510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K110968

B. Purpose for Submission:

Modification of the previously cleared ProFlu+ Rt-PCR assay (K092500). Two new Influenza A probes located directly upstream of the current ProFlu+ Influenza A probe and containing a degenerate nucleotide (C or T) at the 3' terminus were added to the supermix to improve the detection of the 2009 H1N1 influenza viruses containing point mutation in the matrix gene at the probe binding.

C. Measurand:

Respiratory specimen virus nucleic acid (RNA) target sequences. The targeted viruses have been associated with respiratory infections in adults and/or children. Viral types detected: Influenza A, Influenza B, Respiratory Syncytial Virus Type A, Respiratory Syncytial Virus Type B

D. Type of Test:

Multiplex nucleic acid assay for qualitative determination of Influenza A, Influenza B, Respiratory Syncytial Virus Type A, Respiratory Syncytial Virus Type B) in nasopharyngeal swabs obtained from individuals with signs and symptoms of respiratory tract infections.

E. Applicant:

Gen-Probe Prodesse, Inc.

F. Proprietary and Established Names

ProFlu+™ assay
Common Name: Respiratory Viral Panel (RVP) multiplex nucleic acid assay

G. Regulatory Information:

1. Regulation section:	866.3980 - Respiratory viral panel multiplex nucleic acid assay
2. Classification:	Class II
3. Product code:	OCC, OOI
4. Panel:	Microbiology (83)

H. Intended Use:

1. Intended use:

The ProFlu™+ Assay is a multiplex Real-Time PCR (RT-PCR) *in vitro* diagnostic test for the rapid and qualitative detection and discrimination of Influenza A Virus, Influenza B Virus, and Respiratory Syncytial Virus (RSV)

nucleic acids isolated and purified from nasopharyngeal (NP) swab specimens obtained from symptomatic patients. This test is intended for use to aid in the differential diagnosis of Influenza A, Influenza B and RSV viral infections in humans and is not intended to detect Influenza C.

Negative results do not preclude influenza or RSV virus infection and should not be used as the sole basis for treatment or other management decisions. Conversely, 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 and clinical presentation must be considered in order to obtain the final diagnosis of respiratory viral infection.

Performance characteristics for Influenza A Virus were established when Influenza A/H3 and A/H1 were the predominant Influenza A viruses in circulation (2006 – 2007 respiratory season). Performance characteristics for Influenza A were confirmed when Influenza A/H1, Influenza A/H3, and Influenza A/2009 H1N1 were the predominant Influenza A viruses in circulation (2008 and 2009). When other Influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health 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:

   Roche MagNA Pure LC System with software version 3.0.11 or bioMérieux NucliSENS easyMAG System with Software version 1.0.1 or 2.0. Cepheid SmartCycler II Real Time Instrument with Dx software version 1.7b or 3.0 a/b.

I. Device Description:

The ProFlu+ Assay enables detection and differentiation of Influenza A Virus, Influenza B Virus, Respiratory Syncytial Virus (Types A and B), and Internal Control.

An overview of the procedure is as follows:

1. Collect nasopharyngeal swab specimens from symptomatic patients using a polyester, rayon or nylon tipped swab and place into viral transport medium (refer to Materials Required but not Provided).
2. Add an Internal Control (IC) to every sample to monitor for inhibitors present in the specimens.
3. Perform isolation and purification of nucleic acids using a MagNA Pure LC System (Roche) and the MagNA Pure Total Nucleic Acid Isolation Kit (Roche) or a NucliSENS easyMAG System (bioMérieux) and the Automated Magnetic Extraction Reagents (bioMérieux).
4. Add purified nucleic acids to Influenza A/Influenza B/RSV Mix along with enzymes included in the ProFlu+ Detection Kit. The Influenza A/Influenza B/RSV Mix contains oligonucleotide primers and target-specific oligonucleotide probes. The primers are complementary to highly conserved regions of genetic sequences for these respiratory viruses. The probes are dual-labeled with a reporter dye and a quencher (see table below).
5. Perform reverse transcription of RNA into complementary DNA (cDNA) and subsequent amplification of DNA in a Cepheid SmartCycler II instrument. In this process, the probe anneals specifically to the template followed by primer extension and amplification. The ProFlu+ Assay is based on Taqman reagent chemistry, which utilizes the 5' – 3' exonuclease activity of the Taq polymerase to cleave the probe thus separating the reporter dye from the quencher. This generates an increase in fluorescent signal upon excitation from a light source. With each cycle, additional reporter dye molecules are cleaved from their respective probes, further increasing fluorescent signal. The amount of fluorescence at any given cycle is dependent on the amount of amplification products present at that time. Fluorescent intensity is monitored during each PCR cycle by the real-time instrument.

