

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

A. 510(k) Number:

k073029

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 detected:

Influenza A, Influenza B, Respiratory Syncytial Virus Type A, Respiratory Syncytial Virus Type B

D. Type of Test:

Multiplex nucleic acid assay, qualitative determination of 4 respiratory virus types (Influenza A, Influenza B, Respiratory Syncytial Virus Type A, Respiratory Syncytial Virus Type B) in nasopharyngeal swabs using nucleic acid isolation, amplification and detection on the Cepheid SmartCycler II Real Time Instrument with Dx Software version 1.7b, which generates signals based on the acquisition of spectrofluorometric data.

E. Applicant:

Prodesse Incorporated

F. Proprietary and Established Names:

ProFlu™ Plus

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
4. Panel:
Microbiology (83)

H. Intended Use:

1. Intended use(s):

The ProFlu+™ Assay is a multiplex Real Time 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.

A negative test is presumptive and it is recommended these results be confirmed by cell culture. 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.

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 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:
Cepheid SmartCycler II Real Time Instrument

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 the MagNA Pure LC Instrument (Roche) and the MagNA Pure Total Nucleic Acid Isolation Kit (Roche).
4. Add purified nucleic acids to ProFlu+ Supermix along with enzymes included in the ProFlu+ Detection Kit. The ProFlu+ Supermix 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 attached to the 5'-end and a quencher dye attached to the 3'-end (see Table below).

- 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 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 Orange 560	540 nm	561 nm	TET
Respiratory Syncytial Virus B	Polymerase	Cal Orange 560	540 nm	561 nm	TET
Influenza B Virus	Non-structural NS1 and NS2	Cal Red 610	595 nm	615 nm	Texas Red
Internal Control	NA	Quasar 670	647 nm	667 nm	Cy5

Materials Provided

ProFlu+ Assay consists of two separate boxes: Detection Kit (Cat. # H44VK00) and Control Kit (Cat. # H44VK55).

Box 1: Detection Kit (Cat. # H44VK00)

Reagents	Description	Quantity/ Tube	Cap Color	Cat. #	Reactions/ Tube
ProFlu+ Supermix	<ul style="list-style-type: none"> ➤ Taq DNA polymerase ➤ 5 oligonucleotide primer pairs ➤ 5 oligonucleotide probes ➤ Buffer containing dNTPs (dATP, dCTP, dGTP, dTTP), ➤ MgCl₂ and stabilizers ➤ Bovine serum albumin 	1030 µL	Brown	HSM77	50 (2 tubes provided)
M-MLV Reverse Transcriptase	➤ 10U/µL	30 µL	Red	GLS26	100
RNase Inhibitor	➤ 40U/µL	100 µL	Blue	GLS27	100
Internal RNA Control III	➤ Non-infectious <i>in vitro</i> transcribed RNA	30 µL	Yellow	GCT12	100

Box 2: Control Kit (Cat. # H44VK55)

Reagents	Description	Quantity/ Tube	Cap Color	Cat. #	Controls/ Tube
Influenza A RNA Control III	➤ Non-infectious <i>in vitro</i> transcribed RNA of specific viral sequences	300 µL	White	HCT 75	15
Influenza B RNA Control III	➤ Non-infectious <i>in vitro</i> transcribed RNA of specific viral sequences	300 µL	Green	HCT 76	15
RSV A RNA Control III	➤ Non-infectious <i>in vitro</i> transcribed RNA of specific viral sequences	300 µL	Purple	HCT 77	15
RSV B RNA Control III	➤ Non-infectious <i>in vitro</i> transcribed RNA of specific viral sequences	300 µL	Clear	HCT 78	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)
- Cepheid PCR reaction tubes, 25µL
- Parafilm® M or MagNA Pure LC Cartridge Seal

Reagents

- Roche MagNA Pure LC Total Nucleic Acid Isolation Kit (*Roche Cat. # 03038505001*) for 192 isolations
- Micro Test™ M4 Viral Transport Medium (*Remel, Inc. Cat. # 12500*) or *BD Universal Viral Transport medium (UTM Becton, Dickinson and Co. Cat. # 220220)*
- Molecular Grade Water (RNase/DNase Free)*
- Extraction Control (e.g. previously characterized positive sample)*

