

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k063765

B. Purpose for Submission:

New device

C. Measurand:

Respiratory specimen virus nucleic acid (RNA or DNA) target sequences. Viruses targeted have been associated with respiratory infections in adults and/or children. Viral types and subtypes detected:

Influenza A, Influenza A H1, Influenza A H3, Influenza B, Respiratory Syncytial Virus Type A, Respiratory Syncytial Virus Type B, Parainfluenza virus 1, Parainfluenza virus 2, Parainfluenza virus 3, Human Metapneumovirus, Rhinovirus, Adenovirus.

D. Type of Test:

Multiplex nucleic acid assay, qualitative determination of 12 respiratory virus type and subtype target sequences in nasopharyngeal swabs using nucleic acid isolation, amplification and detection on the Luminex[®] xMAP instrument, which generates signals based on the acquisition of spectrofluorometric data.

E. Applicant:

Luminex Molecular Diagnostics Inc.

F. Proprietary and Established Names:

xTAG[™] RVP (Respiratory Viral Panel)

Common Name: Respiratory Viral Panel (RVP) Multiplex Nucleic Acid Detection Assay

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3980, Respiratory viral panel multiplex nucleic acid assay

2. Classification:

Class II

3. Product code:

OCC, OEM, OEP

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use(s):

The xTAG[™] Respiratory Viral Panel (RVP) is a qualitative nucleic acid multiplex test intended for the simultaneous detection and identification of multiple respiratory virus nucleic acids in nasopharyngeal swabs from individuals suspected of respiratory tract infections. The following virus types and subtypes are identified using RVP: Influenza A, Influenza A subtype H1, Influenza A subtype H3, Influenza B, Respiratory Syncytial Virus subtype A, Respiratory Syncytial Virus subtype B, Parainfluenza 1, Parainfluenza 2, and Parainfluenza 3 virus,

Human Metapneumovirus, Rhinovirus, and Adenovirus. The detection and identification of specific viral nucleic acids from individuals exhibiting signs and symptoms of respiratory infection aids in the diagnosis of respiratory viral infection if used in conjunction with other clinical and laboratory findings. It is recommended that specimens found to be negative after examination using RVP be confirmed by cell culture. Negative results do not preclude respiratory virus infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

Positive results do not rule out bacterial infection, or co-infection with other viruses. The agent detected may not be the definite cause of disease. The use of additional laboratory testing (e.g. bacterial culture, immunofluorescence, radiography) and clinical presentation must be taken into consideration in order to obtain the final diagnosis of respiratory viral infection.

Due to seasonal prevalence, performance characteristics for Influenza A/H1 were established primarily with retrospective specimens.

The RVP assay cannot adequately detect Adenovirus species C, or serotypes 7a and 41. The RVP primers for detection of rhinovirus cross-react with enterovirus. A rhinovirus reactive result should be confirmed by an alternate method (e.g. cell culture).

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

2. Indication(s) for use:
Same as Intended Use
3. Special conditions for use statement(s):
For prescription use only
4. Special instrument requirements:
Luminex[®] Instrument (100 IS and 200 systems)

I. Device Description:

The xTAG[™] RVP is a PCR-based system for detecting the presence / absence of viral DNA / RNA in clinical specimens. The oligonucleotide primer / probe components of the xTAG[™] RVP have been designed to specifically target unique regions in the RNA / DNA of each molecular species listed in the following Table:

| Respiratory viral targets |
|-------------------------------------|
| Influenza A (Matrix Gene) |
| Influenza A H1 (Hemagglutinin Gene) |
| Influenza A H3 (Hemagglutinin Gene) |
| Influenza B |
| Respiratory Syncytial Virus Type A |
| Respiratory Syncytial Virus Type B |
| Parainfluenza virus 1 |
| Parainfluenza virus 2 |
| Parainfluenza virus 3 |
| Human Metapneumovirus |
| Rhinovirus |
| Adenovirus |

Amplified products are sorted and analyzed on the Luminex[®] xMAP instrument, which generates signals based on the acquisition of spectrofluorometric data. The raw signals are median fluorescence intensities (MFI) which are acquired in a Luminex[®] Output.csv file that is subsequently analyzed by the software component of the xTAG[™] RVP to establish the presence or absence of all viral types / subtypes for which a Luminex[®] microsphere population has been dedicated. The xTAG[™] RVP primary components are:

1. PCR Primer Mix. The oligonucleotide primers incorporated in this mix have been designed to amplify conserved regions of the viral types / subtypes listed in the Table above and an internal control. Reverse transcription / PCR amplification of cDNA / DNA is the first step in the RVP assay. The PCR amplification product is then subjected to a Target Specific Primer Extension (TSPE) reaction.

2. Target Specific (TS) Primer Mix. Each of the oligonucleotide primers incorporated in this mix has been designed to extend (in the presence of thermostable DNA polymerase) only if the targeted cDNA / DNA sequence is present in the PCR amplification product. If a TS primer is extended, it will incorporate biotinylated dNTPs. After this TSPE reaction is completed and the reaction mix is treated to remove free dNTPs, the biotin that has been incorporated into TSPE reaction products will conjugate to a streptavidin – phycoerythrin reporter molecule that is added to the reaction mix. If a TS primer does not undergo this TSPE reaction, it will not be conjugated to this reporter molecule. Each TS primer also contains a proprietary “tag”, which is a short oligonucleotide sequence designed to hybridize with a high degree of specificity to its complementary “anti-tag”. Each anti-tag is coupled to a specific Luminex[®] microsphere population (“beads”). The TSPE Primer Mix will include oligonucleotides designed to discriminate the viral types / subtypes listed in the Table above.

3. Coupled Bead Mix. This is a suspension containing a defined set of Luminex[®] microspheres. Each microsphere population in this set is spectrally distinguishable from all other microsphere populations in the set when read on the Luminex[®] xMAP system. This feature is the basis on which MFI signals recorded in the Luminex[®] Output.csv file are sorted. The intensity of each recorded signal (Note: one MFI signal is recorded for each bead population in the Bead Mix) is a function of the degree to which the

streptavidin-phycoerythrin reporter molecule has been incorporated into the bead population. This, in turn, is a function of the highly specific tag-anti-tag hybridization between the coupled beads and the TS primers which have incorporated biotinylated dNTPs.

4. Data Analysis Software. This is proprietary software designed and developed by Luminex Molecular Diagnostics Inc. The software component of the system applies analysis algorithms to the MFI signals recorded in the Luminex® Output.csv file and reports a qualitative result for each viral type / subtype / control discriminated by the assay.

Other reagents required to perform testing with the device include **ancillary reagents** for which specific lots have been qualified by Luminex Molecular Diagnostics (LMD) and incorporated in the LMD quality system, for use with the xTAG™ RVP. The xTAG™ RVP product performance requires that only qualified ancillary reagent lots be used with the device. Any lots not specifically qualified by LMD for use with xTAG™ RVP are not validated for use with this assay, and may cause erroneous results. To find an up to date list of Qualified Ancillary Reagents log onto Luminex website Support page https://oraweb.luminexcorp.com/OA_HTML/jtflogin.jsp and search “RVP”. Ancillary reagents should be used only according to the instructions for use contained in the RVP package insert. Any assay problems or failures that are suspected to involve ancillary reagents should be reported to Luminex Molecular Diagnostics Inc. The following is a list of ancillary reagents that are not supplied and are included in LMD’s reagent qualification program:

| |
|--|
| QIAGEN OneStep RT-PCR Enzyme Mix (5x QIAGEN OneStep RT-PCR Buffer, dNTP Mix and RNase-Free Water) |
| TaKaRa Taq™ Hot Start Polymerase (10X PCR Buffer and 2.5 mM TaKaRa dNTPS) |
| Shrimp Alkaline Phosphatase |
| Exonuclease I |
| Bacteriophage Lambda DNA |
| Streptavidin, R-Phycoerythrin conjugate |
| <i>E. coli</i> phage MS 2 |
| *Universal Transport Medium (UTM) Copan Innovations, Cat No 330C |
| *Distilled Water DNase/RNase-Free Water Invitrogen Corp, Cat No: 10977-015 |
| *Biomérieux Nuclisens miniMAG extraction Kit |
| *Biomérieux NucliSENS® easyMAG™ System and reagents |
| QIAamp® MiniElute™ Virus Spin Kit |

* these reagents are not part of the ancillary reagent qualification program, and are not supplied with the kit

The xTAG™ RVP has been designed to generate unique PCR products for each of the targets described above with the exception of RSV targets. RSV subtypes detected by the xTAG™ RVP are discriminated at the TSPE step. The discrimination of Parainfluenza subtypes occurs at both the PCR and TSPE step. The detection of Influenza A subtypes is achieved by amplifying conserved regions of the matrix gene common to all subtypes and target specific regions of the hemagglutinin gene (2 sets of

PCR primers for the 2 listed subtypes).

J. Substantial Equivalence Information:

1. Predicate device name(s):
None
2. Predicate 510(k) number(s):
None
3. Comparison with predicate:
Not applicable

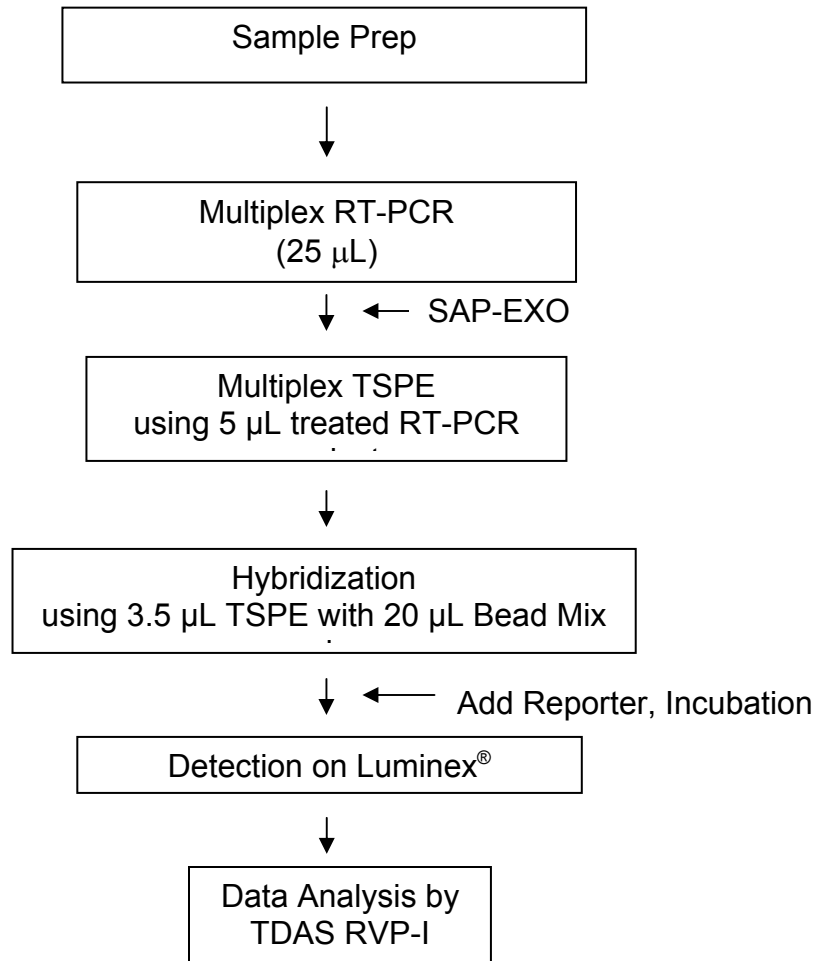
K. Standard/Guidance Document Referenced (if applicable):

- Special controls guidance documents will be promulgated.
- Guidance on Class II Special Controls Guidance Document: Reagents for Detection of Specific Novel Influenza A Viruses (March 2006) - <http://www.fda.gov/cdrh/oivd/guidance/1596.pdf>.
- Guidance on In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path (April 2006) - <http://www.fda.gov/cdrh/oivd/guidance/1594.pdf>.
- Guidance on Informed Consent for In Vitro Diagnostic Device Studies Leftover Human Specimens that are Not Individually Identifiable (April 2006) - <http://www.fda.gov/cdrh/oivd/guidance/1588.pdf>.
- Draft Guidance on Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens (Dec 2005) – <http://www.fda.gov/cdrh/oivd/guidance/1560.html>.
- Software Guidance for the content of premarket submissions for software contained in medical devices (May 2005) – <http://www.fda.gov/cdrh/ode/guidance/337.html>.
- General Guidance on Software Validation (Jan 2002) – <http://www.fda.gov/cdrh/comp/guidance/938.html>.
- CLSI EP17-A: Guidance for Protocols for Determination of Limits of Detection and Limits of Quantitations (Vol. 2, No. 34) (Oct 2004).
- CLSI MM13-A: Guidance for the Collection, Transport, Preparation and Storage of Specimens for Molecular Methods (Vol. 25, No. 31) (Dec 2005).
- CLSI EP7-A2: Guidance for Interference Testing in Clinical Chemistry (Vol. 25, No.27 Second Ed) (Nov 2005).
- CLSI EP12-A: Guidance for User Protocol for Evaluation of Qualitative Test Performance (Vol. 22, No. 14) (Sept 2002).
- CLSI MM6-A: Guidance for the Quantitative Molecular Methods for Infectious Diseases (Vol. 23, No.28) (Oct 2003).
- CLSI EP5-A2: Guidance for Evaluation of Precision Performance of Quantitative Measurement Methods (Vol. 24, No. 25 Second Ed.) (Aug 2004).
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L. Test Principle:

xTAG™ RVP incorporates multiplex Reverse Transcription Polymerase Chain Reaction (RT-PCR) and multiplex Target Specific Primer Extension (TSPE) with Luminex Molecular Diagnostic's proprietary Universal Tag sorting system on the Luminex® xMAP® platform (see figure below). XTAG™ RVP is compatible with both the Luminex® 100 IS and 200 systems.

Summary of Steps in Assay Performed Using XTAG™ RVP:



- Viral nucleic acids are extracted from the sample, and a multiplex RT-PCR reaction is carried out under optimized conditions in a single multiplex PCR resulting in amplicons for each of the viruses/subtypes present in the sample. The amplicon sizes range from 107 bp to 402 bp to enable efficient incorporation of biotin-dCTP during the Target Specific Primer Extension (TSPE) reaction. Each PCR product is treated with Shrimp Alkaline Phosphatase (SAP) to inactivate any remaining nucleotides (especially dCTP), and with Exonuclease I (EXO) to degrade any primers left over from the PCR reaction.
- Multiplex Target Specific Primer Extension (TSPE) is then used to detect viral DNA present in the sample. In this step, each virus is detected by a Target-Specific Primer (TSP) with a unique DNA tag. For each TSP, the 3' end of the primer is a perfect match for its target, but will have a 3' mismatch on any other target. A DNA polymerase is used that will only extend the primer when there is a perfect match on the 3' end, so that the primer is only extended if its target DNA is present in the sample. Biotin-dCTP is incorporated into the extending chain if extension occurs.
- After TSPE, the reaction is added directly to microwells containing bead-immobilized anti-tags, which are the complements of the DNA tags on the primers. The beads which contain the anti-tags are spectrally distinguishable from each other. A fluorescent reporter molecule (streptavidin - phycoerythrin) is bound to the biotin on the extended primers. Each tagged primer hybridizes only to its unique anti-tag complement;

therefore, each colored bead represents a specific virus, through the bead/anti-tag/tagged primer association. The beads are then analyzed by the Luminex[®] instrument (100 IS and 200 systems). The Luminex[®] 100 IS and 200 systems contain two lasers: one identifies the color-coded bead, and the other identifies the presence or absence of extended primer through the phycoerythrin reporter. Thus, the presence of a virus in a sample is identified by the presence of phycoerythrin signal attached to the TSP for that virus.

