Prodesse™ ProFlu™+ Assay

Instructions for Use

For detection and discrimination of Influenza A Virus, Influenza B Virus and Respiratory Syncytial Virus.

Rx Only

100
-or-
1500

REF

303111/303123

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Intended Use

The Prodesse® 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, collect specimens with appropriate infection control precautions for novel virulent Influenza viruses and send to state or local health department for testing. Do not attempt viral culture in these cases unless a BSL 3+ facility is available to receive and culture specimens.

Summary and Explanation

Influenza is an acute respiratory illness caused by infection with the Influenza virus, primarily types A and B.[1]

- **Influenza A** viruses are further categorized into subtypes based on the two major surface protein antigens: hemagglutinin (H) and neuraminidase (N).[2]
- **Influenza B** viruses are not categorized into subtypes.[2]

Epidemics occur yearly; although both types A and B circulate in the population, type A is usually dominant. These yearly epidemics are partly due to antigenic variation in the H and N surface proteins of the virus. Transmission of influenza is primarily via airborne droplet (coughing or sneezing). Symptoms arise on average 1 to 2 days post-exposure and include fever, chills, headache, malaise, cough and coryza. Gastrointestinal symptoms such as nausea, vomiting and diarrhea can occur, primarily in young children. Complications due to influenza include pneumonia causing increased morbidity and mortality in pediatric, elderly and immunocompromised populations. In the United States, it is estimated that influenza results in greater than 200,000 hospitalizations and up to 36,000 deaths annually.[3]

Respiratory syncytial virus (RSV) is the leading cause of lower respiratory tract infections in infants and children. There are 2 types of RSV (A and B) based on antigenic and surface protein variations. Most yearly epidemics contain a mix of type A and B viruses, but one subgroup can dominate during a season. RSV infection can cause severe respiratory illness among all ages but is more prevalent in pediatric, elderly and immunocompromised populations. RSV can infect up to 80% of children less than 1 years of age.[4] Bronchiolitis and pneumonia are the major clinical complications in infants and young children, resulting in an estimated 51,000-82,000 hospital admissions per year in the US.[5] RSV infection is also an important cause of severe respiratory disease and substantial number of deaths in the elderly[6] with an estimated annual cost of $150 to $680 million for RSV pneumonia hospitalizations.[7]

Principles of the Procedure

The ProFlu+ assay enables detection and differentiation of Influenza A Virus, Influenza B Virus, Respiratory Syncytial Virus (Types A and B), and Universal 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 a Universal Internal Control (UIC) 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+ 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
Universal Internal Control	NA	Quasar 670	647 nm	667 nm	Cy5

Materials Provided

ProFlu+ Assay Kit (100 Reactions) (Cat. # 303111)

Reagents	Description	Quantity/ Vial	Cap Color	Cat. #	Reactions/ Vial
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	402983	50 (2 vials provided)
M-MLV Reverse Transcriptase	➲ 10 U/µL	36 µL	Red	GLS26	100
RNase Inhibitor II	➲ 40 U/µL	120 µL	Green	GLS33	100
Inf. A/Inf. B/RSV A Control	➲ Non-infectious in vitro transcribed RNA of specific viral sequences	500 µL	White	403528	25
RSV B Control	➲ Non-infectious in vitro transcribed RNA of specific viral sequences	300 µL	Clear	403527	15
Universal Internal Control (UIC)	➲ Non-infectious *in vitro* transcribed RNA ➲ Non-infectious DNA plasmid	30 µL	Lilac	403097	100

ProFlu+ Assay Kit (1500 Reactions) (Cat. # 303123)

Reagents	Description	Quantity/ Vial	Cap Color	Cat. #	Reactions/ Vial
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	402983	50 (30 vials provided)
M-MLV Reverse Transcriptase	➲ 10 U/µL	36 µL	Red	GLS26	100 (15 vials provided)
RNase Inhibitor II	➲ 40 U/µL	120 µL	Green	GLS33	100 (15 vials provided)
Inf. A/Inf. B/RSV A Control	➲ Non-infectious in vitro transcribed RNA of specific viral sequences	500 µL	White	403528	25 (3 vials provided)
RSV B Control	➲ Non-infectious in vitro transcribed RNA of specific viral sequences	300 µL	Clear	403527	15 (5 vials provided)
Universal Internal Control (UIC)	➲ Non-infectious *in vitro* transcribed RNA ➲ Non-infectious DNA plasmid	30 µL	Lilac	403097	100 (15 vials provided)

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

Note: For information on any hazard and precautionary statements that may be associated with reagents, refer to the Safety Data Sheet Library at www.hologic.com/sds**.**

- ☐ †Roche MagNA Pure LC Total Nucleic Acid Isolation Kit *(Roche Cat. # 03038505001)* for 192 isolations or bioMérieux NucliSENS easyMAG reagents *(Buffer 1 Cat. # 280130, Buffer 2 Cat. # 280131, Buffer 3 Cat. # 280132, Magnetic Silica Cat. # 280133, and Lysis Buffer Cat. #. 280134)*
- ☐ Micro Test M4 Viral Transport Medium *(Remel, Inc. Cat. No. R12500),* Micro Test M5 Viral Transport Medium *(Remel, Inc. Cat. No. R12515),* Micro Test M6 Viral Transport Medium *(Remel, Inc. Cat. No. R12530),* Micro Test M4RT Viral Transport Medium *(Remel, Inc. Cat. No. R12505),* Copan Universal Transport Medium *(Copan Diagnostics, Inc., Cat. No. 330C)*, or BD Universal Viral Transport vial, 3mL *(Becton, Dickinson and Co. Cat. No. 220220)*
- ☐ Molecular Grade Water (RNase/DNase Free)
- ☐ Extraction *Control (e.g. previously characterized positive sample)*