Analyte	Gene Targeted	Probe Fluorophore	Absorbance Peak	Emission Peak	Instrument Channel
Influenza A Virus	Matrix	FAM	495 nm	520 nm	FAM
Respiratory Syncytial Virus A	Polymerase	CAL Fluor Orange 560	540 nm	561 nm	TET
Respiratory Syncytial Virus B	Polymerase	CAL Fluor Orange 560	540 nm	561 nm	TET
Influenza B Virus	Non-structural NS1 and NS2	CAL Fluor Red 610	595 nm	615 nm	Texas Red
Internal Control	NA	Quasar 670	647 nm	667 nm	Cy5

Materials Provided

ProFlu+ Assay consists of a Detection Kit and Control Kit.

Detection Kit (100 Reactions)

Reagents	Description	Quantity/ Tube	Cap Color	Reactions/ Tube
Influenza A/ Influenza B/ RSV Mix	➲ Taq DNA polymerase ➲ oligonucleotide primers ➲ oligonucleotide probes ➲ Buffer containing dNTPs (dATP, dCTP, dGTP, dTTP), ➲ $MgCl_2$ and stabilizers ➲ Bovine serum albumin	1030 µL	Brown	50 (2 tubes provided)
M-MLV Reverse Transcriptase	➲ 10U/µL	36 µL	Red	100
RNase Inhibitor II	➲ 40U/µL	120 µL	Green	100
Internal RNA Control III	➲ Non-infectious *in vitro* transcribed RNA	30 µL	Yellow	100

Control Kit

Reagents	Description	Quantity/ Tube	Cap Color	Reactions/ Tube
Influenza A RNA Control III	➲ Non-infectious *in vitro* transcribed RNA of specific viral sequences	300 µL	White	15
Influenza B RNA Control III	➲ Non-infectious *in vitro* transcribed RNA of specific viral sequences	300 µL	Blue	15
RSV A RNA Control III	➲ Non-infectious *in vitro* transcribed RNA of specific viral sequences	300 µL	Purple	15
RSV B RNA Control III	➲ Non-infectious *in vitro* transcribed RNA of specific viral sequences	300 µL	Clear	15

Materials Required but not Provided

Plasticware and consumables

- ☐ Polyester, rayon or nylon tipped nasopharyngeal swabs
- ☐ RNase/DNase-free 1.5 mL polypropylene microcentrifuge tubes
- ☐ Sterile RNase/DNase-free filter or positive displacement micropipettor tips
- ☐ MagNA Pure LC System Disposables (Reagent Tubs, Reaction Tips, Tip Trays, Cartridges) or easyMAG System Disposables (Sample Strips and Tips)
- ☐ Biohit Pipette Tips for use with easyMAG System
- ☐ Greiner Break Four uncoated plates for use with easyMAG System
- ☐ Cepheid PCR reaction tubes, 25 µL
- ☐ Parafilm M or MagNA Pure LC Cartridge Seals

Reagents

- ☐ Roche MagNA Pure LC Total Nucleic Acid Isolation Kit*)* for 192 isolations or bioMérieux NucliSENS easyMAG reagents

- ☐ Micro Test M4 Viral Transport Medium Micro Test M5 Viral Transport Medium, Micro Test M6 Viral Transport Medium, Micro Test M4RT Viral Transport Medium, Copan Universal Transport Medium, or BD Universal Viral Transport vial, 3mL
- ☐ Molecular Grade Water (RNase/DNase Free)
- ☐ Extraction *Control (e.g. previously characterized positive sample)*

NOTE: Only qualified lots of the MagNA Pure LC Total Nucleic Acid Isolation Kit can be used with the ProFlu+ Assay. Lots not specifically qualified by Gen-Probe Prodesse, Inc. for use with the ProFlu+ Assay are not verified for use with this assay, and may cause erroneous results.
A list of these qualified extraction reagents is available at www.gen-probe.com. Please notify the reagent manufacturer of issues with this ancillary reagent and Gen-Probe Prodesse, Inc. of the impact on the performance of the ProFlu+ Assay.

Equipment

- ☐ – 70°C Freezer
- ☐ Roche MagNA Pure LC System with software version 3.0.11 or bioMérieux NucliSENS easyMAG System with Software version 1.0.1 or 2.0
- ☐ Biohit multi-channel pipettor for use with easyMAG System
- ☐ Cepheid SmartCycler II Real Time Instrument with Dx Software version 1.7b, 3.0a, or 3.0b
- ☐ Micropipettors (range between 1-10 µL, 10-200 µL and 100-1000 µL)
- ☐ Mini-centrifuge with adapter for Cepheid Reaction Tubes
- ☐ Cepheid cooling block
- ☐ Ice/Ice Bucket or -20°C Cold Block
- ☐ Biosafety Cabinet

Interpretation of Specimen Results
The SmartCycler Dx software automatically determines the specimen results. The interpretation of the assay specimen results is as follows:

