

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k113731

B. Purpose for Submission:

To obtain a Substantial Equivalence Determination for a new device

C. Measurand:

Viral RNA/DNA sequences of the following respiratory viruses:

Adenovirus species B/E (combined result) and species C

Human Rhinovirus

Human Metapneumovirus

Influenza A and Influenza A subtypes H1, H3 and 2009 H1N1

Influenza B

Parainfluenza Virus 1, 2, and 3

Respiratory Syncytial Virus subtypes A and B

D. Type of Test:

A multiplexed nucleic acid test intended for use with the eSensor instrument for the qualitative *in vitro* detection and identification of multiple respiratory viral pathogen nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals suspected of respiratory tract infections

E. Applicant:

Clinical MicroSensors, Inc d.b.a GenMark Diagnostics

F. Proprietary and Established Names:

eSensor[®] Respiratory Viral Panel (RVP)

eSensor[®] XT-8 System

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
OCC	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay	Microbiology (83)
OEM	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay (human metapneumovirus)	Microbiology (83)

Product Code	Classification	Regulation Section	Panel
OOU	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay (parainfluenza)	Microbiology (83)
OEP	Class II	21 CFR 866.3980 Respiratory Viral Panel Multiplex Nucleic Acid Assay (influenza a virus subtype differentiation)	Microbiology (83)
OQW	Class II	21 CFR 866.3332 Reagents for detection of specific novel influenza A viruses	Microbiology (83)
NSU	Class II	21 CFR 862.2570 Instrumentation for Clinical Multiplex Test Systems	Clinical Chemistry (75)
OUL	Class I	21 CFR 862.2310 Thermocycler Generic	Clinical Chemistry (75)
JJH	Class I	21 CFR 862.2310 Clinical Sample Concentrator	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

The eSensor Respiratory Viral Panel (RVP) is a qualitative nucleic acid multiplex *in vitro* diagnostic test intended for use on the eSensor XT-8 system for the simultaneous detection and identification of multiple respiratory viral nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals exhibiting signs and symptoms of respiratory infection.

The following virus types and subtypes are identified using the eSensor RVP: Influenza A, Influenza A H1 Seasonal Subtype, Influenza A H3 Seasonal Subtype, Influenza A 2009 H1N1 subtype, Influenza B, Respiratory Syncytial Virus subtype A, Respiratory Syncytial Virus subtype B, Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Human Metapneumovirus, Human Rhinovirus, Adenovirus species B/E, and Adenovirus species C.

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 patient 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 and viral culture, immunofluorescence and radiography) and clinical presentation must be taken into consideration in the final diagnosis of respiratory viral infection.

Performance characteristics for Influenza A were established during the 2010/2011 influenza season when Influenza A 2009 H1N1 and H3N2 were the predominant Influenza A viruses in

circulation. When other Influenza A viruses emerge, performance characteristics may vary.

If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

eSensor XT-8 System

I. Device Description:

The eSensor Respiratory Viral Panel (RVP) reagent kit including primers and signal probes for the following respiratory viral pathogens: Adenovirus (with species specific oligonucleotides for type C, B and E), Influenza A (with subtyping for hemagglutinin genes H1, 2009 H1 and H3), Influenza B, human Metapneumovirus, Parainfluenza Virus (serotypes 1, 2, and 3), Respiratory Syncytial virus (sub-types A and B), and Rhinovirus. The eSensor XT-8 consumable has a plurality of electrode locations that are coated with analyte specific capture probe oligonucleotide for multiplex amplicon detection. The eSensor XT-8 System accepts the consumable and completes the hybridization and detection of each electrode using an assay specific protocol. A summary of the eSensor Respiratory Viral Panel assays and targets is presented in the following table:

Organism (Abbreviation)	Assay Type	Gene Target
Adenovirus C (Adv C)	Adenovirus (DNA)	Hexon
Adenovirus B/E (Adv B/E)		
Influenza A (Flu A)	Orthomyxovirus (RNA)	Matrix Protein
Influenza A H1 (Flu A H1)		Hemagglutinin (HA)
Influenza A H3 (Flu A H3)		Hemagglutinin (HA)
Influenza A 2009 H1N1 (2009 H1N1)		Hemagglutinin (HA)
Influenza B (Flu B)		RNA polymerase subunit PB1
Parainfluenza Virus 1 (PIV 1)	Paramyxovirus (RNA)	Hemagglutinin- neuraminidase (HN)
Parainfluenza Virus 2 (PIV 2)		
Parainfluenza Virus 3 (PIV 3)		
Human Metapneumovirus	Paramyxovirus	Nucleocapsid (N)

Organism (Abbreviation)	Assay Type	Gene Target
(hMPV)	(RNA)	
Respiratory Syncytial Virus A (RSV A)		
Respiratory Syncytial Virus B (RSV B)		
Human Rhinovirus (HRV)	Picornavirus (RNA)	5' Untranslated Region (UTR)

eSensor Respiratory Viral Panel (RVP) Reagent Kit

The RVP Reagent Kit contains all materials required to complete approximately 60 tests. The contents of a test kit are described below:

Box	Component	Packaging and Quantity	Storage Conditions
eSensor Respiratory Viral Panel Cartridge Packs	eSensor RVP Cartridges	6 foil bags with 8 cartridges in each cartridge pack	10-25 °C
	eSensor Respiratory Viral Panel Product Insert	1 Copy	Dry place
eSensor Respiratory Viral Panel Amplification Reagents	RVP Enzyme Mix (reverse transcription)	2 vials for a total of approx. 60 reactions	-15 to -30 °C (in a designated pre-PCR location)
	RVP PCR Mix (amplification reagent)	2 vials for a total of approx. 70 reactions	
	MS2 Internal Control	2 vials with 300 µL each	
eSensor Respiratory Viral Panel Detection Reagents	Exonuclease (amplicon digestion)	2 vials for a total of approx. 60 reactions	-15 to -30 °C (in a designated pre-PCR location)
	RVP Signal Buffer XT Buffer-1 and -2 (hybridization and detection)	2 vials per reagent for a total of approx. 60-70 reactions.	

The Assay Cartridge (eSensor XT-8 Cartridge)

The eSensor XT-8 cartridge device consists of a printed circuit board (PCB) with a multi-layer laminate and a plastic cover that forms a hybridization chamber. The cartridge is fitted with a pump and check valves that circulate the hybridization solution when inserted into the eSensor XT-8 instrument. The PCB chip consists of an array of 72 gold-plated working electrodes, a silver reference electrode, and two gold-plated auxiliary electrodes. Each working electrode has a connector contact pad on the opposite side of the chip for electrical connection to the eSensor XT-8 instrument. Each electrode is modified with a multi-component; self-assembled monolayer that includes oligonucleotide capture probes specific for each polymorphic site on the test panel and insulator molecules. The cartridge also contains an electrically erasable and programmable read only memory component

(EEPROM) that stores information related to the cartridge (e.g., assay identifier, cartridge lot number, and expiration date).

The eSensor XT-8 Instrument

The eSensor XT-8 is a clinical multiplex instrument that has a modular design consisting of a base module and one, two, or three cartridge-processing towers containing eight, 16, or 24 cartridge slots, respectively. The cartridge slots operate independently of each other. Any number of cartridges can be loaded at one time, and the remaining slots are available for use while the instrument is running. The base module controls each processing tower, provides power, and stores and analyzes data. The instrument is designed to be operated solely with the touch screen interface. Entering patient accession numbers and reagent lot numbers can be performed by the bar code scanner or the touch screen.

Each processing tower consists of eight cartridge modules, each containing a cartridge connector, a precision-controlled heater, an air pump, and electronics. The air pumps drive the pump and valve system in the cartridge, eliminating fluid contact between the instrument and the cartridge. The pneumatic pumping enables recirculation of the hybridization solution allowing the target DNA and the signal probes to hybridize with the complementary capture probes on the electrodes. The pump in the cartridge is connected to a pneumatic source from the eSensor XT- 8 instrument and provides unidirectional pumping of the hybridization mixture through the channel during hybridization. Using this process to circulate the hybridization solution minimizes the unstirred boundary layer at the electrode surface and continuously replenishes the volume above the electrode that has been depleted of complementary targets and signal probes.

The XT-8 instrument provides electrochemical detection of bound signal probes by ACV and subsequent data analysis and test report generating functions. All hybridization, ACV scanning and analysis parameters are defined by a scanning protocol loaded into the XT-8 software, and then specified for use by the EEPROM on each cartridge.

Materials Required But Not Provided Equipment

The XT-8 instrument is only for use with XT-8 test cartridges and is not integrated or connected with other laboratory systems.

- GenMark eSensor XT-8 instrument
- Vortex Mixer (with platform head for 96-well PCR plate mixing)
- Dry Heat Block
- Cold Block or Ice
- Adjustable Pipettes
- PCR Thermal Cycler compatible with 0.2 ml reaction tubes and 96-well reaction plates
- Microcentrifuge (with adaptor if using PCR tubes or strips)
- Plate Centrifuge (with adaptor if using 96-well PCR plates)
- Dead Air Hood
- bioMérieux NucliSENS[®] easyMAG[®] extraction system

Consumables

- RNase/DNase-free PCR tubes and caps (0.2 mL, thin-walled), strips of 8 tubes with **individual** caps, or 96-well PCR plates and seals
- Water, Molecular Biology Grade, RNAase/DNase-free
- Disposable Gloves
- Microfuge Tubes, RNase/DNase-free
- Microfuge Tube Racks
- Pipette Tips, Aerosol Resistant, RNase/DNase-free
- Nucleic acid decontaminating solutions or 10% bleach for appropriate surfaces
- bioMérieux NucliSENS® easyMAG® Consumables (buffers and disposables)

J. Substantial Equivalence Information:

1. Predicate device name(s):

Luminex® xTAG™ Respiratory Viral Panel (RVP)
eSensor XT-8 System

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

k081483

3. Comparison with predicate:

Similarities		
Item	eSensor Respiratory Viral Panel (RVP)	Luminex xTAG RVP
Intended Use	<p>The eSensor® Respiratory Viral Panel (RVP) is a qualitative nucleic acid multiplex <i>in vitro</i> diagnostic test intended for use on the eSensor XT-8™ system for the simultaneous detection and identification of multiple respiratory viral nucleic acids in nasopharyngeal swabs (NPS) obtained from individuals exhibiting signs and symptoms of respiratory infection.</p> <p>The following virus types and subtypes are identified using the eSensor RVP: Influenza A, Influenza A H1 Seasonal Subtype, Influenza A H3 Seasonal Subtype, Influenza A 2009 H1N1 subtype, Influenza B, Respiratory Syncytial Virus subtype A, Respiratory Syncytial Virus subtype B,</p>	<p>The xTAG RVP (Respiratory Viral Panel) 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, Para influenza 1, Para influenza 2, and Para influenza 3 virus, Human Metapneumovirus, Rhinovirus, and Adenovirus. The detection and identification of specific viral nucleic acids from individuals</p>

Similarities		
Item	eSensor Respiratory Viral Panel (RVP)	Luminex xTAG RVP
	<p>Parainfluenza Virus 1, Parainfluenza Virus 2, Parainfluenza Virus 3, Human Metapneumovirus, Human Rhinovirus, Adenovirus species B/E, and Adenovirus species C.</p> <p>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.</p> <p>Negative results do not preclude respiratory viral infection and should not be used as the sole basis for diagnosis, treatment or other patient 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 and viral culture, immunofluorescence and radiography) and clinical presentation must be taken into consideration in the final diagnosis of respiratory viral infection.</p> <p>Performance characteristics for Influenza A were established during the 2010/2011 influenza season when Influenza A 2009 H1N1 and H3N2 were the predominant Influenza A viruses in circulation. When other Influenza A viruses emerge, performance characteristics may vary.</p> <p>If infection with a novel Influenza A virus is suspected based on current clinical and</p>	<p>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 after examination using RVP be confirmed by cell culture. 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 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</p>

Similarities		
Item	eSensor Respiratory Viral Panel (RVP)	Luminex xTAG RVP
	epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.	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.
Organisms Detected	Influenza A, Influenza A H1 Seasonal Subtype, Influenza A H3 Seasonal Subtype, Influenza A 2009 H1N1 strain, Human Metapneumovirus, Human Rhinovirus	Same except for the differences listed in the “Differences” table row 1.
Specimen Type	Nasopharyngeal swabs (NPS)	Same
Controls	Internal control added to each sample. External control processed with each batch of samples.	Same
Analyte	RNA/DNA	Same
Assay type	Qualitative	Same
Extraction	EasyMag® extraction system	Same

Differences		
Item	eSensor Respiratory Viral Panel (RVP)	Luminex xTAG RVP
Detection technology	eSensor XT-8 Micro-array hybridization and solid phase electrochemical detection	Luminex® 100/200™ instrument Fluorescence-activated sorting of labeled beads coupled to streptavidin-conjugated biotinylated products
Software	Application software and embedded firmware (controls hardware functions) on XT-8 in addition to Assay Analysis Module (AAM) for RVP-IVD	IS or xPONENT software; xTAG Data Analysis Software RVP (US)
Time to result	Approximately 6 hours	Approximately 8 hours
Assay call method	Automated test interpretation and report generation. User can access	Semi-automated test interpretation. User must review all “no call”

Differences		
Item	eSensor Respiratory Viral Panel (RVP)	Luminex xTAG RVP
	raw data.	results to determine cause and retesting strategy.

K. Standard/Guidance Documents Referenced:

- User Protocol for Evaluation of Qualitative Test Performance, Clinical and Laboratory Standards Institute Approved Guideline, EP12-A2
- Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices, FDA Guidance Document (May 11, 2005)
- Establishing Performance Characteristics of In Vitro Diagnostic Devices for Detection or Detection and Differentiation of Influenza Viruses (July 15, 2011)
- Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Testing for Detection and Differentiation of Influenza A Virus Subtypes Using Multiplex Assays (October 9, 2009)
- Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay (October 9, 2009)
- Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Testing for Human Metapneumovirus (hMPV) Using Nucleic Acid Assays (October 9, 2009)
- Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Instrumentation for Clinical Multiplex Test Systems (March 10, 2005)
- Guidance for Industry, FDA Reviewers and Compliance on Off-The-Shelf Software Use in Medical Devices (September 9, 1999)

L. Test Principle:

The eSensor RVP is a multiplex microarray-based genotyping test system that accepts a nasopharyngeal specimen. It is based on the principles of competitive DNA hybridization using a sandwich assay format, wherein a single stranded target binds concurrently to sequence-specific solution-phase signal probe and solid-phase electrode-bound capture probe. The test employs reverse transcription polymerase chain reaction amplification, exonuclease digestion and hybridization of target DNA/RNA. In the process, the double-stranded PCR amplicons are digested with exonuclease to generate single stranded DNA suitable for hybridization. Hybridization occurs in the eSensor XT-8 Cartridge (described below) where the single-stranded target DNA is mixed with a hybridization solution containing labeled signal probes.

eSensor technology uses a solid-phase electrochemical method for determining the presence of one or more of a defined panel of virus target sequences. Purified DNA/RNA is isolated from the patient specimen and the extracted nucleic acid is reverse transcribed and/or amplified using virus specific primers with an RT-PCR enzyme mix. The amplified DNA is converted to single-stranded DNA via exonuclease digestion and is then combined with a signal buffer containing ferrocene labeled signal probes that are specific for the different viral targets. The mixture of amplified sample and signal buffer is loaded onto a cartridge

containing single-stranded oligonucleotide capture probes bound to gold-plated electrodes. The cartridge is inserted into the XT-8 instrument where the single-stranded targets hybridize to the complementary sequences of the capture probes and signal probes. The presence of each target is determined by voltammetry, which generates specific electrical signals from the ferrocene-labeled signal probe. The eSensor RVP provides a qualitative result, the presence (Positive) or absence (Target Not Detected) of the viruses contained in the panel, along with the internal MS2 control, based upon whether the underlying electrical signals are above or below a pre-defined cut-off signal intensity.

Results are interpreted based on the following tables:

Virus Result	Explanation	Action
Positive	The run was successfully completed AND The analyte indicated as POSITIVE was detected.	Report results
Target Not Detected	The run was successfully completed AND The internal control or at least one other analyte was detected AND The result for the analyte indicated was NEGATIVE	Report results
Error	Electrode or instrument failure	Retest sample. See Table Below: Summary of Sample Retest Recommendations by Report Type
Fail (Internal Control Failure)	The internal control was unsuccessful OR The run was unsuccessful	
Indeterminate results between Influenza A subtypes (Influenza A only, no subtype)	The run was successfully completed AND The result for Influenza A was POSITIVE AND The results for Influenza A H1, H3 and 2009 H1N1 were NEGATIVE	
Target Not Detected (for external positive control)	The run was successfully completed AND The internal control or at least one other analyte was detected AND The results for the external positive controls were NEGATIVE	

The table below describes the interpretation of possible eSensor RVP results for Influenza A.

Assay Final Result	Flu A	H1	H3	2009 H1N1	Required Follow-up
Target Not Detected	Negative	Negative	Negative	Negative	None
Influenza A H1	Positive	Positive	Negative	Negative	
Influenza A H3	Positive	Negative	Positive	Negative	
Influenza A 2009 H1N1	Positive	Negative	Negative	Positive	Retest to
	Negative	Negative	Negative	Positive	

Assay Final Result	Flu A	H1	H3	2009 H1N1	Required Follow-up
					confirm result, see Table 8
Influenza A H1 and Influenza A H3	Positive	Positive	Positive	Negative	Multiple Flu A subtype infections are possible but rare. Retest to confirm result
Influenza A H1 and Influenza A 2009 H1N1	Positive	Positive	Negative	Positive	
Influenza A H3 and Influenza A 2009 H1N1	Positive	Negative	Positive	Positive	
Influenza A (no subtype detected)	Positive	Negative	Negative	Negative	See below

For Influenza A (no subtype detected)

If the Influenza A assay is positive, but none of the hemagglutinin subtyping assays are positive, then the interpretation is ‘Indeterminate result between Influenza A subtypes (no subtype detected)’. This result could occur when the titer of the virus in the specimen is low and not detected by the subtyping assays. This result could also indicate the presence of a novel Influenza A strain. In both cases, the sample in question should be retested. If the retest provides a different result, test the sample a third time to ensure the accuracy of the result. If the retest provides the same result, then the function of the device should be verified by testing with appropriate external control materials (known positive samples for Influenza A H1, Influenza A H3 and Influenza A 2009 H1N1), and a negative control reaction should also be run to test for PCR-product contamination. If the eSensor RVP accurately identifies the external and negative controls, contact local or state public health authorities for confirmatory testing.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Repeatability was evaluated using representative cultures for each viral panel member (see table below) at a concentration of 1x LoD. The study was executed at a single site by a single operator on a single day. One representative culture was spiked into viral transport media then extracted using the NucliSENS easyMAG system. A total of 20 extraction replicates were run per analyte. Viral strains used in the repeatability study are provided below:

TARGET	STRAIN	SOURCE	LOT
Influenza A H1	H1N1 Brisbane/59/07	Zeptomatrix 0810036CF	305254
Influenza A H3	H3N2	ATCC VR-547	7683464
Influenza A 2009 H1N1	NY/2009	Zeptomatrix 080109CFN	305984
Influenza B	Florida/02/06	Zeptomatrix 0810037CF	305445
RSV A	A2	ATCC VR-1540	58224956
RSV B	9320	ATCC VR-955	58189459
PIV 1	C35	ATCC VR-94	58023566
PIV 2	Greer	ATCC VR-92	5006417
PIV 3	C 243	ATCC VR-93	58102987
Metapneumovirus	B2	Zeptomatrix 0810061CFN	305462
Rhinovirus	3	ATCC VR-483	2W
Adenovirus B	Type 7	ATCC VR-7	7578661
Adenovirus C	Type 1	ATCC VR-1	4703343
Adenovirus E	Type 4	ATCC VR-1572	7588003

The repeatability study results are presented below:

Virus	Strain	Viral Titer Tested (TCID ₅₀ /mL)	% Positives	Mean Signal (nA)	Standard Deviation (nA)	%CV (SD/Mean)
Flu A	H1N1 Brisbane/59/07	4.17E-01	100%	253.6	23.4	9.2
Flu A H1	H1N1 Brisbane/59/07	4.17E-01	100%	212.1	14.0	6.6
Flu A	H3N2	1.58E+03	100%	264.1	21.5	8.1
Flu A H3	H3N2	1.58E+03	100%	50.9	10.6	20.9
Flu A 2009 H1N1	NY/2009	1.05E-01	95%	43.2	21.1	48.0
Flu B	Florida/02/06	3.16E-02	100%	75.5	27.7	36.7
HMPV	B2	4.17E+00	100%	43.1	17.4	40.4
HRV	3	1.58E-03	97.5%	43.8	23.7	54.1
PIV 1	C35	2.81E-03	100%	200.5	16.7	8.3
PIV 2	Greer	2.81E-01	100%	259.0	68.6	26.5
PIV 3	C 243	2.81E+01	100%	20.6	9.9	48.1
RSV A	A2	2.81E+00	100%	31.5	25.0	79.3
RSV B	9320	1.58E+00	100%	158.8	22.3	14.0
ADV B/E	Type 4	1.58E+00	100%	147.6	24.1	16.3
ADV C	Type 1	8.89E-01	100%	196.3	14.7	7.5

Reproducibility

A multisite reproducibility study was carried out to evaluate the reproducibility of the eSensor RVP using a representative subset of targeted analytes. Sources of variation due to lot, run, day, site and operator were included in the study design.

