

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

**A. 510(k) Number:**

K071591

**B. Purpose for Submission:**

This is an application for a qualitative immunochromatographic assay used with the RAMP<sup>®</sup> 200 reader for the qualitative detection of Influenza A and Influenza B nucleoprotein antigens in human nasal wash/aspirate, nasopharyngeal aspirate, and nasopharyngeal swab specimens.

**C. Measurand:**

Influenza A and Influenza B nucleoprotein antigens

**D. Type of Test:**

Qualitative immunochromatographic test that utilizes the RAMP<sup>®</sup> 200 reader for the differential determination of Influenza A and Influenza B in nasal wash/aspirate, nasopharyngeal aspirate, and nasopharyngeal swab samples.

**E. Applicant:**

Response Biomedical Corp.

**F. Proprietary and Established Names:**

RAMP Influenza A/B Assay

**G. Regulatory Information:**

1. Regulation section:

21 CFR section 866.3330, Influenza virus serological reagents.

2. Classification:

Class I

3. Product code:

GNX

4. Panel:

Microbiology (83)

**H. Intended Use:**

1. Intended use(s):

The RAMP<sup>®</sup> Influenza A/B Assay is a qualitative immunochromatographic assay used to identify the presence of Influenza A and Influenza B nucleoprotein antigens in nasal wash, nasal aspirate, nasopharyngeal aspirate, and nasopharyngeal swab specimens from symptomatic patients. It is an *in vitro* diagnostic assay aids in the rapid differential diagnosis of influenza viral infections in symptomatic patients. A negative test is presumptive and it is recommended these results be confirmed by cell culture. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other management decisions.

**The test performance characteristics for Influenza B were established primarily with retrospective, frozen specimens. Users may wish to further evaluate the sensitivity performance of this test for Influenza B using fresh samples.**

2. Indication(s) for use:

Same as intended use.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

To be used only with the RAMP<sup>®</sup> 200 reader.

**I. Device Description:**

The RAMP Influenza A/B Assay is a qualitative immunochromatographic test that utilizes the RAMP<sup>®</sup> 200 reader for the differential determination of Influenza A and Influenza B in nasal wash/aspirate, nasopharyngeal aspirate, and nasopharyngeal swab samples. A wash/aspirate or swab sample is mixed with Sample Buffer using Assay Tip containing fluorescent-dyed latex particles coated with monoclonal antibodies targeting the Influenza A and B nucleoproteins, and applied into the sample well of the Test Cartridge. The sample migrates along the strip. Fluorescent-dyed latex (test) particles, coated with anti-Influenza A and anti-Influenza B nucleoprotein monoclonal antibodies

bind to Influenza A or B nucleoprotein antigens, respectively, if present in the sample. As the sample migrates along the strip, Influenza-bound particles are captured at either the Influenza A or the Influenza B detection zone which is coated with either anti-Influenza A or anti-Influenza B nucleoprotein monoclonal antibodies, respectively. Excess fluorescent- dyed particles are captured at the internal standard zone. RAMP® 200 reader then measures the amount of fluorescence emitted by the complexes bound at the two detection zones (Influenza A and Influenza B) and at the internal standard zone. The RAMP® 200 reader calculates a ratio (RAMP Ratio) of each influenza zone (A or B) fluorescence reading to the internal standard zone fluorescence reading. The reader compares these ratios to pre-defined threshold limits to determine a positive or negative result for Influenza A and Influenza B in the tested sample.

#### J. Substantial Equivalence Information:

1. Predicate device name(s):

BinaxNOW Influenza A & B Test

2. Predicate K number(s):

(K062109)

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	<b>RAMP Influenza A/B Assay</b>	<b>BinaxNOW Influenza A &amp; B Test</b>
<b>Features/Technical Information</b>		
Intended Use	<p>The RAMP® Influenza A/B Assay is a qualitative immunochromatographic assay used with the RAMP® 200 reader to identify the presence of Influenza A and Influenza B nucleoprotein antigens in nasal wash, nasal aspirate, nasopharyngeal aspirate, and nasopharyngeal swab specimens from symptomatic patients. It is an <i>in vitro</i> diagnostic assay aids in the rapid differential diagnosis of influenza viral infections in symptomatic patients. A negative test is presumptive and it is recommended these results be confirmed by cell culture. Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other management decisions.</p>	<p>The BinaxNOW Influenza A &amp; B Test is an <i>in vitro</i> immunochromatographic assay for the qualitative detection of Influenza A and B nucleoprotein antigens in nasopharyngeal (NP) Swab, nasal swab, and nasal wash/aspirate specimens. It is intended to aid in the rapid differential diagnosis of Influenza A and B viral infections. Negative test results should be confirmed by cell culture.</p>

Qualitative/Quantitative	Qualitative	Qualitative
Test Principle	Immunochromatographic fluorescence immunoassay	Immunochromatographic colloidal gold immunoassay
Antibodies Used	Two mouse monoclonal antibodies recognizing the nucleoprotein of the Influenza A virus and two mouse monoclonal antibodies recognizing the nucleoprotein of the Influenza B virus.	Unknown antibodies recognizing the nucleoprotein of the Influenza A and influenza B viruses.
Specimen Type	Nasal wash, nasal aspirate, nasopharyngeal aspirate, and nasopharyngeal swab specimens	Nasal wash, nasal swab, nasopharyngeal aspirate, and nasopharyngeal swab specimens
Reagent Stability	In sealed pouch: Up to stated expiration date stored at 15-30°C. Do not remove from pouch until ready to use.	In sealed pouch: Up to stated expiration date stored at 15-30°C. Do not remove from pouch until ready to use.
Test Time	15 minutes	15 minutes
Waste Handling	Dispose of Test Cartridge as per correct institutional biohazard procedure.	Dispose of Test Cartridge as per correct institutional biohazard procedure.
Self Contained	Yes	Yes
Portable	Yes	Yes
Patient Usage	No	No
Standard Curve	Not Applicable	Not Applicable
External Controls	RAMP Influenza A/B Test kit contains Positive and Negative Control swabs. These swabs will monitor the performance of the entire system.	BinaxNOW Influenza A&B Test kit contains Positive and Negative Control swabs. These swabs will monitor the performance of the entire assay.
Analytes	Influenza A and Influenza B	Influenza A and Influenza B
Product Type	<i>In Vitro</i> Diagnostic	<i>In Vitro</i> Diagnostic

Differences		
Item	Device	Predicate
	<b>RAMP Influenza A/B Assay</b>	<b>BinaxNOW Influenza A &amp; B Test</b>
<b>Features/Technical Information</b>		
Sample Preparation	Use provided disposable dropper to mix the sample in provided pre-measured sample buffer vial.	Elution of swab in sample buffer. None for aspirate.
Test Procedure	Mix sample with provided transfer device and add sample to Test Cartridge.	Add sample to Test Device in a drop-wise manner expelling all material from the Binax supplied pipette.