Equipment

- 70°C Freezer
- Roche MagNA Pure LC System with software version 3.0.11
- Cepheid SmartCycler II Real Time Instrument with Dx Software version 1.7b
- Micropipettors (range between 1-10 µL, 10-200 µL and 100-1000 µL)
- Mini-centrifuge with adapter for Cepheid Reaction Tubes
- Cepheid cooling block

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'	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
Sample ID	Unresolved	Fail		NEG	NEG	NEG	Unresolved – PCR inhibition or reagent failure
Sample ID	ND ³	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:

- Predicate device name(s):
xTAG™ RVP (Respiratory Viral Panel)
Common Name: Respiratory Viral Panel (RVP) Multiplex Nucleic Acid Detection Assay
- Predicate 510(k) number(s):
k063765
- Comparison with predicate:
Both assays detect Influenza A and B, and respiratory syncytial virus using

nucleic acid amplification techniques. Both assays use nasal pharyngeal swabs as the collection device and the MagNA Pure LC system for nucleic acid isolation. The detection system with both assays involves spectrophotometric detection. The assays differ in that the predicate also detects Influenza A subtypes H1 and H3, Parainfluenza 1, Parainfluenza 2, and Parainfluenza 3 virus, Rhinovirus, and Adenovirus.

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)
- Guidance on In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path (April 2006)
- Guidance on Informed Consent for In Vitro Diagnostic Device Studies Leftover Human Specimens that are Not Individually Identifiable (April 2006)
- 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).

L. Test Principle:

See I

M. Performance Characteristics (if/when applicable):

Expected Values

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% 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 ProFlu+ detected one Influenza A and Influenza B dual-positive out of the 901 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.

Clinical Performance

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). Samples used for this study were nasopharyngeal (NP) swab specimens that were collected for routine influenza or RSV testing by each site.

The reference method was rapid culture (shell vial) followed by direct fluorescent antibody (DFA) screening and identification.

A total of 891 NP swab samples were tested with the ProFlu+ Assay and by culture. Five (5) samples that initially gave unresolved results remained unresolved upon retesting with the ProFlu+ Assay and are not included in the analysis below. All 5 samples were culture negative.

A total of 23 samples were DFA Respiratory Virus Screen positive (screening reagent detects Influenza A and B, RSV, Parainfluenza 1, 2 and 3 and Adenovirus), but contained too few cells to obtain a specific positive identification. 21 of these 23 samples were also positive by the ProFlu+ Assay (9 Influenza A positive, 11 Influenza B positive and 1 RSV positive) and genetic sequencing analysis confirmed the identification of the specific virus. The other 2 DFA screen positive samples were negative by the ProFlu+ Assay and sequence analysis confirmed that they were negative for Influenza A, Influenza B and RSV; these 2 samples were considered true negatives. Discrepant analysis for samples where ProFlu+ Assay and culture results were in disagreement was performed using RT-PCR with virus specific primers obtained from literature followed by sequencing.

Results from Prospective Study

Influenza A Comparison Results

		<i>Reference Method</i>		Total	Comments
		Positive	Negative		
ProFlu+ Assay	Positive	127	52 ^a	179	Sensitivity 100% (97.1% - 100%) 95% CI
	Negative	0	647	647	Specificity 92.6% (90.4% - 94.3%) 95% CI
	Total	127	699	826	

^a Forty-three (43) samples positive for Influenza A by sequence analysis, 8 samples negative for Influenza A by sequence analysis, and 1 sample unavailable for sequence analysis.

Influenza B Comparison Results

		<i>Reference Method</i>		Total	Comments
		Positive	Negative		
ProFlu+ Assay	Positive	45	11 ^a	56	Sensitivity 97.8% (88.7% - 99.6%) 95% CI
	Negative	1 ^b	769	770	Specificity 98.6% (97.5% - 99.2%) 95% CI
	Total	46	780	826	

^a Eleven (11) samples positive for Influenza B by sequence analysis.

^b One (1) sample negative for Influenza B by sequence analysis.

RSV Comparison Results

		<i>Reference Method</i>		Total	
		Positive	Negative		
ProFlu+ Assay	Positive	34 ^a	40 ^a	74	Sensitivity 89.5% (75.9% - 95.8%) 95% CI
	Negative	4 ^b	748	752	Specificity 94.9% (93.2% - 96.2%) 95% CI
	Total	38	788	826	

^a Thirty-four (34) samples positive for RSV by sequence analysis, 3 samples negative for RSV by sequence analysis, and 3 samples unavailable for sequence analysis.