† **Note: Use only qualified lots of the MagNA Pure LC Total Nucleic Acid Isolation Kit with the ProFlu+ assay. Lots not specifically qualified by Hologic for use with the ProFlu+ assay are not validated for use with this assay, and may cause erroneous results.**

A list of these qualified extraction reagents is available at www.hologic.com. Please notify the reagent manufacturer of any issues with this ancillary reagent and Hologic for any 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

Warnings and Precautions

- For *in vitro* diagnostic use only.
- Limit use of this product to personnel trained in the techniques of Real-Time PCR.
- **Once the RT-PCR master mix is made, the run must be started within one hour.**
- Do not update the SmartCycler Dx Software beyond version 3.0b until Hologic communicates that the updated software version is validated for use with the ProFlu+ assay.
- Performance characteristics of this assay have only been determined with nasopharyngeal swab specimens.
- Handle all specimens as if infectious using safe laboratory procedures such as those outlined in CDC/NIH *Biosafety in Microbiological and Biomedical Laboratories* and in the CLSI Document M29 *Protection of Laboratory Workers from Occupationally Acquired Infections.* Thoroughly clean and disinfect all surfaces with 10% bleach. Autoclave any equipment or materials that have contacted clinical specimens before discarding.
- If infection with a novel Influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, collect specimens with appropriate infection control precautions for novel virulent influenza viruses and send to state or local health department for testing. Do not attempt viral culture in these cases unless a BSL 3+ facility is available to receive and culture specimens.
- Real Time RT-PCR testing in general requires meticulous effort by the operator in reducing the chance of cross-contamination of samples during all steps of the procedure including: extraction, transfer of purified nucleic acids, and preparation of the RT-PCR reactions.
- When handling kit reagents while performing this assay, and when handling materials including samples, reagents, pipettes, and other equipment, use personal protective equipment such as (but not limited to) gloves and lab coats.
- Use micropipettes with aerosol barrier or positive displacement tips for all procedures.
- Always pre-plan, organize and segregate workflow. Proceed with laboratory workflow in a unidirectional manner, beginning in the Pre-Amplification Area and moving to the Amplification/Detection Area.
    - Begin pre-amplification activities with reagent preparation and proceed to specimen preparation.
    - Always dedicate supplies and equipment to a specified area; no cross-movement allowed between areas.
    - Do not use equipment and supplies used for reagent preparation for specimen preparation activities or for pipetting or processing other sources of target nucleic acid.
    - Keep all amplification supplies and equipment in the Amplification/Detection Area at all times.
    - Always wear disposable gloves in each area and change them before entering a different area.
    - Do not open sample tubes following PCR.
- Take care to preserve the purity of kit reagents. Avoid contamination from Positive Controls and specimens by following good laboratory practices.
- Do not use kit after its expiration date.
- Do not mix reagents with different lot numbers or substitute reagents from other manufacturers.
- Safety Data Sheets (SDS) are available on manufacturer's website at www.hologic.com.

Reagent Storage, Handling and Stability

- Store all reagents (opened and unopened) at ≤ -70°C until the expiration date listed on the kit.
- Always check the expiration date on the reagent vials. For Intermediate stock of the Universal Internal Control, use the expiration date of the originating stock control vial. Do not expose controls to more than one (1) freeze-thaw cycle.
- ProFlu+ assay Kits are shipped frozen, arrive frozen and must be stored frozen after receipt. If the kit contents are not frozen, contact Customer Service for assistance.
- An internal study demonstrated that performance of Influenza A/Influenza B/RSV Mix is not affected for up to 5 freeze-thaw cycles and, M-MLV Reverse Transcriptase and RNase Inhibitor II are not affected for up to 10 freeze-thaw cycles.
- Visually examine reagents for adequate reagent volume before beginning any test procedures.
- Protect the Influenza A/Influenza B/RSV Mix from light.
- **Thaw controls and aliquots of controls and keep on ice at all times during preparation and use.**

Recommendation

Aliquoting of kit components to maintain less than 5 freeze/thaw cycles for the Influenza A/Influenza B/RSV Mix *and less than 10 freeze/thaw cycles for* M-MLV Reverse Transcriptase and RNase Inhibitor II *is recommended for labs with smaller batch sizes.*

Specimen Collection, Handling and Storage

Collecting the Specimen

To obtain nasopharyngeal swab samples:

1. Insert a flexible-shaft polyester, rayon or nylon tipped swab containing a dry tip into one nostril and into the nasopharyngeal area.
2. Press the swab gently against the nasal wall to allow the swab to absorb secretions.
3. Rotate the swab two to three times and withdraw it.
4. Place the swab into a tube containing 2 to 3 mL of viral transport medium (Remel M4, M4RT, M5, M6; Copan UTM; or Becton Dickenson UVT).
5. Break off the shaft of the swab and cap the tube.

Note

Using a smaller volume of the viral transport medium may result in inhibition.

Transporting Specimens

Ensure that when transporting human respiratory specimens, all applicable regulations for the transport of etiologic agents are met. Transport human respiratory specimens refrigerated at 2-8°C.

Storing Specimens

Store specimens refrigerated (2-8°C) for up to 72 hours before processing. Store any leftover specimens at ≤ -70°C.

Storing Purified Nucleic Acid

Store purified nucleic acids at ≤ -70°C.

Note

Inadequate or inappropriate specimen collection, storage and transport are likely to yield false negative results.

Recommendation

Training in specimen collection is highly recommended because of the importance of specimen quality.

Reagent and Control Preparation

Reagents

Note

Prepare reagents from the Roche MagNA Pure LC Total Nucleic Acid Isolation Kit or the bioMérieux easyMAG Automated Magnetic Extraction Reagents following the manufacturer's instructions.