Automated Processing	Instrument transport of Test Cartridge within reader is the only moving step. No internal liquid handling. One-step immunochromatographic assay requiring no additional washes.	Added sample develops on Test Device providing visual result.
Read Results	Read results on instrument screen, or print with optional printer.	Visual read for presence or absence of control and test lines.
Instrument	RAMP 200	None
Automated Analysis	Yes	No. Operator must read result precisely 15 minutes after sample application. Results read before or after 15 minutes may not be accurate.
Quality Controls	<p>Provided in every Test Cartridge. Built in performance controls for routine QC requirements. A comparison of the internal standard and the assay result indicates that sufficient properly mixed sample was applied to the test device and that unbound fluorescent label washed sufficiently from the detection zone, and the device was inserted and read properly by the instrument. Background fluorescence measurement serves as a negative control. Reader controls also prevent a used or expired cartridge from being read by the reader. Antibody quality, system function and assay timing are checked on each assay run. An unacceptable result from the control displays a warning message without a test result on the instrument indicating that the test should be repeated.</p>	<p>Provided in every Test Device. Built-in procedural controls. An untested device has a blue line at the "Control" position. If the test flows and the reagents work, this blue line will always turn pink in a tested device. The clearing of background color from the result window is a negative background control. The background color in the window should be light pink to white within 15 minutes. Background color should not hinder reading of the test.</p>
System Quality Control	A built-in Internal Quality Control (IQC) function performs an automated test of all Reader analytical systems on a scheduled or operator-determined basis to provide a record of proper Reader functionality.	Not Applicable

Calibrator	Lot card contains calibration and expiration information and is supplied with every kit. System will not process cartridge if lot card information is not stored in instrument.	Not Applicable
Result Interpretation	Negative or Positive for Influenza A and B; automated analysis, result displayed on LCD screen of instrument	Negative or Positive for Influenza A or B; result visually read by presence or absence of test lines at 15 minutes following sample application. Results read before or after 15 minutes may be inaccurate.

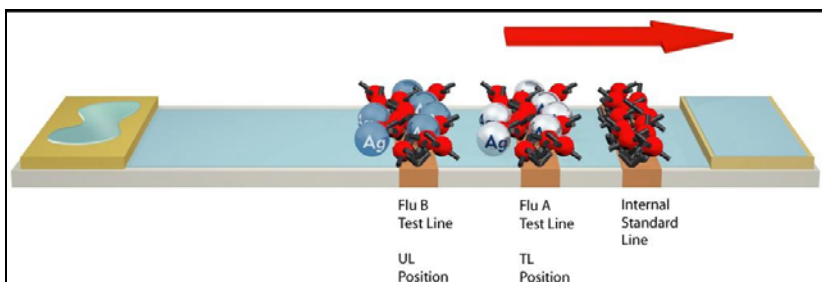
**K. Standard/Guidance Document Referenced (if applicable):**

Not applicable.

**L. Test Principle:**

The RAMP Influenza A/B Assay is a qualitative immunochromatographic test that utilizes the RAMP® 200 reader for the differential determination of Influenza A and Influenza B in nasal wash/aspirate, nasopharyngeal aspirate, and nasopharyngeal swab samples. A wash/aspirate or swab sample is mixed with Sample Buffer using Assay Tip containing fluorescent-dyed latex particles coated with monoclonal antibodies targeting the Influenza A and B nucleoproteins, and applied into the sample well of the Test Cartridge. The sample migrates along the strip. Fluorescent-dyed latex (test) particles, coated with anti-Influenza A and anti-Influenza B nucleoprotein monoclonal antibodies bind to Influenza A or B nucleoprotein antigens, respectively, if present in the sample. As the sample migrates along the strip, Influenza-bound particles are captured at either the Influenza A or the Influenza B detection zone which is coated with either anti-Influenza A or anti-Influenza B nucleoprotein monoclonal antibodies, respectively. Excess fluorescent- dyed particles are captured at the internal standard zone. RAMP® 200 reader then measures the amount of fluorescence emitted by the complexes bound at the two detection zones (Influenza A and Influenza B) and at the internal standard zone. The RAMP® 200 reader calculates a ratio (RAMP Ratio) of each influenza zone (A or B) fluorescence reading to the internal standard zone fluorescence reading. The reader compares these ratios to pre-defined threshold limits to determine a positive or negative result for Influenza A and Influenza B in the tested sample.

The RAMP® 200 is a general use fluorometer that analyzes results produced by immunoassays that use a fluorophore having an excitation wavelength of 560 nm and an emission wavelength of 610 nm.



## M. Performance Characteristics (if/when applicable):

### 1. Analytical performance:

#### a. *Precision/Reproducibility:*

The reproducibility of the RAMP Influenza A/B Assay was evaluated using a panel of 6 simulated samples that included high negative, low positive (the assay LoD) and moderate positive (2 X the assay LoD) Influenza A or Influenza B samples. The influenza strains used to prepare the simulated samples were Influenza A/Hong Kong/8/68 and Influenza B/Lee/40. The influenza strains were re-titrated prior to testing. Panel samples were tested in triplicates at each of the 3 testing sites by 2 operators for 5 days using 1 RAMP Influenza A/B kit lot (6 samples X 3 replicates X 2 operators X 5 days X 3 sites = 540). The overall percent agreement for the RAMP Influenza A/B Assay was 100%, with no significant differences within run (same operator on same day), between run, operators, and sites. The RAMP Flu A/B assay is a qualitative assay based on numerical RAMP Ratio values. The overall RAMP Ratio %CV across all sites ranged from 9.6% to 15.1% depending upon analyte type and concentration tested.

	Panel Member ID	Influenza A High Negative	Influenza A Low Positive	Influenza A Medium Positive	Influenza B High Negative	Influenza B Low Positive	Influenza B Medium Positive	Total Agreement All (%)
	Viral Titer (EID <sub>50</sub> /ml)	50	300	600	60	316	632	
Site 1	Agreement with Expected result	30/30	30/30	30/30	30/30	30/30	30/30	180/180 (100%)
	Mean RAMP Ratio Value	692	2224	4252	414	1192	2045	
	% CV	11.6%	6.3%	7.9%	16.4%	8.9%	7.9%	
Site 2	Agreement with Expected result	30/30	30/30	30/30	30/30	30/30	30/30	180/180 (100%)
	Mean RAMP Ratio Value	661	1993	3859	384	1076	1952	
	% CV	7.7%	8.1%	9.3%	15.6%	9.5%	7.0%	

Site 3	Agreement with Expected result	30/30	30/30	30/30	30/30	30/30	30/30	180/180 (100%)
	Mean RAMP Ratio Value	704	2326	4545	431	1212	2164	
	% CV	14.5%	9.9%	13.5%	11.3%	9.5%	10.5%	
	Total Agreement with Expected result	90/90 (100%)	90/90 (100%)	90/90 (100%)	90/90 (100%)	90/90 (100%)	90/90 (100%)	540/540 (100%)
	95% CI	96%-100%	96%-100%	96%-100%	96%-100%	96%-100%	96%-100%	99%-100%
	Overall Mean RAMP Ratio	685	2181	4219	410	1160	2054	
	Overall % CV	11.9%	10.4%	12.6%	15.1%	10.6%	9.6%	

*b. Linearity/assay reportable range:*  
Not applicable.