Variance Component	Instances
Instruments	3
Sites	3
Users Per Site	2
Reagent Lots	3
Non-consecutive Days of Testing	6
Concentrations Tested Per Analyte	3x LoD, 1x LoD, negative

The following five viral strains were selected for the multisite reproducibility study:

Analyte	Strain	Source	Lot	Stock Concentration (TCID ₅₀ /mL)
Flu A H3	Aichi/2/68/H3N2	ATCC, VR-547	58167241	1.60E+08
RSV A	A2	ATCC, VR-1540	58224956	2.80E+06
PIV 3	C243	ATCC, VR-93	58102987	2.80E+07
hMPV	B2	Zeptomatrix, 810159CF	307012	5.01E+05
ADV B	Type 7	ATCC, VR-7	7578661	8.90E+05

A culture stock of each selected virus was diluted in VTM to construct the positive samples of the reproducibility study panel. The positive samples of the reproducibility study panel were prepared so that each viral strain would be tested at two concentrations: a “low positive” (C₉₅ concentration, expected to be positive approximately 95% of the time), and a “moderate positive” (3 x LoD, expected to be positive approximately 100% of the time). The positive samples for a particular virus strain also served as “negative” samples for the other four virus strains in the reproducibility study panel. All reproducibility study panel samples were randomized and blinded prior to shipment to the study sites.

Summary reproducibility study results are presented in the tables below:

ADV B/E Results

ADV B Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
MOD POS (3x LoD) 47.4 TCID ₅₀ /ml	Site 1	36/36	0/36	100.0%	90.3%-100%	109.1	10.9	10.0
	Site 2	36/36	0/36	100.0%	90.3%-100%	102.6	11.5	11.2
	Site 3	36/36	0/36	100.0%	90.3%-100%	102.2	14.5	14.1
	All Sites	108/108	0/108	100.0%	96.6%-100%	104.7	12.7	12.1
LOW POS (1x LoD) 15.8 TCID ₅₀ /ml	Site 1	36/36	0/36	100.0%	90.3%-100%	92.7	11.4	12.3
	Site 2	36/36	0/36	100.0%	90.3%-100%	89.9	10.9	12.1
	Site 3	36/36	0/36	100.0%	90.3%-100%	84.5	16.9	20.0
	All Sites	108/108	0/108	100.0%	96.6%-100%	89.1	13.7	15.3
Negative	Site 1	1/288	287/288	99.7%	98.1%-100%	1.6	6.0	N/A
	Site 2	0/288	288/288	100.0%	98.7%-100%	1.2	0.4	N/A
	Site 3	0/288	288/288	100.0%	98.7%-100%	1.2	0.4	N/A
	All Sites	1/864	863/864	99.9%	99.4%-100%	1.3	3.5	N/A

Influenza A Results

Flu A Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
MOD POS (3x LoD) 1.3 TCID ₅₀ /ml	Site 1	36/36	0/36	100.0%	90.3%-100%	243.0	23.6	9.7
	Site 2	36/36	0/36	100.0%	90.3%-100%	246.4	29.7	12.0
	Site 3	36/36	0/36	100.0%	90.3%-100%	235.0	32.6	13.9
	All Sites	108/108	0/108	100.0%	96.6%-100%	241.5	29.0	12.0
LOW POS (1x LoD) 0.4 TCID ₅₀ /ml	Site 1	36/36	0/36	100.0%	90.3%-100%	248.3	28.6	11.5
	Site 2	36/36	0/36	100.0%	90.3%-100%	244.7	26.4	10.8
	Site 3	36/36	0/36	100.0%	90.3%-100%	232.8	23.2	10.0
	All Sites	108/108	0/108	100.0%	96.6%-100%	242.0	26.7	11.1
Negative	Site 1	3/288	285/288	99.0%	97.0%-99.8%	1.2	2.4	N/A
	Site 2	1/288	287/288	99.7%	98.1%-100%	1.0	2.2	N/A
	Site 3	2/288	286/288	99.3%	97.5%-99.9%	1.0	0.9	N/A
	All Sites	6/864	858/864	99.3%	98.5%-99.7%	1.1	1.9	N/A

Influenza A H3 Results

Flu A H3 Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
MOD POS (3x LoD) 4.7×10^3 TCID ₅₀ /ml	Site 1	36/36	0/36	100.0%	90.3%-100%	86.5	23.9	27.7
	Site 2	36/36	0/36	100.0%	90.3%-100%	77.4	26.7	34.5
	Site 3	36/36	0/36	100.0%	90.3%-100%	86.0	30.5	35.4
	All Sites	108/108	0/108	100.0%	96.6%-100%	83.3	27.3	32.7
LOW POS (1x LoD) 1.6×10^3 TCID ₅₀ /ml	Site 1	36/36	0/36	100.0%	90.3%-100%	81.5	26.1	32.0
	Site 2	36/36	0/36	100.0%	90.3%-100%	68.2	29.6	43.4
	Site 3	36/36	0/36	100.0%	90.3%-100%	84.9	21.3	25.1
	All Sites	108/108	0/108	100.0%	96.6%-100%	78.2	26.6	34.1
Negative	Site 1	0/288	288/288	100.0%	98.7%-100%	0.4	0.3	N/A
	Site 2	0/288	288/288	100.0%	98.7%-100%	0.3	0.3	N/A
	Site 3	1/288	287/288	99.7%	98.1%-100%	0.4	0.4	N/A
	All Sites	1/864	863/864	99.9%	99.4%-100%	0.4	0.4	N/A

Respiratory Syncytial Virus A Results

RSV A Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
MOD POS (3x LoD) 8.4 TCID ₅₀ /ml	Site 1	36/36	0/36	100.0%	90.3%-100%	166.3	19.2	11.5
	Site 2	36/36	0/36	100.0%	90.3%-100%	156.7	31.7	20.2
	Site 3	36/36	0/36	100.0%	90.3%-100%	156.4	22.9	14.7
	All Sites	108/108	0/108	100.0%	96.6%-100%	159.8	25.3	15.8
LOW POS (1x LoD) 2.8 TCID ₅₀ /ml	Site 1	36/36	0/36	100.0%	90.3%-100%	146.6	22.7	15.5
	Site 2	36/36	0/36	100.0%	90.3%-100%	124.6	41.0	32.9
	Site 3	35/36	1/36	97.2%	85.5%-99.9%	128.2	33.4	26.1
	All Sites	107/108	1/108	99.1%	94.9%-100%	133.1	34.3	25.8
Negative	Site 1	4/288	284/288	98.6%	96.5%-99.6%	0.7	4.0	N/A
	Site 2	0/288	288/288	100.0%	98.7%-100%	0.2	0.1	N/A
	Site 3	0/288	288/288	100.0%	98.7%-100%	0.2	0.2	N/A
	All Sites	4/864	860/864	99.5%	98.8%-99.9%	0.4	2.3	N/A

Human Metapneumovirus Results

hMPV Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
MOD POS (3x LoD) 13 TCID₅₀/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	91.2	26.1	28.6
	Site 2	36/36	0/36	100.0%	90.3%-100%	92.5	37.1	40.1
	Site 3	36/36	0/36	100.0%	90.3%-100%	100.4	22.7	22.6
	All Sites	108/108	0/108	100.0%	96.6%-100%	94.7	29.3	30.9
LOW POS (1x LoD) 4 TCID₅₀/ml	Site 1	36/36	0/36	100.0%	90.3%-100%	56.4	30.0	53.2
	Site 2	35/36	1/36	97.2%	85.5%-99.9%	51.0	31.2	61.2
	Site 3	36/36	0/36	100.0%	90.3%-100%	63.8	28.1	44.0
	All Sites	107/108	1/108	99.1%	94.9%-100%	57.1	30.0	52.5
Negative	Site 1	0/288	288/288	100.0%	98.7%-100%	0.1	0.0	N/A
	Site 2	8/288	280/288	97.2%	94.6%-98.8%	0.7	4.1	N/A
	Site 3	0/288	288/288	100.0%	98.7%-100%	0.1	0.1	N/A
	All Sites	8/864	856/864	99.1%	98.2%-99.6%	0.3	2.4	N/A

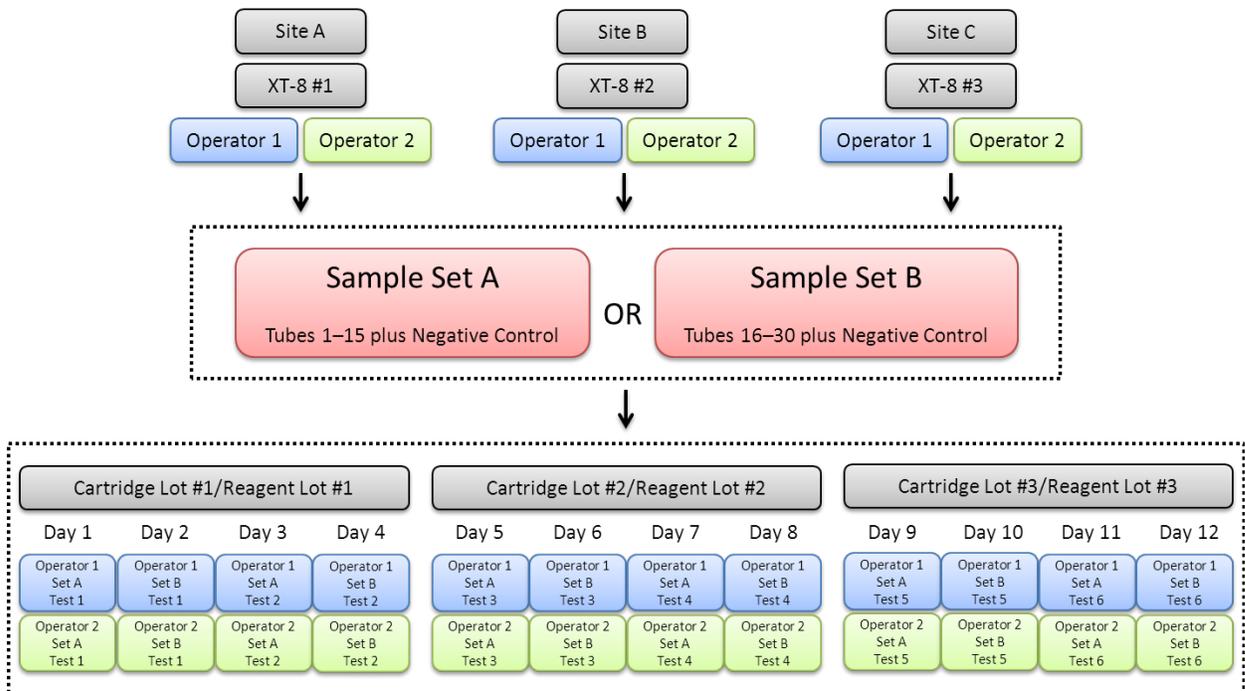
Parainfluenza Virus 3 Results

PIV3 Concentration	Site	# Positive	# Negative	% Agreement with Expected Results	95% CI	Mean (nA)	Std Dev	% CV
MOD POS (3x LoD) 84 TCID ₅₀ /ml	Site 1	36/36	0/36	100.0%	90.3%-100%	178.1	27.4	15.4
	Site 2	36/36	0/36	100.0%	90.3%-100%	193.8	29.8	15.4
	Site 3	36/36	0/36	100.0%	90.3%-100%	160.8	27.4	17.0
	All Sites	108/108	0/108	100.0%	96.6%-100%	177.6	31.1	17.5
LOW POS (1x LoD) 28 TCID ₅₀ /ml	Site 1	36/36	0/36	100.0%	90.3%-100%	139.0	34.8	25.1
	Site 2	36/36	0/36	100.0%	90.3%-100%	162.4	27.9	17.2
	Site 3	36/36	0/36	100.0%	90.3%-100%	126.9	38.9	30.7
	All Sites	108/108	0/108	100.0%	96.6%-100%	142.8	37.0	25.9
Negative	Site 1	0/288	288/288	100.0%	98.7%-100%	0.2	0.1	N/A
	Site 2	0/288	288/288	100.0%	98.7%-100%	0.2	0.1	N/A
	Site 3	0/288	288/288	100.0%	98.7%-100%	0.2	0.1	N/A
	All Sites	0/864	864/864	100.0%	99.6%-100%	0.2	0.1	N/A

An additional reproducibility study was performed to supplement the original study. The new study was requested to provide at least 90 replicates per analyte per concentration and to include data for lot to lot variance evaluation. The additional study was performed at three sites to evaluate the following sources of variation:

- Site instrument to site instrument
- Operator run to operator run
- Extraction to extraction
- Day to day
- Lot to Lot

A schematic of the study design is shown below:



Although the “*Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay*” indicates that every claimed analyte should be represented by the reproducibility study samples, the Agencies thinking regarding the reproducibility study design for multiplexed microbiology device is evolving based on the feedback we obtained from the FDA public meeting “Advancing Regulatory Science for Highly Multiplexed Microbiology/MCM Devices held on October 13, 2011.” Instead of including every claimed analyte in the additional reproducibility study again, testing a representative number of claimed analytes was assessed to be sufficient in this case. A list of representative analytes was reviewed prior to initiation of the additional study. The five representative analytes used in the study are listed below:

Analyte	Strain	Source
Flu A H3	Aichi/2/68/H3N2	ATCC, VR-547
RSV A	A2	ATCC, VR-1540
PIV 3	C243	ATCC, VR-93
hMPV	B2	Zeptomatrix, 810159CF
ADV B	Type 7	ATCC, VR-7

The detailed numerical results from each analyte concentration level were also reported and the variance calculations, based on the raw data, are presented below for each of the variance components included in the study.

Adenovirus B				Influenza A (H3N2)			
Low Positive				Low Positive			
N	Grand Mean	SD	%CV	N	Grand Mean	SD	%CV
108	89.06	13.66	15.34	108	241.95	26.74	11.05
Variance Component		SD estimate	%CV	Variance Component		SD estimate	%CV
Site		2.06	2.31	Site		1.40	0.58
Lot		6.04	6.78	Lot		13.86	5.73
Day		3.07	3.45	Day		5.23	2.16
Operator		3.96	4.44	Operator		9.60	3.97
Residual Variance		11.71	13.15	Residual Variance		21.92	9.06
Moderate Positive				Moderate Positive			
N	Grand Mean	SD	%CV	N	Grand Mean	SD	%CV
108	104.65	12.67	12.10	108	241.47	28.99	12.00
Variance Component		SD estimate	%CV	Variance Component		SD estimate	%CV
Site		3.30	3.16	Site		0.00	0.00
Lot		4.57	4.36	Lot		6.35	2.63
Day		0.81	0.78	Day		9.94	4.12
Operator		0.00	0.00	Operator		0.00	0.00
Residual Variance		11.76	11.24	Residual Variance		26.81	11.10
Negative				Negative			
N	Grand Mean	SD		N	Grand Mean	SD	
864	1.31	N/A		864	1.09	N/A	
Variance Component		SD estimate		Variance Component		SD estimate	
Site		0.00		Site		0.00	
Lot		0.00		Lot		0.11	
Day		0.00		Day		0.14	
Operator		0.17		Operator		0.00	
Residual Variance		3.46		Residual Variance		1.94	

Parainfluenza Virus 3			
Low Positive			
N	Grand Mean	SD	%CV
108	142.77	36.98	25.90
Variance Component		SD estimate	%CV
Site		14.86	10.41
Lot		3.97	2.78
Day		19.65	13.76
Operator		6.20	4.34
Residual Variance		28.43	19.91
Moderate Positive			
N	Grand Mean	SD	%CV
108	177.57	31.07	17.50
Variance Component		SD estimate	%CV
Site		13.83	7.79
Lot		0.00	0.00
Day		15.41	6.68
Operator		7.31	4.12
Residual Variance		23.78	13.39
Negative			
N	Grand Mean	SD	
864	0.18	N/A	
Variance Component		SD estimate	
Site		0.0	
Lot		0.05	
Day		0.03	
Operator		0.00	
Residual Variance		0.12	

Respiratory Syncytial Virus A			
Low Positive			
N	Grand Mean	SD	%CV
108	133.14	34.29	25.76
Variance Component		SD estimate	%CV
Site		0.00	0.00
Lot		1.20	1.66
Day		3.89	6.02
Operator		16.15	9.03
Residual Variance		30.68	12.28
Moderate Positive			
N	Grand Mean	SD	%CV
108	159.82	25.32	15.84
Variance Component		SD estimate	%CV
Site		0.00	0.00
Lot		2.66	1.66
Day		9.63	6.02
Operator		12.43	9.03
Residual Variance		19.63	12.28
Negative			
N	Grand Mean	SD	
864	0.37	N/A	
Variance Component		SD estimate	
Site		0.18	
Lot		0.07	
Day		0.27	
Operator		0.06	
Residual Variance		2.31	

Human Metapneumovirus			
Low Positive			
N	Grand Mean	SD	%CV
108	57.10	29.99	52.52
Variance Component		SD estimate	%CV
Site		0.0	0.00
Lot		22.17	38.82
Day		7.11	12.46
Operator		10.07	17.64
Residual Variance		20.94	36.67
Moderate Positive			
N	Grand Mean	SD	%CV
108	94.72	29.26	30.89
Variance Component		SD estimate	%CV
Site		0.00	
Lot		17.02	
Day		10.81	
Operator		17.23	
Residual Variance		17.87	
Negative			
N	Grand Mean	SD	
864	0.31	N/A	
Variance Component		SD estimate	
Site		0.00	
Lot		0.07	
Day		1.21	
Operator		0.46	
Residual Variance		2.01	

Influenza A H3			
Low Positive			
N	Grand Mean	SD	%CV
108	78.16	26.64	34.08
Variance Component		SD estimate	%CV
Site		7.36	9.41
Lot		13.78	17.63
Day		7.69	9.84
Operator		1.03	1.31
Residual Variance		22.09	28.26
Moderate Positive			
N	Grand Mean	SD	%CV
108	83.32	27.25	32.71
Variance Component		SD estimate	%CV
Site		1.34	1.61
Lot		14.67	17.60
Day		7.42	8.91
Operator		0.00	0.00
Residual Variance		23.33	28.00
Negative			
N	Grand Mean	SD	
864	0.39	N/A	
Variance Component		SD estimate	
Site		0.04	
Lot		0.18	
Day		0.07	
Operator		0.02	
Residual Variance		0.32	

b. Linearity/assay reportable range:

N/A

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

Assay Controls

Negative Control

A negative control is not included in the eSensor RVP kit but should be included with each viral nucleic acid isolation run, and with each eSensor RVP run. RNase/DNase-free water should be used as the control sample (blank transport media may also be used). These negative controls are to be run through the assay process from viral nucleic acid isolation to RT-PCR and XT-8 detection. The results are identified as the negative controls on the XT-8 reports and the software will determine whether the negative controls are valid. If a negative control fails, all results of the samples in the same run are invalid and the samples should be re-extracted and re-run.

External Controls (Positive Controls for Viral Targets)

External positive controls are not provided in the eSensor RVP kit. However, known strains of the targeted viruses should be included in routine quality control procedures (“external controls”). These external controls are viral target positive controls and should be included with each batch of patient specimens. These controls may be previously characterized positive samples or obtained commercially. External controls should be prepared and tested in the same manner as patient samples. Results from external controls should be examined before the results from the patient samples. If an external positive control does not perform as expected, all results of the batch of samples are invalid and the samples should be re-run.

Internal Control (IC)

An internal control (Bacteriophage MS2; IC) is supplied. This allows the user to control for the viral nucleic acid isolation procedure and to check for possible RT-PCR inhibition. IC must be added to each sample during viral nucleic acid isolation; this includes patient samples, negative controls, and external viral controls. The XT-8 will analyze samples for IC signal. Failure to detect IC signal in a sample or a control, in the absence of signal from any of the RVP analytes, will be identified and the user will be required to retest the sample. Specific instructions on adding the IC to a sample for extraction are detailed in the “Viral Nucleic Acid Isolation” section of the product package insert.