^b One (1) sample positive for RSV by sequence analysis and 3 samples negative for RSV by sequence analysis.

Results from Retrospective Study

Influenza A Comparison Results

		<i>Reference Method</i>		Total	Comments
		Positive	Negative		
ProFlu+ Assay	Positive	5	2 ^a	7	Sensitivity 100% (56.6% - 100%) 95% CI
	Negative	0	53	53	Specificity 96.4% (87.7% - 99.0%) 95% CI
	Total	5	55	60	

^a One (1) samples positive for Influenza A by sequence analysis and 1 sample negative for Influenza A by sequence analysis

Influenza B Comparison Results

		<i>Reference Method</i>		Total	Comments
		Positive	Negative		
ProFlu+ Assay	Positive	17	0	17	Sensitivity 89.5% (68.6% - 97.1%) 95% CI
	Negative	2 ^a	41	43	Specificity 100% (91.4% - 100%) 95% CI
	Total	19	41	60	

^a Two (2) samples positive for Influenza B by sequence analysis.

RSV Comparison Results

		<i>Reference Method</i>		Total	
		Positive	Negative		
ProFlu+ Assay	Positive	23	1 ^a	24	Sensitivity 100% (85.7% - 100%) 95% CI
	Negative	0	36	36	Specificity 97.3% (86.2% - 99.5%) 95% CI
	Total	23	37	60	

^a One sample positive for RSV by sequence analysis.

Reproducibility

The reproducibility of the ProFlu+ Assay was evaluated at 3 laboratory sites. Reproducibility was assessed using a panel of 10 simulated samples that included medium and low (near the assay limit of detection) Influenza A, Influenza B, or RSV positive and negative samples. Panels and controls were tested at each site by 2 operators for 5 days (10 samples and 5 controls X 2 operators X 5 days X 3 sites = 450). The overall percent agreement for the ProFlu+ Assay was 98%.

Panel Member ID	Site 1			Site 2			Site 3			Total Agreement with expected result (%)	95% Confidence Interval
	Agreement with expected result	AVE C _T	%CV	Agreement with expected result	AVE C _T	%CV	Agreement with expected result	AVE C _T	%CV		
Negative (2 Panel Members)	20/20	30.5	3.2%	20/20	31.2	7.1%	19*/20	32.2	2.4%	59/60 (98%)	91% - 100%
Influenza A Low Positive	10/10	36.0	3.3%	9/10	36.4	3.9%	7/10	37.8	5.3%	26/30 (87%)	70% - 95%
Influenza A Medium Positive	10/10	32.6	1.4%	10/10	33.4	4.0%	10/10	33	2.5%	30/30 (100%)	89% - 100%
Influenza B Low Positive	10/10	32.7	1.4%	10/10	32.6	1.4%	10/10	32.2	1.9%	30/30 (100%)	89% - 100%
Influenza B Medium Positive	10/10	30.5	1.3%	10/10	30.1	0.7%	10/10	29.7	0.8%	30/30 (100%)	89% - 100%
RSV A Low positive	8/10	30.1	8.3%	8/10	32.5	6.2%	8/10	30.7	6.8%	24/30 (80%)	63% - 90%
RSV A medium positive	10/10	29.5	3.0%	10/10	29.5	3.0%	10/10	29.2	2.7%	30/30 (100%)	89% - 100%
RSV B low positive	10/10	31.9	3.5%	10/10	32.3	5.5%	10/10	31.8	5.1%	30/30 (100%)	89% - 100%
RSV B medium positive	10/10	29.5	1.9%	10/10	29.5	4.0%	10/10	28.7	4.2%	30/30 (100%)	89% - 100%
Influenza A RNA Control	10/10	33.5	1.6%	10/10	32.9	4.2%	10/10	34.4	0.9%	30/30 (100%)	89% - 100%
Influenza B RNA Control	10/10	32.8	1.4%	10/10	32.1	3.1%	10/10	33.8	1.3%	30/30 (100%)	89% - 100%
RSV A RNA Control	10/10	33.7	1.8%	10/10	32.3	3.1%	10/10	34.8	1.5%	30/30 (100%)	89% - 100%
RSV B RNA Control	10/10	32.1	1.6%	10/10	31.9	4.3%	10/10	35.2	2.5%	30/30 (100%)	89% - 100%
Negative Control	10/10	28.9	4.0%	10/10	29.6	5.2%	10/10	30.2	1.4%	30/30 (100%)	89% - 100%
Total Agreement All	148/150 (99%)			147/150 (98%)			144/150 (96%)			439/450 (98%)	96% - 99%