*c. Traceability, Stability, Expected values (controls, calibrators, or methods):*  
Not applicable.

*d. Detection limit:*

The analytical sensitivity (limit of detection or LoD) of the RAMP Influenza A/B Assay was determined using quantified (EID<sub>50</sub>/mL) cultures of 2 Influenza A (1 H1N1 and 1 H3N2) and 2 Influenza B strains serially diluted in either viral transfer media (VTM) to simulate a swab sample type or phosphate buffered saline (PBS) solution to simulate a wash/aspirate sample type. The influenza strains were re-titered prior to testing. Each viral strain was tested in replicates of 20 per concentration of virus.

Analytical sensitivity (LoD) as defined as the lowest concentration, at which ≥ 95% of all replicates tested positive, ranged from 3.0x10<sup>2</sup> to 6.4x10<sup>2</sup> EID<sub>50</sub>/mL for the Influenza A strains and 2.8x10<sup>2</sup> to 7.1x10<sup>4</sup> EID<sub>50</sub>/mL for the Influenza B strains in VTM and PBS.

Viral Strain	LoD Concentration
Influenza A/Hong Kong/8/68 (H3N2), ATCC VR-544 in VTM	3.0 X 10 <sup>2</sup> EID <sub>50</sub> /ml
Influenza A/Hong Kong/8/68 (H3N2), ATCC VR-544 in PBS	5.0 X 10 <sup>2</sup> EID <sub>50</sub> /ml
Influenza A/PR/8/34 (H1N1), ATCC VR-95 in VTM	2.0 X 10 <sup>2</sup> EID <sub>50</sub> /ml
Influenza A/PR/8/34 (H1N1), ATCC VR-95 in PBS	6.4 X 10 <sup>2</sup> EID <sub>50</sub> /ml



Influenza B/Lee/40, ATCC VR-101 in VTM	$3.2 \times 10^2$ EID <sub>50</sub> /ml
Influenza B/Lee/40, ATCC VR-101 in PBS	$2.8 \times 10^2$ EID <sub>50</sub> /ml
Influenza B/Allen/45, ATCC VR-102 in VTM	$5.3 \times 10^4$ EID <sub>50</sub> /ml
Influenza B/Allen/45, ATCC VR-102 in PBS	$7.1 \times 10^4$ EID <sub>50</sub> /ml

e. *Analytical reactivity:*

The analytical reactivity of the RAMP Influenza A/B Assay was evaluated against multiple strains of Influenza A (H1N1 and H3N2 subtypes) and Influenza B. The panel consisted of 3 Influenza A subtype H1N1, 2 Influenza A subtype H3N2, and 3 Influenza B strains. 5 replicates were tested for each viral strain. The concentrations for analytical reactivity testing ranged from  $3.0 \times 10^2$  to  $3.0 \times 10^3$  EID<sub>50</sub>/mL for the Influenza A strains and  $1.6 \times 10^1$  to  $9.5 \times 10^3$  EID<sub>50</sub>/mL for the Influenza B strains. The influenza strains were re-titered prior to testing.

Viral Strain	Analytical Reactivity Concentration	RAMP Assay Result
Influenza A/FM/1/47 (H1N1) ATCC VR-97	$3.0 \times 10^3$ EID <sub>50</sub> /ml	5/5 Flu A Positive
Influenza A/NWS/33 (H1N1) ATCC VR-219	$3.0 \times 10^2$ EID <sub>50</sub> /ml	5/5 Flu A Positive
Influenza A/New Jersey/8/76 (H1N1), ATCC VR-897	$3.0 \times 10^2$ EID <sub>50</sub> /ml	5/5 Flu A Positive
Influenza A/Aichi/2/68 (H3N2) ATCC VR-547	$9.0 \times 10^2$ EID <sub>50</sub> /ml	5/5 Flu A Positive
Influenza A/Victoria/3/75 (H3N2), ATCC VR-822	$9.0 \times 10^2$ EID <sub>50</sub> /ml	5/5 Flu A Positive
Influenza B/GL/1739/54 ATCC VR-103	$9.5 \times 10^3$ EID <sub>50</sub> /ml	5/5 Flu B Positive
Influenza B/Taiwan/2/62 ATCC VR-295	$1.6 \times 10^1$ EID <sub>50</sub> /ml	5/5 Flu B Positive
Influenza B/Hong Kong/5/72 ATCC VR-823	$6.3 \times 10^3$ EID <sub>50</sub> /ml	5/5 Flu B Positive

f. *Analytical specificity:*

The analytical specificity of the RAMP Influenza A/B Assay was evaluated by testing a panel consisting of 17 bacteria and 15 viruses that may be present in the nasal cavity or nasopharynx. Bacterial isolates were tested at  $10^6$  cfu/mL and viral isolates were tested at approximately  $10^5$  TCID<sub>50</sub>/mL at n=3 replicates each. The bacterial and viral organisms were re-grown and re-titered prior to cross-reactivity testing. Analytical specificity of the RAMP Influenza A/B Assay was 100%.

Strain/Isolate	Concentration	RAMP Flu A result	RAMP Flu B result
Adenovirus, Type 1	$10^5$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Adenovirus, Type 7a	$10^{5.15}$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Respiratory Syncytial Virus (RSV)	$10^5$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Human coronavirus, strain OC43	$10^5$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Human coronavirus Strain 229E	$10^{5.23}$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Cytomegalovirus	$10^{5.15}$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Enterovirus, Type 68	$10^{5.15}$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Epstein Barr Virus	$10^5$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Measles	$10^5$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Mumps virus	$10^5$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Human Parainfluenza, Type 1	$10^{4.75}$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Human Parainfluenza, Type 2	$10^5$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Human Parainfluenza, Type 3	$10^5$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Human metapneumovirus	$10^5$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
Human Rhinovirus, Strain 1A	$10^5$ TCID <sub>50</sub> /mL	3/3 Neg	3/3 Neg
<i>Bordetella pertussis</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Corynebacterium Sp.</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Escherichia coli</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Haemophilus influenzae</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Lactobacillus casei</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Legionella pneumophila</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Moraxella catarrhalis</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Mycobacterium tuberculosis avirulent</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Mycoplasma pneumoniae</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Neisseria meningitidis</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Neisseria sicca</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Pseudomonas aeruginosa</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Streptococcus pneumoniae</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Streptococcus pyogenes</i> (Group A)	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Streptococcus salivarius</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Staphylococcus epidermidis</i>	$10^6$ cfu/mL	3/3 Neg	3/3 Neg
<i>Staphylococcus aureus</i> (Protein A producer)	$10^6$ cfu/mL	3/3 Neg	3/3 Neg

Note: RAMP Influenza A/B Assay potential cross-reactivity with *Chlamydia pneumoniae* has not been determined.

g. *Assay cut-off:*

The lot-specific Threshold 1 (Normal cut-off) values and the fixed Threshold 2 (Extreme cut-off) values are programmed into the lot-specific Lot Cards and thus the RAMP Ratio values are invisible to the end user. Only negative or positive results are displayed. The Lot Card provides the reader with information specific to the lot, including lot number, expiration date, thresholds and standard curve information.