Reagent Kit Stability

An analytical study was performed to establish stability of the eSensor RVP assay components. Functional performance of RVP reagents were evaluated with three stability test panels (IVT-1, IVT-2, IVT-3) composed of *in vitro* transcripts. Each stability test panel consisted of a unique combination of claimed viruses, so that the three stability test panels covered all the claimed viruses. All viruses in a particular stability test panel were tested at least once during each stability study. Virus targets were present at a concentration of 2.0×10^4 copies/ μ l. Since each viral target has a unique LoD concentration, a concentration of 2.0

x 10⁴ copies/ul represents a range of 10x to 1 x 10⁷x LoD.

Viral Target	IVT-1 (RVP Set 1)	IVT-2 (RVP Set 2)	IVT-3 (RVP Set 3)
Flu A	X	X	X
Flu A H1	X	X	X
Flu A H3		X	X
Flu A 2009 H1N1			X
Flu B			X
RSV A	X		
RSV B		X	
PIV 1	X		
PIV 2		X	X
PIV 3		X	
hMPV			X
HRV		X	
ADV B			X
ADV C	X		
Total positive targets per RVP Set	5	7	8

Kits were stored according to the package insert (10-25°C) for up to 6 months. Testing was performed after time 0, 3 and 6 months of storage. One test is defined as the extraction of one sample using the NucliSENS EasyMag kit, amplification and analysis on the eSensor. A total of 16 tests were performed per time point and all three sets IVT panels were tested along with a negative control at each time point. A successful time point was determined as a statistically equivalent number of correct calls between the time point tested and the data from time 0. The Fisher's exact test for independence was used to determine statistical equivalence.

Shelf life was determined by acceptable performance at the last time point tested. Acceptable performance was defined as no statistical difference in correct call rates between the particular time point data and the data at time zero. Final results for the reagent stability studies are shown below.

Time point	T0			T1			T2		
	Control			3 Months			6 Months		
	% Correct Calls (95%CI)* *	# of No Calls	# of Mis-Calls	% Correct Calls (95%CI) **	# of No Calls	# of Mis-Calls	% Correct Calls (95%CI) **	# of No Calls	# of Mis-Calls
Master Lot 1	107/108= 99.1 (94.9, 99.8)	0	1	106/108= 98.1 (93.5, 99.5)	1	1	107/108= 99.1 (94.9, 99.8)	1	0
Master Lot 2	105/108= 97.2 (92.1, 99.0)	1	2	104/108= 96.3 (90.9, 98.6)	1	3	105/108= 97.2 (92.1, 99.0)	1	2
Master Lot 3	107/108= 99.1 (94.9, 99.8)	0	1	107/108= 99.1 (94.9, 99.8)	0	1	107/108= 99.1 (94.9, 99.8)	0	1
Total	319/324= 98.5 (96.4, 99.3)	1	4	317/324= 97.8 (95.6, 98.9)	2	5	319/324= 98.5 (96.4, 99.3)	2	3
Time point	T3			T4			T5		
	9 Months			12 Months			15 Months		
	% Correct Calls (95%CI)* *	# of No Calls	# of Mis-Calls	% Correct Calls (95%CI) **	# of No Calls	# of Mis-Calls	% Correct Calls (95%CI) **	# of No Calls	# of Mis-Calls
Master Lot 1	99/108= 91.7 (84.9, 95.5)	6	3	107/108= 99.1 (94.9, 99.8)	0	1	107/108= 99.1 (94.9, 99.8)	0	1
Master Lot 2	107/108= 99.1 (94.9, 99.8)	1	0	108/108= 100 (96.6, 100.0)	0	0	105/108= 97.2 (92.1, 99.0)	0	3
Master Lot 3	108/108= 100 (96.6, 100.0)	0	0	108/108= 100 (96.6, 100.0)	0	0	107/108= 99.1 (94.9, 99.8)	1	0
Total	314/324= 96.9 (94.4, 98.3)	7	3	323/324= 99.7 (98.3, 99.9)	0	1	319/324= 98.5 (96.4, 99.3)	1	4

The no call and miscall results were attributed to an electrical problem with the RVP

cartridge/XT-8 interface. The electrical failures are not reagent or amplicon related.

A statistical analysis of the data was performed using the Fisher's exact test method. There were no statistically significant ($p > 0.05$) difference in the call rates up to time point T2.

These data are acceptable to support reagent kit stability claims as published in the product labeling. The product labeling states:

Box	Component	Packaging & Quantity	Storage
eSensor Respiratory Viral Panel Cartridges	eSensor RVP Cartridges	6 foil bags with 8 cartridges in each foil bag	10-25 °C
	eSensor Respiratory Viral Panel Package Insert	1 copy	Dry place (retain for reference)
eSensor Respiratory Viral Panel Amplification Reagents	RVP Enzyme Mix	2 vials with 40 µL each	-15 to -30 °C (in a designated pre-PCR location)
	RVP PCR Mix	2 vials with 1000 µL each	
	MS2 Internal Control	2 vials with 300 µL each	
eSensor Respiratory Viral Panel Detection Reagents	RVP Signal Buffer	2 vials with 2200 µL each	-15 to -30 °C (in a designated post-PCR location)
	Exonuclease	2 vials with 145 µL each	
	Buffer-1	2 vials with 350 µL each	
	Buffer-2	2 vials with 700 µL each	

Double Stranded Amplicon Stability

An analytical study was performed to establish performance for assay intermediates after storage. Functional performance was evaluated with three stability test panels (IVT-1, IVT-2, IVT-3) of *in vitro* transcripts. Each stability test panel consisted of a unique combination of claimed viruses, so that the three stability test panels covered all the claimed viruses. All viruses in a particular stability test panel were tested at least once during each stability study. Virus targets were present at a concentration of 2.0×10^4 copies/ul. Since each viral target has a unique LoD concentration, a concentration of 2.0×10^4 copies/ul represents a range of $10x$ to 1×10^7x LoD of an amplicon. Three sets of IVT viral panels were used which covered all analytes in the RVP (same sets as presented above). After amplification, all replicates of the same sample set were pooled and labeled. Aliquots from each RVP amplicon set were removed and stored according to the test plan in the figure below. The remaining volume was used for testing the T0 time point. A schematic of the test plan is shown below.

Time Point	Testing Condition	Number of Cartridges	Amplicon Tested
T0 (0 days)	N/A	16	4 Replicates of: RVP Set 1; RVP Set 2 RVP Set 3; RVP Set 4
T1_2-8°C (7 days)	2-8°C	16	4 Replicates of: RVP Set 1; RVP Set 2 RVP Set 3; RVP Set 4
T2_2-8°C (9 days)		16	4 Replicates of: RVP Set 1; RVP Set 2 RVP Set 3; RVP Set 4
T1_<-15°C (4 weeks)	<-15°C	16	4 Replicates of: RVP Set 1; RVP Set 2 RVP Set 3; RVP Set 4
T2_<-15°C (5 weeks)		16	4 Replicates of: RVP Set 1; RVP Set 2 RVP Set 3; RVP Set 4

After the specified storage time, aliquots were removed from storage, exonuclease digested and mixed with hybridization buffer prior to RVP cartridge detection. A summary of the performance at three time points using a -15 to -30 °C storage condition are shown below:

Sample Detection	T0			T1 (4 weeks)			T2 (5 weeks)		
	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls
IVT-1	28/28=100 (87.9, 100.0)	0	0	28/28=100 (87.9, 100.0)	0	0	28/28=96.4 (87.9, 100.0)	0	0
IVT-2	40/40=100 (91.2, 100.0)	0	0	40/40=100 (91.2, 100.0)	0	0	39/40=100 (91.2, 100)	1*	0
IVT-3	36/36=100 (90.4, 100.0)	0	0	36/36=100 (90.4, 100.0)	0	0	35/36=100 (90.4, 100.0)	1*	0
MS2	4/4=100 (51.0, 100.0)	0	0	4/4=100 (51.0, 100.0)	0	0	4/4=100 (51.0, 100.0)	0	0
Total	108/108=100 (96.6, 100.0)	0	0	108/108=100 (96.6, 100.0)	0	0	106/108=98.1 (93.5, 99.5)	2*	0

The no call results at the 5 week time point were attributed to an electrical problem with the RVP cartridge/XT-8 interface. The electrical failures are not reagent or amplicon related.

A summary of the performance at three time points using a 2-8 °C storage condition are shown below:

Sample Detection	T0			T1 (7 days)			T2 (9 days)		
	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls
IVT-1	28/28=100 (87.9, 100.0)	0	0	28/28=100 (87.9, 100.0)	0	0	28/28=96.4 (87.9, 100.0)	0	0
IVT-2	40/40=100 (91.2, 100.0)	0	0	40/40=100 (91.2, 100.0)	0	0	40/40=100 (91.2, 100)	0	0
IVT-3	36/36=100 (90.4, 100.0)	0	0	36/36=100 (90.4, 100.0)	0	0	36/36=100 (90.4, 100.0)	0	0
MS2	4/4=100 (51.0, 100.0)	0	0	4/4=100 (51.0, 100.0)	0	0	4/4=100 (51.0, 100.0)	0	0
Total	108/108=100 (96.6, 100.0)	0	0	108/108=100 (96.6, 100.0)	0	0	108/108=100 (96.6, 100.0)	0	0

The time points tested exhibited no statistically significant difference in the positive detection rate. These data are acceptable to support amplicon stability claims as published in the product labeling. The product labeling states:

...store the amplified samples refrigerated at 2-8 °C for up to 1 week or frozen at -15 to -30 °C for up to one month before processing.

Single Stranded amplicon Stability

An analytical study was performed to establish performance for assay intermediates after storage. Functional performance was evaluated with three stability test panels (IVT-1, IVT-2, IVT-3) of *in vitro* transcripts. Each stability test panel consisted of a unique combination of claimed viruses, so that the three stability test panels covered all the claimed viruses. All viruses in a particular stability test panel were tested at least once during each stability study. Virus targets were present at a concentration of 2.0×10^4 copies/ μ l. Since each viral target has a unique LoD concentration, a concentration of 2.0×10^4 copies/ μ l represents a range of 10x to 1×10^7 x LoD of an amplicon. Three sets of IVT viral panels were used which covered all analytes in the RVP (same sets as presented above). Once amplification and exonuclease digestion were complete, all replicates from the same sample set were pooled and labeled. Aliquots from each RVP amplicon set were removed and stored according to the test plan in the figure below. The remaining volume was used for testing the T0 time point. Final time point performance will be considered acceptable is the number of correctly called samples is statistically equivalent to the T0 time point.

After amplification and exonuclease cleavage, all replicates of the same sample sets were pooled and labeled. Aliquots from each RVP amplicon set were removed and stored according to the test plan in the figure below. The remaining volume was used for testing the T0 time point. A schematic of the test plan is shown below.

Time Point	Condition	Number of Cartridges	Amplicon Tested
T0 (0 days)	+2-8°C And <-15 °C	16	4 Replicates of: RVP Set 1 RVP Set 2 RVP Set 3 RVP Set 4
T1_2-8 °C (7 days)	+2-8°C	16	4 Replicates of: RVP Set 1 RVP Set 2 RVP Set 3 RVP Set 4
T2_2-8 °C (9 days)		16	4 Replicates of: RVP Set 1 RVP Set 2 RVP Set 3 RVP Set 4
T1_<-15 °C (4 weeks)	<-15 °C	16	4 Replicates of: RVP Set 1 RVP Set 2 RVP Set 3 RVP Set 4
T2_<-15 °C (5 weeks)		16	4 Replicates of: RVP Set 1 RVP Set 2 RVP Set 3 RVP Set 4
T3_<-15 °C (10 weeks) FIO		16	4 Replicates of: RVP Set 1 RVP Set 2 RVP Set 3 RVP Set 4
Total Number of Cartridges Required		96	

After the specified storage time, aliquots were removed from storage mixed with hybridization buffer prior to RVP cartridge detection. Performance was tested at three time points using two different storage conditions (-15 to -30 °C and 2 to 8°C) are shown below.

The overall detection rate for exonuclease cleaved amplicons stored at 2-8°C is shown below:

Sample Detection	T0			T1 (7 days)			T2 (9 days)		
	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls
IVT-1	28/28=100 (87.9, 100.0)	0	0	28/28=100 (87.9, 100.0)	0	0	27/28=96.4 (82.3, 99.4)	1*	0
IVT-2	40/40=100 (91.2, 100.0)	0	0	39/40=97.5 (87.1, 99.6)	1*	0	40/40=100 (91.2, 100)	0	0
IVT-3	36/36=100 (90.4, 100.0)	0	0	36/36=100 (90.4, 100.0)	0	0	36/36=100 (90.4, 100.0)	0	0
MS2	4/4=100 (51.0, 100.0)	0	0	4/4=100 (51.0, 100.0)	0	0	4/4=100 (51.0, 100.0)	0	0
Total	108/108=100 (96.6, 100.0)	0	0	107/108=99.1 (94.9, 99.8)	1*	0	107/108=99.1 (94.9, 99.8)	1*	0

No calls were a result of a software error and an electrical connection problem between the RVP cartridge and the XT-8 instrument. These failures were non-reagent or amplicon related.

The overall detection rate for exonuclease cleaved amplicons stored at -15 to -30°C is shown

below:

Sample Detection	T0			T1 (4 weeks)			T2 (5 weeks)		
	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls
IVT-1	28/28=100 (87.9, 100.0)	0	0	27/28=96.4 (82.3, 99.4)	1*	0	28/28=100 (87.9, 100.0)	0	0
IVT-2	40/40=100 (91.2, 100.0)	0	0	39/40=97.5 (87.1, 99.6)	1*	0	40/40=100 (91.2, 100.0)	0	0
IVT-3	36/36=100 (90.4, 100.0)	0	0	36/36=100 (90.4, 100.0)	0	0	36/36=100 (90.4, 100.0)	0	0
MS2	4/4=100 (51.0, 100.0)	0	0	4/4=100 (51.0, 100.0)	0	0	4/4=100 (51.0, 100.0)	0	0
Total	108/108=100 (96.6, 100.0)	0	0	106/108=98.1 (93.5, 99.5)	2*	0	108/108=100 (96.6, 100.0)	0	0

No calls were a result of an electrical connection problem between the RVP cartridge and the XT-8 instrument. These failures were non-reagent or amplicon related.

The time points tested exhibited no statistically significant difference in the positive detection rate. These data are acceptable to support exonuclease cleaved amplicon stability claims as published in the product labeling. The product labeling states:

Proceed immediately with the next step, or store the exonuclease-digested samples refrigerated at 2-8 °C for up to 1 week or frozen at -15 to -30 °C for up to 1 month before processing.

Open pouch stability

An analytical study was performed to establish performance of cartridges in previously opened storage pouches. Functional performance was evaluated with three stability test panels (IVT-1, IVT-2, IVT-3) of *in vitro* transcripts. Each stability test panel consisted of a unique combination of claimed viruses, so that the three stability test panels covered all the claimed viruses. All viruses in a particular stability test panel were tested at least once during each stability study. Virus targets were present at a concentration of 2.0×10^4 copies/ μ l. Since each viral target has a unique LoD concentration, a concentration of 2.0×10^4 copies/ μ l represents a range of 10x to 1×10^7 x LoD of an amplicon. Three sets of IVT viral panels were used which covered all analytes in the RVP (same sets as presented above). At time T0 all RVP cartridges were opened. All cartridges except those designated for T0 testing were stored in a re-sealable plastic bag and placed in controlled incubators for the scheduled storage time. After storage, cartridges were used to analyze test panels according to product labeling instructions. Final time point performance will be considered acceptable if the number of correctly called samples is statistically equivalent to the T0 time point. A schematic of the storage conditions and test time points is shown below:

Time Point and Condition	Number of Cartridges	Amplicon Tested
T0 0 hours	20	5 Replicates of: RVP Set 1; RVP Set 2 RVP Set 3; RVP Set 4
T1 2 weeks 30 °C, Ambient Humidity	20	5 Replicates of: RVP Set 1; RVP Set 2 RVP Set 3; RVP Set 4
T2 4 weeks 30 °C, Ambient Humidity	20	5 Replicates of: RVP Set 1; RVP Set 2 RVP Set 3; RVP Set 4
T3 8 weeks 30 °C, Ambient Humidity	20	5 Replicates of: RVP Set 1; RVP Set 2 RVP Set 3; RVP Set 4
T4 9 weeks 30 °C, Ambient Humidity	20	5 Replicates of: RVP Set 1; RVP Set 2 RVP Set 3; RVP Set 4
Total Number of Cartridges Required	100	

A total of 100 tests were run for each time point and a summary of the results is shown below:

Sample Detection	T0			T1 (2 weeks)			T2 (4 weeks)		
	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls
IVT-1	35/35=100 (90.1, 100.0)	0	0	35/35=100 (90.1, 100.0)	0	0	35/35=100 (90.1, 100.0)	0	0
IVT-2	49/50=98.0 (89.5, 99.7)	1	0	50/50=100 (92.9, 100.0)	0	0	50/50=100 (92.9, 100.0)	0	0
IVT-3	44/45=97.8 (88.4, 99.6)	1	0	45/45=100 (92.1, 100.0)	0	0	45/45=100 (92.1, 100.0)	0	0
MS2	5/5=100 (56.6, 100.0)	0	0	5/5=100 (56.6, 100.0)	0	0	5/5=100 (56.6, 100.0)	0	0
Total	133/135=98.5 (94.5, 99.6)	2*	0	135/135=100 (97.2, 100.0)	0	0	135/135=100 (97.2, 100.0)	0	0

Sample Detection	T0			T3 (8 weeks)			T4 (9 weeks)		
	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls
IVT-1	35/35=100 (90.1, 100.0)	0	0	35/35=100 (90.1, 100.0)	0	0	33/35=94.3 (81.4, 98.4)	2	0
IVT-2	49/50=98.0 (89.5, 99.7)	1	0	50/50=100 (92.9, 100.0)	0	0	48/50=96.0 (86.5, 98.9)	2	0
IVT-3	44/45=97.8 (88.4, 99.6)	1	0	43/45=96.6 (85.2, 98.8)	2*	0	43/45=96.6 (85.2, 98.8)	2	0
MS2	5/5=100 (56.6, 100.0)	0	0	5/5=100 (56.6, 100.0)	0	0	5/5=100 (56.6, 100.0)	0	0
Total	133/135=98.5 (94.5, 99.6)	2*	0	133/135=98.5 (94.5, 99.6)	2*	0	129/135=95.6 (90.6, 97.9)	6*	0

No call results were due to hardware/software problems include electrical contacts issues between the reader and the cartridge and software curve fitting errors.

The “no call” results during week 9 of testing were followed up and further test results pointed to hardware/software problems but the results were not conclusive.

The time points tested exhibited no statistically significant difference in the positive detection

rate. These data are acceptable to support open package stability claims (at ambient storage conditions) as published in the product labeling. The product labeling states:

Cartridges must be used within 4 weeks of opening the pouch.

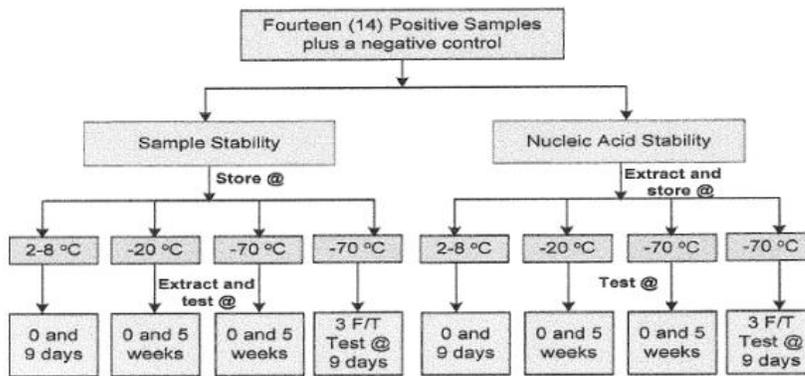
Extracted and Non-extracted Sample Storage and Freeze Thaw Stability

An analytical study was conducted to demonstrate that freezing samples does not affect the accuracy of test results compared to freshly collected specimens or freshly prepared samples. Fourteen viral targets were selected for the sample stability portion of the study, each positive for one viral target. All viral reference strains for this study were acquired from commercial sources and grown and titered prior to being used in the study. The viral strains used in this study are shown below:

RVP Target	Strain
Influenza A H1	H1N1 Brisbane/59/07
Influenza A H3	H3N2 ATCC VR-547
Influenza A 2009 H1N1	NY/2009
Influenza B	Florida/02/06
RSV A	A2
RSV B	9320
PIV 1	C35
PIV 2	Greer
PIV 3	C 243
Metapneumovirus	B2
Rhinovirus	3
Adenovirus B	Type 7
Adenovirus C	Type 1
Adenovirus E	Type 4

Fourteen samples each containing a unique viral strain at 3x LoD were prepared in negative patient nasopharyngeal swab (NPS) samples. Internal control was added to all samples per the product labeling prior to extraction. Prior to the initiation of the study an aliquot of each sample was extracted and used in the RNA/DNA stability portion of the study.