Controls

Note

- *For aliquots of the Positive Control and Intermediate stock of the Universal Internal Control, use the expiration date of the originating stock control vial.*
- *Controls and aliquots of controls must be thawed and kept on ice/cold block at all times during preparation and use. It is recommended to prepare controls in a sample prep area, such as a Biological Safety Cabinet.*
- *Run positive controls in accordance with federal, state, or institution recommendations and requirements.*
- *A single positive control or both positive controls may be included in each run.*

Positive Control (PC)

1. Thaw Positive Controls (the white and clear cap vials) on ice.
2. Make 25 aliquots of 20 µL of the Inf. A/Inf. B/RSV A Control and 15 aliquots of 20 µL of the RSV B Control, label and store at ≤ -70°C. **Ensure that aliquots do not undergo more than one (1) freeze-thaw cycle.**
3. **The Positive Controls are used at the provided concentration.**

Note

Do not spike Positive Controls with the Universal Internal Control. Do not take Positive Control through the nucleic acid isolation procedure.

Universal Internal Control (UIC)

1. Thaw Universal Internal Control (the lilac cap vial) on ice.
2. Create Intermediate stock vials of the Universal Internal Control using the following dilution scheme:

26 µL Universal Internal Control	+	*65 µL RNase Inhibitor*	+	*2509 µL molecular grade water*	=	*2600 µL total volume*

3. Make aliquots of 110 µL, label, and store at ≤ -70°C (this is enough volume to add to 5 samples at 20 µL per sample). Make aliquots of larger or smaller volumes based on the number of samples expected to be processed in a single run. **Ensure that aliquots do not undergo more than one (1) freeze-thaw cycle**.
4. Add the appropriate volume of Intermediate stock of the Universal Internal Control to each sample prior to nucleic acid isolation (see ***Step 1*** of the ***Assay Procedure***).
5. **Save RNase Inhibitor II for use in *Step 4 (b)* of the *Assay Procedure*.**

Negative Control (NC)

1. Use viral transport medium as the Negative Control.
2. Add the appropriate volume of Intermediate stock of the Universal Internal Control to the Negative Control prior to nucleic acid isolation (see ***Step 1*** of the ***Assay Procedure***).

Extraction Control (EC)

Good laboratory practice recommends including a positive extraction control (e.g. previously characterized positive sample) in each nucleic acid isolation run. Treat the extraction control like a sample during assay performance and analysis.

Assay Procedure

Assay Overview

Get Ready: Create the Assay Protocol for the Cepheid SmartCycler instrument using the Dx Software *(first time only).*

1. ***Prepare the Samples and Negative Control.***
2. ***Isolate the Nucleic Acid –*** *MagNA Pure LC System using the Total Nucleic Acid Isolation (TNAI) Kit*

   OR
3. ***Isolate the Nucleic Acid –*** *NucliSENS easyMAG System using the Automated Magnetic Extraction Reagents.*
4. ***Set up the RT-PCR Reaction.***
5. ***Run the ProFlu+ assay.***
6. ***Print report.***

Note

- *Instructions provided for the Cepheid SmartCycler II Real Time Instrument with Dx Software version 3.0a / 3.0b (Instructions for version 1.7b noted).*
- *Do NOT deviate from the protocol settings defined in this section.*

Get Ready: Create the Assay Protocol for the Cepheid SmartCycler instrument using the Dx Software (first time only)

Note

- *The protocol is only created for first time use; it does not need to be recreated with each sample run.*
- *Refer to SmartCycler Dx Software Operator Manual for assistance in defining assay protocols.*
- *To **Define** and **Edit** assay protocols, the user must have administrative access rights. Otherwise, the fields will not be accessible for data entry and editing (they will be grayed out).*
- *Cepheid Dx Software interprets the data and reports the run as either VALID or INVALID, based on the results of the Negative Control. Enter the Positive Controls as if they were samples.*
- *You must interpret the control results and determine if the run is VALID or INVALID. You must meet Positive and Negative Control criteria for the run to be VALID (see **Interpretation of Control Results**).*

1. Create the ProFlu+ assay protocol:

- ***a.*** Launch the Cepheid Dx software application.
- ***b.*** Click the **Define Assay** box at the top of the screen.
- ***c.*** Click the **New Assay** box at the bottom of the screen.
- ***d.*** Enter **ProFlu+ Assay** for the assay protocol in the window that opens.
- ***e.*** Click **OK**.
- ***f.*** Enter **Thermocycler Parameter** in the Protocol section (bottom half of ***Define Assay*** screen).

Stage 1			**Stage 2**			**Stage 3** Repeat **5** times			**Stage 4** Repeat **45** times		
Hold			**Hold**			**2- Temperature Cycle**			**2- Temperature Cycle**		
Temp	Secs	Optics	Temp	Secs	Optics	Temp	Secs	Optics	Temp	Secs	Optics
42	**1800**	**OFF**	**95**	**600**	**OFF**	**95**	**30**	**OFF**	**95**	**10**	**OFF**
						55	**60**	**ON**	**55**	**60**	**ON**

Stages 5 – 10 remain UNUSED

2. Enter information in **BOLD** in the **Analysis Settings** tab as follows:

- ***a.*** Select **FTTC25** for the **Dye Set**.
- ***b.*** **Analysis Type: Qualitative** (default).
- ***c.*** **Customize Result Text: Target-based Result Text** (default).