A. Threshold 1 (Normal cut-off):

- 1) Each RAMP Influenza A/B kit has lot-specific Threshold 1 levels. The Threshold 1 values (separate values for Influenza A and Influenza B) for each lot are based on QC testing data of the specific combination of component lots (Sample Buffer, Test Cartridge, and Assay Tips) used in the Influenza A/B kit lot. QC testing is performed on each kit lot to ensure that the lot meets release specifications as well as to provide the data to set the Threshold 1 values.

The algorithm used to determine the lot-specific Threshold 1 values is the same for each lot and uses defined calculations based on the results of the QC testing data of that lot, and the Threshold Factors that were based on the clinical trial results to optimize sensitivity and specificity of the RAMP Influenza A/B test (ROC Optimization).

B. Threshold 2 (Extreme cut-off):

- 1) During development of the RAMP Influenza A/B Assay, it was observed that when testing clinical samples with extremely high concentrations of Influenza A, there appeared to be interference in the Influenza B Assay potentially resulting in a False Positive Influenza B result in addition to the true positive Influenza A result. Testing was performed on the four Influenza A/B kit lots manufactured for the clinical trials to characterize this interference between multiple lots of the Influenza A/B Assays. Based on this testing, it was observed that the interference was very reproducible between kit lots. Threshold 2 values of 20,000 for Influenza A and 10,000 were arbitrarily set. The pre-determined "extreme" Threshold 2 values are fixed values and are applied to all test kit lots.
- 2) Based on this characterization of the interference of high levels of Influenza A antigen in the Influenza B Assay, an algorithm was derived to provide Threshold 1 for clinical samples that are negative or have low to moderate levels of Influenza A, but to switch to Threshold 2 if a double positive (Influenza A and Influenza B) was determined and the level of Influenza A was extremely high.

Because a simultaneous double infection of Influenza A and Influenza B in the same patient is exceedingly rare, the algorithm and Threshold 2 values

were biased towards minimizing the possibility of displaying a double Influenza A positive and Influenza B positive result.

*h. Interfering Substances:*

Whole blood and a number of other potentially interfering substances (medications and over the counter (OTC) products) that may be present naturally or artificially introduced in the nasal cavity or nasopharynx were evaluated in the RAMP Influenza A/B Assay. The substances were added to a negative sample (viral transport media), an Influenza A LoD positive sample (300 EID<sub>50</sub>/mL), an Influenza A 2x LoD positive sample (600 EID<sub>50</sub>/mL), an Influenza B LoD positive sample (316 EID<sub>50</sub>/mL) and an Influenza B 2x LoD positive sample (632 EID<sub>50</sub>/mL) and tested at n=3 replicates each in the RAMP Influenza A/B Assay. The influenza strains used to prepare the samples were Influenza A/Hong Kong/8/68 and Influenza B/Lee/40. These influenza strains were re-titered prior to interference testing.

None of the substances tested at the concentrations indicated interfere with the test results of negative or positive Influenza A and Influenza B samples in the RAMP Influenza A/B Assay. The RAMP Flu A/B assay is a qualitative assay based on numerical RAMP Ratio values. The Percent of Mean Control Ratio values were calculated using these RAMP Ratios.

Substance Tested	Conc. Tested	RAMP Results									
		Negative		Influenza A LoD		Influenza A 2x LoD		Influenza B LoD		Influenza B 2x LoD	
		RAMP Flu A/B results	Percent of Mean Control Ratio FluA/FluB	RAMP Flu A/B results	Percent of Mean Control Ratio	RAMP Flu A/B results	Percent of Mean Control Ratio	RAMP Flu A/B results	Percent of Mean Control Ratio	RAMP Flu A/B results	Percent of Mean Control Ratio
<b>Control (No interfering substance)</b>	<b>N/A</b>	3/3 Neg	100% / 100%	3/3 Flu A Pos	100%	3/3 Flu A Pos	100%	3/3 Flu B Pos	100%	3/3 Flu B Pos	100%
<b>Halls Throat Drop</b>	<b>15% w/v</b>	3/3 Neg	120% / 105%	3/3 Flu A Pos	101%	3/3 Flu A Pos	103%	3/3 Flu B Pos	99%	3/3 Flu B Pos	120%
<b>Cepacol Throat Drop</b>	<b>15% w/v</b>	3/3 Neg	91% / 101%	3/3 Flu A Pos	98%	3/3 Flu A Pos	101%	3/3 Flu B Pos	105%	3/3 Flu B Pos	118%
<b>Fisherman's Friend Throat Drop</b>	<b>15% w/v</b>	3/3 Neg	120% / 105%	3/3 Flu A Pos	101%	3/3 Flu A Pos	103%	3/3 Flu B Pos	99%	3/3 Flu B Pos	120%
<b>4-Acetamidophenol</b>	<b>10 mg/mL</b>	3/3 Neg	110% / 117%	3/3 Flu A Pos	99%	3/3 Flu A Pos	100%	3/3 Flu B Pos	104%	3/3 Flu B Pos	117%
<b>Acetylsalicylic Acid</b>	<b>15 mg/mL</b>	3/3 Neg	89% / 106%	3/3 Flu A Pos	98%	3/3 Flu A Pos	97%	3/3 Flu B Pos	110%	3/3 Flu B Pos	118%
<b>Chlorphiramine</b>	<b>5 mg/mL</b>	3/3 Neg	107% / 110%	3/3 Flu A Pos	101%	3/3 Flu A Pos	95%	3/3 Flu B Pos	112%	3/3 Flu B Pos	118%
<b>Diphenylhydramine</b>	<b>5 mg/mL</b>	3/3 Neg	109% / 126%	3/3 Flu A Pos	99%	3/3 Flu A Pos	100%	3/3 Flu B Pos	115%	3/3 Flu B Pos	116%
<b>Phenylpropanolamine HCl</b>	<b>20 mg/mL</b>	3/3 Neg	126% / 100%	3/3 Flu A Pos	91%	3/3 Flu A Pos	96%	3/3 Flu B Pos	113%	3/3 Flu B Pos	113%
<b>Oseltamivir Phosphate (Tamiflu)</b>	<b>50 mg/mL</b>	3/3 Neg	106% / 101%	3/3 Flu A Pos	105%	3/3 Flu A Pos	105%	3/3 Flu B Pos	106%	3/3 Flu B Pos	111%
<b>Rimantadine HCl</b>	<b>500 ng/mL</b>	3/3 Neg	103% / 103%	3/3 Flu A Pos	105%	3/3 Flu A Pos	94%	3/3 Flu B Pos	111%	3/3 Flu B Pos	113%
<b>Ribavirin (Rebetol)</b>	<b>100 mg/mL</b>	3/3 Neg	137% / 136%	3/3 Flu A Pos	111%	3/3 Flu A Pos	106%	3/3 Flu B Pos	118%	3/3 Flu B Pos	120%
<b>Scope Mouthwash</b>	<b>20% v/v</b>	3/3 Neg	88% / 99%	3/3 Flu A Pos	96%	3/3 Flu A Pos	94%	3/3 Flu B Pos	98%	3/3 Flu B Pos	112%
<b>Good and Kind Mouthwash</b>	<b>20% v/v</b>	3/3 Neg	97% / 118%	3/3 Flu A Pos	97%	3/3 Flu A Pos	106%	3/3 Flu B Pos	99%	3/3 Flu B Pos	125%