An outline of the study design is shown below:



Non-extracted Sample Stability

For each positive sample a total of 5 aliquots were prepared and labeled with the appropriate storage conditions. At each time point for each storage condition, a total of 15 extractions were performed. The 9 day and 5 week storage time points were tested at 2 temperatures according to the schematic above.

Overall detection results for unextracted samples after 9 days of storage at 2-8°C.

Target	Sample Stability								
	T0			9 days			9 days + 3 Freeze Thaws		
	Control			Storage at 2-8°C			Storage at -70°C		
	Correct Calls (%)	# of No Calls	# of Mis-Calls	Correct Calls (%)	# of No Calls	# of Mis-Calls	Correct Calls (%)	# of No Calls	# of Mis-Calls
ADV B	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
ADV C	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
ADV E	2/3 (66.7%)	1	0	2/3 (66.7%)	0	1	2/3 (66.7%)	0	1
FLU B	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
Flu A	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
H1	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
H1N1	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
H3	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
HMPV	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
HRV	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
PIV 1	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
PIV 2	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
PIV 3	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
RSV A	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
RSV B	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
Final Call	44/45 (97.8%) (88.4, 99.6)**	1*	0	44/45(97.8%) (88.4, 99.6)**	0	1*	44/45(97.8%) (88.4, 99.6)**	0	1*

One no call and two missed calls noted in the table above were due to hardware errors unrelated to the biochemical portion of the assay.

Mean signals (n=3) for each positive viral target call after 9 days in cold storage and the control sample data are shown below:

Target	Sample Stability								
	T0			9 days			9 days + 3 Freeze Thaws		
	Control			Storage at 2-8°C			Storage at -70°C		
	Signal (nA)	Std Dev (nA)	%CV*	Signal (nA)	Std Dev (nA)	%CV*	Signal (nA)	Std Dev (nA)	%CV*
ADV B	111.1	3.2	2.8	110.9	8.5	7.6	73.2	20.3	27.7
ADV C	194.4	35.5	18.3	172.8	56.1	32.4	117.2	45.9	39.2
ADV E	125.9	25.0	19.8	167.7	75.1	44.8	60.9	39.6	65.1
FLU B	117.2	14.7	12.5	128.3	13.3	10.3	63.0	30.9	48.9
Flu A	254.3	19.9	7.8	261.5	26.9	10.3	149.7	25.6	17.1
H1	209.1	52.9	25.3	212.1	6.4	3.0	171.0	7.8	4.6
H1N1	107.7	9.3	8.6	15.5	7.4	47.7	12.0	5.1	42.4
H3	70.7	39.6	56.0	97.8	9.7	9.9	40.9	11.0	27.0
HMPV	82.6	14.6	17.7	61.2	16.1	26.3	44.7	17.2	38.4
HRV	110.2	5.0	4.5	55.5	28.6	51.5	21.8	11.6	53.3
PIV 1	218.9	43.4	19.8	178.5	85.5	47.9	157.6	28.2	17.9
PIV 2	276.2	41.0	14.9	190.8	38.2	20.0	275.5	17.2	6.2
PIV 3	80.6	14.3	17.8	55.9	25.9	46.3	59.0	6.1	10.4
RSV A	117.4	23.1	19.7	77.1	21.4	27.8	5.8	2.8	48.9
RSV B	182.4	13.4	7.4	164.7	17.2	10.5	105.4	33.9	32.2

Overall detection results after 5 weeks of storage at -20°C and -70°C.

Target	Sample Stability								
	T0			5 week incubation					
	Control			Storage at -70°C			Storage at -20°C		
	Correct Calls (%)	# of No Calls	# of Mis-Calls	Correct Calls (%)	# of No Calls	# of Mis-Calls	Correct Calls (%)	# of No Calls	# of Mis-Calls
ADV B	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
ADV C	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
ADV E	2/3 (66.7%)	1	0	3/3 (100%)	0	0	3/3 (100%)	0	0
FLU B	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
Flu A	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
H1	3/3 (100%)	0	0	2/3 (66.7%)	0	1	3/3 (100%)	0	0
H1N1	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
H3	3/3 (100%)	0	0	3/3 (100%)	0	0	2/3 (66.7%)	0	1
HMPV	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
HRV	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
PIV 1	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
PIV 2	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
PIV 3	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
RSV A	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
RSV B	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
Final Call	44/45=97.8 (88.4, 99.6)**	1*	0	44/45=97.8 (88.4, 99.6)**	0	1*	44/45=97.8 (88.4, 99.6)**	0	1*

Missed calls were attributed to electrical connection problems between the eSensor cartridge and the XT-8 instrument.

Mean signals (n=3) for each positive viral target call after 5 weeks in cold storage and the control sample data are shown below:

Target	T0			Sample Stability					
	Control			5 week incubation					
	Signal (nA)	Std Dev (nA)	%CV*	Storage at -70°C			Storage at -20°C		
				Signal (nA)	Std Dev (nA)	%CV*	Signal (nA)	Std Dev (nA)	%CV*
ADV B	111.1	3.2	2.8	117.9	12.2	10.4	68.7	21.9	31.9
ADV C	194.4	35.5	18.3	215.0	37.1	17.3	205.0	14.7	7.2
ADV E	125.9	25.0	19.8	179.5	21.5	12.0	166.5	21.4	12.9
FLU B	117.2	14.7	12.5	129.9	10.2	7.8	143.3	12.9	9.0
Flu A	254.3	19.9	7.8	261.0	6.4	2.5	265.4	31.9	12.0
H1	209.1	52.9	25.3	162.0	51.8	32.0	201.4	54.7	27.1
H1N1	107.7	9.3	8.6	26.9	8.7	32.2	27.0	19.9	73.5
H3	70.7	39.6	56.0	113.2	32.9	29.0	130.1	30.2	23.2
HMPV	82.6	14.6	17.7	79.7	11.7	14.6	76.4	12.7	16.7
HRV	110.2	5.0	4.5	64.2	5.9	9.1	82.8	15.6	18.8
PIV 1	218.9	43.4	19.8	242.1	9.9	4.1	203.1	14.2	7.0
PIV 2	276.2	41.0	14.9	240.7	33.4	13.9	252.1	43.1	17.1
PIV 3	80.6	14.3	17.8	71.2	12.9	18.1	84.8	11.0	13.0
RSV A	117.4	23.1	19.7	85.6	12.0	14.0	108.1	24.5	22.7
RSV B	182.4	13.4	7.4	165.0	26.3	15.9	172.3	33.3	19.3

Extracted Sample Stability

A test of the stability of extracted nucleic acid was run in parallel with the sample stability testing employing the same storage and testing conditions. Each target dilution was extracted 6 times at time 0 (T0) to generate sets of RNA/DNA used to establish extracted stability performance. At the start of the study (T0 time point) these sample extracts were then stored at the specified conditions and tested according to the study schematic at the beginning of this section.

Overall detection results for extracted samples after 9 days of storage at 2-8°C.

Target	Nucleic Acid								
	T0			9 days			9 days + 3 Freeze Thaws		
	Control			Storage at 2-8°C			Storage at -70°C		
	Correct Calls (%)	# of No Calls	# of Mis-Calls	Correct Calls (%)	# of No Calls	# of Mis-Calls	Correct Calls (%)	# of No Calls	# of Mis-Calls
ADV B	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
ADV C	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
ADV E	2/3 (66.7%)	1	0	3/3 (100%)	0	0	3/3 (100%)	0	0
FLU B	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
Flu A	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
H1	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
H1N1	3/3 (100%)	0	0	3/3 (100%)	0	0	1/3 (33.3%)	0	2
H3	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
HMPV	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
HRV	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
PIV 1	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
PIV 2	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
PIV 3	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
RSV A	3/3 (100%)	0	0	2/3 (66.7%)	0	1	3/3 (100%)	0	0
RSV B	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
Final Call	44/45=97.8 (88.4, 99.6)**	1*	0	44/45=97.8 (88.4, 99.6)**	0	1*	43/45=95.6 (85.2, 98.8)**	0	2*

All no calls and miscalls were due to signals below the threshold of <3 nA (false negative) no

hardware failure was reported.

Overall detection results for extracted samples after 9 days of storage at 2-8°C.

Target	Nucleic Acid Stability								
	T0			5 week					
	Control			Storage at -70°C			Storage at -20°C		
	Correct Calls (%)	# of No Calls	# of Mis-Calls	Correct Calls (%)	# of No Calls	# of Mis-Calls	Correct Calls (%)	# of No Calls	# of Mis-Calls
ADV B	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
ADV C	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
ADV E	2/3 (66.7%)	1	0	3/3 (100%)	0	0	3/3 (100%)	0	0
FLU B	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
Flu A	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
H1	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
H1N1	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
H3	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
HMPV	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
HRV	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
PIV 1	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
PIV 2	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
PIV 3	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
RSV A	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
RSV B	3/3 (100%)	0	0	3/3 (100%)	0	0	3/3 (100%)	0	0
Final Call	44/45=97.8 (88.4, 99.6)**	1*	0	45/45=100 (92.1, 100.0)**	0	0	45/45=100 (92.1, 100.0)**	0	0

The single no call result was due to signal below the threshold of <3 nA (false negative) no hardware failure was reported.

The time points tested exhibited no statistically significant difference in the positive detection rate. These data are acceptable to support extracted and unextracted sample stability claims as published in the product labeling. Product labeling states:

Transport and Storage- Clinical specimens can be stored between 2 °C and 8 °C for up to 7 days after collection in viral transport media. Specimens can also be stored at <-15 °C for up to 1 month prior to extraction and undergo up to two freeze/thaw cycles.

Note: Purified nucleic acids have been validated for storage between 2 °C and 8 °C for up to 7 days, at <-15 °C for up to 1 month, and can undergo up to two freeze/thaw cycles.

Reagent Kit Freeze Thaw (FT) Stability

An analytical study was performed to determine the stability of the RT-PCR and detection reagents used in the eSensor RVP when subjected to storage and freeze/thaw (FT) cycles. Reagents were evaluated for functional performance after 4 and 6 freeze thaw cycles at 4 and 6 weeks. The same set of IVT mixes (IVT1-3) were used in this study and have been previously described above. At each time point and freeze/thaw cycle, samples were spiked

with internal control, extracted and amplified then analyzed using the eSensor RVP. An outline of the time points and freeze thaw cycles is shown in the schematic below:

Time Point and Freeze/Thaw (FT) Cycle	Number of Cartridges	IVT Tested
FT0; Control (0 day)	4 replicates for each IVT Set 1 DCM 17 total	4 Replicates of: RVP Set 1; RVP Set 2 RVP Set 3; MS2
FT1	NA	NA
FT2	NA	NA
FT3	NA	NA
FT4 (4 weeks)	4 replicates for each IVT Set 16 total	4 Replicates of: RVP Set 1; RVP Set 2 RVP Set 3; MS2
FT5	NA	NA
FT6 (6 weeks)	4 replicates for each IVT Set 16 total	4 Replicates of: RVP Set 1; RVP Set 2 RVP Set 3; MS2

Two reagent packs designated for FT4 and FT6 were opened at the beginning of the study and underwent three and five FT cycles respectively prior to the final thaw at the time of testing. For each FT cycle all reagents (except the RVP enzyme mix) were thawed at 37°C for 15-20 minutes before use or before the next FT cycle. The RVP enzyme mix was thawed on wet ice between FT cycles. A total of 16 reactions per time point plus one negative control were prepared. Final time point performance was considered acceptable if the numbers of correctly called samples were statistically equivalent for each tested condition.

Overall detection results for IVT mixes tested with reagent kits upon opening (FT0) and stored at 2 test conditions (FT4: 4 weeks of storage at -15°C to -30°C and 3 FT cycles and FT6: 6 weeks of storage at -15°C to -30°C and 5 FT cycles) as shown below:

Sample Detection	FT0			FT4 (4 week)			FT6 (6 week)		
	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls	% Correct Calls (95% CI)**	# of No Calls	# of Mis-Calls
IVT-1	28/28=100 (87.9, 100.0)	0	0	27/28=96.4 (82.3, 99.4)	1*	0	28/28=100 (87.9, 100.0)	0	0
IVT-2	39/40=97.5 (87.1, 99.6)	1*	0	39/40=97.5 (87.1, 99.6)	1*	0	40/40=100 (91.2, 100.0)	0	0
IVT-3	36/36=100 (90.4, 100.0)	0	0	36/36=100 (90.4, 100.0)	0	0	36/36=100 (90.4, 100.0)	0	0
MS2	2/4=50 (15, 85.0)	0	2*	4/4=100 (51.0, 100.0)	0	0	3/4=75 (30.1, 95.4)	0	1*
Total	105/108=97.2 (92.1, 99)	1*	2*	106/108=98.1 (93.5, 99.5)	2*	0	107/108=99.1 (94.9, 99.8)	0	1*

Miscalls were attributed to electrical connection problems between the eSensor cartridge and the XT-8 instrument. Miscalls for the MS2 samples (negative controls) were attributed to carry-over contamination.

The time points tested exhibited no statistically significant difference in the positive detection rate. These data are acceptable to support reagent kit freeze thaw stability claims as published in the product labeling. The product labeling states:

- Freeze remaining reagents immediately after use.
- Store frozen reagents in a non-frost-free freezer (-15 to -30 °C).
- Reagents may be frozen and thawed up to 5 times.

d. Detection limit:

A range finding study was executed initially to estimate the LoD of each claimed virus using pooled negative NPS specimens in viral transport media as the sample matrix. Re-grown and quantified reference strains of one representative strain for each claimed virus was tested in the study. Each virus strain was diluted in log intervals into transport media (a swab was not used to transfer the virus strain into transport media). Three replicates of each virus strain were tested per concentration starting from nucleic acid extraction. The LoD was chosen as the lowest concentration of each virus that generated a positive result by the eSensor RVP assay for all three replicates. The initial estimates of LoD are shown below:

Virus	Dilution	Spiked Conc. (TCID50/mL)
Flu A H1	1:10 ⁶	4.17E-01
Flu A H3	1:10 ⁵	1.58E+03
Flu A 2009 H1N1	1:10 ⁷	1.05E-01
Flu B	1:10 ⁸	3.16E-02
RSV A	1:10 ⁶	2.81E+00
RSV B	1:10 ⁵	1.58E+00
PIV 1	1:10 ⁷	2.81E-03
PIV 2	1:10 ⁷	2.81E-01
PIV 3	1:10 ⁶	2.81E+01
HMPV	1:10 ⁵	4.17E+00
HRV	1:10 ⁷	1.58E-03
ADV B	1:10 ⁷	8.89E-02
ADV C	1:10 ⁸	8.89E-01
ADV E	1:10 ⁶	1.58E+00

The above estimated LoD concentrations were further tested to verify the LoD estimates from the previous range finding study in a LoD confirmation study. The LoD confirmation study tested 20 replicates of each virus strain at the estimated LoD concentrations. The LoD is confirmed if greater than or equal to 95% detection rate is achieved (i.e., at least 19/20 replicates test positive). Results from the first LoD confirmation study are shown below:

Virus	Dilution	Spiked Conc. (TCID50/mL)	# of POS samples	# of NEG samples	% Positives
Flu A H1	1:10 ⁶	4.17E-01	20	0	100
Flu A H3	1:10 ⁵	1.58E+03	20	0	100
Flu A 2009 H1N1	1:10 ⁷	1.05E-01	18	2	90
Flu B	1:10 ⁸	3.16E-02	20	0	100
RSV A	1:10 ⁶	2.81E+00	20	0	100
RSV B	1:10 ⁵	1.58E+00	20	0	100
PIV 1*	1:10 ⁷	2.81E-03	16	3	84
PIV 2	1:10 ⁷	2.81E-01	13	7	65
PIV 3	1:10 ⁶	2.81E+01	20	0	100
HMPV	1:10 ⁵	4.17E+00	20	0	100
HRV	1:10 ⁷	1.58E-03	19	1	95
ADV B	1:10 ⁷	8.89E-02	20	0	100
ADV C	1:10 ⁸	8.89E-01	7	13	35
ADV E	1:10 ⁶	1.58E+00	15	5	75

*19 of 20 data points available due to cartridge failure.

During the first confirmation study it was observed that five virus strains (PIV 1, PIV 2, ADV C, ADV E and Flu A 2009 H1N1) had detection rates below the target of 95%. A second LoD confirmation study was conducted testing 20 additional extraction replicates at the confirmed LoD concentrations for all the virus strains from the first study except for the five virus strains that had below 95% detection rates. The concentration of these five virus strains were increased (a lower dilution of the same stock) and tested in the second LoD confirmation study. The results of the second LoD confirmation study are shown below:

Virus	Dilution	Spiked Conc. (TCID ₅₀ /mL)	# of POS samples	# of NEG samples	% Positives
Flu A H1	1:10 ⁶	4.17E-01	19	1	95
Flu A H3	1:10 ⁵	1.58E+03	20	0	100
Flu A 2009 H1N1	1:10 ⁷	1.05E-01	18	2	90
Flu B	1:10 ⁸	3.16E-02	14	6	70
RSV A	1:10 ⁶	2.81E+00	19	1	95
RSV B	1:10 ⁵	1.58E+00	20	0	100
PIV 1	1:10 ⁶	2.81E-02	20	0	100
PIV 2	1:10 ⁶	2.81E+00	20	0	100
PIV 3	1:10 ⁶	2.81E+01	20	0	100
HMPV	1:10 ⁵	4.17E+00	20	0	100
HRV	1:10 ⁷	1.58E-03	20	0	100
ADV B	1:10 ⁷	8.89E-02	20	0	100
ADV C	1:10 ⁶	8.89E+01	20	0	100
ADV E	1:10 ⁵	1.58E+01	20	0	100

Based on the combined results from the two confirmation studies, Flu B was retested at an increased concentration of 3.16×10^{-1} TCID₅₀/mL and the Flu B LoD was confirmed in a third separate study.

A summary of the final LoD claims are listed below:

Viral Target	Strain	LoD Spiked Conc. (TCID ₅₀ /mL)	# of POS / Total Samples	% Positive
Flu A	H1N1 Brisbane/59/07	4.17×10^{-1}	40/40	100%
Flu A H1	H1N1 Brisbane/59/07	4.17×10^{-1}	39/40	97.5%
Flu A	H3N2	1.58×10^3	40/40	100%
Flu A H3	H3N2	1.58×10^3	40/40	100%
Flu A 2009 H1N1	NY/2009	1.05×10^{-1}	76/80	95%
Flu B	Florida/02/06	3.16×10^{-1}	40/40	100%
hMPV	B2	4.17×10^0	40/40	100%
HRV	3	1.58×10^{-3}	39/40	97.5%
PIV1	C35	2.81×10^{-2}	40/40	100%
PIV2	Greer	2.81×10^0	40/40	100%
PIV3	C 243	2.81×10^1	40/40	100%
RSV A	A2	2.81×10^0	39/40	97.5%
RSV B	9320	1.58×10^0	40/40	100%
ADV B/E	Type 4	1.58×10^1	40/40	100%
ADV C	Type 1	8.89×10^1	40/40	100%

e. Analytical reactivity:

An analytical reactivity (inclusivity) study was performed to determine whether the eSensor RVP is able to detect a variety of strains (inclusivity panel) that represent the temporal and geographic diversity of each of the RVP targeted viruses. This study expanded upon the limit of detection studies by determining whether different strains of the same virus can be detected at or close to the established LoD concentrations.

This study involved inclusivity test panels of diverse virus strains spiked into simulated NPS samples. All virus identities were confirmed prior to the study. All virus titers were confirmed except for three HRV strains where extracted RNA samples provided by Biota, Inc. were tested directly in the eSensor RVP. A negative assay control was included with each eSensor RVP extraction run.

Inclusivity virus strains were initially tested near the limit of detection (3x LoD) for each strain as determined in the LoD study. If a specimen containing a particular strain was positive (detected) at the 3 x LoD level, no further testing of that strain was required. If a strain was not detected at the 3 x LoD concentration, the strain was retested at a higher concentration. If necessary, the same approach was followed until a positive result was obtained or the maximum concentration possible for that strain stock had been tested.

The inclusivity panel represented the evolutionary, temporal, and geographical diversity of the RVP analytes. For example, the inclusivity panel included 14 different Adenovirus serotypes, 32 Influenza A strains (subtypes H3, H1 and 2009 H1) isolated from around the world as early as 1930 and as recent as 2009, and 17 strains of the group that comprised of Human Rhinovirus A, B and C. However, for some analytes, the inclusivity panel is restricted to only two strains either because of limited strain diversity, or due to limited availability.

Each viral strain was diluted in M5 transport medium to a titer of 3x LoD for the corresponding viral target and extracted in triplicate using the bioMerieux NucliSENS easyMAG System. Following extraction, each replicate was tested once using the eSensor RVP. In the case that a viral strain is not detected at 3X LoD, 1000 fold serial dilutions were made from the viral stock and then each dilution was extracted in triplicate and tested using the eSensor RVP.