Note

❖ *Gray boxes are default settings.*

Channel	Dye Name	Channel Name*	Usage	Curve Analysis	Thresh Setting	Manual Thresh	Auto Thresh	Auto Min. Cycle	Auto Max. Cycle	Valid Min. Cycle	Valid Max. Cycle	Bkgnd Sub	Bkgnd Min. Cycle	Bknd Max. Cycle	Boxcar Avg	EndPt Thresh	NC IC %	IC Delta
1	**FAM**	**Influenza A**	**Target****	**Primary**	**Manual**	**60.0**	**NA**	**5**	**10**	**13.0**	**50.0**	**ON**	**5**	**50**	**0**	**60**	**NA†**	**NA**
2	**TET**	**RSV**	**Target****	**Primary**	**Manual**	**50.0**	**NA**	**5**	**10**	**13.0**	**50.0**	**ON**	**5**	**50**	**0**	**50**	**NA†**	**NA**
3	**TxR**	**Influenza B**	**Target****	**Primary**	**Manual**	**20.0**	**NA**	**5**	**10**	**13.0**	**50.0**	**ON**	**5**	**50**	**0**	**20**	**NA†**	**NA**
4	**Cy5**	**Universal Internal Control**	**Internal Control**	**Primary**	**Manual**	**10.0**	**NA**	**5**	**10**	**13.0**	**50.0**	**ON**	**5**	**50**	**0**	**10**	**NA†**	**NA**

** Dx 1.7b = Target*
*** Dx 1.7b = Assay*
† Dx1.7b = 10

3. Enter information in **BOLD** in the **Control Settings** tab**.**

a. Select **NC Fails if any target criterion is positive**.

b. *Enter Positive Control(s) and Extraction Control as samples. Do not use the Positive Control Settings. Enter **0** Replicates to inactivate Positive Controls PC1-3.* A 3079 error (Fluorescence Signal Too High) in the Positive Control invalidates the run; this is avoided if the Positive Control and/or the Extraction Control is entered as a sample and the results for each of the target channels are individually evaluated.

c. *Use only one Negative Control (NC1). Enter **0** Replicates to inactivate the Negative Controls NC2 and NC3.*

Note

❖ *Gray boxes are default settings.*

Control ID	Control Name	Replicate	Influenza A Valid Min Cycle	Influenza A Valid Max Cycle	Influenza A EndPt Thresh	RSV Valid Min Cycle	RSV Valid Max Cycle	RSV EndPt Thresh	Influenza B Valid Min Cycle	Influenza B Valid Max Cycle	Influenza B EndPt Thresh	UIC +/-	UIC Valid Min Cycle	UIC Valid Max Cycle	UIC EndPt Thresh
PC1	Pos Cntrl 1	0	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10	+	13.0	45.0	10
PC2	Pos Cntrl 2	0	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10	+	13.0	45.0	10
PC3	Pos Cntrl 3	0	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10	+	13.0	45.0	10
NC1	**Neg Cntrl 1**	**1**	**13.0**	**50.0**	**60**	**13.0**	**50.0**	**50**	**13.0**	**50.0**	**20**	**+**	**20.0**	**40.0**	**10**
NC2	Neg Cntrl 2	0	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10	+	13.0	45.0	10
NC3	Neg Cntrl 3	0	13.0	45.0	10	13.0	45.0	10	13.0	45.0	10	+	13.0	45.0	10

4. Click the **Advanced** tab and select **Require Lot Number**. **Probe Check Settings** tab, **Advance to New Stage** tab, and **Standards** tab are not used for the ProFlu+ assay protocol.

5. Select **Save Assay**.

1. Prepare Samples, Extraction Control (if included), and Negative Control (Pre-Amplification Area)

a. **Add Universal Internal Control to all samples and Extraction Control (if included).**

i. Thaw on ice, the appropriate number of aliquots of Intermediate stock of the Universal Internal Control (enough volume needed for each sample, the Extraction Control, and the Negative Control).

ii. Remove 180 µL of sample from the original sample tube and pipet into a labeled 1.5 mL microcentrifuge tube. Alternately, pipet 180 µL of sample directly into sample cartridge or sample vessel.

iii. Remove 180 µL of Extraction Control from the original sample tube and pipet into a labeled 1.5 mL microcentrifuge tube. Alternately, pipet 180 µL of Extraction Control directly into sample cartridge or sample vessel.

iv. Add 20 µL of Intermediate stock of the Universal Internal Control to each sample and Extraction Control. **Pipet up and down a minimum of 5 times to mix using a new pipet tip for each tube.**

v. Keep tubes on ice.

vi. Store any remaining sample at ≤ -70°C.

b. **Add Universal Internal Control to the Negative Control.**

i. Include one (1) Negative Control in each run.

ii. Add 180 µL of viral transport medium to a labeled 1.5 mL microcentrifuge tube. Alternately, pipet 180 µL of viral transport medium directly into sample cartridge or sample vessel.

iii. Add 20 µL of Intermediate Stock of Universal Internal Control to the viral transport medium**. Pipet up and down a minimum of 5 times to mix.**

iv. Keep tube on ice.

v. Discard remaining volume of Universal Internal Control – DO NOT reuse.

Do not reuse Universal Internal Control.

2. Isolate the Nucleic Acid (Pre-Amplification Area I) - MagNA Pure LC System using the Total Nucleic Acid Isolation (TNAI) Kit

a. **Start the instrument and software.**

i. Power on the MagNA Pure LC instrument and then the computer.

ii. Start the MagNA Pure LC software.

iii. From the *Main Menu* screen, select **Sample Ordering** and enter sample information in **Sample Name** column.

iv. Select the **Total NA Variable_elution_volume.blk** protocol.

v. Follow the software instructions and specify the number of samples.

vi. Type **200 µL** for the sample volume and verify that **50 µL** elution volume is selected.

vii. Select **Stage Setup**; the software automatically calculates the amount of each reagent that is required.

b. **Fill the reagent tubs.**

Before starting the isolation procedure, fill all reagent tubs outside the instrument with the required volume of each reagent listed on the **Start Information** screen.