<b>Cepacol Mouth Wash</b>	<b>20% v/v</b>	3/3 Neg	88% / 85%	3/3 Flu A Pos	103%	3/3 Flu A Pos	104%	3/3 Flu B Pos	98%	3/3 Flu B Pos	118%
<b>Flonase Nasal Spray</b>	<b>15% v/v</b>	3/3 Neg	103% / 104%	3/3 Flu A Pos	109%	3/3 Flu A Pos	100%	3/3 Flu B Pos	95%	3/3 Flu B Pos	113%
<b>Rhinocort Nasal Spray</b>	<b>15% v/v</b>	3/3 Neg	85% / 88%	3/3 Flu A Pos	109%	3/3 Flu A Pos	101%	3/3 Flu B Pos	90%	3/3 Flu B Pos	122%
<b>Nasonex Nasal Spray</b>	<b>15% v/v</b>	3/3 Neg	104% / 90%	3/3 Flu A Pos	103%	3/3 Flu A Pos	106%	3/3 Flu B Pos	92%	3/3 Flu B Pos	113%
<b>Oxymetazoline HCl</b>	<b>0.05% v/v</b>	3/3 Neg	116% / 95%	3/3 Flu A Pos	101%	3/3 Flu A Pos	103%	3/3 Flu B Pos	97%	3/3 Flu B Pos	108%
<b>Phenylephrine HCl</b>	<b>10 mg/mL</b>	3/3 Neg	126% / 119%	3/3 Flu A Pos	101%	3/3 Flu A Pos	99%	3/3 Flu B Pos	106%	3/3 Flu B Pos	117%
<b>Guaiaacol Glycerol Ether (Benylin)</b>	<b>20 mg/mL</b>	3/3 Neg	166% / 153%	3/3 Flu A Pos	118%	3/3 Flu A Pos	113%	3/3 Flu B Pos	118%	3/3 Flu B Pos	124%
<b>Dextromethorphan</b>	<b>2 mg/mL</b>	3/3 Neg	95% / 101%	3/3 Flu A Pos	115%	3/3 Flu A Pos	115%	3/3 Flu B Pos	126%	3/3 Flu B Pos	134%
<b>Salbutamol Sulfate</b>	<b>400 ng/mL</b>	3/3 Neg	102% / 92%	3/3 Flu A Pos	98%	3/3 Flu A Pos	99%	3/3 Flu B Pos	111%	3/3 Flu B Pos	116%
<b>Whole Blood*</b>	<b>2% v/v</b>	3/3 Neg	126% / 91%	3/3 Flu A Pos	75%	3/3 Flu A Pos	90%	3/3 Flu B Pos	106%	3/3 Flu B Pos	98%

\*A warning is added to the package insert to reflect that samples contaminated with whole blood in greater concentrations (visibly bloody samples) may interfere in the interpretation of the assay.

*i. Transport Media Compatibility:*

Eight transport media (six commercial transport media and two clinical site prepared transport media) were evaluated for compatibility in the RAMP Influenza A/B Assay by testing a negative sample (transport media only), an Influenza A LoD positive sample (300 EID<sub>50</sub>/mL), an Influenza A 2x LoD positive sample (600 EID<sub>50</sub>/mL), an Influenza B LoD positive sample (316 EID<sub>50</sub>/ml), and an Influenza B 2x LoD positive sample (632 EID<sub>50</sub>/ml) at n=3 replicates each in the RAMP Influenza A/B Assay. The influenza strains used to prepare the samples were Influenza A/Hong Kong/8/68 and Influenza B/Lee/40. These influenza strains were re-titered prior to media testing.

All negative samples (media only) tested negative. All inoculated samples (media with Influenza A or Influenza B) tested positive for the appropriate virus. The RAMP Flu A/B assay is a qualitative assay based on numerical RAMP Ratio values. The %CVs were also calculated for the RAMP Ratios. None of the tested transport media interfered with the performance of the RAMP Influenza A/B Assay.

Transport Media Tested	RAMP Results									
	Negative		Influenza A LoD		Influenza A 2x LoD		Influenza B LoD		Influenza B 2x LoD	
	Results	Mean RAMP Ratio FluA/FluB	Results	Mean RAMP Ratio	Results	Mean RAMP Ratio	Results	Mean RAMP Ratio	Results	Mean RAMP Ratio
Copan Universal Transport Media (UTM)	Neg (3/3)	388 / 295	Flu A Pos (3/3)	1559	Flu A Pos (3/3)	2630	Flu B Pos (3/3)	1143	Flu B Pos (3/3)	1928
Remel M4 Media	Neg (3/3)	495 / 307	Flu A Pos (3/3)	1629	Flu A Pos (3/3)	3068	Flu B Pos (3/3)	1254	Flu B Pos (3/3)	2339
Remel M4-RT Media	Neg (3/3)	530 / 364	Flu A Pos (3/3)	1711	Flu A Pos (3/3)	2984	Flu B Pos (3/3)	1218	Flu B Pos (3/3)	2349
Remel M5 Media	Neg (3/3)	409 / 242	Flu A Pos (3/3)	1548	Flu A Pos (3/3)	2684	Flu B Pos (3/3)	1189	Flu B Pos (3/3)	1759
Starplex Transport Media	Neg (3/3)	366 / 269	Flu A Pos (3/3)	1529	Flu A Pos (3/3)	2673	Flu B Pos (3/3)	1110	Flu B Pos (3/3)	1844
Phosphate Buffered Saline (PBS) Solution	Neg (3/3)	345 / 272	Flu A Pos (3/3)	1632	Flu A Pos (3/3)	2939	Flu B Pos (3/3)	1132	Flu B Pos (3/3)	2055
St. Louis Children's Hospital In-house Media	Neg (3/3)	319 / 306	Flu A Pos (3/3)	1684	Flu A Pos (3/3)	3297	Flu B Pos (3/3)	1371	Flu B Pos (3/3)	2309
Texas Children's Hospital In-house Media	Neg (3/3)	332 / 263	Flu A Pos (3/3)	1669	Flu A Pos (3/3)	2559	Flu B Pos (3/3)	1202	Flu B Pos (3/3)	2019
Total Mean RAMP Ratio		398 / 290		1620		2854		1203		2075
RAMP Ratio SD		77 / 37		68		258		83		232
RAMP Ratio % CV		Flu A 19% Flu B 13%		4%		9%		7%		11%

