

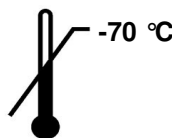
The information previously contained on these webpages were authorized under the 2009 H1N1 Influenza Emergency Use Authorizations (EUAs). As of June 23, 2010, the EUAs have been terminated and this information is no longer current.



Instructions for Use

For detection of 2009 H1N1 Influenza Virus aided by an algorithm that relies on seasonal influenza A/H1 virus and seasonal influenza A/H3 virus results.

**Emergency Use Authorization
For *in vitro* Diagnostic Use**



REF
H33VK00

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Intended Use

The ProFlu-ST Assay is a multiplex real-time RT-PCR assay intended for use in CLIA High Complexity Laboratories with the ability to perform rRT-PCR using the Cepheid SmartCycler II System for the *in vitro* qualitative detection of 2009 H1N1 influenza viral RNA aided by an algorithm that relies on seasonal influenza A/H1 virus and seasonal influenza A/H3 virus results in nasopharyngeal swabs (NPS) from patients who are diagnosed with influenza A by currently available FDA-cleared or authorized devices.

The ProFlu-ST Assay targets the nucleoprotein (NP) gene of the 2009 H1N1 influenza virus, which may react with other swine origin Influenza A strains.

Testing for the 2009 H1N1 influenza virus should not be performed unless the patient meets clinical and epidemiologic criteria for testing suspect specimens. The identification of 2009 H1N1 influenza virus should be performed along with clinical and epidemiological assessment.

Negative results do not preclude influenza virus infection and should not be used as the sole basis for treatment or other patient management decisions.

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 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. In March of 2009, a novel Influenza A virus (2009 H1N1 influenza virus) emerged in North America and globally.³ The 2009 H1N1 influenza virus is considered a reassortant virus composed of two genes from influenza viruses that normally circulate in swine in Europe and Asia in addition to bird (avian) and human genes.⁴ The 2009 H1N1 influenza virus is also considered an Influenza Virus of Swine Origin (S-OIV).

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. The symptoms for the 2009 H1N1 virus are similar to those of seasonal influenza strains, however diarrhea and vomiting may be more commonly reported with the 2009 H1N1 virus.⁴ Complications due to influenza include pneumonia causing increased morbidity and mortality in pediatric, elderly, and immunocompromised populations. Young children, pregnant women, and those with underlying health conditions may be at greater risk for severe complications related to infection with the 2009 H1N1 influenza virus. Antiviral drugs such as oseltamivir (trade name Tamiflu[®]), zanamivir (Relenza[®]), amantadine, and rimantadine are approved for use in the United States for treating influenza. Knowledge of the infecting virus can impact treatment decisions. The CDC currently recommends the use of oseltamivir or zanamivir for treatment of patients with 2009 H1N1 influenza virus as this virus is resistant to amantadine and rimantadine.⁵

Principles of the Procedure

The ProFlu-ST Assay enables detection of 2009 H1N1 Influenza Virus aided by an algorithm that relies on seasonal influenza A/H1 virus and seasonal influenza A/H3 virus results.



Note

When starting from purified nucleic acid samples that have been previously processed for testing with the ProFlu+ Assay, begin at set up of the RT-PCR reaction (see [Step 4](#) of the [Assay Procedure](#)).

An overview of the procedure is as follows:

1. Collect respiratory swab specimens from symptomatic patients using a polyester, rayon, or nylon tipped swab and place into viral transport medium (refer to **Materials Required but not Provided**).
2. Add an Internal Control (IC) to every sample to monitor for inhibitors present in the specimens.
3. Perform isolation and purification of nucleic acids using a MagNA Pure LC Instrument (Roche) and the MagNA Pure Total Nucleic Acid Isolation Kit (Roche) or a NucliSENS® easyMAG™ System (bioMérieux) and the Automated Magnetic Extraction Reagents (bioMérieux), or comparable nucleic acid extraction instrument and reagent kit.
4. Add purified nucleic acids to the supermix along with enzymes included in the ProFlu-ST kit. The supermix contains oligonucleotide primers and target-specific oligonucleotide probes. The probes are dual-labeled with a reporter dye and a quencher dye (see table below).
5. Perform reverse transcription of RNA into complementary DNA (cDNA) and subsequent amplification of DNA in a Cepheid SmartCycler® II instrument or comparable Real Time thermocycler. In this process, the probe anneals specifically to the template followed by primer extension and amplification. The ProFlu-ST 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 |
|---------------------------|-------------------------|-------------------|-----------------|---------------|
| Seasonal H1 Influenza A | Hemagglutinin | FAM | 495 nm | 520 nm |
| Seasonal H3 Influenza A | Hemagglutinin | Cal Orange 560 | 540 nm | 561 nm |
| 2009 H1N1 Influenza Virus | Nucleoprotein | Cal Red 610 | 595 nm | 615 nm |
| Internal Control | <i>E.coli</i> MS2 Phage | Quasar 670 | 647 nm | 667 nm |