Note

Use only the reagent amount needed for the number of samples entered into the software. Reagents are not stable for long-term storage in tubs. Vortex Magnetic Glass Particles (MGPs) and load the exact amount of MGPs (as listed on the **Start Information** *Screen) into the instrument just before the run starts.*

c. **Load reagent tubs and disposables into the instrument.**

Use the information on the **Start Information** screen to place all disposable plastics and reagent tubs necessary for the batch run on the Reagent/Sample Stage.

Recommendation

Use a colored "Positioning Frame" (provided with the TNAI kit) on the Reagent Tub Rack to help correctly load reagents.

d. **Load samples, Extraction Control, and Negative Control into the MagNA Pure Sample Cartridge.**

i. Transfer all 200 µL of each sample to individual wells in the MagNA Pure Sample Cartridge.

ii. Transfer all 200 µL of the Extraction Control and Negative Control to different wells in the MagNA Pure Sample Cartridge.

iii. Cover cartridge with Parafilm or MagNA Pure LC Cartridge Seal and keep cartridge on ice until ready to load the instrument.

e. **Load the samples.**

Transfer the Sample Cartridge containing the samples, Extraction Control, and Negative Control into the MagNA Pure LC instrument.

f. **Start the run.**

i. Start the Batch Run by confirming the correct placement of all disposable plastics and reagents by clicking the respective text boxes on the **Start Information** screen.

ii. Select **OK** to start the automated isolation procedure. The instrument automatically dispenses all reagents and processes the samples.

g. **Store the eluted total nucleic acid.**

After completing the run, immediately place the Storage Cartridge containing the eluted nucleic acids on ice or transfer eluted nucleic acid to 1.5 mL tubes and store for longer durations at ≤ -70°C.

Note

Do not store purified nucleic acids in the Storage Cartridge on the Cooling Unit 1.

3. Isolate the Nucleic Acid (Pre-Amplification Area I) - NucliSENS easyMAG System using the Automated Magnetic Extraction Reagents

a. **Start Instrument and Software.**

Power on the easyMAG instrument and once the LED on the instrument turns green, turn on the computer and log into the software.

b. **Prepare the software for a run.**

To prepare for a run, touch "**Settings**"  in the main toolbar to default to the "Application Settings" icon and choose the following run settings:

Default Protocol: Generic 1.0.6 or 2.0.1 (for software version 1.0.1 or 2.0, respectively)
Run Name Prefix: N/A (leave as default)
Sample ID prefix: N/A (leave as default)
Sample Type: Primary (on-board lysis)
Default On-board Lysis Dispensing: Yes
Default On-board Lysis Incubation: Yes
Sample Addition Guidance: Off
Reagent Tracking: Off

c. **Input buffer information.**

Touch "**Instrument**"  to default to the "**Reagent Inventory**"  and input the buffer barcodes by first scanning the instrument position (A, B, C, or D) and then its corresponding buffer. For example, scan position A and then scan the bottle of Lysis buffer in that position and then move on to position B and its corresponding bottle.

d. **Create a worklist.**

i. Touch "**Daily Use**"  to default to "**Define Extraction Request"**  and select the following settings:

Sample ID: Manually enter the sample name.
Matrix: Other
Protocol: Generic 1.0.6 or 2.0.1 (for software version 1.0.1 or 2.0, respectively)
Volume (mL): 0.200 (input volume of sample)
Eluate (µL): 55
Type: Primary
Priority: Normal

ii. Press **Enter** on the keyboard or touch "**New Extraction Request**"  after each manual sample addition. The settings above remain as the default settings for each subsequent entry as long as you do not navigate to other pages.

e. **Create a run and add samples from the worklist.**

i. Touch "**Organize Runs**"  and then "**Create Run**"  to display the *New Run Window.* In this screen, name the run appropriately and verify that the **auto-number** box is unchecked (not selected) and that **Yes** is selected for both the On-Board Lysis Dispensing and On-Board Lysis Incubation options.

ii. Touch **OK.** The *New Run Window* closes and the "**Organize Runs**" screen is displayed. Use the positioning arrow  to assign samples to run.

iii. Touch "**Load Run"**  and select the run. Touch "**Print worklist"**  to print the list. Use the worklist to keep track of the order of the samples to load into the sample vessels.

f. **Load the samples and tips and barcode the sample strip(s).**

Add all of the 200 µL from each sample into the proper vessels of the sample strip(s) as noted in the worklist. Insert tips into sample vessel(s) in the correct order as noted in the worklist and scan the sample strip(s) position on the instrument and then the sample strip itself. For example, scan position A and then the sample strip in that position, then B and then C, if necessary. After scanning the sample strip(s), the indicator changes from red to green on the screen.

g. **On-Board Lysis Dispensing.**

Once the samples and tips are loaded and the strip(s) scanned, close the lid and touch **"Dispense Lysis"**  . The instrument dispenses 2 mL of Lysis Buffer and incubates for 10 minutes.

h. **Prepare the magnetic silica to add to the sample vessels.**

During the 10-minute lysis incubation, use the Biohit multi-channel pipettor to prepare the magnetic silica. Perform this procedure for each sample vessel used in the run (1, 2, or 3 times).

i. Set the pipettor to **Program 1** and place a Biohit pipette tip on position 1. Program 1 provides the means to aspirate and dispense 550 µL of liquid. The magnetic silica is prepared in a 1:1 ratio of Molecular Biology Grade Water to Magnetic Silica.

ii. Using Program 1 of the pipettor, press the **start** button to aspirate and then again to dispense 550 µL of water into a microcentrifuge tube. Vortex the tube of magnetic silica briefly to mix and use Program 1 of the pipettor to aspirate and then dispense 550 µL of magnetic silica into the same microcentrifuge tube as the water. Eject the tip, cap the tube and vortex to mix.