* 1 negative sample Unresolved (IC = FAIL). C_T values for Influenza A, Influenza B and RSV were negative, however.

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the ProFlu+ Assay was determined using quantified (TCID₅₀/mL) cultures of 4 Influenza A (2 H1N1 and 2 H3N2), 2 Influenza B, 2 Respiratory Syncytial Virus Type A, and 2 Respiratory Syncytial Virus Type B strains serially diluted in nasopharyngeal clinical matrix. Each viral strain was extracted using the Roche MagNA Pure LC and 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 $10^2 - 10^{-1}$ TCID₅₀/mL.

Viral Strain	LoD Concentration
Influenza A/Port Chalmers/1/73 (H3N2)	10^1 TCID ₅₀ /mL
Influenza A/CA/7/04 (H3N2)	10^0 TCID ₅₀ /mL
Influenza A/New Caledonia/12/99 (H1N1)	10^2 TCID ₅₀ /mL
Influenza A/WS/33 (H1N1)	10^0 TCID ₅₀ /mL
Influenza B/Lee/40	10^1 TCID ₅₀ /mL
Influenza B/Wisconsin/2/06	10^0 TCID ₅₀ /mL
Respiratory Syncytial Virus Type A Strain Long	10^0 TCID ₅₀ /mL
Respiratory Syncytial Virus Type A Strain A-2	10^1 TCID ₅₀ /mL
Respiratory Syncytial Virus Type B Strain Wildtype B-1	10^{-1} TCID ₅₀ /mL
Respiratory Syncytial Virus Type B Strain Wash/18537/62	10^1 TCID ₅₀ /mL

Reactivity

The reactivity of the ProFlu+ Assay was evaluated against multiple strains of Influenza A (H1N1, H3N2, and H5N1 subtypes), Influenza B, and Respiratory Syncytial Viruses. The panel consisted of 12 Influenza A subtype H1N1, 13 Influenza A subtype H3N2, 2 Influenza A subtype H5N1, 11 Influenza B, and 5 Respiratory Syncytial Virus strains. Each viral strain was extracted using the Roche MagNA Pure LC and tested in triplicate. All viral cultures of the panel were detected by the ProFlu+ Assay.

Viral Strain	Concentration	Influenza A (FAM)	RSV (TET)	Influenza B (Tex Red)
Influenza A/Fujian/156/00 (H1N1)	10^2 TCID ₅₀ /mL	+	-	-
Influenza A/Hawaii/15/01 (H1N1)	10^2 TCID ₅₀ /mL	+	-	-
Influenza A/Kentucky/2/06 (H1N1)	10^2 TCID ₅₀ /mL	+	-	-
Influenza A/Jiangxi/160/05 (H1N1)	10^2 TCID ₅₀ /mL	+	-	-
Influenza A/Henan/8/05 (H1N1)	10^2 TCID ₅₀ /mL	+	-	-
Influenza A/Taiwan/42/06 (H1N1)	10^2 TCID ₅₀ /mL	+	-	-
Influenza A/Virginia/1/06 (H1N1)	10^2 TCID ₅₀ /mL	+	-	-
Influenza A/Hong Kong/2652/06 (H1N1)	10^2 TCID ₅₀ /mL	+	-	-
Influenza A/New Caledonia/12/99 (H1N1)	10^2 TCID ₅₀ /mL	+	-	-