- All viruses are identified in a single multiplex reaction. The data generated by the Luminex[®] 100 IS and 200 systems is analyzed by the Software component of the kit (TDAS RVP-I) to provide a summary report summarizing of viruses present in the sample, if any. This summary report contains the qualitative output of the test (i.e. calls for each of the 12 analytes + 2 controls probed in each sample). Detailed reports including median fluorescence intensity (MFI) values are also available.

Interpretation of Results:

TDAS RVP-I will display, for each sample, the calls for each target. Possible calls for a given target of a specific sample are:

- POS: the viral target is detected (i.e. analyte signal falls within the positive zone: MFI ≥ 300)
- NEG: the viral target is not detected (i.e. analyte signal falls within the negative zone: MFI < 150)
- *No Call: there is a failure in one or more assay parameters / controls.

Similarly, TDAS RVP-I will display, for each sample, the call for the Internal Control target and the Run Control target:

- PRES: the recommended Internal / Run Control is detected (MFI ≥ 300)
- ABS: the recommended Internal / Run Control is not detected (MFI < 300)
- *No Call: inability to determine presence or absence of the Internal / Run Control due to an assay-specific criterion not being met.

*The distinction between a “No Call” resulting from a target / assay / control failure or ambiguous result (“Invalid Result”), and a “No Call” resulting from an “Equivocal Signal” for a particular target is made in the TDAS RVP-I “Notes and Explanations” column that accompanies each sample output. Scenarios resulting in either of these 2 categories of “No Calls” are summarized in the table below:

| Scenario resulting in a TDAS “No Call” output for any given viral target | TDAS Warning Message(s) in summary view* | Reason for Viral Target “No Call” | Re-test Recommendations |
|--|---|-----------------------------------|---|
| Signal for viral target falls within the equivocal zone ($150 \leq \text{MFI} < 300$) and internal control call is PRES. | “Target(s) failed: value(s) not within predefined ranges” | equivocal signal | Re-run from RNA step (or re-extract or obtain new specimen at laboratory’s discretion) |
| Signal for viral target falls within the equivocal zone ($150 \leq \text{MFI} < 300$) and internal control call is ABS and at least one other target has a signal in the positive zone ($\text{MFI} \geq 300$). | Target(s) failed: value(s) not within predefined ranges” | equivocal signal | Re-run from RNA step (or re-extract or obtain new specimen at laboratory’s discretion) |
| Signal for one or both Influenza A subtypes (H1 and H3) falls within the positive zone ($\text{MFI} \geq 300$) and Influenza A matrix signal falls within the negative zone ($\text{MFI} < 150$). This results in a No Call for both matrix signal and subtype* | “Targets failed: incompatible signals between targets” | invalid result | Re-run from RNA step (or re-extract or obtain new specimen at laboratory’s discretion) |
| None of the viral target signals fall within the positive zone ($\text{MFI} \geq 300$) and internal control call is ABS. | “Sample failed: unexpected control call(s)” | invalid result | Re-extract (or obtain new specimen at laboratory’s discretion) |
| One or more viral targets or controls with low bead count. | “Assay failed: low bead count(s) for negative control sample” “Sample failed: low bead count for internal control” “Target failed: low bead count” | invalid result | Re-run from bead hybridization step (or re-run from RNA step or re-extract or obtain new specimen at laboratory’s discretion) |
| Plate failure due to unexpected signals in the last position on the assay plate (reserved by TDAS for the negative control). Note: Signal > 150 MFI units in any negative control sample on a plate, for one or more viral analytes, is indicative of carryover contamination of the plate. In such a case, it is strongly recommended that the samples on that plate be rerun, starting from the PCR step. | “Assay failed: unexpected value(s) encountered or sample is empty for negative control sample” “Assay failed: a negative control signal exceeds acceptable value”. | invalid result | Re-run from RNA step Re-run from RNA step or re-extract all samples at laboratory’s discretion |

*RVP detection of Influenza A, subtypes H1 and H3 is achieved through (1) detection of the Flu A matrix gene which is common to all subtypes, and also (2) detection of subtype-specific regions of the hemagglutinin gene. Interpretation of results is discussed further below, using the example of Influenza A.

**Re-test Recommendations: It is recommended that the sample be re-tested once according to the instructions provided in the table. If a re-test needs to be carried out due to a “No Call” (due to either an equivocal or invalid result) being returned for a particular sample or target, the re-test results should be considered the final RVP result for that analyte. For detection of Influenza A H1 and H3 subtypes, there are specific precautions that must be followed which are described below. For all other analytes, if the final RVP result is a “no call” then follow-up testing is recommended.

Any assay problems or failures that are suspected to involve ancillary reagents should be reported to Luminex Molecular Diagnostics Inc.

NOTE: if the influenza A matrix signal falls within the positive zone (MFI \geq 300) and all subtype (H1 and H3) signals fall within the negative zone (MFI $<$ 150), a POS call will be generated for influenza A and a NEG call will be generated for each of the H1, H3 subtypes. This is not considered an “Ambiguous Result”. It may be indicative of an atypical variant of influenza A. See Interpretation and Reporting of Influenza A results below.

Interpretation of Influenza A Results:

The RVP assay has been designed to probe for 3 distinct analytes associated with Influenza A virus: 1) a conserved sequence in the matrix gene (Influenza A target); 2) a conserved sequence specific to the H1 subtype of the hemagglutinin gene and 3) a conserved sequence specific to the H3 subtype of the hemagglutinin gene. A clear positive signal (MFI greater than or equal to 300) in the matrix gene is establishing an Influenza A infection. A clear negative signal (MFI less than 150) for each of the listed Influenza A analytes (Influenza A matrix, H1 and H3) should be interpreted as negative for Influenza A. A sample result that involves any other combination of signals for these 3 Influenza A analytes should be considered either equivocal or ambiguous. Further investigation of such equivocal / ambiguous results is recommended. In the particular case where the Influenza A target is detected with no clear positive result for either hemagglutinin target, special precautions must be followed (see reporting below).

Reporting Influenza A Results:

- Report negative test results for Influenza A as “Matrix gene target not detected, and hemagglutinin gene targets not detected. It is recommended that specimens found to be negative after examination using a respiratory viral panel nucleic acid detection assay be confirmed by cell culture. Negative results do not preclude respiratory virus infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.”
- Report positive test results as “Positive for matrix gene target - Influenza A positive, and (where applicable) hemagglutinin gene target (specify hemagglutinin target detected, e.g. H1, or H3). This result does not rule out co-infections with pathogens that were not screened for by RVP. A positive result for a hemagglutinin gene target does not identify a specific influenza A strain (e.g. H1N1). The agent detected may not be the definite cause of disease. Results should be used in conjunction with other clinical and laboratory findings.”
- When Influenza A target is detected with no clear positive result for either H1 or H3, the sample should be re-tested from the extraction step together with positive controls for these two analytes. Extract prepared from the sample should be run in duplicate. In the case where the re-test on both replicates does not type for H1 or H3 and analyte controls are properly typed, necessitates notification of appropriate local, state or federal public health authorities to determine necessary measures for verification of results in accordance with the MMWR notice (<http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5613a4.htm> and <http://www.cste.org/ps/2007pdfs/novelfluandssjan10final23.pdf>). The purpose of the surveillance program described in these documents is to determine whether untypeable Influenza A specimens represent novel strains of Influenza A. In the event that remnant

sample is not available, then extracted material should be forwarded to CDC per the procedures outlined above.

- A “No Call” due to an equivocal or invalid result as shown in the table above, should not be reported but re-tested as per recommendations in this Table.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Three separate precision studies were performed to assess the following:

- Reproducibility of the assay in the specimens near the clinical cutoff of the assay
- Reproducibility of the assay using virus concentrations expected to be found in clinical specimens (clinically significant concentration)
- Reproducibility in dual co-infected specimens.

a) Reproducibility near the assay cut-offs was assessed across 3 sites using replicates of samples containing viral material from culture-derived isolates in the matrix simulating intended use specimen type. The panel contained samples prepared to represent low positive (LP) and high negative (HN) analyte levels relative to the RVP cut-offs. Each simulated sample within the panel was divided into aliquots, blinded and stored frozen (-70°C) prior to testing. Thus, aliquots of the same blinded panel of samples were tested at the three different sites. Each site used a different extraction method and for each of the 3 extraction methods evaluated, 2 aliquots of a given sample dilution were extracted per day, for each of 3 days (i.e. a total of six extractions per site). At each site, both extracts from a given day were assayed in singlicate on the same RVP run. Calls (Positive, Equivocal, Negative) generated for the viral analyte in question are summarized in Tables below.

Summary of Flu A and H3 calls in simulated Influenza A-H3 samples:

| Virus / Titer | All Days (3 extraction days x 2 extractions per day) | | | | | | | |
|--|--|----------------|---------------|----------------|---------------------------------|----------------|---------------------------------|--------------|
| Flu A-H3 (Strain: A/Victoria/3/75 (H3N2), DHI Lot #121106) | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | %CV* |
| Flu A Low Positive (LP) (2 TCID ₅₀ per reaction) | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 1531.38 | 1731.75 | 1969.25 | 29.25 |
| | Site 2 | 6 / 6 | 0 / 6 | 0 / 6 | 1428.75 | 1746.75 | 1828.88 | 40.89 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 541.50 | 800.00 | 899.13 | 39.94 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 870.38 | 1463.00 | 1814.38 | 48.45 |
| H3 Low Positive (LP) (200 TCID ₅₀ per reaction) | | 6 / 6 | 0 / 6 | 0 / 6 | 1679.00 | 1767.00 | 2017.75 | 14.89 |
| | Site 2 | 6 / 6 | 0 / 6 | 0 / 6 | 1567.38 | 1718.25 | 1912.63 | 23.70 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 902.88 | 1077.25 | 1243.00 | 18.44 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 1256.00 | 1661.00 | 1793.75 | 30.04 |
| Flu A High Negative (HN) (0.2 TCID ₅₀ per reaction) | Site 1 | 0 / 6 | 2 / 6 | 4 / 6 | 76.75 | 133.00 | 157.00 | N/A** |
| | Site 2 | 0 / 6 | 6 / 6 | 0 / 6 | 180.00 | 192.50 | 200.88 | N/A |
| | Site 3 | 0 / 6 | 0 / 6 | 6 / 6 | 12.63 | 40.00 | 66.25 | N/A |
| | Total | 0 / 18 | 8 / 18 | 10 / 18 | 56.75 | 133.00 | 176.25 | N/A |
| H3 High Negative (HN) (2 TCID ₅₀ per reaction) | Site 1 | 0 / 6 | 0 / 6 | 6 / 6 | 64.25 | 68.00 | 73.25 | N/A |
| | Site 2 | 0 / 6 | 0 / 6 | 6 / 6 | 100.00 | 119.50 | 127.75 | N/A |
| | Site 3 | 0 / 6 | 0 / 6 | 6 / 6 | 15.13 | 32.50 | 49.50 | N/A |
| | Total | 0 / 18 | 0 / 18 | 18 / 18 | 46.88 | 68.00 | 95.50 | N/A |

* For reproducibility Tables, %CV = Standard Deviation / Mean*100

** For reproducibility Tables, N/A = not applicable.

Summary of Flu A and H1 calls in simulated Influenza A-H1 samples:

| Virus / Titer | All Days (3 extraction days x 2 extractions per day) | | | | | | | |
|---|--|----------------|---------------|----------------|---------------------------------|---------------|---------------------------------|--------------|
| Flu A-H1 (Strain: A/PR/8/34 (H1N1), Zeptomatrix lot #303543) | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | %CV |
| Flu A Low Positive (LP) (0.02TCID ₅₀ per reaction) | Site 1 | 6 | 0 | 0 | 465.00 | 660.50 | 802.38 | 29.41 |
| | Site 2 | 5 | 1 | 0 | 391.25 | 433.50 | 503.50 | 27.06 |
| | Site 3 | 2 | 3 | 1 | 216.75 | 241.75 | 479.75 | 60.22 |
| | Total | 13 / 18 | 4 / 18 | 1 / 18 | 288.38 | 433.50 | 570.38 | 45.13 |
| H1 Low Positive (LP) (0.06 TCID ₅₀ per reaction) | Site 1 | 6 | 0 | 0 | 1038.50 | 1151.50 | 1324.13 | 18.24 |
| | Site 2 | 6 | 0 | 0 | 697.13 | 938.00 | 1088.13 | 36.99 |
| | Site 3 | 6 | 0 | 0 | 666.88 | 890.50 | 933.00 | 33.31 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 826.63 | 990.5 | 1110.75 | 33.00 |
| Flu A High Negative (HN) (0.001 TCID ₅₀ per reaction) | Site 1 | 0 | 0 | 6 | 37.75 | 59.00 | 83.63 | N/A |
| | Site 2 | 0 | 0 | 6 | 92.38 | 98.00 | 101.75 | N/A |
| | Site 3 | 0 | 0 | 6 | 4.00 | 10.25 | 22.50 | N/A |
| | Total | 0 / 18 | 0 / 18 | 18 / 18 | 20.50 | 59.00 | 94.13 | N/A |
| H1 High Negative (HN) (0.004 TCID ₅₀ per reaction) | Site 1 | 0 | 1 | 5 | 58.00 | 95.50 | 135.25 | N/A |
| | Site 2 | 0 | 3 | 3 | 102.50 | 136.00 | 175.50 | N/A |
| | Site 3 | 0 | 0 | 6 | 22.50 | 50.25 | 77.25 | N/A |
| | Total | 0 / 18 | 4 / 18 | 14 / 18 | 52.50 | 87.00 | 140.00 | N/A |

Summary of Flu B calls in simulated Influenza B samples:

| Virus / Titer | All Days (3 extraction days x 2 extractions per day) | | | | | | | |
|--|--|----------------|---------------|----------------|---------------------------------|----------------|---------------------------------|--------------|
| Flu B (Strain: Influenza B/Malaysia/2506/04) | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | %CV |
| Low Positive (LP) (0.001 TCID ₅₀ per reaction) | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 1272.00 | 1440.00 | 1684.13 | 20.83 |
| | Site 2 | 6 / 6 | 0 / 6 | 0 / 6 | 1009.00 | 1258.00 | 1528.00 | 41.44 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 918.75 | 1036.50 | 1201.50 | 18.78 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 1034.25 | 1263.00 | 1528.00 | 31.11 |
| High Negative (HN) (0.00002 TCID ₅₀ per reaction) | Site 1 | 0 / 6 | 0 / 6 | 6 / 6 | 18.50 | 22.00 | 26.25 | N/A |
| | Site 2 | 0 / 6 | 1 / 6 | 5 / 6 | 76.63 | 93.25 | 120.38 | N/A |
| | Site 3 | 0 / 6 | 0 / 6 | 6 / 6 | 4.00 | 31.00 | 81.25 | N/A |
| | Total | 0 / 18 | 1 / 18 | 17 / 18 | 18.50 | 55.00 | 90.88 | N/A |

