from the ones probed by RVP FAST.

For rhinovirus and hMPV, a composite reference method (a predetermined algorithm that combines results of more than one test) was used as comparator. This method consisted of 2 separate, well-characterized nucleic acid amplification tests (NAATs) followed by bi-directional sequencing. To the extent possible, the NAATs used in the composite reference method targeted different genomic regions from the ones probed by RVP FAST. If at least one of the two NAATs was positive by bi-directional sequencing, the specimen was considered to be positive by comparator. If both NAATs were negative by bi-directional sequencing, then the specimen was coded as negative by comparator. C

Nucleic acid testing (NAATs), including RVP FAST, was performed on left-over specimen that had been extracted from the fresh or frozen state. Total extracted nucleic acid material was stored at -70°C prior to testing. A total of 30 specimens were rerun by RVP FAST. Of these, 1 was due to internal control failure ("Sample failed: unexpected control call(s)"); 23 were due to indeterminate Flu A H1 or H3, defined as POS call for H1 or H3 hemagglutinin gene in the absence of a POS call for the matrix gene ("Target failed: incompatible signals between targets"); 2 were due to Flu A unsubtypeable results ("Warning: Influenza A detected but subtype could not be determined. Refer to Kit Package insert for further instructions") and 4 specimens were due low bead count results in the primary negative control ("Assay failed: low bead count(s) for the primary negative control sample").

Diagnostic sensitivity (and specificity) of RVP FAST was established by determining the fraction of comparator positive (or negative) results that were also found positive (or negative) by RVP FAST. Sensitivity was calculated by dividing the total number of "true positive" RVP FAST results (TP) by the sum of the TP and "false negative" (FN) RVP FAST results. Specificity was calculated by dividing the total number of "true negative" RVP FAST results (TN) by the sum of the TN and "false positive" (FP) RVP FAST results. An RVP FAST result was considered to be a TP or TN result only in the event that it agreed with the comparator method result for the analyte in question. The prospective performance data (all sites combined) are presented by analyte in the Tables 44-51:

Table 44. Influenza A

RVP FAST	**Primary Comparator**		
	Positive	Negative	TOTAL
Positive	129	47	176
Negative	8	989	997
TOTAL	137	1036	1173

Sensitivity: 94.2% (88.8% - 97.4%)
Specificity: 95.5% (94.0% - 96.6%)

Note: There were 18 specimens that tested negative by cleared DSFA for Flu A, Flu B, Adenovirus and RSV but that were not assessed by culture followed by DFA. As requested by FDA, data from all 18 specimens were excluded from the calculations of sensitivity and specificity for these targets (1191-18 = 1173).

Table 45. Influenza A/H1

RVP FAST	**Primary Comparator**		
	Positive	Negative	TOTAL
Positive	52	21	73
Negative	2	1116	1118
TOTAL	54	1137	1191

Sensitivity: 96.3% (87.3% - 99.5%)
Specificity: 98.2% (97.2% - 98.9%)

Table 46. Influenza A/H3

RVP FAST	Primary Comparator		
	Positive	Negative	TOTAL
Positive	74	24	98
Negative	3	1090	1093
TOTAL	77	1114	1191

Sensitivity: 96.1% (89.0% - 99.2%)
Specificity: 97.8% (96.8% - 98.6%)

Table 47. Influenza B

RVP FAST	Primary Comparator		
	Positive	Negative	TOTAL
Positive	50	13	63
Negative	3	1107	1110
TOTAL	53	1120	1173

Sensitivity: 94.3% (84.3% - 98.8%)
Specificity: 98.8% (98.0% - 99.4%)
Note: There were 18 specimens that tested negative by cleared DSFA for Flu A, Flu B, Adenovirus and RSV but that were not assessed by culture followed by DFA. As requested by FDA, data from all 18 specimens were excluded from the calculations of sensitivity and specificity for these targets (1191-18 = 1173).

Table 48. Respiratory Syncytial Virus

RVP FAST	Primary Comparator		
	Positive	Negative	TOTAL
Positive	110	24	134
Negative	11	1028	1039
TOTAL	121	1052	1173

Sensitivity: 90.9% (84.3% - 95.4%)
Specificity: 97.7% (96.6% - 98.5%)

Note: There were 18 specimens that tested negative by cleared DSFA for Flu A, Flu B, Adenovirus and RSV but that were not assessed by culture followed by DFA. As requested by FDA, data from all 18 specimens were excluded from the calculations of sensitivity and specificity for these targets (1191-18=1173).

Table 49. Rhinovirus

RVP FAST	Primary Comparator		
	Positive	Negative	TOTAL
Positive	43	85	128
Negative	2	1047	1049
TOTAL	45	1132	1177

Sensitivity: 95.6% (84.9% - 99.5%)
Specificity: 92.5% (90.8% - 94.0%)

Note: A total of 14 specimens were excluded from the calculations of sensitivity and specificity for Rhinovirus as comparator results were not available for this target (1191-14 = 1177).

Table 50. Adenovirus

RVP FAST	**Primary Comparator**		
	Positive	Negative	TOTAL
Positive	12	8	20
Negative	2	1151	1153
TOTAL	14	1159	1173

Sensitivity: 85.7% (57.2% - 98.2%)
Specificity: 99.3% (98.6% - 99.7%)

Note: There were 18 specimens that tested negative by cleared DSFA for Flu A, Flu B, Adenovirus and RSV but that were not assessed by culture followed by DFA. As requested by FDA, data from all 18 specimens were excluded from the calculations of sensitivity and specificity for these targets (1191-18 = 1173).