Viral Strain	Concentration	Influenza A (FAM)	RSV (TET)	Influenza B (Tex Red)
Influenza A/WS/33 (H1N1)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/PR/8 (H1N1)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/Brazil/1137/99 (H1N1)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/Fujian/411/02 (H3N2)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/New York/55/2004 (H3N2)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/California/07/04 (H3N2)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/Victoria/512/05 (H3N2)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/Hong Kong/218/06 (H3N2)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/Anhui/1239/05 (H3N2)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/Hong Kong/2831/05 (H3N2)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/Hiroshima/52/05 (H3N2)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/Kentucky/03/06 (H3N2)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/Port Chalmers/1/73 (H3N2)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/Bahamas/2686/99 (H3N2)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/Brazil/02/99 (H3N2)	10 ³ TCID ₅₀ /mL	+	-	-
Influenza A/Costa Rica/07/99 (H3N2)	10 ² TCID ₅₀ /mL	+	-	-
Influenza A/HK 486 (H5N1)	0.14 ng/μL*	+	-	-
Influenza A/VN 1203 (H5N1)	0.27 ng/μL**	+	-	-
Influenza B/Florida/7/04	10 ² TCID ₅₀ /mL	-	-	+
Influenza B/Hawaii/11/05	10 ² TCID ₅₀ /mL	-	-	+
Influenza B/Michigan/2/06	10 ² TCID ₅₀ /mL	-	-	+
Influenza B/Wisconsin/2/06	10 ² TCID ₅₀ /mL	-	-	+
Influenza B/Hawaii/33/04	10 ² TCID ₅₀ /mL	-	-	+
Influenza B/Ohio/1/05	10 ² TCID ₅₀ /mL	-	-	+
Influenza B/Florida/2/06	10 ² TCID ₅₀ /mL	-	-	+
Influenza B/St Petersburg/14/06	10 ² TCID ₅₀ /mL	-	-	+
Influenza B/Michigan/4/06	10 ² TCID ₅₀ /mL	-	-	+
Influenza B/Lee/40	10 ² TCID ₅₀ /mL	-	-	+
Influenza B/Malaysia/2506/04	10 ² TCID ₅₀ /mL	-	-	+
RSV A Strain A2	10 ² TCID ₅₀ /mL	-	+	-
RSV A Strain Long	10 ² TCID ₅₀ /mL	-	+	-
RSV B Strain Wildtype B-1	10 ² TCID ₅₀ /mL	-	+	-
RSV B Strain Wash/18537/62	10 ² TCID ₅₀ /mL	-	+	-
RSV B Strain 9320	10 ² TCID ₅₀ /mL	-	+	-

*estimated concentration 2.8 x 10⁵ TCID₅₀/mL

**estimated concentration 7.5 x 10⁴ TCID₅₀/mL

NOTE: Although the ProFlu+ 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.

Analytical Specificity

The analytical specificity of the ProFlu+ Assay was evaluated by testing a panel of 50 cultures consisting of 22 viral, 27 bacterial, and 1 yeast strain representing common respiratory pathogens or flora commonly present in nasopharynx. Bacteria and yeast were tested at concentrations of 10^6 to 10^7 CFU/mL. Viruses were tested at concentrations of 10^3 to 10^6 TCID₅₀/mL. Samples were extracted using the Roche MagNA Pure LC and tested in triplicate. Analytical specificity of the ProFlu+ Assay was 100%.

Strains	Concentration	Influenza A (FAM)	RSV (TET)	Influenza B (Tex Red)
Influenza A/Port Chalmers	10^4 TCID ₅₀ /mL	+	-	-
Influenza B/Wisconsin	10^4 TCID ₅₀ /mL	-	-	+
RSV A/Strain Long	10^4 TCID ₅₀ /mL	-	+	-
RSV B Strain Wash	10^4 TCID ₅₀ /mL	-	+	-
Adenovirus 1/Adenoid 71	10^6 TCID ₅₀ /mL	-	-	-
Coronavirus 229E	10^6 TCID ₅₀ /mL	-	-	-
Coxsackievirus B4	10^4 TCID ₅₀ /mL	-	-	-
Coxsackievirus B5/10/2006	10^5 TCID ₅₀ /mL	-	-	-
Cytomegalovirus	10^4 TCID ₅₀ /mL	-	-	-
Echovirus 2	10^6 TCID ₅₀ /mL	-	-	-
Echovirus 3	10^4 TCID ₅₀ /mL	-	-	-
Echovirus 6	10^5 TCID ₅₀ /mL	-	-	-
Echovirus 11	10^5 TCID ₅₀ /mL	-	-	-
Enterovirus 68	10^3 TCID ₅₀ /mL	-	-	-
Enterovirus 70	10^3 TCID ₅₀ /mL	-	-	-
HSV Type 1 Maclynre strain	10^5 TCID ₅₀ /mL	-	-	-
HSV Type 2 G strain	10^4 TCID ₅₀ /mL	-	-	-
Human Rhinovirus 39	10^3 TCID ₅₀ /mL	-	-	-
Human Rhinovirus	10^4 TCID ₅₀ /mL	-	-	-
Measles/7/2000	10^4 TCID ₅₀ /mL	-	-	-
Mumps virus	10^4 TCID ₅₀ /mL	-	-	-
Parainfluenza Type 1	10^4 TCID ₅₀ /mL	-	-	-
Parainfluenza Type 2	10^5 TCID ₅₀ /mL	-	-	-
Parainfluenza Type 3	10^5 TCID ₅₀ /mL	-	-	-
Parainfluenza Type 4	10^4 TCID ₅₀ /mL	-	-	-
Varicella Zoster	10^4 TCID ₅₀ /mL	-	-	-
<i>Bordetella pertussis</i>	10^6 CFU/mL	-	-	-
<i>Bordetella</i>	10^7 CFU/mL	-	-	-