Summary of hMPV calls in simulated hMPV samples:

| Virus / Titer | All Days (3 extraction days x 2 extractions per day) | | | | | | | |
|---|--|----------------|---------------|----------------|---------------------------------|---------------|---------------------------------|--------------|
| hMPV (CAN 97-83; in-house) | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | %CV |
| Low Positive (LP) (0.002 TCID ₅₀ per reaction) | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 662.38 | 701.25 | 757.38 | 82.67 |
| | Site 2 | 5 / 6 | 1 / 6 | 0 / 6 | 377.25 | 601.50 | 742.50 | 63.98 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 538.88 | 646.00 | 690.50 | 24.50 |
| | Total | 17 / 18 | 1 / 18 | 0 / 18 | 523.88 | 662.00 | 757.38 | 69.75 |
| High Negative (HN) (0.0001 TCID ₅₀ per reaction) | Site 1 | 0 / 6 | 0 / 6 | 6 / 6 | 20.25 | 30.00 | 55.13 | N/A |
| | Site 2 | 0 / 6 | 0 / 6 | 6 / 6 | 76.00 | 82.00 | 89.13 | N/A |
| | Site 3 | 0 / 6 | 0 / 6 | 6 / 6 | 31.75 | 46.00 | 54.63 | N/A |
| | Total | 0 / 18 | 0 / 18 | 18 / 18 | 30.00 | 59.00 | 80.00 | N/A |

Summary of RSV A calls in simulated RSV A samples:

| Virus / Titer | All Days (3 extraction days x 2 extractions per day) | | | | | | | |
|--|--|----------------|---------------|----------------|---------------------------------|----------------|---------------------------------|--------------|
| RSV A (Strain: A2, Zeptomatrix lot 303544) | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | %CV |
| Low Positive (LP) (10 TCID ₅₀ per reaction) | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 2171.38 | 3150.00 | 3990.63 | 40.60 |
| | Site 2 | 6 / 6 | 0 / 6 | 0 / 6 | 1004.00 | 1291.25 | 1442.00 | 32.56 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 834.00 | 1193.00 | 1507.00 | 64.94 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 1067.00 | 1509.25 | 2721.13 | 65.22 |
| High Negative (HN) (0.8 TCID ₅₀ per reaction) | Site 1 | 1 / 6 | 2 / 6 | 3 / 6 | 110.50 | 144.00 | 158.00 | N/A |
| | Site 2 | 1 / 6 | 2 / 6 | 3 / 6 | 104.00 | 145.25 | 255.50 | N/A |
| | Site 3 | 0 / 6 | 0 / 6 | 6 / 6 | 21.50 | 25.50 | 39.25 | N/A |
| | Total | 2 / 18 | 4 / 18 | 12 / 18 | 52.00 | 111.00 | 153.88 | N/A |

Summary of RSV B calls in simulated RSV B samples:

| Virus / Titer | All Days (3 extraction days x 2 extractions per day) | | | | | | | |
|---|--|----------------|---------------|----------------|---------------------------------|---------------|---------------------------------|--------------|
| RSV B (Strain: B WV/14617/ '85 [B-1 wild type], ATCC) | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | %CV |
| Low Positive (LP) (0.1 TCID ₅₀ per reaction) | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 639.75 | 818.50 | 962.38 | 45.96 |
| | Site 2 | 5 / 6 | 1 / 6 | 0 / 6 | 474.00 | 602.00 | 814.75 | 45.05 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 609.50 | 735.50 | 968.75 | 29.21 |
| | Total | 17 / 18 | 1 / 18 | 0 / 18 | 556.75 | 683.00 | 926.13 | 41.49 |
| High Negative (HN) (0.0008 TCID ₅₀ per reaction) | Site 1 | 0 / 6 | 1 / 6 | 5 / 6 | 61.50 | 91.75 | 110.75 | N/A |
| | Site 2 | 0 / 6 | 0 / 6 | 6 / 6 | 72.63 | 87.00 | 100.63 | N/A |
| | Site 3 | 0 / 6 | 0 / 6 | 6 / 6 | 22.25 | 39.75 | 56.13 | N/A |
| | Total | 0 / 18 | 1 / 18 | 17 / 18 | 53.13 | 71.75 | 95.00 | N/A |

Summary of Para 1 calls in simulated Parainfluenza-1 samples:

| Virus / Titer | All Days (3 extraction days x 2 extractions per day) | | | | | | | |
|---|--|----------------|---------------|----------------|---------------------------------|---------------|---------------------------------|--------------|
| Para 1 (Strain: C-35, DHI Lot 081006B) | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | %CV |
| Low Positive (LP) (100 TCID ₅₀ per reaction) | Site 1 | 5 / 6 | 0 / 6 | 1 / 6 | 863.00 | 924.50 | 1099.25 | 50.83 |
| | Site 2 | 5 / 6 | 1 / 6 | 0 / 6 | 347.13 | 502.75 | 633.63 | 65.52 |
| | Site 3 | 5 / 6 | 0 / 6 | 1 / 6 | 769.50 | 798.25 | 848.38 | 73.11 |
| | Total | 15 / 18 | 1 / 18 | 2 / 18 | 482.88 | 798.25 | 940.25 | 63.02 |
| High Negative (HN) (2 TCID ₅₀ per reaction) | Site 1 | 0 / 6 | 0 / 6 | 6 / 6 | 35.00 | 45.00 | 64.75 | n/a |
| | Site 2 | 0 / 6 | 0 / 6 | 6 / 6 | 68.00 | 83.00 | 95.75 | n/a |
| | Site 3 | 0 / 6 | 0 / 6 | 6 / 6 | 2.13 | 11.50 | 20.50 | n/a |
| | Total | 0 / 18 | 0 / 18 | 18 / 18 | 21.50 | 52.00 | 70.88 | n/a |

Summary of Para 2 calls in simulated Parainfluenza-2 samples:

| Virus / Titer | All Days (3 extraction days x 2 extractions per day) | | | | | | | |
|---|--|------------|-------------|------------|---------------------------------|------------|---------------------------------|-------|
| Para 2 (Strain: Greer, DHI Lot 062706) | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | %CV |
| Low Positive (LP) (6 TCID ₅₀ per reaction) | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 600.25 | 726.50 | 1116.00 | 44.73 |
| | Site 2 | 3 / 6 | 1 / 6 | 2 / 6 | 179.00 | 308.50 | 387.75 | 83.16 |
| | Site 3 | 5 / 6 | 1 / 6 | 0 / 6 | 453.50 | 595.50 | 910.00 | 50.25 |

| | | | | | | | | |
|--|--------------|----------------|---------------|----------------|---------------|---------------|---------------|--------------|
| | Total | 14 / 18 | 2 / 18 | 2 / 18 | 332.75 | 544.50 | 930.75 | 59.26 |
| High Negative (HN) (0.4 TCID ₅₀ per reaction) | Site 1 | 0 / 6 | 1 / 6 | 5 / 6 | 54.50 | 69.00 | 112.38 | N/A |
| | Site 2 | 0 / 6 | 0 / 6 | 6 / 6 | 78.50 | 86.50 | 96.38 | N/A |
| | Site 3 | 0 / 6 | 0 / 6 | 6 / 6 | 18.25 | 52.50 | 67.63 | N/A |
| | Total | 0 / 18 | 1 / 18 | 17 / 18 | 51.50 | 73.25 | 96.38 | N/A |

Summary of Para 3 calls in simulated Parainfluenza-3 samples:

| Virus / Titer | All Days (3 extraction days x 2 extractions per day) | | | | | | | |
|---|---|-------------------|--------------------|-------------------|---------------------------------------|-------------------|---------------------------------------|--------------|
| Para 3 (Strain: C-243, DHI Lot 052506) | Site | # Positive | # Equivocal | # Negative | 25th Percentile MFI | Median MFI | 75th Percentile MFI | %CV |
| Low Positive (LP) (0.2 TCID ₅₀ per reaction) | Site 1 | 4 / 6 | 2 / 6 | 0 / 6 | 293.88 | 405.50 | 543.00 | 43.89 |
| | Site 2 | 3 / 6 | 3 / 6 | 0 / 6 | 239.50 | 291.00 | 348.50 | 54.53 |
| | Site 3 | 3 / 6 | 2 / 6 | 1 / 6 | 200.00 | 285.00 | 461.88 | 50.45 |
| | Total | 10 / 18 | 7 / 18 | 1 / 18 | 236.00 | 327.00 | 482.63 | 47.44 |
| High Negative (HN) (0.02 TCID ₅₀ per reaction) | Site 1 | 0 / 6 | 0 / 6 | 6 / 6 | 21.25 | 22.25 | 27.38 | N/A |
| | Site 2 | 0 / 6 | 0 / 6 | 6 / 6 | 63.88 | 70.75 | 75.00 | N/A |
| | Site 3 | 0 / 6 | 0 / 6 | 6 / 6 | 6.25 | 19.00 | 25.75 | N/A |
| | Total | 0 / 18 | 0 / 18 | 18 / 18 | 21.25 | 27.50 | 62.38 | N/A |

Summary of Rhino calls in simulated Rhinovirus samples:

| Virus / Titer | All Days (3 extraction days x 2 extractions per day) | | | | | | | |
|--|---|-------------------|--------------------|-------------------|---------------------------------------|-------------------|---------------------------------------|--------------|
| Rhinovirus (Type 54: ATCC) | Site | # Positive | # Equivocal | # Negative | 25th Percentile MFI | Median MFI | 75th Percentile MFI | %CV |
| Low Positive (LP) (0.0006 TCID ₅₀ per reaction) | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 775.25 | 906.50 | 972.50 | 27.97 |
| | Site 2 | 6 / 6 | 0 / 6 | 0 / 6 | 512.00 | 670.00 | 769.50 | 33.92 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 827.38 | 1215.25 | 1283.25 | 30.47 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 666.00 | 827.00 | 1049.38 | 35.55 |
| High Negative (HN) (0.00004 TCID ₅₀ per reaction) | Site 1 | 0 / 6 | 0 / 6 | 6 / 6 | 36.25 | 50.00 | 54.75 | N/A |
| | Site 2 | 0 / 6 | 0 / 6 | 6 / 6 | 78.75 | 94.00 | 96.88 | N/A |
| | Site 3 | 0 / 6 | 0 / 6 | 6 / 6 | 24.88 | 67.50 | 121.75 | N/A |
| | Total | 0 / 18 | 0 / 18 | 18 / 18 | 36.25 | 60.75 | 96.88 | N/A |

Summary of Adeno Calls in simulated Adenovirus samples:

| Virus / Titer | All Days (3 extraction days x 2 extractions per day) | | | | | | | |
|---|---|-------------------|--------------------|-------------------|---------------------------------------|-------------------|---------------------------------------|--------------|
| Adenovirus (cultured patient isolate – Species C) | Site | # Positive | # Equivocal | # Negative | 25th Percentile MFI | Median MFI | 75th Percentile MFI | %CV |
| Low Positive (LP) (0.8 TCID ₅₀ per reaction) | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 865.63 | 925.25 | 992.75 | 9.52 |
| | Site 2 | 5 / 6 | 0 / 6 | 1 / 6 | 708.75 | 834.00 | 905.63 | 44.23 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 758.88 | 971.00 | 1204.50 | 41.80 |
| | Total | 17 / 18 | 0 / 18 | 1 / 18 | 813.63 | 888.25 | 1007.50 | 36.16 |
| High Negative (HN) (0.05 TCID ₅₀ per reaction) | Site 1 | 0 / 6 | 2 / 6 | 4 / 6 | 121.00 | 126.75 | 178.63 | N/A |
| | Site 2 | 2 / 6 | 3 / 6 | 1 / 6 | 162.75 | 225.00 | 303.75 | N/A |
| | Site 3 | 1 / 6 | 3 / 6 | 2 / 6 | 146.75 | 222.00 | 263.50 | N/A |
| | Total | 3 / 18 | 8 / 18 | 7 / 18 | 124.50 | 189.00 | 259.00 | N/A |

For all analytes assessed in the reproducibility study described above, a total of 55 (out of 468) replicates were miscalled. Of these 55 missed calls, 23 were from low positive samples which generated either an equivocal (n=16/23) or negative (n=7/23) call for the analyte in question. The remaining 32 missed calls were from high negative samples for which 27/32 generated equivocal calls and 5/32 generated positive calls.

b) Reproducibility of the assay using virus concentrations expected to be found in clinical specimens (clinically significant concentration). A separate reproducibility study was carried out on simulated samples prepared at titers representative of what is typically encountered in clinical samples. An aliquot of each sample was extracted once and 6 replicates were prepared from each extract for evaluation by RVP. Median MFI values across all extractions methods for each viral analyte evaluated in this study (excluding adenovirus) ranged from 1140 to 7381. The strain of adenovirus evaluated in this study (Type 5, Adenoid 75, ATCC VR-5) is a member of species C with a median MFI value (387) which was significantly lower than that observed for other analytes. Results of this study are summarized in Tables below.