Sample ID[1]	Assay Result	IC Result	Warning / Error Code	Influenza A Result	RSV Result	Influenza B Result	Interpretation of Results
Sample ID	Negative	Pass		**NEG**	**NEG**	**NEG**	Influenza A, B and RSV nucleic acid **not detected**
Sample ID	Positive	NA*		**POS**	NEG	NEG	Influenza A nucleic acid **detected**
Sample ID	Positive	NA*		NEG	**POS**	NEG	RSV nucleic acid **detected**
Sample ID	Positive	NA*		NEG	NEG	**POS**	Influenza B nucleic acid **detected**
Sample ID	Positive	NA*		**POS**	**POS**	NEG	Influenza A and RSV nucleic acid **detected**
Sample ID	Positive	NA*		**POS**	NEG	**POS**	Influenza A and

							Influenza B nucleic acid **detected**
Sample ID	Positive	NA*		NEG	**POS**	**POS**	RSV and Influenza B nucleic acid **detected**
Sample ID	Positive	NA*		**POS**	**POS**	**POS**	Influenza A, Influenza B and RSV nucleic acid detected. Triple infections are rare, repeat testing from the purified nucleic acid or collect and test a new sample.
Sample ID	Unresolved	Fail		**NEG**	**NEG**	**NEG**	Unresolved – PCR inhibition or reagent failure. Repeat testing from the purified nucleic acid or collect and test a new sample.
Sample ID	ND	ND	3079[2]	ND	ND	ND	Not Determined – error code 3079
Sample ID	Invalid		4098[3]	ND	ND	ND	Not Determined – error code 4098

[1] *Columns and data not used for interpretation are not included*

[2] *Error Code 3079: Warning/Error Code 3079 is periodically observed with Influenza A positives (Influenza A Positive Control, Influenza A positive NP swab samples). Warning/Error Code 3079 occurs when the fluorescence (RFU) signal is too high. In this case, all results for that sample are reported by the Dx software as ND (Not Determined). If a Ct value ≥13 is reported in the* ***Influenza A, RSV, and/or Influenza B Ct*** *columns, the sample results can be recorded as POS for the specific analyte(s).*

[3] *An Invalid assay run will display Error Code 4098*

* *Detection of the Internal Control in the Cy5 detection channel is not required for positive result. High viral load can lead to reduced or absent Internal Control signal.*

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Luminex Molecular Diagnostics, xTAG™ Respiratory Viral Panel (RVP)
   Gen-Probe Prodesse, ProFlu+ Assay

2. Predicate 510(k) number(s):
   K063765, K073029

3. Comparison with predicate:

Features	**New ProFlu+ Assay**	**Current ProFlu+ Assay**	**Luminex RVP**
510(k)	TBD	K073029	K063765
Regulation	866.3980	866.3980	866.3980
Product Code	OCC	OCC	OCC, OEM, OEP
Device Class	Class II	Class II	Class II
Intended Use	For the *in vitro* qualitative detection and differentiation of influenza A, influenza	For the *in vitro* qualitative detection and differentiation of influenza A, influenza	Direct and differential qualitative detection of influenza types A

Features	New ProFlu+ Assay	Current ProFlu+ Assay	Luminex RVP
	B, and RSV viral nucleic acids.	B, and RSV viral nucleic acids.	and B, RSV types A and B, Parainfluenza types 1, 2 and 3, Adenovirus and Rhinovirus viral nucleic acids.
Technology/Detection	Real Time RT-PCR Detection	Real Time RT-PCR Detection	RT-PCR Detection: Amplified products are coupled to microspheres and detected using spectrofluorometric analysis.
Specimen Types	NP swabs	NP swabs	NP swabs
Nucleic Acid Isolation	Roche MagNA Pure LC System and bioMerieux NucliSENS easyMAG	Roche MagNA Pure LC System and bioMerieux NucliSENS easyMAG	NucliSENS® miniMAG extraction Kit (bioMerieux) QIAamp® MiniElute® Virus Spin Kit (Qiagen)
Instrument /Assay Platform	Cepheid SmartCycler II System	Cepheid SmartCycler II System	Luminex 100 or 200
Assay Controls	Influenza A, Influenza B, RSV A, RSV B positive RNA transcript controls and an Internal RNA control provided	Influenza A, Influenza B, RSV A, RSV B positive RNA transcript controls and an Internal RNA control provided	Bacteriophage lambda positive control and *E. coli* MS2 phage Internal Control –ancillary reagents not provided

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

- User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline (CLSI EP12-A 2002)
- Protocols for Determination of Limits of Detection and Limits of Quantitation (CLSI EP-17A 2004)
- Molecular Diagnostic Methods for Infectious Diseases; Approved Guideline (CLSI MM3-A2 2006)
- Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline (CLSI MM9-A 2004)
- Collection, Transport, Preparation, and Storage of Specimens for Molecular Methods (CLSI MM13A 2006)
- Medical devices – Quality management systems – Requirements for regulatory purpose (ISO 13485:2003)
- Medical devices - Application of risk management to medical devices (ISO 14971:2007)
- Stability Testing of In Vitro Diagnostic Reagents (CEN 13640:2002)