iii. Set the pipettor to **Program 2** and place a Biohit pipette tip on position 1. Program 2 transfers 8 volumes of the previous mix to the 8 vessels of a strip on an ELISA plate (1 strip/sample vessel). Press the **start** button to aspirate the mix. Press the **start** button again to dispense the remaining mixture back into the tube containing the mix to reset the pipette.

iv. Press the **start** button 8 separate times to dispense the remaining mix in each of 8 vessels of an ELISA plate strip and eject the tip.

v. After the 10 minute lysis incubation is finished, set the pipettor to **Program 3** and place 8 Biohit pipette tips on the multichannel pipettor (or however many samples are present in the specific sample strip). Make sure that the filter tips are very well connected with the multichannel pipettor to prevent leakage errors. Program 3 first mixes the magnetic silica mixture in the ELISA plate and then aspirates it for delivery to the vessels of the sample strip where it is mixed.

vi. Press the **start** button once and the pipette mixes the silica in the ELISA plate and aspirates it for addition to the sample vessel. Verify that each tip has the same volume of silica mix before placing in the sample vessel. Place the pipettor over the sample vessel strip so the tips are below the liquid level of each sample and press the **start** button again to aspirate 800 µL out of each sample vessel and perform 3 mix cycles with 1000 µL. As it is mixing, be sure to hold the pipette steady below the liquid/air interface to avoid introducing bubbles to the sample.

vii. Repeat for each sample strip in the run.

i. **Start the run.**

Touch "**Start**"  to begin the run. The instrument performs 5 washes and heated elution. Transfer the purified nucleic acids to appropriate storage tubes (1.5 mL microcentrifuge tubes) on ice within 30 minutes of extraction to avoid contamination by the magnetic silica stuck to the front wall of the sample vessel(s). Use immediately or store at ≤ -70°C.

4. Set up the RT-PCR Reaction (Pre-Amplification II)


Note

Start the SmartCycler ProFlu+ assay run within 1 hour of making the RT-PCR Master mix.

a. **Thaw the Positive Controls.**

i. Include at least one Positive Control (Inf. A/Inf. B/RSV A Control or RSV B Control) with each run. Thaw one (1) aliquot of Positive Control(s) on ice.

ii. Keep tubes on ice.

b. **Prepare the RT-PCR Master mix.**


Note

The RT-PCR Master mix must be prepared FRESH for each RT-PCR run.

i. Calculate the amount of each reagent needed based on the number of reactions (samples + controls):

19.45 µL	Influenza A/Influenza B/RSV Mix
+0.30 µL	M-MLV Reverse Transcriptase
+0.25 µL	RNase Inhibitor
20.00 µL	per reaction

ii. Thaw the Influenza A/Influenza B/RSV Mix on ice and **mix by pipetting up and down a minimum** of 5 times before use.

iii. Remove M-MLV Reverse Transcriptase and RNase Inhibitor enzymes from the freezer and keep on ice during use. Spin down M-MLV Reverse Transcriptase and pipette from the top.

iv. Prepare the RT-PCR Master mix by combining the reagents listed above in a 1.5 mL microcentrifuge tube. **Pipet up and down a minimum of 5 times to mix.**

v. Keep the RT-PCR Master mix on ice and protected from light before adding to SmartCycler tubes.

Recommendation

If setup is prolonged, keep the cold blocks on ice while adding reagents to the SmartCycler tubes.

c. **Add 20 µL of RT-PCR Master mix to the SmartCycler tubes.**

i. Load the required number of tubes into the Cepheid Cooling Block.

ii. Pipet the RT-PCR Master mix into the upper part of the SmartCycler tubes. Discard any unused RT-PCR Master mix.

d. **Add 5 µL of each sample's nucleic acid to individual SmartCycler tubes containing RT-PCR Master mix.**

i. After adding the sample's nucleic acid to the SmartCycler tube, **pipet up and down 2 to 3 times in the upper part of the tube.**

ii. Close the tube. Use a new pipette tip for each sample.

e. **Add 5 µL of Positive Control(s) to the SmartCycler tube(s) (one control per tube) containing RT-PCR Master mix.**

i. After adding one or more Positive Control(s) to the SmartCycler tube(s), **pipet up and down 2 to 3 times in the upper part of the tube using a new pipet tip for each control tube.**

ii. Close the tube.

iii. Discard remaining volume of Positive Control(s) – DO NOT reuse.

Do not reuse Positive Controls aliquots.

f. **Add 5 µL of the Extraction Control nucleic acid to a separate SmartCycler tube containing RT-PCR Master mix.**

i. After adding the Extraction Control nucleic acid to the SmartCycler tube, **pipet up and down 2 to 3 times in the upper part of the tube.**

ii. Close the tube.

g. **Add 5 µL of the Negative Control nucleic acid to the last SmartCycler tube containing RT-PCR Master mix.**

i. After adding the Negative Control nucleic acid to the SmartCycler tube, **pipet up and down 2 to 3 times in the upper part of the tube.**

ii. Close the tube.

h. **Centrifuge all tubes.**

i. Appropriately label the SmartCycler tubes on the caps.

ii. Centrifuge all tubes for 5 to 10 seconds using the Cepheid microcentrifuge specially adapted to fit the SmartCycler tubes.

iii. Return tubes to the cooling block.

i. **Keep the tubes on the Cooling Block before loading them into the SmartCycler instrument.**

5. Run the ProFlu+ Assay (Amplification/Detection Area)

a. Create a new run by clicking **Create Run** at the top of the screen. The *Create Run* screen is displayed.

b. Under **Run Name** in the left panel, enter a unique run identifier.

c. Click the **Assay** arrow in the left panel and select the **ProFlu+ Assay** protocol from the drop-down menu.

d. Under **Assay Information** in the left panel, enter the **Lot Number** and **Expiration Date** of the ProFlu+ assay Kit.

e. In the left panel, enter the number of specimens (including the Positive and the Extraction Controls, but excluding the Negative Control) and click **Apply**. The **Site Table** is displayed and the SmartCycler Dx Software automatically selects the **I-Core** sites.

f. In the **Site Table** under the **Sample ID** column, enter the Sample Identifier or Positive Control Identifier for the appropriate I-Core sites.

g. Insert each reaction tube into an I-Core site of the SmartCycler by pressing down firmly on all tubes and close each lid. Verify that the Negative Control (NC1) is loaded into the correct I-Core site.

h. Select the **Start Run** button located at the bottom left corner of the screen. Verify that the LED is on for the appropriate I-Core sites.