Reproducibility in medium titer Influenza samples:

| Virus / TCID ₅₀ per reaction | 6 replicates prepared from each extract (1 extract per method) | | | | | | | |
|--|--|----------------|---------------|---------------|---------------------------------------|----------------|---------------------------------------|-------------|
| | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | CV |
| Flu A / 10 | Site 1 | 6 | 0 | 0 | 6707.5 | 7005 | 7288.25 | 13.1 |
| | Site 2 | 6 | 0 | 0 | 4178.5 | 4438.5 | 4876.625 | 22.0 |
| | Site 3 | 6 | 0 | 0 | 1789.25 | 2640.75 | 3215.875 | 33.1 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 3071.625 | 4438.5 | 6623 | 46.4 |
| H1 / 10 | Site 1 | 6 | 0 | 0 | 4444.75 | 4824 | 4961.375 | 13.9 |
| | Site 2 | 6 | 0 | 0 | 2080 | 3152.75 | 3343.875 | 33.6 |
| | Site 3 | 6 | 0 | 0 | 1362.625 | 2168 | 2549.625 | 35.6 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 2106.75 | 3152.75 | 4351 | 46.2 |
| Flu A / 100 | Site 1 | 6 | 0 | 0 | 5415.375 | 5704.25 | 5885.5 | 5.6 |
| | Site 2 | 6 | 0 | 0 | 7350.125 | 7768.75 | 8105.25 | 6.1 |
| | Site 3 | 6 | 0 | 0 | 6374.75 | 7430 | 7634.375 | 18.6 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 5836.5 | 7305.5 | 7634.375 | 17.4 |
| H3 / 100 | Site 1 | 6 | 0 | 0 | 907.75 | 990 | 1050.125 | 8.7 |
| | Site 2 | 6 | 0 | 0 | 2570.75 | 2809 | 3039 | 13.9 |
| | Site 3 | 6 | 0 | 0 | 654 | 946 | 1238 | 43.9 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 907.75 | 1140.5 | 2467 | 61.9 |
| Flu B / 0.5 | Site 1 | 6 | 0 | 0 | 4234.5 | 4283 | 4354.375 | 2.9 |
| | Site 2 | 6 | 0 | 0 | 762.875 | 1267 | 1829.25 | 52.8 |
| | Site 3 | 6 | 0 | 0 | 5897 | 6460 | 7067.25 | 16.5 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 1996.375 | 4283 | 5542.125 | 54.7 |

Reproducibility in medium titer RSV, Parainfluenza, Adenovirus, hMPV and Rhinovirus samples:

| Virus / TCID ₅₀ per reaction | 6 replicates prepared from each extract (1 extract per method) | | | | | | | |
|--|--|----------------|---------------|---------------|---------------------------------------|----------------|---------------------------------------|-------------|
| | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | CV |
| RSV A /100 | Site 1 | 6 | 0 | 0 | 5726.875 | 5946.25 | 6102.25 | 6.3 |
| | Site 2 | 6 | 0 | 0 | 3360.375 | 3599 | 3690.25 | 10.1 |
| | Site 3 | 6 | 0 | 0 | 2821.25 | 4090.5 | 4653.25 | 33.8 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 3544.5 | 4339.5 | 5681.75 | 30.5 |
| RSV B /100 | Site 1 | 6 | 0 | 0 | 4549.375 | 4706 | 4815.375 | 5.3 |
| | Site 2 | 6 | 0 | 0 | 2907 | 2964.75 | 2980.125 | 8.9 |
| | Site 3 | 6 | 0 | 0 | 3661.5 | 4563.25 | 4638.5 | 17.1 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 3010.5 | 4467.75 | 4655.875 | 23.1 |
| Para 1 /100 | Site 1 | 6 | 0 | 0 | 3190 | 3209.5 | 3244 | 3.6 |
| | Site 2 | 6 | 0 | 0 | 970.5 | 1548.5 | 1835.5 | 48.7 |
| | Site 3 | 6 | 0 | 0 | 1751.75 | 1826.5 | 1875 | 6.2 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 1635 | 1881.5 | 3185.375 | 42.7 |
| Para 2 /100 | Site 1 | 6 | 0 | 0 | 5359.25 | 5425.5 | 5638.75 | 6.3 |
| | Site 2 | 6 | 0 | 0 | 1539.125 | 1859.25 | 2237.5 | 42.7 |
| | Site 3 | 6 | 0 | 0 | 2179.875 | 2257.5 | 2307.75 | 10.1 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 2048.625 | 2311 | 5314 | 53.0 |
| Para 3 /25 | Site 1 | 6 | 0 | 0 | 2951.375 | 3075.25 | 3135.75 | 4.3 |
| | Site 2 | 3 | 0 | 3 | 116.75 | 234.5 | 600.5 | 87.2 |
| | Site 3 | 6 | 0 | 0 | 5977.75 | 6785.5 | 8248.75 | 35.5 |
| | Total | 15 / 18 | 0 / 18 | 3 / 18 | 723.125 | 2988.75 | 5106.875 | 87.5 |
| Adeno /5000 | Site 1 | 2 | 4 | 0 | 247.375 | 284.75 | 300 | 13.9 |
| | Site 2 | 6 | 0 | 0 | 362.625 | 387 | 484.5 | 20.0 |
| | Site 3 | 6 | 0 | 0 | 546.625 | 684 | 719 | 20.7 |
| | Total | 14 / 18 | 4 / 18 | 0 / 18 | 303.875 | 387 | 541.375 | 41.0 |
| hMPV /0.5 | Site 1 | 6 | 0 | 0 | 4764.875 | 4958 | 5072.375 | 13.4 |
| | Site 2 | 6 | 0 | 0 | 7405.125 | 7670.5 | 7717.25 | 5.6 |
| | Site 3 | 6 | 0 | 0 | 7583.75 | 8175.25 | 8743.875 | 20.6 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 5033.125 | 7381.25 | 7788 | 24.0 |
| Rhino /100 | Site 1 | 6 | 0 | 0 | 2787.125 | 2836 | 2890.5 | 2.4 |
| | Site 2 | 6 | 0 | 0 | 3133.25 | 3219 | 3413.5 | 6.5 |
| | Site 3 | 6 | 0 | 0 | 3366.25 | 3631 | 4159.75 | 26.1 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 2871.5 | 3147.5 | 3515 | 18.3 |

Simulated samples used in the reproducibility evaluation summarized in Tables above were constructed from the following materials: Flu A-H1 (strain A/WS/33 (H1N1), ATCC VR-1520); Flu A-H3 (in-house strain, similar to: A/swine/Ontario/00130/97(H3N2)); Flu B (strain B/Malaysia/2506/040; RSV-A (ATCC VR-26); RSV-B (strain B WV/14617/85 (B-1 wild type), ATCC VR-1400); hMPV (CAN97-83); PARA-1 (strain 35, ATCC VR-1380); PARA-2 (strain Greer, ATCC VR-1381); PARA-3 (strain C243, ATCC VR-93); Adenovirus (Type 5, strain Adenoid 75, ATCC VR-5); Rhinovirus (Type 39, strain 209, ATCC VR-340).

c) Reproducibility in Dual Infection Samples. The results below summarize the findings from a reproducibility study on replicates of 4 simulated samples containing

viral material from culture derived isolates: Flu A-H1 (strain A/WS/33 (H1N1), ATCC VR-1520), RSV-A (ATCC VR-26), Adenovirus (Species C, Serotype 5, strain Adenoid 75, ATCC VR-5). Each sample was prepared to mimic dually-infected specimens where one viral target was present at high titer (depicted as “H” for high) relative to the second viral target (depicted as “M” for medium). TCID₅₀ units per reaction are summarized in tables below. Each sample was extracted 3 times (once by each method assessed) and 6 replicates were prepared from the given extract for testing by RVP. Results for a given *analyte* are summarized in tables below. There were a total of 18 (out of 180) replicates that were miscalled (9/18 gave equivocal calls and 9/18 gave negative calls).

Summary of Calls in a Flu A-H1 (H) / RSV A (M) Dual Positive Simulated Sample:

| Virus (Titer) / TCID ₅₀ per reaction | 6 replicates prepared from each extract (1 extract per method) | | | | | | | |
|---|--|----------------|---------------|---------------|---------------------------------|----------------|---------------------------------|-------------|
| | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | CV |
| Flu A (H) / 10,000 | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 4207 | 4357.25 | 4504.875 | 6.9 |
| | Site 2 | 6 / 6 | 0 / 6 | 0 / 6 | 7231 | 7850.5 | 7938.25 | 11.4 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 7286.875 | 8091 | 8736.875 | 13.9 |
| | Total | 18/18 | 0/18 | 0/18 | 4618.75 | 7123.25 | 7938.25 | 27.9 |
| H1 (H) / 10,000 | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 3557.625 | 3589.3 | 3705.25 | 5.8 |
| | Site 2 | 6 / 6 | 0 / 6 | 0 / 6 | 4953.25 | 5523 | 5916.875 | 13.4 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 7825.875 | 8318 | 8559.25 | 7.8 |
| | Total | 18/18 | 0/18 | 0/18 | 3823.25 | 5523 | 7603.875 | 35.0 |
| RSV A (M) / 10,000 | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 353.125 | 372.75 | 408.875 | 9.56 |
| | Site 2 | 2 / 6 | 4 / 6 | 0 / 6 | 229.375 | 243.25 | 385.75 | 51.51 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 1175.375 | 1358 | 1924.25 | 30.55 |
| | Total | 14 / 18 | 4 / 18 | 0 / 18 | 341 | 422.25 | 1164.125 | 85.3 |

Note: There were 2 co-infected Flu A / RSV specimens detected in the clinical study.

If RSV A is present in medium levels in clinical specimens, it may not be detected by RVP in the presence of a high level of Influenza A/H1.

Summary of Calls in a Flu A-H1 (M) / RSV A (H) Dual Positive Simulated Samples:

| Virus (Titer) / TCID ₅₀ per reaction | 6 replicates prepared from each extract (1 extract per method) | | | | | | | |
|---|--|----------------|---------------|---------------|---------------------------------|----------------|---------------------------------|-------------|
| | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | CV |
| Flu A (M) / 10 | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 3340.25 | 3644 | 3804.875 | 19.8 |
| | Site 2 | 6 / 6 | 0 / 6 | 0 / 6 | 4649.625 | 5143.25 | 5321.125 | 22.3 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 7251.5 | 7813 | 8268 | 9.1 |
| | Total | 18/18 | 0/18 | 0/16 | 3753.625 | 5185.25 | 7092.5 | 35.4 |
| H1 (M) / 10 | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 2387.5 | 2422.8 | 2683.375 | 17.6 |
| | Site 2 | 6 / 6 | 0 / 6 | 0 / 6 | 2443 | 2933 | 3225.375 | 31.0 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 5403.875 | 5899.5 | 6107.5 | 11.8 |
| | Total | 18/18 | 0/18 | 0/18 | 2405.875 | 3202.25 | 5126.125 | 42.2 |
| RSV A (H) / 500,000 | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 2234 | 2440.5 | 2688.25 | 26.01 |
| | Site 2 | 6 / 6 | 0 / 6 | 0 / 6 | 5465.5 | 3639.5 | 6099.625 | 7.49 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 6664.5 | 7188.3 | 7609.625 | 11.74 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 3066.375 | 5596.25 | 6459.25 | 38.6 |

Note: There were 2 co-infected Flu A / RSV specimens detected in the clinical study.

Summary of Calls in a Adeno (H) / RSV A (M) Dual Positive Simulated Samples:

| Virus (Titer) / TCID ₅₀ per reaction | 6 replicates prepared from each extract (1 extract per method) | | | | | | | |
|---|--|----------------|---------------|---------------|---------------------------------|---------------|---------------------------------|-------------|
| | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | CV |
| Adeno (H) / 5,000,000 | Site 1 | 0 / 6 | 3 / 6 | 3 / 6 | 144.75 | 149.25 | 169.125 | 15.9 |
| | Site 2 | 6 / 6 | 0 / 6 | 0 / 6 | 993.25 | 1047.5 | 1110.75 | 23.4 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 827.25 | 896.5 | 923.75 | 12.0 |
| | Total | 12 / 18 | 3 / 18 | 3 / 18 | 181.625 | 849.5 | 993.75 | 60.9 |
| RSV A (M) / 500 | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 2844 | 3179.5 | 3359.375 | 13.81 |
| | Site 2 | 2 / 6 | 1 / 6 | 3 / 6 | 136.375 | 179.5 | 286.75 | 68.43 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 6381.25 | 6623.3 | 6934.25 | 7.90 |
| | Total | 14 / 18 | 1 / 18 | 3 / 18 | 346.625 | 3179.5 | 6273.75 | 81.6 |

Note: There was 1 co-infected Adeno / RSV specimen detected in the clinical study.

Poor detection of this strain of Adenovirus is expected in dual infections. If RSV A is present in low levels in clinical specimens, it may not be detected by RVP in the presence of a high level of Adenovirus.

Summary of Calls in a Adeno (M) / RSV A (H) Simulated Samples:

| Virus (Titer) / TCID ₅₀ per reaction | 6 replicates prepared from each extract (1 extract per method) | | | | | | | |
|---|--|----------------|---------------|---------------|---------------------------------|---------------|---------------------------------|-------------|
| | Site | # Positive | # Equivocal | # Negative | 25 th Percentile MFI | Median MFI | 75 th Percentile MFI | CV |
| Adeno (M) / 5000 | Site 1 | 2 / 6 | 1 / 6 | 3 / 6 | 125.375 | 139.25 | 390.875 | 109.8 |
| | Site 2 | 6 / 6 | 0 / 6 | 0 / 6 | 747.375 | 860 | 920.5 | 18.9 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 619 | 683.5 | 884.5 | 26.3 |
| | Total | 14 / 18 | 1 / 18 | 3 / 18 | 479.125 | 683.5 | 920.5 | 51.3 |
| RSV A (H) / 500,000 | Site 1 | 6 / 6 | 0 / 6 | 0 / 6 | 3459.5 | 3588 | 5930.5 | 44.85 |
| | Site 2 | 6 / 6 | 0 / 6 | 0 / 6 | 6091.25 | 6393.5 | 6645.5 | 7.57 |
| | Site 3 | 6 / 6 | 0 / 6 | 0 / 6 | 5543.5 | 6159 | 6630.5 | 14.14 |
| | Total | 18 / 18 | 0 / 18 | 0 / 18 | 5268.75 | 6161.5 | 6663.75 | 25.3 |

Note: There was 1 co-infected Adeno / RSV specimen detected in the clinical study. Poor detection of this strain of adenovirus is expected in dual infections.

Additionally, a single site evaluation of precision carried out using plasmid controls established the baseline variability in the xTAG™ RVP assay (RT-PCR, TSPE, Data Acquisition, Data Analysis). The study involved a total of 21 runs carried out over the span of 22 days and tested variability across ancillary reagents, instruments (3 thermal cyclers and 3 Luminex instruments), and 3 lots of xTAG™ RVP kits. The overall percentage of correct calls observed across samples representing all viral types and subtypes probed by the assay was 100%.

b. Linearity/assay reportable range:

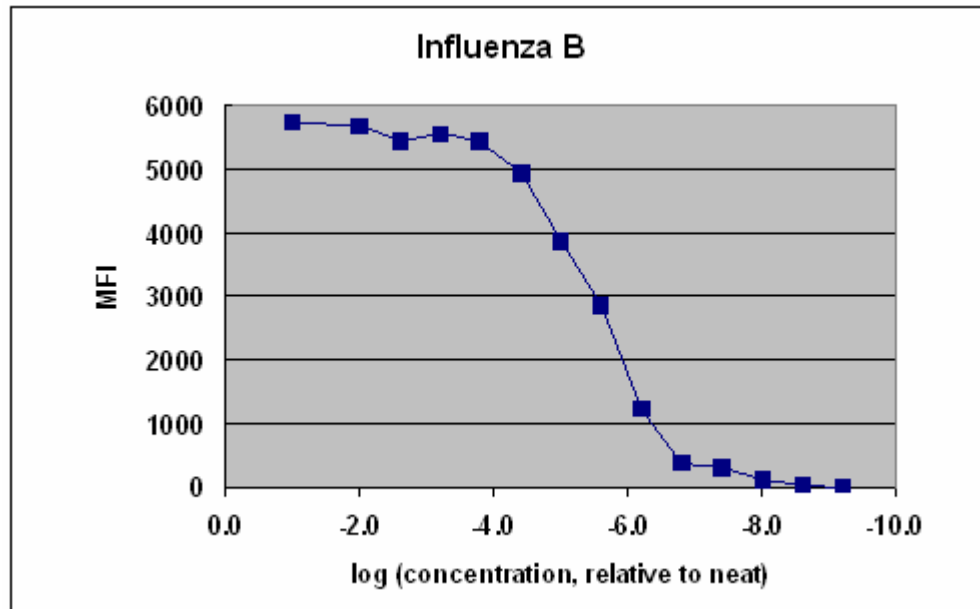
Two types of studies (analytical and clinical) were used to test for the existence of a Hook effect, in which the signal is quenched at very high input concentrations of analyte.