- Format for Traditional and Abbreviated 510(k)s - Guidance for Industry and FDA Staff
  http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm084365.html
- Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens - Draft Guidance for Industry and FDA Staff
  http://www.fda.gov/ohrms/dockets/98fr/05d-0434-gd10001.pdf
- Guidance for Off-the-Shelf Software Use in Medical Devices; Final
  http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm073778.html
- Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests; Draft Guidance for Industry and FDA Reviewers
  http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm071148.html
- Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable - Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff
  http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm078384.html
- Draft Guidance for Industry and FDA Staff: Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Influenza Viruses
  http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm079171.html
- In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path - Guidance for Industry and FDA Staff
  http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm078538.html
- Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Respiratory Viral Panel Multiplex Nucleic Acid Assay
  http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm180307.html

L. Test Principle:

ProFlu+ assay enables detection and differentiation of Influenza A Virus, Influenza B Virus, Respiratory Syncytial Virus (Types A and B), and Internal Control.

Nucleic acids are isolated and purified from nasopharyngeal swab specimens using a MagNA Pure LC System (Roche) and the MagNA Pure Total Nucleic Acid Isolation Kit (Roche) or a NucliSENS easyMAG System (bioMérieux) and the Automated Magnetic Extraction Reagents (bioMérieux). Purified nucleic acids are added to Influenza A/Influenza B/RSV Mix along with enzymes included in the ProFlu+ Detection Kit. The Influenza A/Influenza B/RSV Mix contains oligonucleotide primers and target-specific oligonucleotide probes. The primers are complementary to highly conserved regions of genetic sequences for these respiratory viruses. The

probes are dual-labeled with a reporter dye and a quencher attached to the 3' end or internally.

First RNA is reverse transcribed into complementary DNA (cDNA) which is then subsequently amplified in a Cepheid SmartCycler II instrument. In this process, the probe anneals specifically to the template followed by primer extension and amplification. Taq polymerase cleaves the probe utilizing its 5' – 3' exonuclease activity thus separating the reporter dye from the quencher. This generates an increase in fluorescent signal upon excitation from a light source. With each cycle, additional reporter dye molecules are cleaved from their respective probes, further increasing fluorescent signal. The amount of fluorescence at any given cycle is dependent on the amount of amplification products present at that time. Fluorescent intensity is monitored during each PCR cycle by the real-time instrument.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Please refer to previously FDA-cleared 510(k) Premarket Notification, K092500 for additional information.

b. Linearity/assay reportable range:

Not applicable.

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

There are no changes to the controls for the New ProFlu+ Assay. Please refer to previously FDA-cleared 510(k) Premarket Notification, K092500.

d. Detection limits:

The analytical sensitivity (limit of detection or LoD) of the ProFlu+ Assay was determined using 6 Influenza A strains, 1 strain each of Influenza B, RSV A and RSV B. Each viral strain was extracted using the Roche MagNA Pure LC instrument and tested in replicates of 60 per concentration. Analytical sensitivity (LoD) defined as the lowest concentration at which ≥ 95% of all replicates tested positive are summarized in the table below. The LoDs for the reformulated ProFlu+ Assay were identical to the original ProFlu+ Assay for all strains tested.

Viral Strain	LoD Concentration
Influenza A/ Virginia/1/06 (H1N1)	$1x10^0$ $TCID_{50}$/mL
Influenza A/New Caledonia/12/99 (H1N1)	$1x10^3$ $TCID_{50}$/mL
Influenza A/Port Chalmers/1/73 (H3N2)	$1x10^2$ $TCID_{50}$/mL
Influenza A/California/07/04 (H3N2)	$1x10^0$ $TCID_{50}$/mL
Influenza A/California/04/09 (2009 H1N1)	$1x10^1$ $TCID_{50}$/mL

Influenza A/Clinical Isolate, Chicago, IL (2009 H1N1)	$1x10^1$ $TCID_{50}$/mL
Influenza B/Wisconsin/2/06	$1x10^1$ $TCID_{50}$/mL
RSV A Strain Long	$1x10^1$ $TCID_{50}$/mL
RSV B Strain Wash/18537/62	$1x10^2$ $TCID_{50}$/mL

e. Analytical specificity:

Cross Reactivity

The analytical specificity of the ProFlu+ Assay was evaluated by testing a panel of 58 cultures consisting of 31 viral, 26 bacterial, and 1 yeast strain representing common respiratory pathogens or flora commonly present in nasopharynx. Bacteria and yeast were tested at concentrations of 10^5 to10^8 CFU/mL. Viruses were tested at concentrations of 10^3 to 10^6 $TCID_{50}$/mL, except where noted. Samples were extracted using the Roche MagNA Pure LC instrument and tested in triplicate. Analytical specificity of the ProFlu+ Assay was 100%.