The summary of all isolates tested appears below:

Target	Strain	Concentration Detected (TCID50/ml)	LoD Multiple Detected
Flu A H1	A/New Caledonia/20/1999	4.2	10x
	A/Brisbane/59/07	1.26	3x
	FM/1/47H1	1.26	3x
	A/Denver/1/57	1.26	3x
	A/Solomon Islands/3/2006	1.26	3x

Target	Strain	Concentration Detected (TCID50/ml)	LoD Multiple Detected
	A/Taiwan/42/06	1.26	3x
	A/NWS/33	1260	Flu A- 3x; H1- 3000x
	A/PR/8/34	1.26	Flu A- 3x; H1- not detected*
	A/Mal/302/54	6372	Flu A- 3x H1- 15172x*
	A/Fort Monmouth/1/1947 (H1N1)	5.5	Flu A- 3x H1-13x
Flu A H3	A/Aichi/2/68 H3N2	4743	3x
	A/Brisbane/10/07	4743	3x
	A/Victoria/3/75	4743	3x
	A/Port Chalmers/1/73	4743	3x
	A/Wisconsin/67/05	4743	3x
	A/Hong Kong/8/68	4743	3x
	A/Perth/16/2009	4743	3x
	Alice (vaccine) A/England/42/72	4743	3x
	MRC-2 Recombinant Strain	4743	3x
A/Nanchang/933/95	4743	3x	
Flu A 2009 H1N1	A/NY/02/2009	0.3	3x
	A/New Jersey/8/76	0.3	3x
	A/California/7/2009	0.3	3x
	A/Swine NY/01/2009	0.3	3x
	A/Swine NY/03/2009	0.3	3x
	A/Mexico/4108/09	0.3	3x
	A/Virginia/ATCC1/2009	0.3	3x
	A/Virginia/ATCC2/2009	0.6	Flu A- 3x 2009 H1N1- 6x
	A/Virginia/ATCC3/2009	2.7	Flu A- 3x 2009 H1N1- 27x
A/Iowa/15/30	100	Flu A- 3x; 2009 H1N1-1000x	
Flu A H2N2	A/Japan/305/57, RNA	1.625 ng	FluA
	A/Korea/426/68 (HA, NA) x A/Puerto Rico/8/34, RNA	3.12 ng	FluA
Flu A H1N2	A/NWS/34 (HA) x A/Rockefeller Institute/5/57 (NA), RNA	0.74 ng	FluA H1
Flu A H5N3	A/duck/Singapore/645/97, Wild Type	1.26	FluA
Flu A H10N7	A/chicken/Germany/N/49	1.26	FluA
Flu B	B/Florida/02/06	1	3x
	B/Malaysia/2506/04	1	3x
	B/Lee/40	1	3x
	B/Allen/45	1	3x
	B/GL/1739/54	1	3x

Target	Strain	Concentration Detected (TCID50/ml)	LoD Multiple Detected
	B/Taiwan/2/62	1	3x
	B/Hong Kong/5/72	1	3x
	B/Maryland/1/59	1	3x
RSV A	A2	8.4	3x
	Long	8.4	3x
RSV B	9320	4.8	3x
	WV/14617/85	4.8	3x
	Wash/18537/62	4.8	3x
PIV1	C35	0.084	3x
	Type 1	0.084	3x
PIV2	Greer	8.4	3x
	Type 2	8.4	3x
PIV3	C-243	84	3x
	Type 3	84	3x
HMPV	IA3-2002 G, A1	12.6	3x
	IA14-2003 G, A2	12.6	3x
	Peru2-2002 G, B1	12.6	3x
	Peru6-2003 G, B2	12.6	3x
HRV A	1A	0.9	450x [#]
	A2	0.9	569x
	A7	0.005	3x
	A16	0.005	3x
	18	Detected**	N/A
	A34	0.005	3x
	A57	0.005	3x
	A77	0.005	3x
277G	0.2	100x [#]	
HRV B	B3	0.1	80x
	B14	0.02	14x
	B17	0.4	253x
	B42	0.005	3x
	FO2-2547	0.2	89x [#]
	B83	0.2	127x
	84	Detected**	N/A
HRV C	C ^s	Detected**	N/A
ADV B	Type 3	0.3	3x
	Type 7A	0.3	3x
	Type 11 (lot 306523)	0.3	3x
	De Wit Type 14	0.3	3x
	Ch.79 Type 16	0.3	3x
	Type 21 (lot 307610)	0.3	3x
	Compton Type 34	0.3	3x
	Holden Type 35	0.3	3x
	Wan Type 50	0.3	3x
ADV C	Type 1	267	3x
	Type 2	533	6x
	Type 5	533	6x
	Type 6	533	6x
ADV E	Type 4	47	3x

* *In silico* analysis revealed little homology between the strain sequence and the H1 primers.

HRV strain 3, used for LoD determination, had a TCID₅₀/ml of 0.0016. HRV strains 1A, FO2-2547, 277G were detected at a higher LoD multiple to the reference strain, respectively with their corresponding TCID₅₀/ml values of 0.9, 0.2, and 0.2.

**No concentration available since it was an extracted RNA sample.

§ Only one test done for HRV C due to limited sample availability

Two Flu A isolates from non-contemporary strains (Flu A/NWS/33 and Flu A/PR/8/34) were successfully detected at 3x the LoD. The Flu A/NWS/33 strain subtype was differentiated as Flu A H1 subtype at 3000 x the LoD. *In silico* analysis revealed several mismatches of this strain with the H1 primers which would result in reduced sensitivity for the H1 target. The subtype for isolate Flu A/PR/8/34 strain was not differentiated at any concentration tested. *In silico* analysis revealed low homology between the strain sequence and the H1 primers.

Flu A/Iowa/15/30 strain was detected as FluA only at 3x the LoD; Flu A 2009 H1N1 subtype was detected at 1000x LoD. *In silico* analysis revealed several mismatches of this strain with the 2009 H1N1 primers which would result in reduced sensitivity for the 2009 H1N1 target.

Rhinovirus strains 1A, F02-2547, 277G were detected at 450X, 89X, and 100X the LoD, respectively with their corresponding TCID₅₀/ml values of 0.9, 0.2, and 0.2. The HRV strain 3, which was used for the LoD determination, had a TCID₅₀/ml of 0.0016. The detection levels for HRV reactivity strains were determined relative to this value as described below.

Adenovirus C types 2, 5, and 6 were detected at 6x the LoD. All three strains have a single mismatch with ADVC type 1 capture probe and ADVC type 6 has an additional mismatch with the signal probe which would result in reduced sensitivity for these strains. One mismatch was observed between the three target strains and the ADV C capture probe.

In addition to laboratory testing, bioinformatics resources were also used to predict reactivity of additional species, strains and serotypes with the eSensor RVP system. Simulated reactivity was based on the number and location of mismatches between the target sequence and the assay primer(s).

f. Analytical specificity and cross-reactivity:

An analytical specificity study was carried out to assess the potential for false positive results due to cross-reactivity between eSensor RVP assay targets and other RVP or non-RVP organisms.

Cross-Reactivity Evaluation for Viruses Detected by the eSensor RVP

Cross-reactivity of each viral target (14 viral targets) was evaluated at high concentrations with the eSensor RVP by making three serial dilutions of viral reference strains with viral

transport media (Remel M5) at 10,000x, 1000x and 100x the LoD. The titer of each virus dilution and corresponding LoD values were determined and provided in the table below. Cross-reactivity was not observed with any of the RVP viral targets at the concentrations tested. The table below summarizes the cross-reactivity results:

Viral Target	Strain	LoD Concentration (TCID ₅₀ /mL)	Highest Test Concentration (TCID ₅₀ /mL)	Highest Multiple of LoD Tested	Cross-Reactivity Results
Flu A	H1N1 Brisbane/59/07	4.17 x 10 ⁻¹	4.17 x 10 ³	10,000x	Not Observed
Flu A H1	H1N1 Brisbane/59/07	4.17 x 10 ⁻¹	4.17 x 10 ³	10,000x	Not Observed
Flu A	H3N2	1.58 x 10 ³	1.58 x 10 ⁷	10,000x	Not Observed
Flu A H3	H3N2	1.58 x 10 ³	1.58 x 10 ⁷	10,000x	Not Observed
Flu A 2009 H1N1	NY/2009	1.05 x 10 ⁻¹	1.05 x 10 ³	10,000x	Not Observed
Flu B	Florida/02/06	3.16 x 10 ⁻¹	3.16 x 10 ³	10,000x	Not Observed
hMPV	B2	4.17 x 10 ⁰	4.17 x 10 ⁴	10,000x	Not Observed
HRV	3	1.58 x 10 ⁻³	1.58 x 10 ¹	10,000x	Not Observed
PIV1	C35	2.81 x 10 ⁻²	2.81 x 10 ²	10,000x	Not Observed
PIV2	Greer	2.81 x 10 ⁰	2.81 x 10 ⁴	10,000x	Not Observed
PIV3	C 243	2.81 x 10 ¹	2.81 x 10 ⁵	10,000x	Not Observed
RSV A	A2	2.81 x 10 ⁰	2.81 x 10 ⁴	10,000x	Not Observed
RSV B	9320	1.58 x 10 ⁰	1.58 x 10 ⁴	10,000x	Not Observed
ADV B/E	Type 7 Type 4	8.89 x 10 ⁻² 1.58 x 10 ¹	1.58 x 10 ⁵	10,000x	Not Observed
ADV C	Type 1	8.89 x 10 ¹	8.89 x 10 ⁵	10,000x	Not Observed

As shown in the table below, the signal intensities observed for each tested target and panel analyte indicating that cross-reactivity only exists between the ADV E and ADV B targets. These analytes are reported as a single result due to the experimentally confirmed genetic similarity between these species. All other panel members showed no cross-reactivity at levels below the cut-off of 3 nA for all panel members tested.

RVP Target	Reference Reagent, Signal Results (nA)														
	ADV B	ADV E	ADV C	Flu A	Flu A H1	Flu A H1N1	Flu A H3	Flu B	HMPV	HRV	PIV 1	PIV 2	PIV 3	RSV A	RSV B
ADV B/E	94.3	125.1	0.3	0.5	0.2	0.1	0.3	0.3	0.1	0.3	0.3	0.4	0.2	0.1	0.5
ADV C	0.3	0.1	127.9	0.4	0.1	0.1	0.2	0.2	0.1	0.3	0.2	0.3	0.1	0.1	0.4
Flu A	0.5	0.5	0.4	238.7	0.0	0.0	0.3	0.3	0.6	0.6	0.5	0.4	0.4	0.5	0.5
Flu A H1	0.3	0.1	0.2	0.0	250.4	0.1	0.2	0.2	0.1	0.2	0.2	0.3	0.1	0.1	0.4
Flu A H1N1	0.4	0.1	0.3	0.0	0.2	253.5	0.4	0.5	0.1	0.5	0.4	0.5	0.2	0.1	0.5
Flu A H3	0.5	0.1	0.3	0.3	0.1	0.2	94.6	0.5	0.1	0.4	0.3	0.5	0.2	0.2	0.4
Flu B	0.5	0.1	0.4	0.3	0.1	0.1	0.2	129.6	0.1	0.5	0.3	0.4	0.1	0.1	0.5
HMPV	0.4	0.1	0.4	0.6	0.2	0.1	0.3	0.4	106.2	0.4	0.3	0.4	0.2	0.2	0.6
HRV	0.6	0.2	0.4	0.6	0.1	0.2	0.3	0.5	0.1	132.1	0.4	0.5	0.2	0.2	0.7
PIV 1	0.3	0.1	0.3	0.5	0.1	0.1	0.2	0.3	0.1	0.3	228.2	0.4	0.2	0.2	0.5
PIV 2	0.6	0.1	0.3	0.4	0.1	0.2	0.4	0.5	0.1	0.4	0.3	266.4	0.2	0.3	0.4
PIV 3	0.6	0.1	0.4	0.4	0.1	0.2	0.3	0.5	0.1	0.5	0.4	0.6	260.1	0.2	0.6
RSV A	0.5	0.1	0.3	0.5	0.1	0.1	0.3	0.5	0.1	0.3	0.4	0.5	0.2	191.7	0.9
RSV B	0.4	0.1	0.4	0.5	0.2	0.1	0.3	0.5	0.1	0.4	0.3	0.3	0.2	160.4	

Cross-Reactivity with Other Respiratory Viruses Not Targeted by the eSensor RVP

Cross-reactivity with 5 respiratory viruses known to circulate with low frequency in the general population was assessed. All viral strains were diluted in M5 transport media to a titer of 10^5 pfu/mL (except Echovirus 30 which was tested at 9.89×10^4 pfu/ml) and extracted using the bioMérieux NucliSENS easyMAG extraction method in triplicate. Following extraction, each replicate was tested once in the RVP assay.

Organism	Source	Test Concentrations	Cross-Reactivity Results
Parainfluenza 4	Zeptomatrix	2.92×10^5 pfu/mL	Not Detected
Coronavirus OC43*	Zeptomatrix	5.96×10^4 pfu/mL	Not Detected
Coronavirus 229E	Zeptomatrix	1.36×10^5 pfu/mL	Not Detected
Coronavirus NL63**	Zeptomatrix	9.89×10^4 pfu/mL	Not Detected
Coronavirus HKU1	Clinical Isolate	N/A [§]	Not Detected

*OC43 had one replicate fail the IC control at high (10^5) concentration.

**NL63 was tested at the highest concentration available - 9.89×10^4 pfu/mL.

[§]The Coronavirus HKU1 sample was a clinical isolate identified during the method comparison study. The method used was qualitative so no genome copy number information was available.

Cross-Reactivity with 17 additional viruses that are not targets of the eSensor RVP were also assessed. All viral strains were diluted in M5 transport media to a titer of 10^5 pfu/mL and extracted using the bioMérieux NucliSENS easyMAG extraction method in triplicate reactions prior to detection using the eSensor RVP cartridge.

Organism	Source	Test Concentrations	Cross-Reactivity Results
Adenovirus 18 (A)	Zeptomatrix VPL-030	2.37×10^5 pfu/mL	Not Detected
Adenovirus 9 (D)	Zeptomatrix VPL-030	4.63×10^5 pfu/mL	ADVC False Positive*
Adenovirus 41 (F)	Zeptomatrix VPL-030	8.05×10^5 pfu/mL	ADVC False Positive*
Enterovirus 71	Zeptomatrix 0810047CF	2.92×10^5 pfu/mL	Not Detected

Organism	Source	Test Concentrations	Cross-Reactivity Results
Coxsackievirus A10	Zeptomatrix 0810106CF	1.72E x10 ⁵ pfu/mL	Not Detected
Coxsackievirus A9	Zeptomatrix 0810017CF	2.21x10 ⁵ pfu/mL	Not Detected
Echovirus E6	Zeptomatrix 0810076CF	7.16x10 ⁵ pfu/mL	Not Detected
Coxsackievirus B2	ATCC VR-29	6.22E+08 pfu/mL	Not Detected
Coxsackievirus B3	Zeptomatrix 0810074CF	1.06x10 ⁵ pfu/mL	Not Detected
Coxsackievirus B4	Zeptomatrix 0810075CF	8.04E+06 pfu/mL	2/3 Not Detected 1 HRV Positive
Coxsackievirus B5	Zeptomatrix 081019CF	7.16E+07 pfu/mL	Not Detected
Echovirus 9	Zeptomatrix 081007CF	1.41E+05 pfu/mL	Not Detected
Echovirus 25	Zeptomatrix VPL-030	1.93x10 ⁵ pfu/mL	Not Detected
Echovirus 30	Zeptomatrix 0810078CF	9.89E+04 pfu/mL	Not Detected
Coxsackievirus A21	Zeptomatrix 0810018CF	2.92x10 ⁵ pfu/mL	Not Detected
Coxsackievirus A24	ATCC VR-583	7.00E+05 pfu/mL	Not Detected
Enterovirus 68	ATCC VR-561	1.40E+06 pfu/mL	Not Detected
Poliovirus	ATCC VR-193	1.11x10 ⁵ pfu/mL,	HRV False Positive*
Bocavirus	Clinical Isolate	N/A	Not Detected
Herpesvirus 1: Herpes Simplex	Zeptomatrix 0810005CF	1.01x10 ⁵ pfu/mL	Not Detected
Herpesvirus 3: Varicella Zoster	Zeptomatrix 0810026CF	2.35x10 ⁶ copies/mL [#]	Not Detected
Herpesvirus 4: Epstein Barr	Zeptomatrix 0810008CF	1.06x10 ⁵ pfu/mL	Not Detected
Herpesvirus 5: Cytomegalovirus	Zeptomatrix 0810003CF	6.68x10 ⁵ pfu/mL	Not Detected
Measles	Zeptomatrix	1.37x10 ⁵ pfu/mL	Not Detected
Mumps	Zeptomatrix 0810079CF	1.93x10 ⁵ pfu/mL	Not Detected

*ADV C cross-reactive signal was also obtained from Adenovirus 9 (D) and Adenovirus 41 (F) when it was diluted 1000 folds from the initial testing concentration. Due to the genetic similarity between Adenovirus C, D, and F, the eSensor RVP cannot reliably differentiate them. A positive eSensor RVP Adenovirus specie C result should be followed-up using an alternative method (e.g., sequence analysis) if definitive Adenovirus speciation is needed.

**Due to the genetic similarity between human rhinovirus and poliovirus, the eSensor RVP cannot reliably differentiate them. If a polio infection is suspected, a positive eSensor RVP human rhinovirus (HRV) result should be confirmed using an alternate method (e.g., cell culture).

#Quantification of the viral RNA contained in the Herpesvirus-3 (Varicella Zoster Virus) sample was performed using real-time RT-PCR and provided in copies/mL

Systematic false positives observed during the cross-reactivity study will be communicated to the user by the following three statements in the product labeling:

Due to the genetic similarity between Adenovirus species C, D and F, and between Adenovirus species B and E, the eSensor RVP cannot reliably differentiate between Adenovirus species C, D and F, or between Adenovirus species B and E. A positive eSensor RVP Adenovirus specie C result or a positive Adenovirus species B/E result should be followed-up using an alternative method (e.g., sequence analysis) if definitive Adenovirus speciation is needed.

Due to the genetic similarity between human rhinovirus and poliovirus, the eSensor RVP cannot reliably differentiate them. If a polio infection is suspected, a positive eSensor RVP human rhinovirus (HRV) result should be confirmed using an alternate method (e.g., cell culture).

Due to the genetic similarity between Adenovirus species C, D (serotype 9) and F (serotype 41), the eSensor RVP cannot reliably differentiate between Adenovirus species C, D (serotype 9) and F (serotype 41). A positive eSensor RVP Adenovirus species C result should be followed-up using an alternative method (e.g., sequence analysis) if definitive Adenovirus speciation between species C, D (serotype 9) and F (serotype 41) is needed.

Cross-Reactivity with Bacteria and Fungus

The non-RVP target exclusivity panel consisted of 32 bacteria, 25 viruses, and 2 fungi (*Candida albicans* and *Candida glabrata*). The organisms included in the non-RVP exclusivity panel were selected either because they are related to RVP target organisms, are clinically relevant (colonize the upper respiratory tract or cause respiratory symptoms), are common skin flora or laboratory contaminants, or are microorganisms with a high prevalence of infection (Herpes Simplex virus, etc.).

Bacterial and fungal strains tested for cross-reactivity with the eSensor RVP were diluted in viral transport media to a titer of at least 10^6 CFU/mL. Nucleic acids of these organisms, listed below, were extracted in triplicate with the bioMérieux NucliSENS easyMAG system. Following extraction, each replicate was tested once using the eSensor RVP. A “not detected” result indicates all three replicates yielded the same result.