Clinical Data. Since viral loads in pediatric patients are generally higher than in adult patients, any clinically significant hook effect would be expected to produce a lower detection rate in the younger age group. This was not observed in a comparison of RVP detection rates in specimens obtained from pediatric patients (0-5 yrs) compared with those obtained from adult patients (18+ years). RVP correctly identified 346/374 (92.5%) of the

claimed analytes in pediatric specimens compared to 235/244 (96.3%) in adult specimens. Signal (MFI) distributions were also similar in these 2 populations.

Analytical Data. In the LoD study, a sigmoidal “system response curve” was obtained, when the observed MFI was plotted as a function of input concentration of virus. A typical curve is shown in the Figure below. To generate this curve, a dilution series was prepared, starting with the neat (undiluted) stock, and diluting by successive factors of 4x, down to a lower limit of 6×10^{-10} from the neat / starting concentration (100 TCID₅₀/μl). For example, in this system response curve for Influenza B, a plateau effect is observed as the input amount of analyte is increased. However, even at the highest tested input amounts, the signal was not significantly quenched, indicating the absence of a Hook effect. Specifically, for the analytes which showed a decline in a signal due to high viral loads, the MFI values at these loads were 10 times greater than the clinical cut-off.

Typical System Response Curve:



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

Calibrators

Before using the Luminex® System to read any samples prepared by the xTAG™ assay, the Luminex® system must be prepared and calibrated following the procedures described in the Luminex® User Manual.

Assay Controls

Quality Control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and a laboratory's standard quality control procedures. It is recommended that the user refer to CLSI document C24-A2.

Negative amplification/detection control: It is required that three negative controls be included with each run of xTAG™ RVP: one at the first plate position, one near the middle of the plate, and one at the last plate position. The software uses the DNAase,

RNAase-free water negative control in the last plate position to determine background signal levels. Signal > 150 MFI units in any of the three negative controls, for one or more analyte targets, is indicative of carryover contamination of the plate, and the samples on that plate should be rerun, starting from the PCR step. (Note: Separate areas should be assigned for pre- and post-PCR amplification as a precaution against carryover contamination.) For proper functioning of the assay, it is required to routinely include additional in-process controls in each assay. The following in-process controls are used and detected by XTAG™ RVP:

***E.coli* phage MS 2:** is an internal control added to each sample prior to extraction, to allow the user to ascertain whether the extraction and reverse-transcription steps of the assay are functioning correctly. Failure to generate a PRES (present) call for the MS 2 control indicates a failure at either the extraction step or reverse-transcription step, and may be indicative of the presence of amplification inhibitors which could lead to false negative results.

Bacteriophage Lambda DNA: a PCR/TSPE control (run control) to be included as a separate control sample during the RT-PCR set-up.

External Controls: Matrix negative controls and external positive controls representing viruses probed by the xTAG™ RVP should be included in routine laboratory control procedures in accordance with local, state, and/or federal regulations or accreditation requirements and laboratory's standard quality control procedures. Known strains of the targeted viruses should be included in routine quality control procedures. Analyte positive and negative controls should be included with each batch of patient specimens and should be prepared, extracted and tested in the same manner as these samples. Results from analyte controls should be examined before the results from the patient samples. If a given analyte positive or negative control does not perform as expected, all results for that analyte in the batch of samples are invalid and samples must be re-run.

Stability: The shelf-life of xTAG™ RVP kit is 12 months when the kit reagents are stored at -25°C to -15°C.

Formal evaluations have shown that the RVP assay performs as intended with purified nucleic acid stored for up to 96 hours at -70°C to -80°C and thawed on ice to room temperature just prior to testing by RVP. When working with purified RNA samples, standard precautions to minimize RNA degradation should be used.

d. Detection limit:

Limit of Blank (LoB) - Simulated samples that were positive for individual analytes (viral targets) were prepared from the materials listed in the footer of the Table below. By design, a simulated sample containing one of the analytes was formulated to *not* contain any of the other analytes. There was no detectable “crosstalk” or interference between detection of the different analytes. The limit of blank of the xTAG™ RVP assay was determined for each claimed analyte, through analysis of a large number (N = 431 to 480) of simulated samples which were negative for that analyte (although positive for other analytes). The LoB determinations are described in Table below. Columns 2, 3 of this Table give the 95th percentile of the distribution of the MFI values for each claimed analyte.

Limit of Blank (LoB) for Viral Targets detected by RVP:

| Analyte | LoB | |
|-------------------------------|-------------------------------------|-----|
| | MFI at 95 th percentile) | N |
| Flu A, Matrix gene | 52 | 431 |
| Flu A-H1, Hemagglutinin gene | 82 | 480 |
| Flu A- H3, Hemagglutinin gene | 108 | 480 |
| Influenza B | 56 | 480 |
| RSV-A | 54 | 481 |
| RSV-B | 53 | 480 |
| HMPV | 54 | 480 |
| PARA-1 | 50 | 431 |
| PARA-2 | 62 | 456 |
| PARA-3 | 50 | 479 |
| Adenovirus | 52 | 480 |
| Rhinovirus | 53 | 455 |

Simulated samples used in the determination of LoB were constructed from the following materials: Flu A-H1 (strain A/WS/33 (H1N1), ATCC VR-1520); Flu A- H3 (in-house strain, similar to: A/swine/Ontario/00130/97(H3N2)); Flu B (strain B/Malaysia/2506/040; RSV-A (ATCC VR-26); RSV-B (strain B WV/14617/85 (B-1 wild type), ATCC VR-1400); HMPV (CAN97-83); PARA-1 (strain 35, ATCC VR-1380); PARA-2 (strain Greer, ATCC VR-1381); PARA-3 (strain C243, ATCC VR-93); Adenovirus (Type 5, strain Adenoid 75, ATCC VR-5); Rhinovirus (Type 39, strain 209, ATCC VR-340).

Limit of Detection (LoD) – The LoD was evaluated using samples prepared from regrown and retitered viral reference strains, which are listed in column 2 of the Table below. Serial dilutions of each viral strain (corresponding to a single analyte) were prepared in a simulated clinical matrix. Note that specimens used for these LoD determinations were different from those used for the LoB determination described above. For each reference strain (analyte target), Column 4 gives the LoD in TCID₅₀ /mL that produces the MFI value listed in Column 5.

Limit of Detection (LoD) for Viral Targets detected by RVP:

| Analyte Target | | | RVP Detection Above the Clinical Cut-off (MFI = 300) | |
|----------------|--|--|--|-----------|
| Virus | Strain | Starting Titer (TCID ₅₀ per µL) | TCID ₅₀ /mL (LoD) | MFI (LoD) |
| Flu A | A/PR/8/34 | 5000 | 8x10 ⁻¹ | 662 |
| Flu A-H1 | A/PR/8/34 | 5000 | 3 | 982 |
| Flu A | A/Victoria/3/75 | 50,000 | 1x10 ² | 1203 |
| Flu A- H3 | A/Victoria/3/75 | 50,000 | 8x10 ³ | 891 |
| Influenza B | B/Malaysia/2506/04 | 100 | 6x10 ⁻² | 1225 |
| RSV-A | A2 | 1000 | 6x10 ² | 1139.5 |
| RSV-B | B WW/14617/ ' 85 [B-1 wild type] | 10 | 6 | 903 |
| hMPV | CAN97-83 (group 1B) | 50 | 1x10 ⁻¹ | 689 |
| PARA-1 | C-35 | 500,000 | 1x10 ³ | 450 |
| PARA-2 | Greer | 500,000 | 3x10 ² | 929 |
| PARA-3 | C-243 | 5,000 | 10 | 860 |
| Adenovirus | cultured patient isolate, species C | 1,000 | 40 | 643 |
| Rhinovirus | Type 54 | 50 | 3x10 ⁻² | 895 |

e. Analytical specificity:

Analytical specificity of the xTAG™ RVP was evaluated with respect to potential cross-reactivity with, or interference by, pathogens associated with respiratory tract infections that are not probed by the RVP assay. Additionally, analytical cross-reactivity was assessed using the number of the additional virus strains for each virus/analyte that is detected by RVP assay.

Cross-reactivity:

Cross-Reactivity Evaluation For Viruses detected by RVP. Simulated samples corresponding to each analyte target were prepared at a series of dilutions and tested in the RVP assay. There was no cross-reactivity observed at high multiples of the LoD, as shown in the Table below.

Test for Cross-Reactivity among RVP analytes:

| Analyte | Strain | Highest multiple of LoD showing no cross-reactivity with other claimed analytes |
|-------------|--|---|
| Flu A | A/PR/8/34 | 16,384 x |
| Flu A-H1 | A/PR/8/34 | 16,384 x |
| Flu A | A/Victoria/3/75 | 16,384 x |
| Flu A- H3 | A/Victoria/3/75 | 64 x |
| Influenza B | B/Malaysia/2506/04 | 262,144 x |
| RSV-A | A2 | 64 x |
| RSV-B | B WW/14617/ ' 85 [B-1 wild type] | 64 x |
| HMPV | CAN97-83 (group 1B) | 65,536 x |
| PARA-1 | C-35 | 4,096 x |
| PARA-2 | Greer | 16,384 x |
| PARA-3 | C-243 | 4,096 x |
| Adenovirus | cultured patient isolate, species C | 1,024 x |
| Rhinovirus | strain 54 | 65,536 x |

Cross-Reactivity with Enterovirus - Rhinoviruses and Enteroviruses are closely-related genera of the Picornaviridae family, small, non-enveloped ssRNA positive-strand viruses. Significant cross-reactivity between the rhinovirus-specific primers in the RVP assay and specific enterovirus strains was observed in both analytical and clinical evaluations. *Analytical evaluations* were carried out on a simulated specimen prepared by spiking a reference strain for enterovirus (Coxsackie virus B1 - ATCC VR- 28) into a background of human DNA. Nine separate extractions were performed and each of the 9 extracts was divided into 6 replicates run in the RVP assay (54 replicates of extracted nucleic acid in total). All replicates generated a positive call for Rhinovirus (negative for all other probed viruses). Evaluations on clinical samples are summarized in the Table below.

Cross-Reactivity with Enterovirus in Clinical Specimens:

| Human Enterovirus Class | RVP Detection of Reference Strain | Number of Clinical Specimens Containing Enterovirus* |
|-------------------------|--|--|
| Non-polio enterovirus A | Enterovirus-71 | 2 |
| Non-polio enterovirus B | Coxsackie virus B3, B4; echovirus 6,11 | 1 |
| Non-polio enterovirus D | Enterovirus-68 | 1 |
| Poliovirus | Poliovirus 1,2,3 | 0 |

*determined by sequencing pre-selected archived and prospectively collected specimens

Therefore, enterovirus strains 71 and 68, Coxsackie virus strains B3 and B4, echovirus 6 and 11, and poliovirus 1, 2 and 3 will cross-react in RVP assay, yielding a positive call for rhinovirus.

In the prospective *clinical study* of 554 nasopharyngeal swab (NPS) specimens collected at four different clinical sites in North America, RVP yielded 43 positive calls for rhinovirus, out of which 42 were identified as rhinovirus by a comparator assay, and one was identified as an enterovirus (Coxsackie A6, which is a member of the non-polio enterovirus A class).

Cross-Reactivity with Other Respiratory Viruses - Cross-reactivity with five respiratory viruses known to circulate with low frequency in the general population was assessed in analytical evaluations at 3 sites summarized in the Table below. In these evaluations, each analyte was spiked into a matrix of human DNA at three different concentrations (high, medium and low virus titer), and extracted in 9 separate extractions, resulting in 54 replicates for each of the 5 assessed viruses. Each of the 12 RVP outputs (corresponding to the 12 RVP intended use viruses) was assessed to determine if there was any significant cross-reactivity (54 replicates / sample x 12 RVP outputs / replicate = 648 RVP outputs / sample).

RVP Outputs on Simulated Samples Representing Rare Respiratory Viruses:

| Virus | Strain | Overall RVP Output (648 outputs per sample) |
|------------------|---|---|
| Parainfluenza 4 | Type 4 Strain M-25 ATCC VR-1378 | 647 / 648 negative calls** |
| Coronavirus HKU1 | Transcript (similar to HKU1 strain N18 genotype A (DQ415914)) | 647 / 648 negative calls*** |
| Coronavirus 229E | HcoV Strain 229E ATCC VR-740 | 648 / 648 negative calls |
| Coronavirus OC43 | HcoV Strain OC43 ATCC VR-1558 | 648 / 648 negative calls |
| Coronavirus NL63 | HcoV Strain NL63 In-House | 642 / 648 negative calls* |

*Contamination of 1 sample during the pre-analytical step – contaminant reproducibly detected in all six replicates prepared from total extracted nucleic acid as either equivocal or low positive result just above the cutoff.

**One of the 648 RVP outputs assessed for the Parainfluenza 4 sample resulted in an equivocal call for Influenza B (MFI = 197).

***One of the 648 outputs assessed for the Coronavirus HKU1 sample resulted in an equivocal call for Influenza A (MFI = 150).

Cross-Reactivity with Other Bacteria & Viruses - A total of 20 bacteria and 7 additional viruses that are not targets of the RVP assay were assessed for cross-reactivity with the RVP assay. These were chosen on the basis of (1) being causative agents of respiratory infections which are not targeted by the xTAG™ RVP, and (2) being reported in the

scientific literature as co-infecting species, with the viral agents targeted by the xTAG™ RVP.

Viral cultures were regrown in the appropriate cell host, retitered, and prepared to a titer of approximately 1,000xTCID₅₀. For Bocavirus, a high-titer patient sample was used instead of viral culture. Bacterial culture stocks were grown and extractions were performed on dilutions of stock cultures at densities of approximately 1.5 x 10⁶ bacteria/mL. Pathogens assessed as potential cross-reactive species in the RVP assay are listed in the Table below:

| Pathogen |
|---|
| <i>Bordetella pertussis</i> |
| <i>Chlamydia pneumoniae</i> |
| <i>Haemophilus influenzae</i> |
| <i>Pseudomonas aeruginosa</i> |
| <i>Streptococcus pneumoniae</i> |
| <i>Moraxella catarrhalis</i> |
| <i>Mycobacterium intracellulare</i> |
| <i>Mycoplasma bovis</i> |
| <i>Mycoplasma pneumoniae</i> |
| <i>Klebsiella pneumoniae</i> |
| <i>Legionella pneumophila</i> |
| <i>Neisseria meningitidis</i> |
| <i>Staphylococcus aureus</i> |
| <i>Staphylococcus epidermis</i> |
| <i>Streptococcus Agalactiae Group B</i> |
| <i>Acinetobacter baumannii</i> |
| <i>Streptococcus pyogenes</i> |
| <i>Mycobacterium avium</i> |
| <i>Serratia marcescens</i> |
| <i>Escherichia coli</i> |
| Herpes simplex virus Type 1 |
| Cytomegalovirus |
| Varicella-zoster virus |
| Mumps |
| Easles |
| Epstein Barr virus |
| Human Bocavirus* |

*One false positive call by RVP was made with Bocavirus as false positive for rhinovirus.