Strains	Concentration	Influenza A	RSV	Influenza B
H1N1 IA New Caledonia	10^3 $TCID_{50}$/ml	+	-	-
H3N2 IA Port Chalmers	$10^3$$TCID_{50}$/ml	+	-	-
IB Wisconsin	10^1 $TCID_{50}$/ml	-	-	+
RSV A Long	10^2 $TCID_{50}$/ml	-	+	-
RSV B Wash	10^3 $TCID_{50}$/ml	-	+	-
Adenovirus 1/Adenoid 71	10^6 $TCID_{50}$/mL	-	-	-
Adenovirus 7	10^6 $TCID_{50}$/mL	-	-	-
Coronavirus 229E	10^6 $TCID_{50}$/mL	-	-	-
Coxsackie B4	10^4 $TCID_{50}$/mL	-	-	-
Coxsackie B5/10/2006	10^5 $TCID_{50}$/mL	-	-	-
Cytomegalovirus	10^4 $TCID_{50}$/mL	-	-	-
Echovirus 2	10^6 $TCID_{50}$/mL	-	-	-
Echovirus 3	10^5 $TCID_{50}$/mL	-	-	-
Echovirus 6	10^5 $TCID_{50}$/mL	-	-	-
Echovirus 11	10^6 $TCID_{50}$/mL	-	-	-
Enterovirus 68	10^3 $TCID_{50}$/mL	-	-	-
Enterovirus 70	10^3 $TCID_{50}$/mL	-	-	-
Epstein Barr Virus	10^8 copies/mL	-	-	-
HSV Type 1 MacIntyre Strain	10^5 $TCID_{50}$/mL	-	-	-
HSV Type 2 G strain	10^5 $TCID_{50}$/mL	-	-	-
Human Metapneumovirus A2	10^4 $TCID_{50}$/mL	-	-	-
Human Rhinovirus 1a	10^3 $TCID_{50}$/mL	-	-	-
Human Rhinovirus	10^3 $TCID_{50}$/mL	-	-	-
Measles/7/2000	10^4 $TCID_{50}$/mL	-	-	-
Mumps Virus	10^3 $TCID_{50}$/mL	-	-	-
Parainfluenza Type 1	10^3 $TCID_{50}$/mL	-	-	-
Parainfluenza Type 2	10^5 $TCID_{50}$/mL	-	-	-
Parainfluenza Type 3	10^6 $TCID_{50}$/mL	-	-	-
Parainfluenza Type 4	10^4 $TCID_{50}$/mL	-	-	-
Poliovirus 1	10^6 $TCID_{50}$/mL	-	-	-
Varicella Zoster Virus	10^4 $TCID_{50}$/mL	-	-	-

Strains	Concentration	Influenza A	RSV	Influenza B
Bordetella pertussis	10^8 cfu/mL	-	-	-
Bordetella bronchoiseptica	10^7 cfu/mL	-	-	-
Chlamydia pneumonia	10^6 $TCID_{50}$/mL	-	-	-
Chlamydia trachomatis	10^6 $TCID_{50}$/mL	-	-	-
Legionella micdadei	10^7 cfu/mL	-	-	-
Legionella pneumophila	10^7 cfu/mL	-	-	-
Mycobacterium intracellulare	10^6 cfu/mL	-	-	-
Mycobacterium tuburculosis	10^5 cfu/mL			
Mycoplasma pneumonia	10^6 cfu/mL	-	-	-
Haemophilus influenza	10^8 cfu/mL	-	-	-
Pseudomonas aeruginosa	10^7 cfu/mL	-	-	-
Proteus vulgaris	10^7 cfu/mL	-	-	-
Proteus mirabilis	10^7 cfu/mL	-	-	-
Neisseria gonorrhoeae	10^7 cfu/mL	-	-	-
Neisseria meningitides	10^7 cfu/mL	-	-	-
Neisseria mucosa	10^7 cfu/mL	-	-	-
Klebsiella pneumonia	10^7 cfu/mL	-	-	-
Escherichia coli	10^7 cfu/mL	-	-	-
Moraxella catarrhalis	10^6 cfu/mL	-	-	-
Corynebacterium diptheriae	10^7 cfu/mL	-	-	-
Lactobacillus plantarum	10^7 cfu/mL	-	-	-
Streptococcus pneumoniae	10^5 cfu/mL	-	-	-
Streptococcus pyogenes	10^7 cfu/mL	-	-	-
Streptococcus salivarius	10^6 cfu/mL	-	-	-
Staphylococcus epidermidis	10^7 cfu/mL	-	-	-
Staphylococcus aureus	10^7 cfu/mL	-	-	-
Candida albicans	10^7 cfu/mL	-	-	-

Reactivity

The reactivity of the ProFlu+ Assay was evaluated against multiple strains of Influenza A, Influenza B, and Respiratory Syncytial Viruses. The panel consisted of 13 Influenza A subtype H1N1, 13 Influenza A subtype H3N2, 9 swine-origin Influenza A, 2 Influenza A subtype H5N1, 10 Influenza B, and 2 Respiratory Syncytial Virus strains. Each viral strain was extracted using the Roche MagNA Pure LC and tested in triplicate in each assay.