6. Print Report

a. Click **Report** at the bottom of the screen to open the **Report Preview** screen.

b. Click **Print** at the top of the screen.

Interpretation of Control Results

Validation of Run

Note

You must interpret the Positive Control(s) (PC) and the Extraction Control (if included) results to determine whether the run is VALID; the SmartCycler Dx software automatically interprets the Negative Control results.

For a VALID Extraction run, the following conditions must be met:

Sample ID[1]	Assay Result	UIC Result	Warning / Error Code	Sample Type	UIC Ct	IA or RSV or IB Result	IA or RSV or IB Ct
Extraction Control	Positive	NA	**	SPEC	NA	POS	13-50
Neg Cntrl	Valid[2]	Pass		NC1	20-40	Valid	0

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

[2] *(Typical) an Invalid Assay will display Error Code 4098.*

** *Error Code 3079: Warning/Error Code 3079 is periodically observed with Influenza A positives (Extraction 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 the sample are reported by the Dx software as ND (Not Determined). When this code is observed for the Extraction Control, extraction run validity can be determined based on Ct values of the Extraction Control. The Extraction Control must have a Ct value between 13-50 in the IA or IB or RSV Ct column to be considered VALID.*

For a VALID RT-PCR run, the conditions in the table below must be met. If the run is valid, interpret specimens using the next section: *Interpretation of Specimen Results*.

Sample ID[1]	Assay Result	UIC Result	Warning / Error Code	Sample Type	UIC Ct	IA Result	IA Ct	RSV Result	RSV Ct	IB Result	IB Ct
Inf. A/Inf. B/RSV A Control	Positive	NA	**	SPEC	0	POS	20-40	POS	20-40	POS	20-40
RSV B Control	Positive	NA		SPEC	0	NEG	0	POS	20-40	NEG	0
Neg Control	Valid[2]	Pass		NC1	20-40	Valid	0	Valid	0	Valid	0

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

[2] *(Typical) An Invalid Assay will display Error Code 4098.*

** *Error Code 3079: Warning/Error Code 3079 is periodically observed with Influenza A positives (Inf. A/Inf. B/RSV A Control) 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 between 20-40 is reported in the "**Influenza A Ct**" column for the Inf. A/Inf. B/RSV A Control and Ct values are valid in the RSV and Influenza B Ct columns, results can be recorded as positive and the run considered VALID.*

Invalid Extraction Run

If the conditions for a Valid Extraction run are not met (i.e., the Extraction Control is not positive or the Negative Control is Invalid), repeat the entire extraction run. Start from original sample(s) using a new Extraction Control and a new Negative Control (starting at ***Step 1*** of the ***Assay Procedure*****).**

Invalid RT-PCR Run

If the Positive Control is not positive within the specified Ct range but the Negative Control is valid, prepare all new reactions using remaining purified nucleic acids and a new Positive Control (starting with PCR at ***Step 4*** of the ***Assay Procedure*****).**

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	UIC 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 (Inf. A/Inf. B/RSV AControl, 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 Universal Internal Control in the Cy5 detection channel is not required for positive result. High viral load can lead to reduced or absent Universal Internal Control signal.*

Not Determined Samples

If an assay result of **ND** (Not Determined) is reported with an instrument failure other than Warning/Error Code,3079, repeat testing from the purified nucleic acids (see ***Step 4 (a)*** of the ***Assay Procedure***). Refer to the Cepheid Dx Software Operator Manual for interpretation of Warning Codes.

Quality Control

- Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures. Refer to CLSI document C24-A3, *Statistical Quality Control for Quantitative Measurements: Principles and Definitions:* [Approved Guideline – Third Edition] or other published guidelines for general quality control recommendations. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1205.
- Quality control procedures are intended to monitor reagent and assay performance.

Control Type	Used to Monitor
Positive	Substantial reagent failure including primer and probe integrity
Negative	Reagent and/or environmental contamination
Extraction	Failure in lysis and extraction procedure
Internal	PCR inhibition in individual samples and Reagent failure or process error

- Dilute the Universal Internal Control and test both the Positive Controls and Universal Internal Control prior to running samples with each new kit lot to ensure all reagents and kit components are working properly.
- Good laboratory practice recommends including a positive Extraction Control in each nucleic acid isolation run. Treat the Extraction Control as a sample.
- Never run the Positive Controls through nucleic acid isolation.
- Always include one Negative Control *(containing Universal Internal Control)* and at least one Positive Control in each amplification/detection run performed.
- Failure of Controls (Positive, Negative and/or Extraction) invalidates the run and results should not be reported.
- If the Positive Control is not positive within the specified Ct range but the Negative Control is valid, repeat testing starting from the purified nucleic acid using a new aliquot of the Positive Control. If repeat results are still invalid, do not report results; repeat testing from the original sample or collect and test a new sample.
- If the Extraction Control is not positive within the specified Ct range or the Negative Control is invalid, repeat testing starting from the original sample using a new Extraction Control and a new Negative Control. If repeat results are still invalid, do not report results; collect and test a new sample.