The RVP assay did not cross-react with 26 out of 27 bacterial and viral species that were tested. Further analysis by real-time PCR confirmed the RVP result by demonstrating that the stock of Bocavirus used in cross-reactivity study was contaminated or co-infected with rhinovirus.

Additional supplemental studies were performed with four *E. coli* strains (ATCC # 25922, #35340, #35150 and K12 strain MG1655) that were tested at a concentration of 1x10⁶ cfu/ml. The RVP assay did not return any positive calls for viral targets probed by the RVP assay indicating that *E. coli* does not cross-react with any RVP targets. However, the RVP assay yielded a positive call for Lambda bacteriophage internal control in one of the 4 *E. coli* strains tested (#35150), indicating this strain may be infected with lambdoid phages.

Analytical reactivity:

Influenza A

Sixty-one (61) different Influenza A reference strains were tested in the analytical

reactivity study at medium range concentrations (approximately 20xLoD for subtype H1 strains, and approximately 10xLoD for subtype H3 strains). Results of the testing are given in the Tables below.

RVP assay results on Influenza-A strains, subtype H1:

| Strain ID | Strain | Flu A call | H1 call | H3 call |
|--------------------------|--------|------------|---------|---------|
| A/New Caledonia/20/99 | H1N1 | POS | POS | NEG |
| A/swine/Ontario/52156/03 | H1N2 | POS | POS | NEG |

Concentrations were estimated as multiples of the LoD concentration, based on the LoD obtained for strain A/PR/8/34_H1N1 in the LoD study.

RVP assay results on Influenza-A strains, subtype H3:

| Strain ID | Strain | Flu A call | H1 call | H3 call |
|--|--------|------------|---------|---------|
| A/Aichi/174/2005 | H3N2 | POS | NEG | POS |
| A/Christchurch/90/2004 | H3N2 | POS | NEG | POS |
| A/Italy/384/2005 | H3N2 | POS | NEG | POS |
| A/Japan/1383/2005 | H3N2 | POS | NEG | POS |
| A/New York/401/2001 | H3N2 | POS | NEG | POS |
| A/New York/402/2001 | H3N2 | POS | NEG | POS |
| A/New York/403/2002 | H3N2 | POS | NEG | POS |
| A/New York/404/2002 | H3N2 | POS | NEG | POS |
| A/New York/405/2002 | H3N2 | POS | NEG | POS |
| A/New York/392/2004 | H3N2 | POS | NEG | POS |
| A/New York/206/2005 | H3N2 | POS | NEG | POS |
| A/New York/243/2005 | H3N2 | POS | NEG | POS |
| A/New York/376/2005 | H3N2 | POS | NEG | POS |
| A/New York/258/2005 | H3N2 | POS | NEG | POS |
| A/New York/384/2005 | H3N2 | POS | NEG | POS |
| A/New York/469/2004 | H3N2 | POS | NEG | POS |
| A/New York/464/2005 | H3N2 | POS | NEG | POS |
| A/Ontario/00130/97 | H3N2 | POS | NEG | POS |
| A/Taiwan/0149/00 | H3N2 | POS | NEG | POS |
| A/Wisconsin/67/2005 | H3N2 | POS | NEG | POS |
| A/Wyoming/3/03 | H3N2 | POS | NEG | POS |
| A/Zhejiang/209/2005 | H3N2 | POS | NEG | POS |
| similar to: A/swine/Ontario/00130/97* | H3N2 | POS | NEG | POS |

*spontaneous passage from human to swine, Ontario 1997

Concentrations were estimated as multiples of the LoD concentration, based on the LoD obtained for strain A/Victoria/3/75_H3N2 in the LoD study.

Additionally, a number of avian Influenza A strains were tested in the analytical reactivity study and yielded positive Influenza A results using RVP at the concentrations tested (approximately 25 x LoD):

| Strain ID | Strain | RVP Flu A call | RVP H1 call | RVP H3 call |
|------------------------------|--------|----------------------|-------------------|-------------------|
| A/Hongkong/156/97 | H5N1 | POS | NEG | NEG |
| A/Hongkong/483/97 | H5N1 | POS | NEG | NEG |
| A/Hongkong/486/97 | H5N1 | POS | NEG | NEG |
| A/Vietnam/1194/04 | H5N1 | POS | NEG | NEG |
| A/Vietnam/1203/04 | H5N1 | POS | NEG | NEG |
| A/Vietnam/1204/04 | H5N1 | POS | NEG | NEG |
| A/Vietnam/3212/04 | H5N1 | POS | NEG | NEG |
| A/Vietnam/3218/04 | H5N1 | POS | NEG | NEG |
| A/Turkey/15/2006 | H5N1 | POS | NEG | NEG |
| A/turkey/Turkey/1/05 | H5N1 | POS | NEG | NEG |
| A/chicken/Egypt/03/06_ | H5N1 | POS | NEG | NEG |
| A/swan/Germany/R651/2006 | H5N1 | POS | NEG | NEG |
| A/chicken/Nigeria/BA209/2006 | H5N1 | POS | NEG | NEG |
| A/chicken/Nigeria/BA210/2006 | H5N1 | POS | NEG | NEG |
| A/chicken/Nigeria/BA211/2006 | H5N1 | POS | NEG | NEG |
| A/chicken/Nigeria/SO300/2006 | H5N1 | POS | NEG | NEG |
| A/chicken/Nigeria/SO452/2006 | H5N1 | POS | NEG | NEG |
| A/chicken/Nigeria/SO493/2006 | H5N1 | POS | NEG | NEG |
| A/chicken/Nigeria/SO494/2006 | H5N1 | POS | NEG | NEG |

The first 9 strains summarized in the Table above were obtained from culture derived isolates from human specimens.

NOTE: Although the RVP assay has been shown to detect cultured avian influenza viruses, including avian Influenza A subtype H5N1 virus, the performance characteristics of this test with specimens from humans infected with H5N1 or other avian influenza viruses are unknown.

The following Influenza A strains in the Table below gave equivocal or negative Influenza A results in the analytical reactivity study when tested at the medium range concentrations:

| Strain ID | Subtype | Flu A call | H1 call | H3 call |
|------------------------|---------|------------|---------|---------|
| A/swine/Iowa/1976/1931 | H1N2 | POS | NEG | NEG |
| A/Jiangsu/76/2004 | H3N2 | POS | NEG | EQUIV |

‡ Concentration estimated as 16x LoD concentration, based on the LoD obtained for strain A/PR/8/34_H1N1 in the LoD study.

‡‡ Concentration estimated as 10x LoD concentration, based on the LoD obtained for strain A/Victoria/3/75_H3N2 in the LoD study.

Clinical data: In addition, a total of 71 prospectively collected Influenza A samples (66 positive for H3 subtype and 5 positive for H1 subtype) were bi-directionally sequenced and subjected to BLAST and phylogenetic analysis. These clinical Influenza A (H1) samples were found to be closely related to strains of Influenza A (H1) that were in worldwide circulation during the 2004-2006 period (for example, A/Florida/4/2004; A/New Jersey/10/2005; A/Washington/1/2006). The clinical Influenza A (H3) samples were found to be most closely related to strains of Flu A (H3) that were in worldwide circulation during the 2004-2006 period (for example, A/Aichi/211/2006; A/Cambodia/9/2005; A/California/7/2004).

Influenza B

Analytical data: Four reference strains of Influenza B were examined in an analytical reactivity study, and tested RVP-positive. The B/Malaysia/2506/04 strain was tested and gave positive RVP result at the LoD concentration as well as higher tested concentrations. B/Ohio/01/2005, B/Jiangsu/10/03, and B/Hong Kong/330/2001 were tested at medium range concentrations (approximately 10xLoD, based on the LoD obtained for strain B/Malaysia/2506/04 in the LoD study):

| Strain ID | Flu B call |
|----------------------|------------|
| B/Ohio/01/2005 | POS |
| B/Jiangsu/10/03 | POS |
| B/Hong Kong/330/2001 | POS |
| B/Malaysia/2506/04 | POS |

Clinical data: In addition, seven clinical Influenza B samples, obtained through prospective sample testing, were subjected to bidirectional sequencing and phylogenetic analysis. These clinical specimens were found to be most closely related to the B/Memphis/12/97 strain of Influenza B.

Rhinovirus

Thirty-six (36) specimens from the clinical multi-site study that tested rhinovirus-positive with the RVP assay were bi-directionally sequenced in the 5'-UTR (untranslated region) and subjected to phylogenetic analysis. The phylogenetic analysis resolved the rhinovirus genus into six phylogenetic groups (letters A-F). At least one RVP-positive rhinovirus sample was found in each group except for group D:

| Clinical Sample ID | Phylogenetic Group | Clinical Sample ID | Phylogenetic Group |
|--------------------|--------------------|--------------------|--------------------|
| 02-280 | A | 06-108 | E |
| 02-412 | A | 06-64 | E |
| 02-419 | A | 02-446 | E |
| 06-109 | A | 01-206 | E |
| 02-400 | A | 01-189 | E |
| 01-232 | A | 02-292 | F |
| 02-416 | A | 01-208 | F |
| 01-09 | A | 06-48 | F |
| 02-443 | A | 06-53 | F |
| 07-114 | A | 01-252 | F |
| 06-184 | A | 01-08 | F |
| 02-340 | B | 02-437 | F |
| 02-424 | C | 06-166 | F |
| 07-060 | C | 06-173 | F |
| 02-307 | E | 07-53 | F |
| 02-324 | E | 02-326 | F |
| 06-39 | E | 07-125 | F |
| 06-89 | E | 07-58 | F |

Respiratory Syncytial Virus

Analytical data - RSV-A strain AUS/A2/61 (Australia, 1961; Genbank M11486), and RSV-B strain B WV/14617/ 85 [B-1 wild type] tested RVP-positive at the assay LoD concentration as well as at higher concentrations tested.

Clinical data - One hundred and thirty-eight (138) RSV sequences were recovered from clinical specimens. These sequences were represented by eight distinct “parent sequences” which, in phylogenetic analysis, clustered most closely with the AUS/A2/61 reference strain.

Human Metapneumovirus

Analytical data - Five reference strains representing all four known phylogenetic groups of hMPV were tested, including two reference strains from group 1A, and one reference strain from each of groups 1B, 2A, and 2B. Strains 1A and 1B were tested in concentrations close to the LoD, while 2A and 2B were tested at medium range concentrations. All were found to test positive by the RVP assay:

| Phylogenetic Group | Strain ID |
|--------------------|-------------|
| 1A | RS.MPV05-12 |
| 1A | hMPV 9 |
| 1B | RS.MPV05-02 |
| 2A | hMPV 14 |
| 2B | hMPV 8 |

Clinical data - Twenty-nine (29) hMPV positive clinical specimens from either a prospective clinical study, or from retrospective known hMPV positive specimens were sequenced using bidirectional sequencing. All of these 29 hMPV specimens were collected during the 2005-2006 flu season at North American and European sites. Results show that the hMPVs detected in the clinical specimens using RVP belong to phylogenetic groups 1B, 2A, and 2B.

Adenovirus

Clinical Sample Data - Adenovirus species B, C, D, and E were detected in clinical samples which were RVP-positive for adenovirus. Clinical samples that were RVP-positive for adenovirus were subjected to PCR and bidirectional sequencing. Sequence data was recovered from the N-terminal portion of the adenovirus hexon gene. Blast analysis indicates that the following adenovirus serotypes were detected by RVP in these clinical samples:

- One or more of serotypes #3, 7, 16 (comprising a Species B subgroup)
- One or more of serotypes #14, 21, 50 (comprising another Species B subgroup)
- One or more of serotypes #1, 2, 5, 6 (comprising Species C)
- One or more of serotypes #19, 26, 48, 49, 51 (comprising a Species D subgroup)
- Serotype 4 (the only member of Species E).

In the clinical samples that were positive in a comparator assay, the low end of the MFI distribution for species C adenovirus was observed to fall below the RVP clinical cutoff (MFI=300) for a positive call (Table below). Furthermore, the frequency with which comparator-positive clinical samples generated an MFI below this cutoff was higher for species C compared to what was observed for species B, D and E. Therefore, the RVP does not detect adenovirus C species when present in concentrations close to the assay cutoff.

RVP Detection of Different Species of Adenovirus in Clinical Samples:

| Adenovirus Species | No. of Clinical samples | No. of MFI Determinations | MFI Range | Median MFI | % of determinations with MFI < 300. |
|---------------------------|--------------------------------|----------------------------------|------------------|-------------------|---|
| B | 25 | 27 | 71 - 4191 | 962 | 1/27 = 3.7 % |
| C | 26 | 27 | 40.5 - 1969 | 674 | 5/27 = 18.5 % |
| D | 3 | 3 | 755.5 - 974 | 764 | 0/3 = 0 % |
| E | 1 | 1 | 1035 - 1035 | 1035 | 0/1 = 0 % |

Analytical Data – Analytical testing was performed on 27 reference strains of human adenovirus, distributed across 16 serotypes. All six species (A, B, C, D, E, F) of human adenovirus were represented. Ten of the 16 serotypes have been implicated in respiratory infections in non-immunosuppressed patients. Sample concentrations used were representative of a low positive sample at the LoD for adenovirus (see above). Results are summarized below:

| Species | Serotype | Source | Strain ID | MFI at Adenovirus estimated LoD |
|---------|----------|--|--------------|---------------------------------|
| A | 31 | UI-CPH*** | Ad31 | 330-1530* |
| B | 3 | UI-CPH | Ad3 | 680 |
| | 7a | Zeptomatrix | Adeno 7a | 270 |
| | 7d2 | UI-CPH | Ad7d2-1 | 1310 |
| | 7d2 | UI-CPH | Ad7d2-2 | 1680 |
| | 7d2 | UI-CPH | Ad7d2-3 | 1590 |
| | 7h1 | UI-CPH | Ad7h1 | 760 |
| | 11 | UC-CPH | Ad11 | 1400 |
| | 14 | UI-CPH | Ad14 | 3330 |
| | 35 | UI-CPH | Ad35 | 1880 |
| C | 1 | St. Joseph's Hospital, Hamilton, Ontario | lot # 290606 | 780 |
| | 1 | Zeptomatrix | Adeno 1 | 270 |
| | 1 | UI-CPH | Ad1 | 410 |
| | 2 | UI-CPH | Ad2 | 240 |
| | 5 | Advanced Biotechnologies Inc. | Adeno 5 | 70 |
| | 5 | QCMD**** | ADV05-04 | 850 |
| | 5 | UI-CPH | Ad5 | 100 |
| | 6 | UI-CPH | Ad6 | 240 |
| D | 19 | UI-CPH | Ad19 | 1340 |
| | 22 | UI-CPH | Ad22-1 | 1600 |
| | 22 | UI-CPH | Ad22-2 | 1590 |
| | 25 | UI-CPH | Ad25 | 1380 |
| | 45 | UI-CPH | Ad45 | 850 |
| E | 4 | QCMD | RE-01 | 3700** |
| | 4p3 | UI-CPH | Ad4p3 | 1750 |
| | 4a | UI-CPH | Ad4a | 2280 |
| F | 41 | UI-CPH | Ad41 | 330 |

*examined at 5 different concentrations spanning LoD, and over 100,000-fold input range.