Viral Strain	Concentration	Influenza A	RSV	Influenza B
A/Taiwan/42/06 (H1N1)	$1x10^1$ $TCID_{50}$/mL	+	-	-
A/Henan/8/05 (H1N1)	$1x10^1$ $TCID_{50}$/mL	+	-	-
A/Fuijan/156/00 (H1N1)	$1x10^1$ $TCID_{50}$/mL	+	-	-
Brazil/1137/99 (H1N1)	$1x10^3$ $TCID_{50}$/mL	+	-	-

Viral Strain	Concentration	Influenza A	RSV	Influenza B
A/Kentucky/2/06 (H1N1)	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Hawaii/15/01 (H1N1)	$1x10^{3}$ $TCID_{50}$/mL	+	-	-
A/Brisbane/59/2007 (H1N1)	$1x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Solomon Islands/03/06 (H1N1)	$2x10^{1}$ $TCID_{50}$/mL	+	-	-
A/Jiangxi/160/05 (H1N1)	$2x10^{1}$$TCID_{50}$/mL	+	-	-
A/WS/33 (H1N1)	$5x10^{3.75}$ $TCID_{50}$/mL✪	+	-	-
A1/Mal/302/54 (H1N1)	$5x10^{5.25}$ $TCID_{50}$/mL ✪	+	-	-
A/PR/8/34 (H1N1)	$1x10^{6}$ $TCID_{50}$/mL	+	-	-
VR 546 A1/Denver/1/57 (H1N1)	$5x10^{5.25}$ $TCID_{50}$/mL ✪	+	-	-
A/Hiroshima/52/05 (H3N2)	$2x10^{0}$ $TCID_{50}$/mL	+	-	-
A/Victoria/512/05 (H3N2)	$2x10^{0}$ $TCID_{50}$/mL	+	-	-
VR 822 A/Victoria/3/75 (H3N2)	$2x10^{3}$ $TCID_{50}$/mL ✪	+	-	-
A/Brazil/02/99 (H3N2)	$2x10^{2}$ $TCID_{50}$/mL	+	-	-
A/New York/55/2004 (H3N2)	$2x10^{0}$ $TCID_{50}$/mL	+	-	-
A/Hong Kong/2831/05 (H3N2)	$2x10^{1}$ $TCID_{50}$/mL	+	-	-
A/Bahamas/2686/99 (H3N2)	$2x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Fuijan/411/02 (H3N2)	$2x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Kentucky/03/06 (H3N2)	$2x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Costa Rica/07/99 (H3N2)	$2x10^{2}$ $TCID_{50}$/mL	+	-	-
A/Hong Kong/218/06 (H3N2)	$2x10^{2}$ $TCID_{50}$/mL	+	-	-
VR 544 A/Hong Kong/8/68 (H3N2)	$2x10^{2}$ $CEID_{50}$/mL✪	+	-	-
VR 547 A/Aichi/2/68 (H3N2)	$2x10^{2}$ $CEID_{50}$/mL✪	+	-	-
2009 H1N1 Clinical Isolate #1	$2x10^{2}$ $TCID_{50}$/mL	+	-	-
2009 H1N1 Clinical Isolate #2	$2x10^{3}$ $TCID_{50}$/mL	+	-	-
2009 H1N1 Clinical Isolate #3	$2x10^{2}$ $TCID_{50}$/mL	+	-	-
2009 H1N1 Clinical Isolate #4	$2x10^{1}$ $TCID_{50}$/mL	+	-	-
2009 H1N1 Clinical Isolate #5	$2x10^{0}$ $TCID_{50}$/mL	+	-	-
A/New Jersey/8/76 (Swine-origin)	$2x10^{4}$ $CEID_{50}$/mL✪	+	-	-
A/South Dakota/03/2008 (Swine-origin)	$2x10^{3}$ $TCID_{50}$/mL	+	-	-
A/Wisconsin/10/1998 (swine-origin)	$2x10^{3}$ $TCID_{50}$/mL	+	-	-
A/Iowa/2006 (swine-origin)	$2x10^{3}$ $TCID_{50}$/mL	+	-	-
H5N1/VN/1203 2.7ng/µL RNA	2.7ng/µL	+	-	-
H5N1/HK/486 1.4ng/µL RNA	1.4ng/µL	+	-	-
B/Hawaii/11/05	$1x10^{2}$ $TCID_{50}$/mL	-	-	+
B/Michigan/2/06	$1x10^{2}$ $TCID_{50}$/mL	-	-	+
B/Hawaii/33/2004	$1x10^{2}$ $TCID_{50}$/mL	-	-	+
B/Ohio/1/2005	$1x10^{2}$ $TCID_{50}$/mL	-	-	+
B/Florida/2/06	$1x10^{2}$ $TCID_{50}$/mL	-	-	+
B/St. Petersburg/04/06	$1x10^{2}$ $TCID_{50}$/mL	-	-	+
B/Michigan/4/06	$1x10^{2}$ $TCID_{50}$/mL	-	-	+
B/Malaysia 2506/2004	$1x10^{2}$ $TCID_{50}$/mL	-	-	+
B/Florida/7/2004	$1x10^{2}$ $TCID_{50}$/mL	-	-	+
B/Lee/40	$1x10^{2}$ $TCID_{50}$/mL	-	-	+
RSV A strain A2	$1x10^{2}$ $TCID_{50}$/mL	-	+	-
RSV B strain 9320	$1x10^{2}$ $TCID_{50}$/mL	-	+	-