*j. Sample Collection Swabs Compatibility:*

Four swab materials were evaluated for compatibility in the RAMP Influenza A/B Assay by testing a negative sample (swab alone with no virus present), an Influenza A LoD positive sample (300 EID<sub>50</sub>/mL after extraction), an Influenza A 2x LoD positive sample (600 EID<sub>50</sub>/mL after extraction), an Influenza B LoD positive sample (316 EID<sub>50</sub>/mL after extraction), and an Influenza B 2x LoD positive sample (632 EID<sub>50</sub>/mL after extraction) at n=3 replicates each in the RAMP Influenza A/B Assay. The influenza strains used to prepare the samples

were Influenza A/Hong Kong/8/68 and Influenza B/Lee/40. These influenza strains were re-titered prior to swab testing. Each swab was dosed with 20  $\mu$ L of the appropriate sample and extracted into 3 mL Copan Universal Transport media by vortexing for 30 seconds to get the target concentration for each sample prior to testing in the RAMP Influenza A/B Assay. Each replicate tested was prepared from a separate swab. Swabs made of sterile foam, polyester, nylon, or rayon were evaluated. The RAMP Flu A/B assay is a qualitative assay based on numerical RAMP Ratio values. The %CVs were also calculated based on the RAMP Ratios for the testing

The negative sample (swab alone) tested negative. At least two of three of each swab type inoculated with Influenza A or Influenza B at the LoD tested positive for the virus.

Swab Material Tested	RAMP Results									
	Influenza A Threshold 1= 1023				Influenza B Threshold 2= 649					
	Negative		Influenza A LoD		Influenza A 2x LoD		Influenza B LoD		Influenza B 2x LoD	
	Results	Mean RAMP Ratio FluA/FluB	Results	Mean RAMP Ratio	Results	Mean RAMP Ratio	Results	Mean RAMP Ratio	Results	Mean RAMP Ratio
<b>Foam</b>	Neg (3/3)	441 / 231	Flu A Pos (3/3)	1392	Flu A Pos (3/3)	2354	Flu B Pos (3/3)	1171	Flu B Pos (3/3)	2194
<b>Polyester</b>	Neg (3/3)	370 / 228	Flu A Pos (2/3)	1116	Flu A Pos (3/3)	2342	Flu B Pos (2/3)	735	Flu B Pos (3/3)	1431
<b>Rayon</b>	Neg (3/3)	406 / 252	Flu A Pos (3/3)	1267	Flu A Pos (3/3)	2169	Flu B Pos (2/3)	661	Flu B Pos (3/3)	1342
<b>Nylon</b>	Neg (3/3)	543 / 353	Flu A Pos (3/3)	1581	Flu A Pos (3/3)	2629	Flu B Pos (3/3)	1147	Flu B Pos (3/3)	2002
<b>Total Mean RAMP Ratio</b>		440 / 266		1339		2374		928		1742
<b>RAMP Ratio SD</b>		75 / 59		197		190		268		420
<b>RAMP Ratio % CV</b>		Flu A 17% Flu B 22%		15%		8%		29%		24%



k. *Sample Stability Testing:*

The RAMP Influenza A/B Assay was evaluated to determine the effect of time of storage of the sample at 2-8°C to assess stability of detection of the antigen in the sample. Clinical samples from the British Columbia Centre for Disease Control (BCCDC) equivalent to those tested in the clinical trial were thawed and diluted in viral transport media to simulate samples that were close to the Threshold 1 ratio values. The clinical samples were then tested immediately and after 72 hours storage at 2°C and 8°C.

The results indicate that samples tested in the RAMP Influenza A/B Assay are stable for at least 72 hours when stored at 2-8°C.

Influenza A RAMP Ratio (Results)					
	Time 0	72 hrs at 2°C		72 hrs at 8°C	
Sample ID (dilution) (BCCDC)	Ratio (Result)	Ratio (Result)	Percent of Time 0 Ratio	Ratio (Result)	Percent of Time 0 Ratio
A5VI 27170-A (1/20)	1556 (Flu A Pos)	1816 (Flu A Pos)	117	1486 (Flu A Pos)	96
A5VI 27597-A (1/6)	2466 (Flu A Pos)	2883 (Flu A Pos)	117	2480 (Flu A Pos)	101
A6VI 01262-B (1/6)	364 (Negative)	406 (Negative)	112	339 (Negative)	93
A5VI 27163-A (1/10)	1016 (Flu A Pos)	948 (Flu A Pos)	93	1094 (Flu A Pos)	108
A5VI 27308-A (1/10)	1289 (Flu A Pos)	1224 (Flu A Pos)	95	1216 (Flu A Pos)	94
A5VI 27606-A (1/40)	1943 (Flu A Pos)	2241 (Flu A Pos)	115	2016 (Flu A Pos)	104
A5VI 27559-A (1/20)	2576 (Flu A Pos)	2873 (Flu A Pos)	112	3033 (Flu A Pos)	118
A5VI 27562-A (1/20)	1710 (Flu A Pos)	1720 (Flu A Pos)	101	1777 (Flu A Pos)	104
A5VI 27213-A (1/10)	706 (Negative)	980 (Flu A Pos)	139	976 (Flu A Pos)	138
A5VI 27349-A (1/10)	446 (Negative)	437 (Negative)	98	413 (Negative)	93
10008549 (1/20)	374 (Negative)	460 (Negative)	123	400 (Negative)	107
10008549 (1/40)	320 (Negative)	394 (Negative)	123	416 (Negative)	130
10017303 (1/20)	443 (Negative)	490 (Negative)	111	375 (Negative)	85
10041464 (1/6)	411 (Negative)	378 (Negative)	92	382 (Negative)	93
10045523 (1/6)	438 (Negative)	440 (Negative)	101	331 (Negative)	76
10008549 (1/80)	385 (Negative)	293 (Negative)	76	473 (Negative)	123
<b>Mean % Recovery</b>			108		104