**examined at a single concentration close to 10xLoD.

***UI-CPH – University of Iowa, College of Public Health.

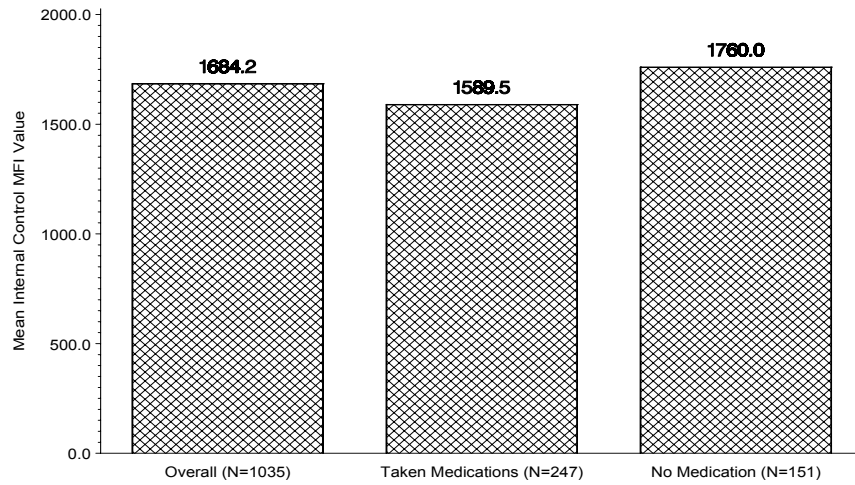
****QCMD - Quality Control for Molecular Diagnostics, Glasgow.

Analytical reactivity study results show that if present at LoD concentrations, the RVP assay does not detect adenovirus serotype 7a (species B), serotype 41 (species F), or species C serotypes 1, 2, 5, and 6.

Interference:

RT-PCR is potentially subject to the inhibition of reverse transcriptase or DNA polymerase activity by endogenous and/or exogenous interferents contained within the sample matrix. In nucleic acid based tests, it typically is necessary to extract and purify DNA/RNA prior to the RT and PCR amplification steps for efficient removal of these potential interferents. Extraction, co-amplification and detection of an internal control is useful for evaluating efficacy of nucleic acid extraction methods, and for estimating the effects of inhibitors or interferents on amplification in a nucleic acid based assay. The RVP assay has been validated using commercially available extraction methods and incorporates the co-amplification of a bacteriophage MS2 internal control. Any inhibitory effects of residual interferents in the total extracted nucleic acid preparation would result in a significant reduction in MFI values for the MS2 internal control. This was not seen when comparing the mean MFI for the MS2 control in 247 specimens

obtained from patients taking medications (mean MFI = 1589.5) with that in 151 specimens obtained from patients who were not medicated (mean MFI = 1760.0):



Virus and Bacteria –

A total of 16 combinations of test analytes and potential interferents were assessed for interference with RVP test results. The potential interferents were chosen on the basis of (1) being causative agents of respiratory infections, but not targeted by the xTAG™ RVP, and (2) being reported in the scientific literature as co-infecting pathogens, with the viral agents targeted by the xTAG™ RVP. After mixing each analyte and potential interferent, the resultant mixture was extracted and assayed by the RVP.

Potential Interferents: Potential bacterial interferents were grown in culture, and spiked into the individual test analytes at a final concentration of approximately 1.5×10^6 bacteria/mL. Cytomegalovirus was grown in culture, and spiked into the individual test analytes at a final concentration of approximately 10^4 TCID₅₀/mL. Bocavirus was sourced from a high titer patient specimen, and was spiked into the individual test analytes at a 1/100 dilution.

Test Analytes: RSV-B was prepared from viral culture (concentration approximately 100xLoD). High-titer patient samples were used to produce analyte-positive material for testing adenovirus, influenza A (H1), and rhinovirus. The concentrations of the test analytes that were assayed by RVP were as follows: RSV B: 30 x LoD; adenovirus: 400 x LoD; Flu A (H1): 70 x LoD; rhinovirus: 10 x LoD. A summary of results is provided in the Table below:

Test of Potentially Interfering Bacteria and Viruses on Function of RVP Assay:

| Analyte (Tested Dilution) | Source | Potential Interfering Bacterium or Virus (high titer) | Tested Interferent Concentration | Results |
|-----------------------------------|-------------------|---|--|-----------------|
| RSV B (30 x LoD) | viral culture | None | 0 | Target Present |
| | | <i>Haemophilus influenzae</i> | 1.5×10^6 bacteria/mL | No Interference |
| | | <i>Streptococcus pneumoniae</i> | 1.5×10^6 bacteria/mL | No Interference |
| | | <i>Bordetella pertussis</i> | 1.5×10^6 bacteria/mL | No Interference |
| | | Cytomegalovirus | 10^4 TCID ₅₀ /mL | No Interference |
| | | Human Bocavirus | 10^{-2} x neat | No Interference |
| Adenovirus (400 x LoD) | patient sample | None | 0 | Target Present |
| | | <i>Bordetella pertussis</i> | 1.5×10^6 bacteria/mL | No Interference |
| | | Cytomegalovirus | 10^4 TCID ₅₀ /mL | No Interference |
| | | <i>Chlamydia pneumoniae</i> | 1.5×10^6 bacteria/mL | No Interference |
| | | Human Bocavirus | 10^{-2} x neat | No Interference |
| Flu A H1 (70 x LoD) | patient sample | None | 0 | Targets Present |
| | | <i>Streptococcus pneumoniae</i> | 1.5×10^6 bacteria/mL | No Interference |
| | | <i>Staphylococcus aureus</i> | 1.5×10^6 bacteria/mL | No Interference |
| | | <i>Bordetella pertussis</i> | 1.5×10^6 bacteria/mL | No Interference |
| | | <i>Chlamydia pneumoniae</i> | 1.5×10^6 bacteria/mL | No Interference |
| Rhinovirus (10 x LoD) | patient sample | None | 0 | Target Present |
| | | <i>Streptococcus pneumoniae</i> | 1.5×10^6 bacteria/mL | No Interference |
| | | <i>Mycoplasma pneumoniae</i> | 1.5×10^6 bacteria/mL | No Interference |
| | | <i>Haemophilus influenzae</i> | 1.5×10^6 bacteria/mL | No Interference |

Analysis of Clinical Dataset for Effects of Potential Interfering Medications -

Note: No interference study was performed by spiking known concentrations of potentially interfering substances (e.g. cold medications, FluMist vaccine, blood, etc) into the sample matrix containing the assay analytes.

Data analysis of a sub-population of patients from the prospective clinical study prescribed various medications (i.e. anti-bacterials, anti-virals, steroids, common cold medications) showed similar sensitivity per target compared to the population not receiving medication. Clinically significant interference by medications would result in lower RVP detection rates in specimens obtained from medicated patients compared with

non-medicated patients. This was not observed in a comparison of RVP detection rates in specimens collected from medicated patients vs. those collected from patients not receiving medications. Although the concentration of the interferents in the total extracted nucleic acid preparation is unknown, the results represent doses that are typically prescribed to the intended use population. RVP correctly identified 156/162 (96.3%) of the claimed analytes in the population receiving medication and 87/88 (98.9%) in the population not receiving medications. Signal (MFI) distributions for these analytes and for the MS2 internal control were also similar. A complete list of medications recorded in patient charts extracted from the clinical dataset is presented in the Table below.

Medications Administered: Patient Populations Included in the Prospective Dataset

| Generic Medication List | | | |
|--|--------------------------|-----------------------------|-------------------------------|
| Acetaminophen | Chlorpheniramine maleate | Glimepiride | Prednisolone Sodium Phosphate |
| Acetylcysteine | Ciprofloxacin | Guaifenesin | Prednisone |
| Acyclovir | Clarithromycin | Homatropine Methylbromide | Pseudoephedrine |
| Albuterol | Clindamycin | Hydrocodone Bitartrate | Pyrazinamide |
| Albuterol Sulfate | Clopidogrel Bisulfate | Hydromorphone Hydrochloride | Racemic epinephrine |
| Aluminium hydroxide, light magnesium carbonate | Cloxacillin | Hydroxyurea | Ranitidine Hcl |
| Amikacin | Codeine | Ibuprofen | Ribavirin |
| Amlodipine Besylate | Co-trimoxazole | Idarubicin | Rifampin |
| Amoxicillin | Dapsone | Imiglucerase | Risedronate Sodium |
| Amoxicillin Clavulanate | Deferasirox | Imipenem | Ritonavir |
| Amphotericin B | Didanosine | Immunoglobulin | Sucalfate |
| Ampicillin | Digoxin | Ipratropium Bromide | Sulfamethoxazole |
| Aspirin | Donepezil Hcl | Lamivudine | Tamsulosin Hydrochloride |
| Atenolol | Dopamine | Lerothyroxine | Tazobactam |
| Atorvastatin Calcium | Efavirenz | Levalbuterol | Tenofovir Disoproxil Fumarate |
| Atovaquone | Emtricitabine | Levofloxacin | Ticarcillin and Clavulanate |
| Azithromycin | Enalapril | Loperamide Hcl | Tiotropium Bromide |
| Aztreonam | Enfuvirtide | Loratadine | Tipranavir |
| Budesonide | Enoxaparin Sodium | Losartan | Tobramycin |
| Caspofungin | Epinephrine | Meropenem | Tolterodine Tartrate |
| Cefazolin | Epoetin Alfa | Methylprednisolone | Trimethoprim |
| Cefdinir | Erythromycin | Metoprolol Succinate | Valsartan |
| Cefepime | Escitalopram Oxalate | Metronidazole | Vancomycin |
| Cefotaxime | Famotidine | Midazolam | Voriconazole |
| Cefotaximine | Fentanyl | Moxifloxacin | Warfarin |
| Cefprozil | Fexofenadine Hcl | Mycophenolate Mofetil | Zidovudine |
| Ceftazidime | Filgrastim | Nystatin | Zithromax |
| Ceftiaxone | Fluconazole | Ofloxacin | |
| Cefuroxime | Fluticasone Propionate | Ondansetron Hydrochloride | |
| Cefuroxime Axetil | Foscarnet | Oseltamivir | |
| Cephalexin | Furosemide | Pantoprazole | |
| Cetirizine | Ganciclovir | Phenylephrine HCl | |
| Cetotaxime | Gentamicin | Piperacillin | |

Note: Flu A H1 positive samples were found only in the medicated population.

Purified Human Genomic DNA – human cellular material might be present in clinical specimens and, consequently, human genomic DNA might be carried through the extraction procedure into the total extracted nucleic acid. To test for an interference effect, purified human genomic DNA (50 ng per reaction) was spiked into synthetic Positive Controls (mixtures of recombinant plasmids containing viral target sequences) and compared with un-spiked synthetic Positive Controls. The human genomic DNA (50 ng/reaction) did not interfere with identification of viral nucleic acids in these experiments.

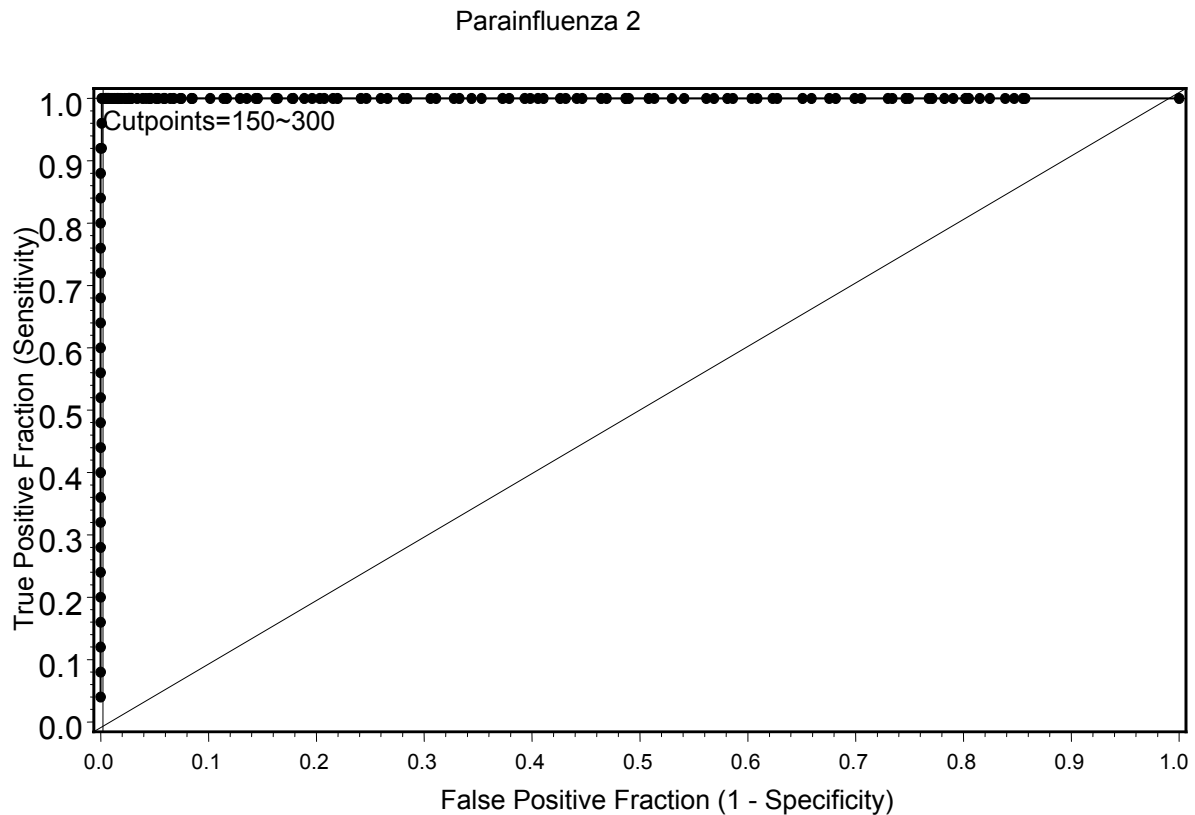
Matrix Effects - Matrix effects were evaluated by assaying synthetic Positive Controls (purified plasmids, phage lambda DNA, and purified MS2 RNA) and simulated clinical samples (cell culture lysates containing low titers of RSV-A or Flu-A H1, and spiked-in MS2 phage) in the presence of potential interferents (viral transport medium (VTM), and nasopharyngeal swabs (NPS)). These potential interferents at $\leq 5\%$ (v/v) exerted no inhibitory effect on the xTAG™ RVP run on purified plasmids. The assay of purified MS2 RNA was unaffected by $\leq 5\%$ (v/v) VTM or NPS. Detection of RSV-A, Flu-A H1, and the internal control (MS2) from extracts of simulated samples was severely inhibited in the presence of $\geq 0.1\%$ VTM and NPS but not in the presence of 0.02% of either of these potential interferents.