✪ Strains not re-cultured and titered. The original culture/titer from ATCC was used in this study.

f. Interference Studies:

Competitive Interference of the ProFlu+ Assay was evaluated using simulated samples containing pairs of target viruses (Influenza A and Influenza B, Influenza A and RSV, Influenza B and RSV) at two different concentrations. One of the concentrations was near the Limit of Detection (LoD) while the other concentration was 1000x the LoD. Samples were extracted using the Roche MagNA Pure LC instrument and tested in triplicate. The presence of two viruses at varying concentrations in a single sample had no effect on the analytical sensitivity (limit of detection or LoD) of the ProFlu+ Assay.

g. Extraction equivalency:

Extraction equivalency of the bioMérieux NucliSENS easyMAG and Roche MagNA Pure LC instruments was evaluated by performing a limit of detection study and a reproducibility study using both instrument systems. For the limit of detection study, Influenza A, Influenza B, RSV A or RSV B virus was diluted into NP swab matrix to the study's limit of detection. Each viral strain dilution was extracted in replicates of 10 on each automated extractor and tested using the ProFlu+ Assay. For the reproducibility study, a panel of 6 simulated samples that included medium and low (near the assay limit of detection) Influenza A and RSV A positive and negative samples was tested. The panel was run on each automated extractor 2 times per day for a total of 5 days.

The bioMérieux NucliSens easyMAG instrument performed equivalently to the Roche MagNA Pure LC instrument with respect to limit of detection and reproducibility (percent agreement). The limits of detection were the same for both instruments and the overall reproducibility percent agreement was 100%.

h. Carry-over/Cross-Contamination

In an internal study there was no evidence of carry-over/cross contamination with the ProFlu+ Assay using either the Roche MagNA Pure LC or the bioMériuex NucliSens easyMAG automated nucleic acid extraction instruments.

2. Comparison studies:

Clinical Comparison Results

The ProFlu+ Assay's supermix was reformulated and performance characteristics were established by comparing the reformulated assay to the original ProFlu+ Assay. All samples positive for Influenza A, Influenza B or RSV using either the current ProFlu+ Assay and/or the reformulated "New" ProFlu+ Assay were confirmed using bidirectional sequencing. The sequencing assays targeted either a different gene than the ProFlu+ Assay or targeted a different region of the same gene as the ProFlu+ Assay. Prospectively collected archived samples from respiratory season years 2008 and 2009 that were collected at two clinical study sites (Columbus, OH and Albuquerque, NM) were used for this study.

"True" influenza A, influenza B or RSV positives were considered as any sample that tested positive for the respective analyte by the original ProFlu+ Assay. "True" influenza A, influenza B or RSV negatives were considered as any sample that tested negative by the original ProFlu+ Assay.

Influenza A Comparison Results

		Current ProFlu+ Assay			
		Positive	**Negative**	**Total**	**Comments**
New Pro Flu+	**Positive**	60	1*	**61**	Percent Positive Agreement 100% (93.98%-100%) 95% CI
	Negative	0	172	**172**	Percent Negative Agreement 99.4% (96.80%-99.90%) 95% CI
	Total	**60**	**173**	**233**	

* Sample was positive for Influenza A using bi-directional sequencing.

Influenza B Comparison Results

		Current ProFlu+ Assay			
		Positive	**Negative**	**Total**	**Comments**
New Pro Flu+ Assay	**Positive**	14	0	**14**	Percent Positive Agreement 100% (78.47% - 100%) 95% CI
	Negative	0	219	**219**	Percent Negative Agreement 100% (98.28% - 100%) 95% CI
	Total	**14**	**219**	**233**	

RSV Comparison Results

		Current ProFlu+ Assay			
		Positive	**Negative**	**Total**	**Comments**
New Pro Flu+	**Positive**	35	2*	**37**	Percent Positive Agreement 100% (90.11% - 100%) 95% CI
	Negative	0	196	**196**	Percent Negative Agreement 99.0% (96.39%-99.72%) 95% CI
	Total	**35**	**198**	**233**	

* Two samples were positive for RSV using bi-directional sequencing.