Influenza B RAMP Ratio (Results)					
	Time 0	72 hrs at 2°C		72 hrs at 8°C	
Sample ID (dilution) (BCCDC)	Ratio (Result)	Ratio (Result)	Percent of Time 0 Ratio	Ratio (Result)	Percent of Time 0 Ratio
A5VI 27170-A (1/20)	186 (Negative)	108 (Negative)	58	181 (Negative)	97
A5VI 27597-A (1/6)	250 (Negative)	246 (Negative)	98	129 (Negative)	52
A6VI 01262-B (1/6)	668 (Flu B Pos)	516 (Flu B Pos)	77	713 (Flu B Pos)	107
A5VI 27163-A (1/10)	209 (Negative)	143 (Negative)	68	137 (Negative)	66
A5VI 27308-A (1/10)	259 (Negative)	136 (Negative)	53	228 (Negative)	88
A5VI 27606-A (1/40)	155 (Negative)	168 (Negative)	108	164 (Negative)	106
A5VI 27559-A (1/20)	129 (Negative)	233 (Negative)	181	236 (Negative)	183
A5VI 27562-A (1/20)	264 (Negative)	248 (Negative)	94	150 (Negative)	57
A5VI 27213-A (1/10)	119 (Negative)	148 (Negative)	124	193 (Negative)	162
A5VI 27349-A (1/10)	182 (Negative)	93 (Negative)	51	223 (Negative)	123
10008549 (1/20)	2657 (Flu B Pos)	2825 (Flu B Pos)	106	2945 (Flu B Pos)	111
10008549 (1/40)	1215 (Flu B Pos)	1468 (Flu B Pos)	121	1433 (Flu B Pos)	118
10017303 (1/20)	443 (Negative)	518 (Flu B Pos)	117	474 (Flu B Pos)	107
10041464 (1/6)	110 (Negative)	137 (Negative)	125	176 (Negative)	160
10045523 (1/6)	150 (Negative)	111 (Negative)	74	223 (Negative)	149
10008549 (1/80)	684 (Flu B Pos)	700 (Flu B Pos)	102	861 (Flu B Pos)	126
<b>Mean % Recovery</b>			97		113

2. Comparison studies:

a. *Method comparison with predicate device:*

Not applicable.

b. *Matrix comparison:*

Not applicable.

3. Clinical studies:

*a. Prospective Clinical studies*

The performance of the RAMP Influenza A/B Assay was compared to cell culture in a prospective study conducted as part of a multi-center trial in North America during the 2006-2007 influenza season when influenza A/H3 (36.5%) and A/H1 (63.5%) were the predominant Influenza A viruses in circulation<sup>1</sup>. Four independent laboratories (located in distinct geographic regions) evaluated the RAMP Influenza A/B Assay in parallel with cell culture. Eight hundred Forty-four (844) fresh specimens (nasal wash/aspirate, nasopharyngeal aspirate, or nasopharyngeal swab samples) prospectively collected during the 2006-2007 influenza season were tested. Across the sites these samples were drawn from an approximately equal mix of pediatric (0 – 21 years) and adult patients (21+ years) with approximately equal numbers of male and female patients. The mean (standard deviation) age of the patients was 28.6 (30.7) years. The results of the prospective clinical trials based on sample type are given in the following tables:

<b>Prospective Fresh Nasopharyngeal Swab</b>	<b>RAMP Influenza A</b>			<b>RAMP Influenza B</b>		
<b>Tissue Culture</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Positive</b>	53	13	66	7	5	12
<b>Negative</b>	19	541	560	12	602	614
<b>Total</b>	72	554	626	19	607	626
			<b>95% CI</b>			<b>95% CI</b>
<b>Sensitivity</b>	53/66	80.3%	68.7-89.1%	7/12	58.3%	27.7-84.8%
<b>Specificity</b>	541/560	96.6%	94.8-97.9%	602/614	98.0%	96.6-99.0%

<b>Prospective Fresh Nasal Wash/Aspirate</b>	<b>RAMP Influenza A</b>			<b>RAMP Influenza B</b>		
<b>Tissue Culture</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Positive</b>	36	9	45	9	0	9
<b>Negative</b>	11	162	173	2	207	209
<b>Total</b>	47	171	218	11	207	218
			<b>95% CI</b>			<b>95% CI</b>
<b>Sensitivity</b>	36/45	80.0%	65.4-90.4%	9/9	100%	66.4-100%
<b>Specificity</b>	162/173	93.6%	88.9-96.8%	207/209	99.0%	96.6-99.9%

The results of the prospective clinical trials based on sample type and stratified by patient age group are given in the following tables:

<b>Prospective Fresh Nasopharyngeal Swab (Age 0-5)</b>	<b>RAMP Influenza A</b>			<b>RAMP Influenza B</b>		
<b>Tissue Culture</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Positive</b>	11	1	12	1	0	1
<b>Negative</b>	12	187	199	6	204	210
<b>Total</b>	23	188	211	7	204	211
			<b>95% CI</b>			<b>95% CI</b>
<b>Sensitivity</b>	11/12	91.7%	61.5-99.8%	1/1	100%	NA
<b>Specificity</b>	187/199	94.0%	89.7%-96.8%	204/210	97.1%	93.9-98.9%

<b>Prospective Fresh Nasopharyngeal Swab (Age 6-21)</b>	<b>RAMP Influenza A</b>			<b>RAMP Influenza B</b>		
<b>Tissue Culture</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Positive</b>	12	3	15	2	2	4
<b>Negative</b>	4	66	70	4	77	81
<b>Total</b>	16	69	85	6	79	85
			<b>95% CI</b>			<b>95% CI</b>
<b>Sensitivity</b>	12/15	80.0%	51.9-95.7%	2/4	50.0%	6.8-93.2%
<b>Specificity</b>	66/70	94.3%	86.0-98.4%	77/81	95.1%	87.8-98.6%

<b>Prospective Fresh Nasopharyngeal Swab (Age 22-59)</b>	<b>RAMP Influenza A</b>			<b>RAMP Influenza B</b>		
<b>Tissue Culture</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Positive</b>	16	7	23	2	2	4
<b>Negative</b>	1	136	137	1	155	156
<b>Total</b>	17	143	160	3	157	160
			<b>95% CI</b>			<b>95% CI</b>
<b>Sensitivity</b>	16/23	69.6%	47.1-86.8%	2/4	50.0%	6.8-93.2%
<b>Specificity</b>	136/137	99.3%	96.0-100%	155/156	99.4%	96.5-100%

<b>Prospective Fresh Nasopharyngeal Swab (Age &gt;=60)</b>	<b>RAMP Influenza A</b>			<b>RAMP Influenza B</b>		
<b>Tissue Culture</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Positive</b>	14	2	16	2	1	3
<b>Negative</b>	2	152	154	1	166	167
<b>Total</b>	16	154	170	3	167	170
			<b>95% CI</b>			<b>95% CI</b>
<b>Sensitivity</b>	14/16	87.5%	61.7-98.4%	2/3	66.7%	9.4-99.2%
<b>Specificity</b>	152/154	98.7%	95.4-99.8%	166/167	99.4%	96.7-100%

<b>Prospective Fresh Nasal Wash/Aspirate (Age 0-5)</b>	<b>RAMP Influenza A</b>			<b>RAMP Influenza B</b>		
<b>Tissue Culture</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Positive</b>	24	4	28	6	0	6
<b>Negative</b>	6	117	123	1	144	145
<b>Total</b>	30	121	151	7	144	151
			<b>95% CI</b>			<b>95% CI</b>
<b>Sensitivity</b>	24/28	85.7%	67.3-96.0%	6/6	100%	54.1-100%
<b>Specificity</b>	117/123	95.1%	89.7-98.2%	144/145	99.3%	96.2-100%