Organism	Source	Test Concentrations	Cross-Reactivity Results
<i>Acinetobacter baumannii</i>	Zeptomatrix 0801597	5.2x10 ⁶ CFU/mL	Not Detected
<i>Bordetella parapertussis</i>	Zeptomatrix 0801461	9.8x10 ⁶ CFU/mL	Not Detected
<i>Bordetella pertussis</i>	Zeptomatrix 0801459	5.8x10 ⁶ CFU/mL	Not Detected
<i>Burkholderia cepacia</i>	Zeptomatrix BacT-050	2.3x10 ⁶ CFU/mL	Not Detected
<i>Candida albicans</i>	Zeptomatrix 0801504	1x10 ⁶ CFU/mL	Not Detected
<i>Candida glabrata</i>	Zeptomatrix 0801535	9.73x10 ⁶ CFU/mL	Not Detected
<i>Chlamydomphila pneumoniae</i> DNA	ABI 08-942-250	1.4x10 ⁷ copies/mL	Not Detected
<i>Corynebacterium diphtheriae</i>	Zeptomatrix BacT-050	3.58x10 ⁶ CFU/mL	Not Detected
<i>Escherichia coli</i>	Zeptomatrix 0801624	1.5x10 ⁶ CFU/mL	Not Detected
<i>Haemophilus influenzae</i>	Zeptomatrix 0801680	2.6x10 ⁶ CFU/mL	Not Detected
<i>Klebsiella pneumoniae</i>	Zeptomatrix 0801506	1.07x10 ⁶ CFU/mL	Not Detected
<i>Lactobacillus acidophilus</i>	Zeptomatrix 0801540	2.12x10 ⁶ CFU/mL	Not Detected
<i>Lactobacillus planarum</i>	Zeptomatrix 0801507	1.75x10 ⁶ CFU/mL	Not Detected
<i>Legionella pneumophila</i>	Zeptomatrix 0801645	2.6x10 ⁶ CFU/mL	Not Detected
<i>Moraxella catarrhalis</i>	Zeptomatrix 0801509	3.9x10 ⁶ CFU/mL	Not Detected
<i>Mycobacterium tuberculosis</i>	Zeptomatrix 0801660	2.2x10 ⁶ CFU/mL	Not Detected
<i>Mycoplasma pneumoniae</i>	Zeptomatrix 0801579	2.47x10 ⁶ CCU/mL	Not Detected
<i>Neisseria meningitidis</i>	Zeptomatrix 0801511	3.37x10 ⁶ CFU/mL	Not Detected
<i>Neisseria sicca</i>	Zeptomatrix 0801754	3.37x10 ⁶ CFU/mL	Not Detected
<i>Porphyromonas gingivalis</i>	Zeptomatrix BacT-050	3.55x10 ⁶ CFU/mL	Not Detected
<i>Proteus vulgaris</i>	Zeptomatrix BacT-050	1.0x10 ⁶ CFU/mL	Not Detected
<i>Pseudomonas aeruginosa</i>	Zeptomatrix 0801519	1.05x10 ⁶ CFU/mL	Not Detected
<i>Serratia marcescens</i>	Zeptomatrix 0801723	6.1x10 ⁶ CFU/mL	Not Detected
<i>Staphylococcus aureus</i> (COL)	Zeptomatrix 0801638	8.4x10 ⁶ CFU/mL	Not Detected
<i>Staphylococcus aureus</i> (MSSA)	Zeptomatrix 0801675	1.2x10 ⁶ CFU/mL	Not Detected
<i>Staphylococcus epidermidis</i> (MSSE)	Zeptomatrix 0801689	2.2x10 ⁶ CFU/mL	Not Detected

Organism	Source	Test Concentrations	Cross-Reactivity Results
<i>Staphylococcus epidermidis</i> (MRSE)	Zeptomatrix 0801651	6.2x10 ⁶ CFU/mL	Not Detected
<i>Staphylococcus haemolyticus</i>	Zeptomatrix 0801591	2.16x10 ⁶ CFU/mL	Not Detected
<i>Streptococcus agalactiae</i>	Zeptomatrix 0801545	2.2x10 ⁶ CFU/mL	Not Detected
<i>Streptococcus dysgalactiae</i>	Zeptomatrix 0801516	6.46x10 ⁶ CFU/mL	Not Detected
<i>Streptococcus mitis</i>	Zeptomatrix 0801695	2.43x10 ⁶ CFU/mL	Not Detected
<i>Streptococcus pneumoniae</i>	Zeptomatrix 0801439	2.8x10 ⁶ CFU/mL	Not Detected
<i>Streptococcus pyogenes</i>	Zeptomatrix 0801512	1.55x10 ⁶ CFU/mL	Not Detected
<i>Streptococcus salivarius</i>	Zeptomatrix BacT-050	6.53x10 ⁶ CFU/mL	Not Detected

No positive test results were observed during testing of the bacterial or fungal species listed above at the tested concentrations.

g. Assay cut-off:

The eSensor RVP provides a qualitative result detecting the presence (Positive) or absence (Target Not Detected) of the viruses contained in the panel, along with the internal MS2 control, based upon underlying target signals and appropriate signal boundaries. Signals are generated from an electrode and are analyzed by electrochemical voltammetry with the units of nanoamperes (nA). Boundary settings were based upon the negative data as a reference point for viral target cutoffs and the internal control (MS2) data as a reference point for the control cutoffs. Each viral target can have signal from either of two measuring electrodes. If the target signal on at least one of the two measuring electrodes is greater than the assay cut-off level, then that target is considered positive.

To establish the cut-off values for the eSensor RVP targets, blank negative samples consisting of three different viral transport media (M4, M5, and UTM) were spiked with MS2 internal control and then extracted 24 times for each transport media, resulting in a total of 72 tests. The signals obtained from these blank negatives were then combined with results from negative electrodes of samples used in a comparator study during assay development. Negative electrode data from the comparator study were obtained from the negative electrodes of samples which were positive for another viral target. The number of data points obtained from this analysis varied between targets due to the differing number of samples which were positive for each viral target. The total data set included between 419 and 585 observations for each target. The mean and standard deviation were calculated for each target, and a cut-off value of 3 nA was established for all viral targets. This level was chosen to be the largest value after applying the formula [Signal Cut-off = Mean (Target Signal from Negative sample) + 5* Observed Std Dev (Target Signal)] was 3.02 nA (RSV A analyte).

For the MS2 control, the cut-off value was determined by analyzing the signals on MS2 electrodes from 68 samples which were tested with no MS2 and the 72 samples from the three transport media (MS2 positive) listed above. After applying the formula [Signal Cut-off = Mean (MS2 Signal from Negative sample) + 5* Observed Std Dev (MS2 Signal)] a 1 nA boundary was then established.

Analyte	Mean + 5 SD	Number of Samples Tested
ADV E	0.33	564
ADV B	1.22	581
Flu A	2.48	419
Flu A H1	0.28	559
Flu A H1N1	1.98	466
Flu A H3	2.32	550
Flu B	1.15	541
PIV 1	0.91	574
PIV 2	1.33	576
PIV 3	0.57	538
RSV A	3.02	498
RSV B	0.94	542

To verify the established cut-off values, signals from 200 archived patient samples positive by either DFA or RT-PCR were compared against the 3 nA cut-off for each viral target. Out of the 200 confirmed positive samples, 191 generated signals above 5 nA; the remaining 9 positives generated signals less than 1.5 nA. Therefore, an assay cut-off at 3 nA for the viral targets was chosen to maximize assay sensitivity and specificity. For the MS2 control, the 1 nA boundary was compared against MS2 signals from 200 archived patient samples, a 1 nA cut-off was chosen based on this data set.

h. Interfering substances

Potentially interfering substances were selected based on the fact that they could pre-exist in the specimen (e.g. blood, nasal secretions or mucus, and nasal and throat medications used to relieve congestion, nasal dryness, irritation, or asthma and allergy symptoms) as well as those that could be introduced during specimen collection and preparation. Each potentially interfering substance was tested individually with the exception of Luffa operculata, Galphimia glauca, Histaminum hydrochloricum, and Sulfur, which were tested together as Zicam® Allergy Relief Nasal spray and Oxymetazoline and Menthol, which were tested together as Afrin® No Drip Severe Congestion nasal spray, thereby bringing the total to 21 potentially interfering test combinations. Viral samples representative of the 14 viral targets on the eSensor RVP were obtained from commercially available cultured cell lines as indicated in Table 31. Seven viral mixes were made, each containing unique viral targets. Viral mixes were added to each potentially interfering substance resulting in a final testing concentration of 3X LoD for each analyte. Each was extracted in triplicate with each extract tested once with the eSensor RVP. Twenty-one (21) combinations of 24 potentially interfering substances were tested in this study. Additionally, nine potentially interfering

microorganisms (viral and bacterial) were also tested in the same manner as described above. The microorganisms and their testing concentrations are listed in Table 31. All substances and microorganisms tested for interference were shown to be compatible with the eSensor RVP. No potentially interfering substance or microorganism was shown to inhibit the eSensor RVP at all tested concentrations^a.

Potentially Interfering Substance	Active Ingredient	Substance Form	Tested Concentration
Sample Matrix	Control for no interfering substance	Liquid	N/A
Viral transport medium	Becton Dickinson VTM	Liquid	N/A
Blood (human)	Blood	Liquid	2% v/v
	Human gDNA	50 ng/rxn	50 ng/rxn
Throat lozenges, oral anesthetic and analgesic	Benzocaine	Dry	30% w/v
	Menthol*	Nasal Spray	1% v/v
Mucin: bovine submaxillary gland, type I-S	Purified mucin protein	Dry	1% w/v
Nasal sprays or drops	Phenylephrine (Neo-Synephrine)	Dry	1.5% v/v
	Oxymetazoline* (also contains Benzalkonium Chloride, Menthol, Eucalyptol, Camphor, benzyl alcohol and phosphate buffers)	Nasal Spray	1% v/v
	Sodium chloride	Dry	0.8% w/v
Antibacterial, systemic	Tobramycin	Dry	5% w/v
Antibiotic, nasal ointment	Mupirocin	Dry	2% w/v
Nasal corticosteroids	Beclomethasone	Dry	1.5% w/v
	Dexamethasone	Dry	1.5% w/v
	Flunisolide	Dry	1.5% w/v
	Triamcinolone	Dry	1.5% w/v
	Budesonide (Pulmicort)	Dry	1.5% w/v
	Fluticasone (Flonase [®])	Dry	3% w/v
Nasal gel	Luffa operculata**	Nasal Gel	1% v/v
	Sulfur**	Nasal Gel	1% v/v
Homeopathic allergy relief medicine	Galphimia glauca**	Nasal Gel	1% v/v
	Histaminum hydrochloricum**	Nasal Gel	1% v/v
FluMist ^{®#}	Live intranasal influenza virus vaccine [#]	Liquid	0.5%-1% v/v
Anti-viral drugs	Zanamivir (Relenza [®])	Dry	550 ng/ml
	Oseltamivir (Tamiflu [®])	Dry	142 ng/ml
Virus	Cytomegalovirus	Culture	1 x 10 ⁵
	Enterovirus 71	Culture	PFU/mL
Bacteria	Streptococcus pneumoniae	Culture	1 x 10 ⁶ CFU/mL
	Bordetella pertussis	Culture	
	Haemophilus influenza	Culture	
	Mycoplasma pneumoniae	Culture	
	Staphylococcus aureus	Culture	
	Neisseria meningitidis	Culture	
	Corynebacterium diphtheriae	Culture	

*Tested together (Afrin No Drip Severe Congestion nasal spray)

** Tested together (Zicam Allergy Relief)

[#] FluMist vaccine: Addition of FluMist Live Intranasal Influenza Vaccine to the transport media control resulted in positive calls for Flu A, Flu A H3, Flu A 2009

H1N1 and Flu B. This was due to the live attenuated influenza virus present in the vaccine.

^a Testing of FluMist at 1% (v/v) resulting in an inhibition in the detection of hMPV. FluMist did not inhibit the detection of hMPV when tested at 0.5% (v/v).

Raw data from the interfering substances study was compared with data from the control runs (VTM only) to generate the table below. The signal ratio is calculated by dividing the mean nA signal of samples with interfering substance by mean nA signal of control samples without interfering substance. The results of this calculation are summarized below:

Potentially Interfering Substance	Percent Interference							
	Flu A	Flu A H1	Flu A H3	Flu A 2009 H1N1	Flu B	RSVA	RSVB	HRV
Remel M5 (Control)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BD VTM	1.11	1.38	1.15	0.64	0.87	0.74	1.17	1.05
Blood gDNA	1.10	1.24	0.99	0.70	1.04	1.76	1.14	1.21
Blood	1.28	1.20	0.95	0.18	0.73	2.16	1.91	0.91
Benzocaine	1.26	1.19	1.13	0.33	0.86	1.98	1.62	0.81
Sodium Chloride	1.17	1.01	1.20	0.77	0.75	1.42	1.40	1.27
Mucin	1.30	0.83	1.07	0.90	0.86	0.80	1.39	1.27
Oxymetazoline-HCl (Afrin)	1.30	1.42	0.89	0.54	0.76	0.62	0.71	1.20
Tobramycin Sulfate salt	0.97	1.02	0.91	0.40	0.72	1.75	0.82	1.05
Mupirocin	1.38	1.33	1.12	0.61	0.88	0.62	2.06	1.03
Flunisolide	1.44	1.22	0.77	1.35	0.92	0.90	1.81	1.18
Dexamethasone	0.97	0.85	0.98	0.85	0.87	0.05	2.23	0.84
Triamcinolone Acetomide	1.25	1.40	1.03	1.08	0.95	1.32	2.13	1.08
(R)-(-)-Phenylephrine-HCl	1.33	1.08	1.00	0.91	0.87	1.52	2.36	1.23
Beclomethasone	1.16	1.23	1.05	0.67	0.87	0.84	2.37	1.11
Budesonide	1.10	1.18	1.01	0.53	0.83	1.37	2.30	0.74
Fluticasone propionate	1.28	1.19	0.96	0.79	0.84	2.27	2.11	1.15
Zicam	1.11	1.21	1.39	0.75	0.86	0.12	2.00	0.82
FluMist (1%)	1.25	1.31	1.99	3.98	1.14	0.17	0.97	0.57
Zanamivir (Relenza)	1.27	1.14	1.00	0.46	0.94	1.04	1.54	1.22
Oseltamivir (Tamiflu)	1.08	1.36	0.86	0.46	0.91	1.37	1.92	1.10

Potentially Interfering Substance	Percent Interference							
	HMPV	PIV 1	PIV2	PIV3	ADVC	ADVB	ADVE	ADVB/E*
Remel M5 (Control)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
BD VTM	1.07	1.51	0.97	0.88	0.96	1.01	1.03	1.03
Blood gDNA	0.80	1.72	0.86	0.64	0.93	0.83	0.96	0.90
Blood	0.70	1.21	0.71	0.39	1.27	0.85	0.90	0.88
Benzocaine	0.89	1.31	0.67	1.03	1.07	0.94	0.98	0.97
Sodium Chloride	0.76	1.66	0.80	0.80	1.11	1.04	0.84	0.93
Mucin	1.20	1.69	0.98	1.23	1.33	0.66	1.12	0.92
Oxymetazoline-HCl (Afrin)	0.87	1.79	0.87	1.43	1.37	0.85	0.81	0.83
Tobramycin Sulfate salt	1.02	1.19	0.74	0.66	1.20	1.05	0.89	0.96
Mupirocin	0.95	1.47	0.94	1.05	1.77	0.98	1.10	1.05
Flunisolide	0.95	1.19	0.75	1.04	1.31	0.94	0.75	0.83
Dexamethasone	1.05	1.28	0.74	1.00	1.76	0.93	0.78	0.84
Triamcinolone Acetomide	1.00	1.37	0.97	1.32	1.79	0.92	0.75	0.83
(R)-(-)-Phenylephrine-HCl	0.85	1.64	0.80	0.72	1.36	0.89	0.88	0.89
Beclomethasone	0.75	1.28	0.78	0.71	1.48	0.92	0.85	0.88
Budesonide	0.94	1.72	0.71	0.66	1.73	0.98	0.79	0.87
Fluticasone propionate	0.71	1.22	0.87	0.71	1.45	0.79	0.92	0.86
Zicam	0.83	1.52	1.08	0.76	1.49	0.86	1.11	1.00
FluMist (1%)	0.05	1.30	0.93	0.19	1.27	0.95	1.18	1.02
Zanamivir (Relenza)	0.85	1.44	1.06	0.99	1.48	0.90	1.17	1.05
Oseltamivir (Tamiflu)	0.68	1.58	0.93	1.11	1.47	1.01	0.83	0.91

An analytical interference study was carried out by testing RVP viral target mixes against a panel of potentially interfering microorganisms. Seven different RVP viral target mixes were used during the study. Each of the mixes contained two RVP panel members at a concentration of 3X LoD. All sample/substance mixtures were extracted in triplicate according to the package insert. Potentially interfering microorganisms and the concentrations tested are shown below:

Type	Potential Interfering Substance	Lot Number	Stock Concentration	Testing Concentration
Viral	Cytomegalovirus (CMV)	305811	4.17E+05	1 x 10 ⁵ pfu/ml
	Enterovirus 71	308126	7.24E+05	
Bacteria	Streptococcus pneumoniae	305705	2.80E+10	1 x 10 ⁶ cfu/ml
	Bordetella pertussis	305539	5.80E+09	
	Haemophilus influenza	305130	2.60E+06	
	Mycoplasma pneumoniae	306813	2.70E+07	
	Staphylococcus aureus	305344	1.20E+08	
	Neisseria meningitidis	306072	3.37E+08	
	Corynebacterium diphtheriae	305956	3.58E+09	

Each potential interfering substance was analyzed for detection interference between the substance and the added RVP viral target. No interfering effects were observed with regard to the call rate of the RVP viral targets, this data is shown below.

Microorganism Tested										
RVP Viral Target	Control (VTM)	Cytomegalovirus	Enterovirus 71	S. pneumoniae	B. pertussis	H. influenza	M. pneumoniae	S. aureus	N. meningitidis	C. diphtheriae
Flu A	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%
Flu A H1	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%
Flu A H3	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%
2009 H1N1	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%
Flu B	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%
RSV A	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%
RSV B	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%
PIV 1	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%
PIV 2	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%
PIV 3	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%
hMPV	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%
HRV	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%
ADV B	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%

Microorganism Tested										
RVP Viral Target	Control (VTM)	Cytomegalovirus	Enterovirus 71	S. pneumoniae	B. pertussis	H. influenza	M. pneumoniae	S. aureus	N. meningitidis	C. diptheriae
ADV C	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%
ADV E	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%	3/3, 100%

Raw data from the interfering microorganisms study was compared with data from the control runs (VTM only) to generate a table of percent interference for each microorganism and analyte combination. Percent interference is calculated by dividing the mean nA signal of samples with interfering substance by mean nA signal of control samples without interfering substance x 100. The results of the percent interference calculations are summarized below:

Microorganism Tested										
RVP Viral Target	Control (VTM)	Cytomegalovirus	Enterovirus 71	S. pneumoniae	B. pertussis	H. influenza	M. pneumoniae	S. aureus	N. meningitidis	C. diptheriae
Flu A	1.00	0.97	1.02	1.06	1.01	1.02	1.02	1.01	1.08	0.95
Flu A H1	1.00	1.09	1.08	1.01	1.15	1.09	1.17	1.20	1.02	1.01
Flu A H3	1.00	1.10	1.03	1.12	0.98	0.86	0.94	1.37	0.98	1.11
2009 H1N1	1.00	0.95	0.97	0.80	1.02	0.91	1.06	1.09	1.12	1.00
Flu B	1.00	1.07	1.02	1.04	0.96	1.07	1.07	1.06	0.85	1.09
RSV A	1.00	1.06	0.99	1.21	1.04	1.13	1.00	1.13	0.82	0.94
RSV B	1.00	0.92	1.02	0.98	0.86	0.94	0.90	0.95	0.83	0.98
PIV 1	1.00	0.98	0.94	1.00	1.03	0.90	0.95	0.92	0.94	0.91
PIV 2	1.00	1.02	0.92	1.01	0.92	0.97	0.93	0.79	0.97	1.04
PIV 3	1.00	0.88	0.95	0.94	1.01	0.89	0.74	0.96	0.95	0.99
hMPV	1.00	0.98	0.85	1.10	1.06	0.92	0.83	1.05	0.97	1.02
HRV	1.00	0.96	0.74	0.86	0.66	0.87	0.66	0.88	0.91	0.78
ADV B	1.00	0.98	0.94	1.02	1.05	1.02	0.74	1.02	1.06	1.06
ADV C	1.00	0.91	0.95	0.98	0.91	0.90	0.86	0.85	0.82	0.91
ADV E	1.00	0.90	0.90	0.90	1.00	1.10	1.01	0.96	0.96	0.99

Based on the analytical study data of potentially interfering substances and microorganism the following limitations have been added to the product labeling:

The effect of interfering substances has only been evaluated for those listed in the labeling. Interference by substances other than those described in the “Interference” section below can lead to erroneous results.

Recent administration of a live intranasal influenza virus vaccine may cause false positive results for Influenza A, H1, H3, 2009 H1N1, and/or Influenza B.

i. Carryover contamination

The carryover/cross-contamination study challenged the extraction, RT-PCR, and detection portions of the assay within and between runs and operators tested over the course of five testing days. A representative strain of Parainfluenza Virus 3 was obtained as a commercially available cultured cell line. Positive Parainfluenza Virus 3 samples were prepared at a concentration of 1.00×10^5 TCID₅₀/mL (3559x LoD) while negative samples were un-inoculated Remel M5 transport media. All samples were extracted using the bioMérieux easyMAG System. Five sets of alternating high concentration positive and negative samples were extracted and tested in a checkerboard pattern. Each set of samples contained 24 tests (12 positive and 12 negative). Total number of tests for the duration of the study was 120 samples (60 positive and 60 negative).