3. Clinical studies:

   Clinical performance characteristics of the ProFlu+ Assay were established during a prospective study at 3 U.S. clinical laboratories and a retrospective study at 1 U.S. site during the 2006-2007 respiratory virus season (February – April). Please refer to previously FDA-cleared 510(k) Premarket Notification, K092500 for additional information.

4. Clinical cut-off**:** N/A

5. Expected values/Reference range:

The prevalence of Influenza and RSV 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 U.S. respiratory season, the combined prevalence of Influenza A and Influenza B was 13.2%[8] and in 2005-2006 the combined prevalence was 12.1%[9]. The prevalence of RSV during the 2005-2006 season was 16.2%[10]. In the 2007 ProFlu+ multi-center clinical study (samples collected between February and April), the prevalence as observed with culture of Influenza A was 15.8%, Influenza B was 5.4% and RSV was 4.2%. As influenza and RSV seasons overlap, dual positive infections can occur. During this study, culture and the ProFlu+ Assay each detected one Influenza A and RSV dual-positive (although not the same sample) and the ProFlu+ Assay detected one Influenza A and Influenza B dual-positive out of the 891 total samples included in the study. Because the incidence of a triple infection of Influenza A, Influenza B, and RSV is low, it is recommended that the samples undergo repeat testing if nucleic acids from all three analytes are detected in a single sample.

The performance of the modified ProFlu+ assay has been demonstrated using a subset of archived prospectively collected clinical samples from 2008 - 2009 influenza seasons. They were selected to include 20 Influenza A 2009 H1N1, 20 Influenza A H1, 20 Influenza A H3, 10 Influenza B, 10 RSV positive and 121 negative samples.

N. Instrument Name:

Roche MagNA Pure LC System with software version 3.0.11 or bioMérieux NucliSENS easyMAG System with Software version 1.0.1 or 2.0. Cepheid SmartCycler II Real Time Instrument with Dx software version 1.7b or 3.0 a/b.

O. System Descriptions:

1. Modes of Operation:

The Roche MagNA Pure LC is an automated nucleic acid isolation and purification system based upon binding of nucleic acids to glass particles and has the capability to process a total of 32 reactions within one run. Nucleic acid is purified in multiple plastic reaction tips and cartridges by several steps that include cell lysis and binding of nucleic acid to magnetic glass particles, wash steps, and a heated elution to unbind the nucleic acid from the glass particles.

The bioMérieux NucliSens easyMAG is an automated nucleic acid isolation and purification system that is based upon the same silica extraction technology as the MagNA Pure. The easyMAG is capable of processing a total of 24 reactions with variable sample types, sample volumes, and elution volumes within a single run.

Nucleic acid is purified within a single cartridge by several steps that include lysis and binding of nucleic acid to high affinity magnetic silica beads, a series of wash steps and heated elution of purified nucleic acid from the silica beads.

The Cepheid Smart Cycler II Real Time instrument with Dx software version 1.7b, 3.0a/b is used to perform polymerase chain reaction (PCR) amplification and detection of nucleic acid. Other nucleic acid amplification tests that use the Smart Cycler II instrument have previously received 510(k) clearance: these tests include Prodesse's ProFAST+ Assay (K101855), ProParaflu+ Assay (K091053), ProGastro Cd Assay (K090239), ProhMPV+ Assay (K082688) and others. The Cepheid SmartCycler instrument is an integrated nucleic acid amplification and detection instrument system based on Cepheid's proprietary microprocessor-controlled I-CORE module. For purified DNA samples, the SmartCycler instrument enables PCR for the amplification of DNA and hybridization of fluorogenic target-specific probes for the detection of the amplified cDNA.

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 manually enters Patient ID/Sample ID.

4. Specimen Sampling and Handling:

   Not applicable

5. Calibration:

   Not applicable

6. Quality Control:

   Positive Control (PC): The ProFlu+ Assay Control kit contains four RNA controls that consist of non-infectious *in vitro* transcribed RNA of specific Influenza A, Influenza B, RSV A or RSV B viral sequences. The PC does not go through nucleic acid isolation and purification, but is included with each RT-PCR run. The PC in conjunction with the IC is used to test for procedural errors in order to verify reagent and system performance.

   Internal Control (IC): The IC is a non-infectious RNA transcript that is distinguished from the viral targets of the assay by means of a unique IC primer and probe set present in the ProFlu+ Assay. The IC is incorporated into every

sample and is carried through all steps of the procedure from nucleic acid isolation and purification through amplification to monitor for inhibitors present in the specimen or reaction tube. The IC also serves as a general process control ensuring that each step of the procedure was performed correctly, assay and instrument parameters were set correctly, and that general reagents were working.

Negative Control (NC): A Negative Control is not provided with the kit, but is required and described in the ProFlu+ Assay Instructions for Use. Viral transport medium spiked with the IC is to be used as the Negative Control and is processed starting from nucleic acid isolation. The Negative Control serves to monitor for contamination.

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.