<b>Prospective Fresh Nasal Wash/Aspirate (Age 6-21)</b>	<b>RAMP Influenza A</b>			<b>RAMP Influenza B</b>		
<b>Tissue Culture</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Positive</b>	12	5	17	3	0	3
<b>Negative</b>	5	41	46	1	59	60
<b>Total</b>	17	46	63	4	59	63
			<b>95% CI</b>			<b>95% CI</b>
<b>Sensitivity</b>	12/17	70.6%	44.0-89.7%	3/3	100%	29.2-100%
<b>Specificity</b>	41/46	89.1%	76.4-96.4%	59/60	98.3%	91.1-100%

<b>Prospective Fresh Nasal Wash/Aspirate (Age 22-59)</b>	<b>RAMP Influenza A</b>			<b>RAMP Influenza B</b>		
<b>Tissue Culture</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Positive</b>	0	0	0	0	0	0
<b>Negative</b>	0	3	3	0	3	3
<b>Total</b>	0	3	3	0	3	3
			<b>95% CI</b>			<b>95% CI</b>
<b>Sensitivity</b>	0/0	NA	NA	0/0	NA	NA
<b>Specificity</b>	3/3	100%	29.2-100%	3/3	100%	29.2-100%

<b>Prospective Fresh Nasal Wash/Aspirate (Age &gt;=60)</b>	<b>RAMP Influenza A</b>			<b>RAMP Influenza B</b>		
<b>Tissue Culture</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Positive</b>	0	0	0	0	0	0
<b>Negative</b>	0	1	1	0	1	1
<b>Total</b>	0	1	1	0	1	1
			<b>95% CI</b>			<b>95% CI</b>
<b>Sensitivity</b>	0/0	NA	NA	0/0	NA	NA
<b>Specificity</b>	1/1	100%	NA	1/1	100%	NA

*b. Retrospective Clinical studies*

In order to supplement the prospective study, performance of the RAMP Influenza A/B Test was also evaluated by two independent laboratories comparing to the original cell culture testing results (cell culture testings were performed originally using fresh specimens) on 75 retrospective frozen clinical nasopharyngeal swab samples and 130 retrospective frozen clinical wash/aspirate samples. The results of the retrospective clinical trials based on sample type are given in the following tables:

<b>Retrospective Frozen Nasopharyngeal Swab</b>	<b>RAMP Influenza A</b>			<b>RAMP Influenza B</b>		
<b>Tissue Culture</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Positive</b>	11	2	13	23	7	30
<b>Negative</b>	0	62	62	0	45	45
<b>Total</b>	11	64	75	23	52	75
			<b>95% CI</b>			<b>95% CI</b>
<b>Positive Percent Agreement</b>	11/13	84.6%	54.5-98.1 %	23/30	76.7%	57.7-90.1%
<b>Negative Percent Agreement</b>	62/62	100%	94.2-100%	45/45	100%	92.1-100%

<b>Retrospective Frozen Nasal Wash/Aspirate</b>	<b>RAMP Influenza A</b>			<b>RAMP Influenza B</b>		
<b>Tissue Culture</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>	<b>Positive</b>	<b>Negative</b>	<b>Total</b>
<b>Positive</b>	35	10	45	33	6	39
<b>Negative</b>	2	83	85	2	89	91
<b>Total</b>	37	93	130	35	95	130
			<b>95% CI</b>			<b>95% CI</b>
<b>Positive Percent Agreement</b>	35/45	77.8%	62.9-88.8%	33/39	84.6%	69.5-94.1%
<b>Negative Percent Agreement</b>	83/85	97.6%	91.8-99.7%	89/91	97.8%	92.3%-99.7%

*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 Influenza varies each year with epidemics occurring during the fall and winter months in the US. Variables that affect the rate of positivity observed in respiratory testing include: the efficiency and timing of specimen collection, handling and transport of the specimen, the time of year, age of the patient, and local disease prevalence. During the 2006-2007 US respiratory season, the combined prevalence of Influenza A and Influenza B was 13.2%<sup>1</sup>. In the 2007 RAMP Influenza A/B multi-center clinical study (samples collected between January and April), the prevalence as observed with culture of Influenza A was 13.2% (111/844), Influenza B was 2.5% (21/844).

**N. Instrument Name:**

RAMP® 200 reader

**O. System Descriptions:**

1. Modes of Operation:

The RAMP® 200 measures the amount of fluorescence emitted by antibody/antigen complexes bound at two detection zones (Influenza A and Influenza B) and at the internal standard zone. The RAMP® 200 calculates a ratio (RAMP Ratio) using the fluorescence reading of each detection zone (A and B) and the internal zone. The reader compares these ratios to predefined threshold limits to determine a positive or negative result for Influenza A and Influenza B in the tested sample.

2. Software:

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

Yes \_\_\_\_X\_\_\_\_ or No \_\_\_\_\_

3. Specimen Identification:

User enters Patient ID/Sample ID using either the touch screen of the Control Module or optional barcode scanner.

4. Specimen Sampling and Handling:

Not applicable

5. Calibration:

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<sup>1</sup> Centers for Disease Control and Prevention. 2007. Update: Influenza activity--United States and worldwide, 2006-07 season, and composition of the 2007-08 influenza vaccine. MMWR 56(31); 789-794.

Each box of test strips is supplied with a Lot Card containing the calibration information for each specific manufacturing lot. The Reader determines the lot information by reading the barcode on the bottom of each Test Cartridge and reports the result based on the fluorescence ratio calculated for the Cartridge and comparing it to the specific lot information loaded into the memory from the associated Lot Card.

6. Quality Control:

When the RAMP<sup>®</sup> 200 is turned on, it performs a Self-Test which verifies the system calibration, the cartridge transport mechanism and the Reader optics. During the self-test the system also determines remaining battery power, verifies the system clock and verifies the instrument calibration. In addition, the RAMP<sup>®</sup> 200 has an Internal Quality Control (IQC) function. IQC can be scheduled to be performed at a pre-specified interval (1-24 hours) and takes less than 1 minute to complete. The IQC function repeats all the start-up checks and ensures appropriate instrument operation.

The RAMP<sup>®</sup> 200 scans the entire length of the Assay strip within the Cartridge. There are 2 areas on the strip to which the fluorescently labeled antibodies bind, the “detection zone” and the “internal standard zone”. The Reader monitors the sample flow across the length of the Assay Strip. Should an operator delay for too long between loading the sample and inserting the Cartridge into the Reader, insert a Cartridge that has already been run, apply too little sample to the Cartridge, or not use the supplied Assay Tip from the kit Pouch, the flow detected will not adhere to expected patterns and an error will be reported. In this way each Cartridge assures appropriate operator technique. Prior to reporting a result, the Reader software checks that the background signal (measured from areas outside of the detection zone and internal standard zones) is not higher than a predetermined value and that the total amount of fluorescence is above a predefined value. If either of these parameters is not met, an error is reported.

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

Not applicable

**Q. Proposed Labeling:**

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

**R. Conclusion:**

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