No carryover/cross-contamination was observed in the eSensor RVP, as 100% of the PIV 3 negative samples were reported as ‘Target Not Detected’.

2. Comparison studies:

a. Method comparison with predicate device:

N/A

b. Matrix comparison:

All analytical studies were performed using viral targets diluted in transport media. A bridging study was conducted to determine the effects of natural clinical matrix (NCM) on assay performance. Excess sample from the clinical study which tested negative for all panel members by the eSensor RVP constituted the NCM in this study. The bridging study compared assay performance of representative viral targets diluted in negative natural clinical matrix (NCM) compared to targets diluted in simulated matrix (SM). Panel members were tested by the eSensor RVP at 3x LoD in NCM and SM.

Positive rate for each target in the natural matrix is the same as the positive rate for the corresponding target in the simulated matrix control,

Sample Matrix	Flu A	Flu A H3	HMPV	ADV B/E	PIV 3	RSV A	IC
	POS Calls						
Natural Clinical Matrix (NCM)	5/5	5/5	5/5	5/5	5/5	5/5	25/25
Simulated Matrix (Control)	5/5	5/5	5/5	5/5	5/5	5/5	25/25

Sample Matrix	Flu A (n=5)				Flu A H3 (n=5)			
	Mean Signal (nA)	Std Dev	CV	% Signal	Mean Signal (nA)	Std Dev	CV	% Signal
Natural Clinical Matrix (NCM)	230.8	18.9	8.2	96.9	93.3	11.3	12.1	108.9
Simulated Matrix (Control)	238.3	17.9	7.5		85.7	26.8	31.3	

Sample Matrix	HMPV (n=5)				ADV B/E (n=5)			
	Mean Signal (nA)	Std Dev	CV	% Signal	Mean Signal (nA)	Std Dev	CV	% Signal
Natural Clinical Matrix (NCM)	76.0	12.0	15.7	81.6	98.9	9.7	9.9	100.5
Simulated Matrix (Control)	93.1	15.5	16.6		98.4	8.3	8.4	

Sample Matrix	RSV A (n=5)				PIV3 (n=5)			
	Mean Signal (nA)	Std Dev	CV	% Signal	Mean Signal (nA)	Std Dev	CV	% Signal
Natural Clinical Matrix (NCM)	167.3	9.7	5.8	94.0	136.0	33.5	24.6	93.9
Simulated Matrix (Control)	178.0	11.5	6.4		144.8	34.9	24.1	

Sample Matrix	MS2 IC (n=25)			
	Mean Signal (nA)	Std Dev	CV	% Signal
Natural Clinical Matrix (NCM)	200.0	38.0	19.0	96.5
Simulated Matrix (Control)	207.3	10.0	4.8	

An analysis of the raw data reveals that the p-values for the PIV 3, Flu A, ADV B and Flu A H3 datasets are not statistically different ($p > 0.05$). The hMPV and RSV A datasets are statistically different ($p < 0.05$) however there is no clinically significance difference (100% agreement of positive calls).

3. Clinical Studies:

Prospective Clinical Study

The study was designed to collect and test prospective nasopharyngeal samples from individuals representing the intended use population. The study was performed in two arms at a total of 3 external clinical study sites representing a geographic diversity within the United States. All clinical specimens in the prospective clinical study were nasopharyngeal (NP) swab specimens, prospectively collected and tested during the 2010/11 influenza season at three North American clinical laboratories. Clinical laboratories were located in Cleveland, Ohio; Providence, RI; and Albuquerque, NM. Demographic details for patient population are summarized in the table below. Study sites enrolled subjects from diverse demographic groups; about 40% of the specimens were obtained from patients enrolled at a hospital. The remaining specimens were collected from outpatients and patients in an emergency department.

A total of 1182 patient samples were collected prospectively across the three clinical sites from January 2011 until May 2011. Out of these patient samples, 1037 were evaluable. A total of 145 samples were excluded for the following reasons: samples not tested within 5 days of specimen collection (72/145), operator and/or easyMAG mechanical errors (62/145), samples not retested (11/145). Of the evaluable prospective clinical specimens, 93% (963/1037) yielded valid results on the first attempt. Invalid results or no results were obtained for the remaining 74 specimens (45 of which generated results on the first run, but required retesting due to a negative control failure caused by operator error). Data generated from the retests was used in the final analysis. All 74 specimens yielded valid results after a single retest when tested according the retest recommendations.

Out of the 1037 samples collected, an even split of patients were male and female. Approximately one quarter of the samples came from children under the age of 1; patients aged 21-65 contributed the largest share of the samples. A summary of the patient demographics is shown below:

General Demographic Data for Prospectively Collected Specimens (N=1037)

Demographic	Site 1	Site 2	Site 3	All Sites
	N = 245 (%)	N = 533 (%)	N = 259 (%)	N = 1037 (%)
SEX				
Male	105 (42.9)	296 (55.5)	117 (45.2)	518 (50.0)
Female	140 (57.1)	237 (44.5)	142 (54.8)	519 (50.0)

Demographic	Site 1	Site 2	Site 3	All Sites
	N = 245 (%)	N = 533 (%)	N = 259 (%)	N = 1037 (%)
AGE (yrs)				
0 – 1	46 (18.8)	197 (37.0)	27 (10.4)	270 (26.0)
> 1 – 5	20 (8.2)	94 (17.6)	22 (8.4)	136 (13.1)
> 5 – 21	19 (7.8)	82 (15.4)	26 (10.0)	127 (12.2)
> 21 – 65	97 (39.6)	106 (19.9)	130 (50.2)	333 (32.1)
> 65	63 (25.7)	54 (10.1)	54 (20.8)	171 (16.5)
SUBJECT STATUS				
Outpatient	7 (2.9)	219 (41.1)	90 (34.7)	316 (30.5)
Hospitalized	131 (53.5)	162 (30.4)	114 (44.0)	407 (39.2)
Emergency Department	107 (43.7)	152 (28.5)	55 (21.2)	314 (30.3)

The performance of the RVP assay was compared to the established gold standard reference method of viral culture for most viral targets. For respiratory viruses in which culture was not available, a composite (multi-test) reference method (a predetermined algorithm that combined the results of a few tests) was used as the comparator method. Composite methods were designed to target a different genomic region for each viral target than the one used by the RVP assay. The comparator methods were analytically validated prior to use in this study. As seen in the table below, viral culture followed by DFA identification testing was used as the comparator method for Influenza A, Influenza B, RSV, Parainfluenza Viruses (PIV1, PIV2, PIV3), and adenovirus. Since viral culture cannot determine the subtype for influenza A, RSVs, and adenoviruses, these viruses were subtyped by an independently developed qRT-PCR assay or qPCR assay followed by bidirectional sequencing to determine the subtypes (Influenza A H1, Influenza A H3, Influenza A 2009 H1N1, RSVA, RSVB, ADVB/E and ADCV). All molecular comparator and subtyping testing was performed on extracted nucleic acids. All clinical samples, regardless of the RVP or culture/DFA result, were sent for HMPV and HRV testing with two validated qRT-PCR assays for each viral target. If at least one of the assays was positive for the specific target, then the positive sample was sequenced using target specific primers.

Samples whose RVP result didn't match the culture/DFA result were sent for discordant testing. A third party performed DNA sequencing on the extracted nucleic acid samples supplied by GenMark using primer pairs for the specific viral target being tested. The results of this discordant testing were used to footnote the sensitivity and specificity tables.

Assays were developed to perform discordant testing for Flu B, PIV1, PIV2, and PIV3. Samples that required discordant testing for Flu B were tested in the comparator qRT-PCR assay for Flu B, and any positive samples were then sequenced using a target specific primer for Flu B. Similarly, any sample that required testing for PIV1, PIV2, or PIV3 were tested in the corresponding comparator qRT-PCR assay for that specific viral target and any positive samples were sequenced using a target specific primer for the specific Parainfluenza virus. A viral target was determined to be present in a specimen only if indicated by sequencing.

Virus (Analyte)	Comparator Method	Subtyping
Influenza A	Viral culture followed by DFA identification ¹	qRT-PCR + Bidirectional Sequencing
Influenza A H1		
Influenza A H3		
Influenza A 2009 H1N1		
RSV A		
RSV B		
Adenovirus B/E		
Adenovirus C		
Influenza B	Viral culture followed by DFA identification ²	N/A
PIV 1		
PIV 2		
PIV 3		
Human Metapneumovirus	2 qRT-PCR (2 methods) with Bidirectional Sequencing ³	N/A
Human Rhinovirus		

¹Validated performance of the eSensor RVP assay detecting Influenza A, RSV or ADV respectively was compared to viral culture followed by fluorescent antibody identification. “True” Influenza A, RSV or ADV positives respectively, were considered as any sample that tested positive for Influenza A, RSV or ADV respectively, by viral culture followed by DFA testing. True positive samples were subtyped using one analytically validated qRT-PCR assay with bi-directional sequence confirmation. The comparator assays were designed to amplify a different sequence from that amplified by the eSensor RVP assay(s). None of the comparator PCR assays overlapped any RVP amplicon sequence even if the same gene was targeted. “True” Influenza A H1, H3, or 2009 H1N1 positives, respectively, were considered as any sample that tested positive for Influenza A by viral culture, and had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched Influenza A/H1, A/H3, or A/2009 H1 sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), respectively, with acceptable E-values. “True” RSV A or RSV B positives, respectively, were considered as any sample that tested positive for Influenza A by viral culture, and had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched RSV A or RSV B sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), respectively, with acceptable E-values. “True” ADV C or ADV B/E positives, respectively, were considered as any sample that tested positive for Influenza A by viral culture, and had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched ADV C or ADV B/E sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), respectively, with acceptable E-values.

²Performance of the eSensor RVP assay detecting Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2 and Parainfluenza Virus 3 respectively was compared to viral

culture followed by fluorescent antibody identification. “True” Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2 or Parainfluenza Virus 3 positives, respectively, were considered as any sample that tested positive for Influenza B, Parainfluenza Virus 1, Parainfluenza Virus 2, or Parainfluenza Virus 3, respectively, by viral culture followed by DFA testing.

³Performance of the eSensor RVP assay detecting Human Rhinovirus or Human Metapneumovirus, respectively, was compared to a predetermined algorithm that used composite comparator methods. The methods consist of two analytically validated PCR assays followed by bi-directional sequencing. “True” Human Rhinovirus or Human Metapneumovirus positives, respectively, were considered as any sample that had bi-directional sequencing data meeting pre-defined quality acceptance criteria that matched Human Rhinovirus or Human Metapneumovirus sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank database (www.ncbi.nlm.nih.gov), respectively, with acceptable E-values.

Depending on the comparator method used for a particular virus, performance is described as sensitivity/specificity or Positive Percent Agreement (PPA)/Negative Percent Agreement (NPA). Sensitivity or positive percent agreement (PPA) was calculated by dividing the number of true positive (TP) results by the sum of true positive and false negative (FN) results, while specificity or negative percent agreement (NPV) was calculated by dividing the number of true negative (TN) results by the sum of true negative and false positive (FP) results. A TP result was one where the positive RVP result matched the positive reference/comparator result, while a TN result was one whereby a negative RVP result matched a negative reference/comparator result. The two-sided 95% confidence interval was also calculated. The detailed performance results from each viral target are summarized below.

eSensor RVP prospective clinical performance

Virus (Analyte)	Sensitivity			Specificity		
	TP/(TP+FN)	Percent	95% CI	TN/(TN+FP)	Percent	95% CI
Influenza A ¹	132/137 ^a	96.4%	91.7% - 98.8%	850/897 ^b	94.8%	93.1% - 96.1%
Influenza A H1*	0/0	NA	NA	1027/1027	100.0%	99.6% - 100.0%
Influenza A H3	74/74	100.0%	95.1% - 100.0%	927/952 ^c	97.4%	96.2% - 98.3%
Influenza A 2009 H1N1	49/49	100.0%	92.7% - 100.0%	956/971 ^d	98.5%	97.5% - 99.1%
Influenza B	64/69 ^e	92.8%	83.9% - 97.6%	947/965 ^f	98.1%	97.1% - 98.9%
Parainfluenza Virus 1*	4/4	100.0%	39.8% - 100.0%	1029/1030 ⁱ	99.9%	99.5% - 100.0%
Parainfluenza Virus 2*	5/6 ^j	83.3%	35.9% - 99.6%	1026/1028 ^k	99.8%	99.3% - 100.0%
Parainfluenza Virus 3	64/68 ^l	94.1%	85.6% - 98.4%	944/966 ^m	97.7%	96.6% - 98.6%
Respiratory Syncytial Virus A	68/68	100.0%	94.7% - 100.0%	905/956 ⁿ	94.7%	93.1% - 96.0%
Respiratory Syncytial Virus B	28/28	100.0%	87.7% - 100.0%	955/996 ^o	95.9%	94.5% - 97.0%
Adenovirus B/E*	13/13	100.0%	75.3% - 100.0%	1012/1021 ^p	99.1%	98.3% - 99.5%
Adenovirus C*	6/6	100.0%	54.1% - 100.0%	993/1028 ^q	96.6%	95.3% - 97.5%

Virus (Analyte)	PPA			NPA		
	TP/(TP+FN)	Percent	95% CI	TN/(TN+FP)	Percent	95% CI
Human Metapneumovirus	55/55	100.0%	93.5% - 100.0%	979/981 ^g	99.8%	99.3% - 100.0%
Human Rhinovirus	132/148	89.2%	83.0% - 93.7%	853/888 ^h	96.1%	94.6% - 97.3%

*These viral targets were supplemented with retrospective samples as shown below.

¹ Influenza A results contain 14 Flu A samples without a positive subtype and 123 samples with either Influenza A H3 or 2009 H1N1 positive results.

^a Flu A was not detected in all 5 RVP False Negative samples using independently developed and validated qPCR assays.

^b Flu A viruses were confirmed positive in 35/47 RVP False Positive samples using bidirectional sequencing.

^c Flu A H3 viruses were confirmed positive in 22/25 RVP False Positive samples using bidirectional sequencing.

^d Flu A 2009 H1N1 viruses were confirmed positive in 14/15 RVP False Positive samples using bidirectional sequencing.

^e Flu B was not detected in 4/5 RVP False Negative samples using bidirectional sequencing.

^f Flu B was confirmed positive in 11/18 RVP False Positive samples using bidirectional sequencing.

^g hMPV was confirmed positive in 1/2 RVP False Positive samples using bidirectional sequencing.

^h HRV was confirmed positive in 7/35 RVP False Positive samples using bidirectional sequencing.

ⁱ PIV 1 was not detected in this RVP False Positive sample by bidirectional sequencing.

^j PIV 2 was not detected in this RVP False Negative sample using independently developed and validated qPCR assays.

^k PIV 2 virus was confirmed positive in 0/2 RVP False Positive samples by bidirectional sequencing.

^l PIV 3 was not detected in 4/4 RVP False Negative samples using independently developed and validated qPCR assays.

^m PIV 3 virus was confirmed positive in 10/22 RVP False Positive samples using bidirectional sequencing.

ⁿ RSV A were confirmed positive in 43/51 RVP False Positive samples using bidirectional sequencing.

^o RSV B was confirmed positive in 35/41 RVP False Positive samples using bidirectional sequencing.

^p ADV B/E was confirmed positive in 8/9 RVP False Positive samples using bidirectional sequencing.

^q ADV C was confirmed positive in 16/35 False Positive samples using bidirectional sequencing.

Mixed Infections

The eSensor RVP system detected a total of 128 mixed infections in the prospective clinical evaluation (1037 tested and analyzed specimens). This represents 18.4% of the total positive specimens (128/696). One hundred fourteen (114/128; 89.1%) were double infections, eleven (11/128; 8.6%) were triple infections, and three (3/128; 2.3%) samples with four or more RVP analytes were identified. Ninety five of the 128 samples contained one or more analytes that the reference/comparator method failed to detect.

Distinct Co-infection Combinations Detected by eSensor RVP					Total Number of Co-infections	Number of Discrepant Co- infections	Discrepant Analyte(s)*
Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5			
ADV B/E	Flu B				2	2	ADV B (2), Flu B (1)
ADV B/E	HRV				2	0	
ADV B/E	PIV3				3	3	ADV B (3)
ADV B/E	RSV A				2	2	ADV B (1), RSV A (2)
ADV B/E	RSV B				1	1	RSV B (1)
ADV B/E	HMPV	HRV	RSV A	RSV B	1	1	RSV A (1), RSV B (1)
ADV C	Flu B				1	1	ADV C (1)
ADV C	HMPV				3	3	ADV C (3)
ADV C	HRV				6	4	ADV C (4), HRV (1)
ADV C	PIV3				1	1	ADV C (1)
ADV C	RSV A				4	4	ADV C (3), RSV A (2) ⁱ
ADV C	RSV B				3	3	ADV C (3), RSV B (2)
ADV C	HRV	PIV3			1	1	ADV C (1)
ADV C	HRV	RSV A			1	0	
Flu A	ADV B/E				1	1	Flu A
Flu A	ADV C				6	6	ADV C (6)
Flu A	Flu B				2	2	Flu A (2), HRV (1)
Flu A	HMPV				2	2	H1N1 (1), H3 (1), HMPV (1)
Flu A	HRV				4	2	H1N1 (1), HRV (2)
Flu A	PIV2				1	1	PIV2 (1)
Flu A	PIV3				2	2	Flu A (1), PIV3 (2)
Flu A	RSV A				1	1	RSV A (1)
Flu A	RSV B				2	2	RSV B (2)
Flu A	HRV	PIV3			2	1	H1N1 (1)
Flu A	RSV A	RSV B			2	2	RSV A (2), RSV B (2)
Flu A	ADV C	HRV	RSV A		1	1	ADV C (1), HRV (1)
Flu A	ADV C	HRV	PIV3		1	1	ADV C (1), Flu A (1), PIV3 (1)
Flu B	HRV				4	2	Flu B (1), HRV (1)
Flu B	PIV3				3	3	Flu B (2), PIV3 (2)
Flu B	RSV A				5	5	Flu B (2), RSV A (5)
Flu B	RSV B				1	1	RSV B (1)
Flu B	HRV	PIV2			1	1	HRV (1), PIV2 (1)
Flu B	HRV	RSV A			2	1	RSV A (1) ⁱ
HMPV	HRV				5	1	HMPV (1)
HMPV	PIV3				1	0	
HMPV	RSV B				1	1	RSV B (1)

Distinct Co-infection Combinations Detected by eSensor RVP					Total Number of Co-infections	Number of Discrepant Co-infections	Discrepant Analyte(s)*
Analyte 1	Analyte 2	Analyte 3	Analyte 4	Analyte 5			
HRV	PIV1				2	1	PIV1 (1)
HRV	PIV2				1	1	HRV (1)
HRV	PIV3				11	4	HRV (4), PIV3 (2)
HRV	RSV A				16	9	HRV (5), RSV A (6)
HRV	RSV B				8	6	HRV (1), RSV B (5)
HRV	PIV3	RSV A			1	1	RSV A (1)
HRV	PIV3	RSV B			1	1	RSV B (1)
PIV3	RSV A				6	6	PIV3 (4), RSV A (5)
PIV3	RSV B				1	1	PIV3 (1), RSV B (1)
Total Number of Co-infections					128	95	117/278 ¹
Total Number of Double Infections					114	85	99/232
Total Number of Triple Infections					11	8	11/33
Total Number of Quadruple Infections					2	2	5/8
Total Number of Quintuple Infections					1	1	2/5

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

¹117/117 discrepant analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 58/117 cases.

^a6/6 discrepant ADV B/E analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 5/6 cases

^b24/24 discrepant ADV C analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 11/24 cases

^c6/6 discrepant Flu B analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 3/6 cases

^d4/4 discrepant Flu A 2009 H1N1 analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 4/4 cases

^e1/1 discrepant Flu A H3 analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 1/1 cases

^f2/2 discrepant HMPV analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 1/2 cases

^g19/19 discrepant HRV analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 3/19 cases

^h12/12 discrepant PIV3 analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 3/12 cases

ⁱ27/27 discrepant RSV A analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 17/27 cases

^j17/17 discrepant RSV B analytes were investigated using an alternate method; bi-directional sequence analysis identified the analyte in question in 11/17 cases

During the prospective clinical trial, there were a number of co-infection combinations which were detected by the reference/comparator methods but not detected by the eSensor RVP assay. These co-infections are summarized below:

Distinct Co-Infection Combinations*		Total Number of Co-Infections	Number of Discrepant Co-infections	Discrepant Analyte(s)
Analyte 1	Analyte 2			
Flu B	HRV	6	3	Flu B (2), HRV (3)
Flu B	RSV B	1	1	Flu B (1), RSV B (1)
HRV	PIV3	13	3	HRV (3), PIV3 (3)

*This table includes only co-infections that were detected by the reference/comparator method but not by RVP; the remaining co-infections detected by the reference/comparator method are already represented in Table above.