NOTE: the inhibition of the xTAG™ RVP by matrix effects is detectable as a decrease in MFI of the internal control (MS2). Thus, it is essential to spike the internal control into the sample before extraction.

Other Potential Interferents – Certain compounds (blood, IgG, hemoglobin, lactoferrin, anticoagulant activity, cryoglobulins), expected to be present in blood-containing specimens, have been reported in the scientific literature to potentially interfere with DNA polymerases. These substances might be found in respiratory specimens because of poor sample collection technique. Additionally, specimens from patients on certain medications might contain low concentrations of compounds inhibitory to the PCR reactions (e.g., Acyclovir and possibly related compounds) or to reverse transcriptase (e.g., Zidovudine and other chain terminating nucleoside analogs). The interfering compounds should be removed by the nucleic acid extraction step, and any residual inhibitory effects are controlled for by the internal control (MS2 phage).

f. Assay cut-off:

The universal “Call zones” established in the RVP assay has been defined empirically using clinical specimens, and are as follows: Positive call ≥ 300 MFI, No Call 150-299 MFI, Negative < 150 MFI for each probed analyte. These “Call zones” provide diagnostically relevant sensitivity and specificity values for the claimed analytes as demonstrated by ROC curve analyses. A typical ROC curve is presented in the figure below:



2. Comparison studies:

a. *Method comparison with gold standard/reference method:*

See clinical studies (Section 3.).

Additionally, 164 clinical specimens (NP swabs) were pre-selected to supplement the prospective clinical dataset for analytes with low prevalence. These specimens were tested by RVP and comparator methods in the same manner described for the prospective data set in the clinical studies. Specimens were extracted and tested at 2 of the 4 clinical sites. Three (3) of these 164 pre-selected NP swabs were co-infected with Rhinovirus (1 Flu A/H1 and 2 Para 1 comparator positive samples).

Positive percent agreement (PPA) and negative percent agreement (NPA) in the pre-selected dataset are summarized in the Table below. Of the 164 pre-selected specimens, there were 147 NP swabs used to supplement H1, Parainfluenza 1, Parainfluenza 2 and Parainfluenza 3. Of these 147 specimens, 93 were from patients >5 yrs (these 93 were included in the RSV A and B testing in population > 5 years). There were an additional 17 samples that were used to supplement the Parainfluenza 2 calculations. In total, there were 164 specimens (147 + 17) in this dataset.

RVP performance in pre-selected banked clinical specimens (n=164 NP swabs):

| Virus (Analyte) | PPA | 95% CI | NPA | 95% CI |
|------------------------|------------------|---------------|--------------------|---------------|
| H1 subtype of Flu A | 16/16 (100%) | 79.4% - 100% | 131 /131 (100%) | 97.2% - 100% |
| RSV A (> 5yrs) | 17/18 (94.4%) | 72.7% - 99.9% | 75/75 (100%) | 95.2% - 100% |
| RSV B (> 5yrs) | 14/14 (100%) | 76.8% - 100% | 78/79 (98.7%) | 93.1% -100% |
| Para1 | 22/22 (100%) | 84.6% - 100% | 123/125 (98.4%) | 94.3% - 99.8% |
| Para2 | 20/20 (100%) | 83.2% - 100% | 144/144 (100%) | 97.5% - 100% |
| Para3 | 36/38 (94.7%) | 82.3% - 99.4% | 109/109 (100%) | 96.7% - 100% |

Performance in Fresh vs Frozen Clinical Specimens:

A set of 163 human pediatric NP swabs were collected and tested at a 5th clinical site (i.e. a site not enrolled in the prospective clinical multi-center study described in clinical studies). These 163 specimens were tested by RVP in the fresh state and then frozen in the un-extracted state at -70°C. Frozen samples were thawed and re-tested by RVP for the purpose of comparing performance of the assay on fresh vs. frozen specimens. Total nucleic acid was extracted, and the positive percent agreement between RVP results from fresh aliquots vs. frozen aliquots was calculated across all claimed analytes. The positive percent agreement between RVP results from fresh aliquots vs. frozen aliquots was 91.4% (95% confidence interval 87.2% - 94.5%), and the negative percent agreement was 99.7% (95% confidence interval 99.4% - 99.9%). Most of the discordant results occurred with samples where positive or negative results in the fresh aliquots were near the assay cut-offs (MFI = 300): 1/22 equivocal discordant positive samples and 2/7 equivocal discordant negative samples were observed.

b. Matrix comparison:

Not applicable

3. Clinical studies:

All clinical specimens were prospectively collected and tested during the 2005/06 influenza season at 4 North American clinical laboratories. All specimens were tested fresh by viral culture and/or DFA for the following targets: Influenza A, Influenza B, RSV, Parainfluenza 1, Parainfluenza 2, Parainfluenza 3 and Adenovirus. Well-characterized RT-PCR amplification followed by bidirectional sequencing was used as the comparator method for Influenza A subtyping, RSV subtyping, hMPV, and Rhinovirus. All amplification primers used in comparator methods targeted regions distinct from those targeted by RVP. In the case of hMPV, a composite analysis using well characterized viral culture, DFA, and RT-PCR amplification/sequencing results was used as a primary comparator method. In the case of Flu A subtyping, results from the CDC assay used as part of the surveillance program described in the MMWR notice and CSTE document described in “References” below were used as the comparator for 9 initially untypeable Flu A specimens.

A total of 544 prospectively collected nasopharyngeal (NP) swabs were analyzed fresh

using DFA and/or viral culture, and specimen remnants were frozen for nucleic acid testing, including RVP. 163 additional specimens were tested fresh by RVP in both the fresh and frozen state to establish equivalence of RVP results under these conditions. All specimens tested after freezing were stored at -70°C before being extracted and tested by RVP. Total extracted nucleic acid material was also stored at -70°C. All extracted material underwent a freeze/thaw cycle before being tested by RVP.

a. and b. Clinical Sensitivity and Specificity:

Clinical sensitivity and specificity of RVP in the 544 prospectively collected specimens:

| Virus (Analyte) | Sensitivity | | 95%CI for Sensitivity | Specificity | | 95% CI for Specificity |
|----------------------|--------------|---------|-----------------------|-------------|---------|------------------------|
| | TP / (TP+FN) | percent | | TN/ (TN+FP) | percent | |
| Human Influenza A | 81/84 | 96.4% | 89.9% - 99.3% | 441/460 | 95.9% | 93.6% - 97.5% |
| H1 subtype of Flu A* | 6/6 | 100% | 54.1% - 100% | 532/532 | 100% | 99.3% - 100% |
| H3 subtype of Flu A | 66/72 | 91.7% | 82.7% - 96.9% | 463/469 | 98.7% | 97.2% - 99.5% |
| Human Influenza B | 54/59 | 91.5% | 81.3% - 97.2% | 469/485 | 96.7% | 94.7% - 98.1% |
| RSV A | 23/23 | 100% | 85.2% - 100% | 501/509 | 98.4% | 96.9% - 99.3% |
| RSV B | 33/33 | 100% | 89.4% - 100% | 492/505 | 97.4% | 95.6% - 98.6% |
| Parainfluenza 1 | 3/3 | 100% | 29.2% - 100% | 540/541 | 99.8% | 99.0% - 100% |
| Parainfluenza 2 | 6/6 | 100% | 54.1% - 100% | 537/538 | 99.8% | 99.0% - 100% |
| Parainfluenza 3 | 16/19 | 84.2% | 60.4% - 96.6% | 523/525 | 99.6% | 98.6% - 100% |
| Rhinovirus | 42/42 | 100% | 91.6% - 100% | 168/184 | 91.3% | 86.3% - 95.0% |
| Adenovirus** | 18/23 | 78.3% | 56.3% - 92.5% | 520/520 | 100% | 99.3% - 100% |
| Metapneumovirus*** | 24/25 | 96.0% | 79.7% - 99.9% | 320/324 | 98.8% | 96.9 - 99.7% |

*Note that the prevalence of influenza subtypes for the 2005/06 season available at the CDC website indicates 8.1% of Influenza A cases were subtype H1, while 91.9% were subtype H3.

**As summarized in the analytical reactivity study, RVP results on reference strains and sequence analysis of clinical samples positive for adenovirus suggest that the low overall sensitivity for adenovirus observed in the clinical study is mainly due to poor detection of serotypes falling within the adenovirus C species.

***hMPV performance presented in the table is calculated against a composite comparator (culture and PCR followed by bidirectional sequencing). The performance was additionally calculated using a well characterized PCR followed by sequencing as a sole comparator method yielding a positive percent agreement of 100.0% (22/22, 95% CI 84.6%-100.0%) and a negative percent agreement of 98.2% (321/327, 95% CI 96.0%-99.3%).

Co-infections detected by RVP vs. comparator methods in prospective clinical specimens:

| Co-infections | True Positive | False positive | False Positive Target(s) | False Negative |
|----------------|---------------|----------------|--------------------------|----------------|
| Flu A / Rhino | 0 | 2 | Flu A (both samples)* | 0 |
| Flu B / Rhino | 1 | 0 | - | 0 |
| RSV A / Rhino | 1 | 0 | - | 0 |
| RSV B / Rhino | 1 | 2 | Rhino (both samples) | 0 |
| Para 1 / Rhino | 1 | 0 | - | 0 |
| Para 2 / Rhino | 1 | 0 | - | 0 |
| Para 3 / Rhino | 0 | 1 | Para 3 | 0 |
| Adeno/ Rhino | 2 | 1 | Rhino | 0 |
| Adeno/ RSV A | 1 | 0 | - | 0 |
| Flu A / RSV B | 0 | 2 | Flu A (both samples)* | 0 |
| Flu B / RSV A | 0 | 1 | RSV A | 0 |
| Flu A / hMPV | 0 | 1 | hMPV | 0 |
| Flu B / hMPV | 1 | 0 | - | 0 |
| RSV B / hMPV | 0 | 1 | hMPV | 0 |
| Flu A / Para 1 | 0 | 1 | Flu A* | 0 |

* False positive Flu A RVP results due to presence of contaminant in these five samples.

Demographic details for the prospective clinical study patient population are summarized in the Table below. RSV sensitivity in prospective specimens older than 5 years was not established.

| SEX | NUMBER OF SUBJECTS |
|------------------------|--------------------|
| Male | 264 (48.5%) |
| Female | 280 (51.5%) |
| Not Determined | 0 (0.0%) |
| AGE (yrs) | |
| 0 - 1 | 137 (25.2%) |
| >1 - 5 | 68 (12.5%) |
| >5 - 21 | 66 (12.1%) |
| >21 - 65 | 144 (26.5%) |
| >65 | 124 (22.8%) |
| Not Determined | 5 (0.9%) |
| SUBJECT STATUS | |
| Outpatients | 305 (56.1%) |
| Hospitalized | 120 (22.1%) |
| Emergency Department | 84 (15.4%) |
| Extended Care Facility | 28 (5.1%) |
| Not Determined | 7 (1.3%) |

- c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable.

4. Clinical cut-off:
Not applicable.

5. Expected values/Reference range:

The prevalence of individual viruses based on RVP results in the clinical study patient population (2005/2006 flu season):

| Age (yrs) | Flu A (matrix) | Flu A H1 | Flu A H3 | FluB | RSV A | RSV B | Para 1 | Para 2 | Para 3 | Rhino | Adeno | hMPV |
|--------------|----------------|----------|----------|-------|-------|-------|--------|--------|--------|-------|-------|------|
| 0-5 | 16 | 3 | 8 | 15 | 27 | 34 | 2 | 3 | 10 | 36 | 12 | 6 |
| >5-21 | 10 | 1 | 5 | 33 | 0 | 0 | 0 | 0 | 1 | 7 | 2 | 1 |
| >21-65 | 30 | 2 | 23 | 16 | 2 | 4 | 0 | 1 | 4 | 7 | 3 | 11 |
| >65 | 44 | 0 | 36 | 6 | 1 | 8 | 1 | 2 | 3 | 6 | 1 | 10 |
| Not Reported | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | 2 | 0 | 0 |
| All Ages | 100 | 6 | 72 | 70 | 31 | 46 | 4 | 7 | 18 | 58 | 18 | 28 |
| Prevalence | 18.4% | 1.1% | 13.2% | 12.9% | 5.7% | 8.5% | 0.7% | 1.3% | 3.3% | 10.7% | 3.3% | 5.1% |

The prevalence of co-infections based on RVP results in the clinical study patient population (2005/2006 flu season):

| Age (yrs) | Flu A / Rhino | Flu B / Rhino | RSV A / Rhino | RSV B / Rhino | Para 1 / Rhino | Para 2 / Rhino | Para 3 / Rhino | Adeno/ Rhino | Adeno/ RSV A |
|--------------|---------------|---------------|---------------|---------------|----------------|----------------|----------------|--------------|--------------|
| 0-5 | 1 | 0 | 0 | 2 | 0 | 1 | 1 | 2 | 0 |
| >5-21 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| >21-65 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| >65 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| Not Reported | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 |
| All Ages | 2 | 1 | 1 | 3 | 1 | 1 | 1 | 3 | 1 |
| Prevalence | 0.4% | 0.2% | 0.2% | 0.5% | 0.2% | 0.2% | 0.2% | 0.5% | 0.2% |

| Age (yrs) | Flu A / RSV B | Flu B / RSV A | Flu A / hMPV | Flu B / hMPV | RSV B / hMPV | Flu A / Para 1 |
|--------------|---------------|---------------|--------------|--------------|--------------|----------------|
| 0-5 | 2 | 1 | 0 | 0 | 1 | 0 |
| >5-21 | 0 | 0 | 0 | 1 | 0 | 1 |
| >21-65 | 0 | 0 | 1 | 0 | 0 | 0 |
| >65 | 0 | 0 | 0 | 0 | 0 | 0 |
| Not Reported | 0 | 0 | 0 | 0 | 0 | 0 |
| All Ages | 2 | 1 | 1 | 1 | 1 | 1 |
| Prevalence | 0.4% | 0.2% | 0.2% | 0.2% | 0.2% | 0.2% |

N. Proposed Labeling:

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

O. Conclusion:

The petition for Evaluation of Automatic Class III Designation for this device is accepted. The device is classified as Class II under regulation 21 CFR 866.3980 with special controls. The special control guidance documents "Class II Special Controls Guidance Document: Respiratory viral panel multiplex nucleic acid assay," "Class II Special Controls Guidance Document: Testing for Human Metapneumovirus (hMPV) using multiplex nucleic acid assays," and "Class II Special Controls Guidance Document: Testing for detection and differentiation of Influenza A virus subtypes using multiplex nucleic acid assays" will be available shortly.