Limitations

- A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- This test does not differentiate Influenza A subtypes (i.e. H1N1, H3N2) or RSV subgroups (i.e., A or B); additional testing is required to differentiate any specific Influenza A subtypes or strains or specific RSV subgroups, in consultation with local public health departments.
- Negative results do not preclude infection and should not be used as the sole basis for treatment or other patient management decisions.
- Analyte targets (viral nucleic acid) may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious, or are the causative agents for clinical symptoms.
- The detection of viral nucleic acid is dependent upon proper specimen collection, handling, transportation, storage, and preparation (including extraction). Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.
- There is a risk of false negative values due to the presence of sequence variants in the viral targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms for amplification.
- False Negative results may occur due to the loss of nucleic acid. The Universal Internal Control has been added to the test to aid in the identification of specimens that contain inhibitors to PCR Amplification. The Universal Internal Control does not indicate whether or not nucleic acid has been lost due to inadequate collection, transport or storage of specimens.

- The performance of this test has not been established for individuals who received nasally administered influenza vaccines.
- A specimen yielding a negative result may contain respiratory viruses other than Influenza A and B or RSV.
- There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
- This test is a qualitative test and does not provide the quantitative value of detected organism present.
- The performance of the test has been evaluated for use with human specimen material only.
- The performance of this test has not been evaluated for sample types other than nasopharyngeal (NP) swab specimens.
- The performance of this test has not been evaluated for immunocompromised individuals.
- The performance of this test has not been established for patients without symptoms of influenza infection.
- An interference study evaluating potentially interfering common cold medications was not performed.

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%[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+ Assay 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.

Performance Characteristics

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[11, 12, 13] followed by sequencing.

Results from Prospective Study

Influenza A Comparison Results

		Reference Method			
		Positive	**Negative**	**Total**	**Comments**
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

		Reference Method			
		Positive	**Negative**	**Total**	**Comments**
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

		Reference Method			
		Positive	**Negative**	**Total**	**Comments**
ProFlu+ Assay	**Positive**	34	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

		Reference Method			
		Positive	**Negative**	**Total**	**Comments**
ProFlu+ Assay	**Positive**	5	2 [a]	**7**	Percent Positive Agreement 100% (56.6% - 100%) 95% CI
	Negative	0	53	**53**	Percent Negative Agreement 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

		Reference Method			
		Positive	**Negative**	**Total**	**Comments**
ProFlu+ Assay	**Positive**	17	0	**17**	Percent Positive Agreement 89.5% (68.6% - 97.1%) 95% CI
	Negative	2 [a]	41	**43**	Percent Negative Agreement 100% (91.4% - 100%) 95% CI
	Total	**19**	**41**	**60**	

[a] *Two (2) samples positive for Influenza B by sequence analysis.*

RSV Comparison Results

		Reference Method			
		Positive	**Negative**	**Total**	**Comments**
ProFlu+ Assay	**Positive**	23	1 [a]	**24**	Percent Positive Agreement 100% (85.7% - 100%) 95% CI
	Negative	0	36	**36**	Percent Negative Agreement 97.3% (86.2% - 99.5%) 95% CI
	Total	**23**	**37**	**60**	

[a] *One (1) sample positive for RSV by sequence analysis.*

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 ProFlu+ Assay	**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 ProFlu+ 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 ProFlu+ Assay	**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 positive for RSV using bi-directional sequencing.

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 Med 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 Med 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 Med 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 Med 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 limiting dilutions of the following virus cultures: 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) is defined as the lowest concentration at which ≥ 95% of all replicates tested positive and are summarized in the table below. The LoD's 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

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, 15 Influenza A subtype H3N2, 9 swine-origin Influenza A, 2 Influenza A subtype H5N1,1 Influenza A H7N9, 10 Influenza B, and 2 Respiratory Syncytial Virus strains. Viral strains were extracted using the Roche MagNA Pure LC or bioMérieux NucliSENS easyMAG and tested in triplicate in each assay. Viruses present at concentrations below those tested for Reactivity may not be detected by the ProFlu+ 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	+	-	-
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)	$2x10^1TCID_{50}$/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/Indiana/10/2011 (H3N2v)*	$2x10^2$ $TCID_{50}$/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/New York/1/2015 (H3N2)	$2x10^1$ $TCID_{50}$/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	+	-	-

Viral Strain	Concentration	Influenza A	RSV	Influenza B
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	+	-	-
A/VN/1203 (H5N1) RNA	2.7ng/µL	+	-	-
A/HK/486 (H5N1) RNA	1.4ng/µL	+	-	-
A/Anhui/1/2013 (H7N9) RNA*	0.02 pg/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.

* Although this test has been shown to detect A/Anhui/1/2013 H7N9 RNA and Influenza A/ Indiana/10/2011 (H3N2v) virus cultured from positive human respiratory specimens, the performance characteristics of this device with clinical specimens that are positive for H7N9 or H3N2v Influenza viruses have not been established. The Prodesse ProFlu+ assay can distinguish between Influenza A and B viruses, but it cannot differentiate Influenza A subtypes.

Analytical Specificity

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	-	-	-

Strains	Concentration	Influenza A	RSV	Influenza B
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	-	-	-
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	-	-	-

Competitive Inhibition

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.

Extraction Equivalency

Extraction equivalency of the bioMérieux NucliSENS easyMAG and Roche MagNA Pure LC instruments was evaluated by performing both a limit of detection study and a reproducibility study. 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 used. 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%.

Carry-over/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.

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

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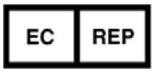
	Upper Storage Temperature Limitation		Authorized Representative
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