Retrospective Clinical Study

Banked samples previously characterized as positive for Influenza A H1, Parainfluenza Virus 1, Parainfluenza Virus 2, Adenovirus B/E, and Adenovirus C were used to supplement the performance studies for these analytes. These frozen banked samples were collected from various sites across the United States or from the Centers for Disease Control and Prevention (CDC). Upon arrival at GenMark, banked samples were blinded and intermixed with negative samples before being sent for testing, which was conducted by multiple sites involved in the prospective analysis of the patient samples. Testing of the banked samples was performed identically to prospectively-collected patient specimens. Results from the banked samples are presented separately from the prospectively collected specimens.

A total of 343 retrospective banked samples were collected for analysis. Out of this sample set, 11 samples were sent which didn't contain a banked viral target so these eleven samples were not tested further. Eight additional samples were excluded as they didn't contain a banked viral target as originally reported by the collection site and confirmed by comparator testing. Two samples reported errors on targets but were not retested as indicated. One sample was not sequenced. One sample had an internal control failure but was not retested as indicated. After these data were excluded, a total of 320 banked samples (including negative samples) for 5 viral targets were tested and analyzed.

With the exception of Flu A H1 samples, these banked samples were also sent to a third party lab for comparator testing, and the results from the third party lab testing were compared to the results obtained by the eSensor RVP. Since the Flu A H1 samples came from the Centers for Disease Control and Prevention and were verified to be Flu A H1, these samples were not tested further. The individual analyte results are presented below.

Influenza A H1 (Banked Samples)			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	29	0	29
Negative	1	290	291
Total	30	290	320
Positive Percent Agreement: 96.7% (95% CI: 82.8% - 99.9%)			
Negative Percent Agreement: 100.0% (95% CI: 98.7% - 100.0%)			

PIV1 (Banked Samples)			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	25	6	31
Negative	0	289	289
Total	25	295	320
Positive Percent Agreement: 100.0% (95% CI: 86.3% - 100.0%)			
Negative Percent Agreement: 98.0% (95% CI: 95.6% - 99.3%)			

PIV2 (Banked Samples)			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	26	10	36
Negative	0	284	284
Total	26	294	320
Positive Percent Agreement: 100.0% (95% CI: 86.8% - 100.0%)			
Negative Percent Agreement: 96.6% (95% CI: 93.8% - 98.4%)			

ADV B/E (Banked Samples)			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	25	5	30
Negative	0	290	290
Total	25	295	320
Positive Percent Agreement: 100.0% (95% CI: 86.3% - 100.0%)			
Negative Percent Agreement: 98.3% (95% CI: 96.1% - 99.4%)			

ADV C (Banked Samples)			
eSensor RVP	Reference		
	Positive	Negative	Total
Positive	16	34	50
Negative	0	270	270
Total	16	304	320
Positive Percent Agreement: 100.0% (95% CI: 80.6% - 100.0%)			
Negative Percent Agreement: 88.8% (95% CI: 84.8% - 91.9%)			

A summary of the individual performance tables is shown below:

Virus	Positive Percent Agreement			Negative Percent Agreement		
	TP/(TP+FN)	%	95% CI	TN/(TN+FP)	%	95% CI
Influenza A H1	29/30	96.7%	82.8% - 99.9%	290/290	100%	98.7% - 100.0%
Parainfluenza Virus 1	25/25	100.0%	86.3% - 100.0%	289/295	98.0%	95.6% - 99.3%
Parainfluenza Virus 2	26/26	100.0%	86.8% - 100.0%	284/294	96.6%	93.8% - 98.4%
Adenovirus B/E	25/25	100.0%	86.3% - 100.0%	290/295	98.3%	96.1% - 99.4%
Adenovirus C	16/16	100.0%	80.6% - 100.0%	270/304	88.8%	84.8% - 91.9%

4. Clinical cut-off: N/A

5. Expected values/Reference range:

A prospective clinical study testing nasopharyngeal (NP) swab specimens was conducted during the 2010/11 influenza season at three North American clinical laboratories. The expected values of individual analytes and mixed co-infections based on eSensor RVP prospective sample testing results are summarized below:

Virus (Analyte)	Age 0-1 (N = 270)	Age >1-5 (N = 136)	Age >5-21 (N = 127)	Age >21-65 (N = 333)	Age >65 (N = 171)	All Ages (N = 1037)
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Influenza A (Un-Subtypable)	2 (0.7)	0 (0.0)	2 (1.6)	5 (1.5)	1 (0.6)	10 (1.0)
Influenza A (Total)	25 (9.3)	22 (16.2)	17 (13.4)	84 (25.2)	31 (18.1)	179 (17.3)
Influenza A H3	12 (4.8)	15 (11.0)	7 (5.5)	43 (12.9)	22(12.9)	99(9.5)

Virus (Analyte)	Age 0-1 (N = 270)	Age >1-5 (N = 136)	Age >5-21 (N = 127)	Age >21-65 (N = 333)	Age >65 (N = 171)	All Ages (N = 1037)
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Influenza A 2009 H1N1	10 (3.7)	8 (5.9)	6 (4.7)	33 (9.9)	7 (4.1)	64 (6.2)
Influenza B	10 (3.7)	17 (12.5)	33 (26.0)	15 (4.5)	7 (4.1)	82 (7.9)
Human Metapneumovirus	18 (6.7)	11 (8.1)	3 (2.4)	15 (4.5)	10 (5.9)	57 (5.5)
Human Rhinovirus	82 (30.4)	27 (19.9)	21 (16.6)	26 (7.8)	11 (6.4)	167 (16.1)
Parainfluenza Virus 1	3 (1.1)	0 (0.0)	1 (0.8)	0 (0.0)	1 (0.6)	5 (0.5)
Parainfluenza Virus 2	1 (0.4)	1 (0.7)	0 (0.0)	3 (0.9)	2 (1.2)	7 (0.7)
Parainfluenza Virus 3	43 (15.9)	15 (11.0)	5 (3.9)	18 (5.4)	5 (2.9)	86 (8.3)
Respiratory Syncytial Virus A	69 (25.6)	21 (15.4)	8 (6.3)	11(3.3)	10 (5.8)	119 (11.4)
Respiratory Syncytial Virus B	28 (10.4)	17 (12.5)	4 (3.2)	14 (4.2)	6 (3.5)	69 (6.7)
Adenovirus B/E	6 (2.2)	8 (5.9)	3 (1.6)	5 (1.8)	0 (0.0)	22 (2.1)
Adenovirus C	21 (7.7)	4 (2.9)	1 (0.8)	9 (2.7)	6 (3.5)	41 (3.9)

Below is the summary of expected values (as determined by eSensor RVP) by site during the prospective clinical sample testing.

Virus (Analyte)	Site 1 (N = 245)	Site 3 (N = 533)	Site 4 (N = 259)	All Sites (N = 1037)
	N (%)	N (%)	N (%)	N (%)
Influenza A (Un-Subtypable)	0 (0.0)	8 (1.5)	2 (0.8)	10 (1.0)
Influenza A (Total)	58 (23.7)	89 (16.7)	32 (12.4)	179 (17.3)
Influenza A H3	32 (13.1)	54 (10.1)	13 (5.0)	99 (9.5)
Influenza A 2009 H1N1	19 (7.8)	28 (5.4)	17 (6.6)	64 (6.2)
Influenza B	4 (1.6)	59 (11.1)	19 (7.3)	82 (7.9)
Human Metapneumovirus	23 (9.4)	25 (4.7)	9 (3.5)	57 (5.5)
Human Rhinovirus	44 (18.0)	99 (18.6)	24 (9.3)	167 (16.1)
Parainfluenza Virus 1	0 (0.0)	4 (0.8)	1 (0.4)	5 (0.5)
Parainfluenza Virus 2	1 (0.4)	6 (1.1)	0 (0.0)	7 (0.7)
Parainfluenza Virus 3	3 (1.2)	68 (12.8)	15 (5.8)	86 (8.3)
Respiratory Syncytial Virus A	17 (6.9)	85 (15.9)	17 (6.6)	119 (11.4)
Respiratory Syncytial Virus B	15 (6.1)	41 (7.7)	13 (5.0)	69 (6.7)
Adenovirus B/E	0 (0.0)	14 (2.6)	8 (3.1)	22 (2.1)
Adenovirus C	16 (6.5)	19 (3.6)	6 (2.3)	41 (3.9)

Below is the summary of expected values (as determined by eSensor RVP) by age group during the prospective clinical sample testing.

Co-Infection	Age 0-1 (N = 270)	Age >1-5 (N = 136)	Age >5- 21 (N = 127)	Age >21- 65 (N = 333)	Age >65 (N = 171)	All Ages (N = 1037)
	N	N	N	N	N	N (%)
ADV B/E + Flu B	0	0	0	2	0	2 (0.2)
ADV B/E + HRV	0	2	0	0	0	2 (0.2)
ADV B/E + PIV3	3	0	0	0	0	3 (0.3)
ADV B/E + RSV A	1	1	0	0	0	2 (0.2)
ADV B/E + RSV B	0	1	0	0	0	1 (0.1)
ADV B/E + HMPV + HRV + RSV A + RSV B	1	0	0	0	0	1 (0.1)
ADV C + Flu B	1	0	0	0	0	1 (0.1)
ADV C + HMPV	3	0	0	0	0	3 (0.3)
ADV C + HRV	3	1	0	1	1	6 (0.6)
ADV C + PIV3	0	0	0	1	0	1 (0.1)
ADV C + RSV A	2	2	0	0	0	4 (0.4)
ADV C + RSV B	1	0	0	1	1	3 (0.3)
ADV C + HRV + PIV3	1	0	0	0	0	1 (0.1)
ADV C + HRV + RSV A	1	0	0	0	0	1 (0.1)
Flu A + ADV B/E	0	0	1	0	0	1 (0.1)
Flu A + ADV C	1	1	0	2	2	6 (0.6)
Flu A + Flu B	0	0	1	1	0	2 (0.2)
Flu A + HMPV	0	0	0	1	1	2 (0.2)
Flu A + HRV	3	0	0	0	1	4 (0.4)
Flu A + PIV2	0	0	0	0	1	1 (0.1)
Flu A + PIV3	2	0	0	0	0	2 (0.2)
Flu A + RSV A	1	0	0	0	0	1 (0.1)
Flu A + RSV B	0	1	0	1	0	2 (0.2)
Flu A + HRV + PIV3	2	0	0	0	0	2 (0.2)
Flu A + RSV A + RSV B	2	0	0	0	0	2 (0.2)
Flu A + ADV C + HRV + RSV A	1	0	0	0	0	1 (0.1)
Flu A + ADV C + HRV + PIV3	1	0	0	0	0	1 (0.1)
Flu B + HRV	1	0	1	1	1	4 (0.4)
Flu B + PIV3	0	2	0	0	1	3 (0.3)
Flu B + RSV A	2	0	2	0	1	5 (0.5)
Flu B + RSV B	0	1	0	0	0	1 (0.1)
Flu B + HRV + PIV2	0	1	0	0	0	1 (0.1)
Flu B + HRV + RSV A	2	0	0	0	0	2 (0.2)
HMPV + HRV	4	1	0	0	0	5 (0.5)
HMPV + PIV3	0	0	0	1	0	1 (0.1)
HMPV + RSV B	0	0	0	1	0	1 (0.1)
HRV + PIV1	2	0	0	0	0	2 (0.2)
HRV + PIV2	1	0	0	0	0	1 (0.1)
HRV + PIV3	9	0	1	1	0	11 (1.1)
HRV + RSV A	11	3	1	1	0	16 (1.6)
HRV + RSV B	6	2	0	0	0	8 (0.8)
HRV + PIV3 + RSV A	1	0	0	0	0	1 (0.1)
HRV + PIV3 + RSV B	0	1	0	0	0	1 (0.1)
PIV3 + RSV A	1	3	0	2	0	6 (0.6)
PIV3 + RSV B	0	0	0	1	0	1 (0.1)

Below is the summary of expected values for co-infection (as determined by eSensor RVP) by site during the prospective clinical sample testing.

Co-Infection	Site 1 (N = 245)	Site 2 (N = 533)	Site 3 (N = 259)	All Sites (N = 1037)
	N	N	N	N (%)
ADV B/E + Flu B	0	0	2	2 (0.2)
ADV B/E + HRV	0	2	0	2 (0.2)
ADV B/E + PIV3	0	2	1	3 (0.3)
ADV B/E + RSV A	0	1	1	2 (0.2)
ADV B/E + RSV B	0	1	0	1 (0.1)
ADV B/E + HMPV + HRV + RSV A + RSV B	0	0	1	1 (0.1)
ADV C + Flu B	0	1	0	1 (0.1)
ADV C + HMPV	1	2	0	3 (0.3)
ADV C + HRV	2	3	1	6 (0.6)
ADV C + PIV3	0	1	0	1 (0.1)
ADV C + RSV A	1	3	0	4 (0.4)
ADV C + RSV B	3	0	0	3 (0.3)
ADV C + HRV + PIV3	0	1	0	1 (0.1)
ADV C + HRV + RSV A	0	1	0	1 (0.1)
Flu A + ADV B/E	0	1	0	1 (0.1)
Flu A + ADV C	3	2	1	6 (0.6)
Flu A + Flu B	0	1	1	2 (0.2)
Flu A + HMPV	1	0	1	2 (0.2)
Flu A + HRV	2	2	0	4 (0.4)
Flu A + PIV2	1	0	0	1 (0.1)
Flu A + PIV3	0	2	0	2 (0.2)
Flu A + RSV A	0	1	0	1 (0.1)
Flu A + RSV B	0	1	1	2 (0.2)
Flu A + HRV + PIV3	0	2	0	2 (0.2)
Flu A + RSV A + RSV B	0	2	0	2 (0.2)
Flu A + ADV C + HRV + RSV A	0	1	0	1 (0.1)
Flu A + ADV C + HRV + PIV3	0	1	0	1 (0.1)
Flu B + HRV	1	3	0	4 (0.4)
Flu B + PIV3	0	2	1	3 (0.3)
Flu B + RSV A	0	2	3	5 (0.5)
Flu B + RSV B	0	1	0	1 (0.1)
Flu B + HRV + PIV2	0	1	0	1 (0.1)
Flu B + HRV + RSV A	0	2	0	2 (0.2)
HMPV + HRV	2	2	1	5 (0.5)
HMPV + PIV3	0	0	1	1 (0.1)
HMPV + RSV B	1	0	0	1 (0.1)
HRV + PIV1	0	2	0	2 (0.2)
HRV + PIV2	0	1	0	1 (0.1)
HRV + PIV3	0	11	0	11 (1.1)
HRV + RSV A	3	12	1	16 (1.6)
HRV + RSV B	1	6	1	8 (0.8)
HRV + PIV3 + RSV A	0	1	0	1 (0.1)
HRV + PIV3 + RSV B	0	1	0	1 (0.1)

Co-Infection	Site 1 (N = 245)	Site 2 (N = 533)	Site 3 (N = 259)	All Sites (N = 1037)
	N	N	N	N (%)
PIV3 + RSV A	2	4	0	6 (0.6)
PIV3 + RSV B	0	0	1	1 (0.1)

N. Instrument Name:

eSensor Respiratory Viral Panel (RVP)

O. System Descriptions:

1. Modes of Operation:

The eSensor XT-8 is a clinical multiplex instrument that has a modular design consisting of a base module and one, two, or three cartridge-processing towers containing 8, 16, or 24 cartridge slots, respectively. The cartridge slots operate independently of each other. Any number of cartridges can be loaded at one time, and the remaining slots are available for use while the instrument is running.

The base module controls each processing tower, provides power, and stores and analyzes data. The base module includes the user interface, and a 15 inch portrait-orientation display and touch panel. The instrument is designed to be operated solely with the touch screen interface. Entering patient accession numbers and reagent lot codes can be performed by the bar code scanner, the touch screen, or uploading a text file from a USB memory stick.

Each processing tower consists of eight cartridge modules, each containing a cartridge connector, a precision-controlled heater, an air pump, and electronics. The air pumps drive the diaphragm pump and valve system in the cartridge, eliminating fluid contact between the instrument and the cartridge. The pneumatic pumping enables recirculation of the hybridization solution allowing the target DNA and the signal probes to hybridize with the complementary capture probes on the electrodes. The diaphragm pump in the cartridge is connected to a pneumatic source from the eSensor XT-8 instrument and provides unidirectional pumping of the hybridization mixture through the microfluidic channel during hybridization. Using microfluidic technology to circulate the hybridization solution minimizes the unstirred boundary layer at the electrode surface and continuously replenishes the volume above the electrode that has been depleted of complementary targets and signal probes.

The XT-8 instrument provides electrochemical detection of bound signal probes by ACV and subsequent data analysis and test report generating functions. All hybridization, ACV scanning and analysis parameters are defined by a scanning protocol loaded into the XT-8 Software, and then specified for use by the EEPROM on each cartridge

2. Software:

FDA has reviewed applicant's Hazard Analysis and software development processes for this

line of product types:

Yes or No

Level of Concern:

Moderate

Software Description:

The eSensor XT-8 system software consists of instrument software and assay specific software called “Assay Analysis Module” or “AAM”. The instrument software consists of: 1) Application Software, which is the user interface; 2) Instrument Firmware; 3) Windows XP Operating System; 4) Printer Software; and 5) On-line User Manual for the instrument. The Application software was developed using C# in the Microsoft Visual Studio .NET 2.0 development framework. All of the hardware functions of the instrument are controlled by embedded firmware under the command of the Application Software. The embedded firmware was developed using the C language with the Greenhills compiler for the CPU firmware and Texas Instrument Code Composer for the DSP firmware.

The AAM is a software plug-in specifically designed to generate detection, contamination, and configuration reports for specific Assays. AAM consists of four sub-components: 1) AAM Instrument Application Interface, 2) AAM Data Analysis Module, 3) AAM Assay Specific Report Module, and 4) AAM Assay Specific Configuration Files. The AAM Instrument Application Interface is used to interface the AAM with XT-8 Application Software. The AAM Data Analysis Module performs signal classification, signal error detection, and statistics. The AAM Assay Specific Report Module performs genotyping, while the Configuration Files are used to specify configurable parameters for the assay specific polymorphisms. Among all the software components, only the AAM Assay Specific Report Module and the Configuration Files are assay specific to Respiratory Viral Panel Test. By keeping these assay-specific AAMs separate from the other modules, they are individually validated and installed on an instrument without having to revalidate the rest of the software such as Instrument software and two AAM sub-components (i.e., AAM Instrument Application Interface and AAM Data Analysis Module), which have been cleared previously by the FDA in K073720 and K090901.

FDA has reviewed the hazard analysis and software documentation for the cleared XT-8 system under 510(k) K073720. The XT-8 Instrument Software (including Application Software, Instrument Firmware, Window XP OS, Printer Software, and On-line User Manual for the instrument) and two AAM sub-components (i.e., AAM Instrument Application Interface and AAM Data Analysis Module) have not been changed in this 510(k). The only changes are to the two “assay specific” modules, AAM Assay Specific Report Module and AAM Assay Specific Configuration Files. The validation study results are provided in Section 7.0. The data provided in Section 7.0 also include a description of software design, evaluation of the hazard analysis and AAM validation testing.

The XT-8 instrument is only for use with XT-8 test cartridges and is not integrated or connected with other laboratory systems.

Device Hazard Analysis:

Acceptable as reviewed in document RVP-21

Architecture Design Chart:

Acceptable as reviewed in document REC-1004

Software Design Specification:

Acceptable as reviewed in document REC-1004

Traceability Analysis:

Acceptable as reviewed in document REC-1527 section 6

Software Development Environment Description:

Acceptable as reviewed in document REC-1004

Verification and Validation Testing:

Acceptable as reviewed in document REC-1527

Revision Level History:

Acceptable as reviewed in document REC-1527 (RVP IVD Version 1.0)

Unresolved Anomalies:

Acceptable as reviewed in document REC-1527 section 3.2

Off the Shelf Software or Software of Unknown Pedigree:

None reported.

3. Specimen Identification:

Specimens are manually identified and sample ID's are entered into the system software.

4. Specimen Sampling and Handling:

The specimen type is a Nasopharyngeal Swab (NPS) Collection. The NPS specimen collection should be performed according to standard technique and placed in viral transport media.

Minimum Sample Volume - 200 µL NPS specimen is required for testing.

Transport and Storage - Clinical specimens can be stored between 2 °C and 8 °C for up to 7 days after collection in viral transport media. Specimens can also be stored at <-15 °C for up to 1 month prior to extraction and undergo 2 freeze thaw cycles.

Note: Storage of purified nucleic acids have been validated for storage between 2 °C and 8 °C for up to 7 days and at <-15 °C for up to 1 month with up to 2 freeze thaws.

5. Calibration:

No routine calibration or user maintenance is required.

6. Quality Control:

See section M.c for a discussion of the quality control materials

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:

None

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.