Strains	Concentration	Influenza A (FAM)	RSV (TET)	Influenza B (Tex Red)
<i>bronchiseptica</i>				
<i>Chlamydophila pneumoniae</i>	10 ⁴ TCID ₅₀ /mL	-	-	-
<i>Chlamydia trachomatis</i>	10 ⁴ TCID ₅₀ /mL	-	-	-
<i>Legionella pneumophila</i>	10 ⁶ CFU/mL	-	-	-
<i>Mycobacterium intracellulare</i>	10 ⁷ CFU/mL	-	-	-
<i>Mycobacterium tuberculosis</i>	10 ⁷ CFU/mL	-	-	-
<i>Mycobacterium avium</i>	10 ⁷ CFU/mL	-	-	-
<i>Haemophilus influenzae</i>	10 ⁶ CFU/mL	-	-	-
<i>Pseudomonas aeruginosa</i>	10 ⁶ CFU/mL	-	-	-
<i>Proteus vulgaris</i>	10 ⁶ CFU/mL	-	-	-
<i>Proteus mirabilis</i>	10 ⁶ CFU/mL	-	-	-
<i>Neisseria gonorrhoeae</i>	10 ⁶ CFU/mL	-	-	-
<i>Neisseria meningitidis</i>	10 ⁶ CFU/mL	-	-	-
<i>Neisseria mucosa</i>	10 ⁷ CFU/mL	-	-	-
<i>Klebsiella pneumoniae</i>	10 ⁶ CFU/mL	-	-	-
<i>Escherichia coli</i>	10 ⁶ CFU/mL	-	-	-
<i>Moraxella catarrhalis</i>	10 ⁷ CFU/mL	-	-	-
<i>Corynebacterium diphtheriae</i>	10 ⁷ CFU/mL	-	-	-
<i>Lactobacillus plantarum</i>	10 ⁶ CFU/mL	-	-	-
<i>Streptococcus pneumoniae</i>	10 ⁶ CFU/mL	-	-	-
<i>Streptococcus pyogenes</i>	10 ⁶ CFU/mL	-	-	-
<i>Streptococcus salivarius</i>	10 ⁶ CFU/mL	-	-	-
<i>Staphylococcus epidermidis</i>	10 ⁶ CFU/mL	-	-	-
<i>Staphylococcus aureus</i>	10 ⁶ CFU/mL	-	-	-
<i>Candida albicans</i>	10 ⁶ CFU/mL	-	-	-

Competitive Inhibition

Competitive inhibition of the ProFlu+ Assay was evaluated using simulated samples with varying concentrations of Influenza A Virus (LoD to 3 logs above LoD) and Respiratory Syncytial Virus A (LoD to 4 logs above LoD) in a single sample. Samples were extracted using the Roche MagNA Pure LC and tested in triplicate. The presence of both Influenza A Virus and Respiratory Syncytial Virus A at varying concentrations in a single sample had no effect on the analytical sensitivity (limit of detection or LoD) of the ProFlu+ Assay.

Carry-over/Contamination

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

N. Proposed Labeling:

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

O. Conclusion:

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