Table 51. Human Metapneumovirus

RVP FAST	**Primary Comparator**		
	Positive	Negative	TOTAL
Positive	35	12	47
Negative	1	1121	1122
TOTAL	36	1133	1169

Sensitivity: 97.2% (85.5% - 99.9%)
Specificity: 98.9% (98.0% - 99.5%)

Note: A total of 22 specimens were excluded from the calculations of sensitivity and specificity for human metapneumovirus as comparator results were not available for this target.

The summary of the prospective performance data (all sites combined) are presented in Table 52:

Table 52. Sensitivity and Specificity of RVP FAST in the Prospective Data Set

Virus (Analyte)	Sensitivity		95%CI for Sensitivity	Specificity		95% CI for Specificity
	TP / (TP+FN)	Percent		TN/ (TN+FP)	Percent	
Human Influenza A	129/137	94.2%	88.8% -	989/1036	95.5%	94.0% -
H1	52/54	96.3%	87.3% -	1093/1111	98.4%	97.5% -
H3	74/77	96.1%	89.0% -	1065/1088	97.9%	96.8% -
Human Influenza B	50/53	94.3%	84.3% -	1107/1120	98.8%	98.0% -
RSV	110/121	90.9%	84.3% -	1028/1052	97.7%	96.6% -
Rhinovirus	43/45	95.6%	84.9% -	1047/1132	92.5%	90.8% -
Adenovirus	12/14	85.7%	57.2% -	1151/1159	99.3%	98.6% -
Metapneumovirus	35/36	97.2%	85.5% -	1121/1133	98.9%	98.2% -

Note: Not all 1191 specimens collected were assessed by the comparator for all targets and results for those targets could therefore not be included.

Prospective Clinical Study Mixed Infection Analysis

Co-infections with more than one virus were reported in 36 of the1191 clinical specimens included in the prospective dataset (3.0%). The prevalence of co-infections in the sample set is summarized in Table 53. The single most common co-infection was Human Rhinovirus with Respiratory Syncytial Virus. These viruses were the most prevalent in the tested population.

Table 53. Prevalence of Co-infections in the Prospective Data Set (Based on RVP FAST Results)

Co-infections	Number Reported	Prevalence
Flu A / Flu B	1	<0.1%
Flu A / Rhino	5	0.4%
RSV / Rhino	13	1.1%
RSV / Adeno	3	0.25%
Adeno / Rhino	5	0.4%
hMPV / Rhino	1	<0.1%
Adeno / hMPV	1	<0.1%
Adeno / Flu A	1	<0.1%

Flu A / RSV	1	<0.1%
Flu A / hMPV	1	<0.1%
Flu B / hMPV	1	<0.1%
Flu A / hMPV / Rhino	1	<0.1%
Flu A / Flu B / Rhino	1	<0.1%
Adeno / hMPV / Rhino	1	<0.1%
Total	**36**	**3.0%**

Retrospective Clinical Study

Since Adenovirus does not show seasonality, the prospective sample set was supplemented with banked, pre-selected, positive clinical specimens (34) collected at selected sites and tested by RVP FAST (Table 54). Pre-selected positive clinical specimens were not blinded prior to testing. These retrospective archived samples were characterized previously at the source sites using a variety of methods including DSFA, viral culture followed by DFA, Luminex X-TAG RVP, and LDT PCR assays

Table 54. Positive Agreement of RVP FAST in the Pre-Selected Data Set (N=34)

.Virus (Analyte)	Positive Agreement		95% CI for Positive Agreement
	TP/(TP+FN)	Percent	
Adenovirus	33/34	97.06%	84.67% - 99.93%

Confirmed Swine Flu (2009/H1N1) Specimens:

An additional 77 clinical specimens (NP swabs) confirmed by CDC real-time PCR Swine Flu (H1N1) assay to be positive for Flu A 2009 H1N1 were tested by RVP FAST. Of these, seventy-five (75) were Flu A unsubtypeable (97.40%, LB 95% CI 90.93%), two (2) specimens were Flu A H1 positive by RVP FAST (2.60%) and none were negative for Flu A (0.00%).

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

The prevalence of individual viruses as detected by RVP FAST in the clinical study patient population presented in Table 55:

Table 55. Prevalence of Individual Viruses

Age (yrs)	Flu A (matrix)	Flu A H1	Flu A H3	Flu B	RSV	Rhino	hMPV	Adeno
0-5	17	10	7	5	80	51	11	8
>5-21	25	16	10	16	4	12	5	5
>21-65	97	39	51	34	21	47	16	5
>65	37	5	29	8	29	18	15	2
All Ages	176	70	97	63	134	128	47	20
Prevalence	14.8%	5.9%	8.1%	5.3%	11.2%	10.7%	3.9%	1.7%

N. Instrument Name:

Luminex LX 100/200 System.

O. System Descriptions:

1. Modes of Operation:

   The Luminex LX100/200 instrument is used to sort and analyze amplified PCR products attached to bead arrays. The instrument generates signals based on the acquisition of spectrofluorometric data. The raw signals are median fluorescence intensities (MFI) which are acquired in a Luminex Output.csv file that is subsequently analyzed by the xTAG Data Analysis Software (TDAS RVP FAST) to establish the presence or absence of all viral types / subtypes for which a Luminex microsphere population has been dedicated.

2. Software:

   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes ____X____ or No _____

3. Specimen Identification:

   User enters Patient ID/Sample ID by typing it in.

4. Specimen Sampling and Handling:

   Not applicable

5. Calibration:
   Not applicable

6. Quality Control:

   The RVP FAST contains an internal and external control. Additional positive and negative controls are recommended as indicated in section L above.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In the "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

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

R. Conclusion:

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