Materials Provided
ProFlu-ST Assay Kit (Cat. # H33VK00)

| Reagents | Description | Quantity/ Tube | Cap Color | Cat. # | Reactions/ Tube |
|--|---|-------------------|--------------|--------|------------------------------|
| ProFlu-ST Supermix | <ul style="list-style-type: none"> ➤ Taq DNA polymerase ➤ Oligonucleotide primers and probes ➤ Buffer containing dNTPs ➤ MgCl₂ and stabilizers | 1030 µL | Brown | HSM33 | 50 (2 tubes provided) |
| M-MLV Reverse Transcriptase | ➤ 10U/µL | 36 µL | Red | GLS26 | 100 |
| RNase Inhibitor II | ➤ 40U/µL | 120 µL | Green | GLS33 | 100 |
| Internal RNA Control III | ➤ Non-infectious <i>in vitro</i> transcribed RNA | 30 µL | Yellow | GCT12 | 100 |
| Influenza A Subtyping RNA Control (Positive Control) | ➤ Non-infectious <i>in vitro</i> transcribed RNA of specific viral sequences | 300 µL | None | HCT30 | 15 |

Materials Required But Not Provided
Plasticware and consumables

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

Reagents

- Roche MagNA Pure LC Total Nucleic Acid Isolation Kit (*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. # R12500*), Micro Test[™] M4RT[®] Viral Transport Medium (*Remel, Inc. Cat. # R12505*), Micro Test[™] M5[®] Viral Transport Medium (*Remel, Inc. Cat. # R12515*), Micro Test[™] M6[™] Viral Transport Medium (*Remel, Inc. Cat. # 12530*), BD Universal Viral Transport (*UVT; Becton, Dickinson and Co. Cat. # 220220*), or Copan Universal Transport Medium (*UTM; Copan Cat. # 330C*)
- Molecular Grade Water (RNase/DNase Free)

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 or 3.0 (a or b)
- Micropipettors (range between 1-10 µL, 10-200 µL and 100-1000 µL)
- Mini-centrifuge with adapter for Cepheid Reaction Tubes
- Cepheid cooling block

Warnings and Precautions

For *in vitro* diagnostic use only.

Use of this product should be limited to personnel who have been trained in the techniques of Real Time PCR.

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.

Diagnostic laboratory work on clinical samples from patients who are suspected cases of 2009 H1N1 influenza infection should be conducted in a BSL2 laboratory. All sample manipulations should be done inside a biosafety cabinet. Viral isolation on clinical specimens from patients who are suspected cases of 2009 H1N1 influenza virus infection should be performed in a BSL2 laboratory⁶

Use micropipettes with aerosol barrier or positive displacement tips for all procedures.

Always pre-plan, organize and segregate workflow. Workflow in the laboratory should proceed 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 pipeting 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 Control 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.

Material Safety Data Sheets (MSDS) are available on Prodesse's website at www.prodesse.com from the **Technical Information** tab.

Reagent Storage, Handling and Stability

Store all reagents (opened and unopened) at $\leq -70^{\circ}\text{C}$ until the expiration date listed on the kit.

Always check the expiration date on the reagent tubes. For Intermediate stock of the Internal Control, use the expiration date of the originating stock control tube. Do not expose controls to more than one (1) freeze-thaw cycle.

ProFlu-ST Kits are shipped frozen, should arrive frozen and should be stored frozen after receipt. If the kit contents are not frozen, contact Prodesse, Inc. for assistance.

Visually examine reagents for adequate reagent volume before beginning any test procedures.

Protect the ProFlu-ST Supermix from light.

Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.



Aliquoting of kit components is recommended when running smaller sample runs.

Recommendation

Specimen Handling and Storage

Specimen Collection

Acceptable specimen types include nasopharyngeal swabs (NPS) placed into approximately 3 mL of viral transport medium (Remel M4, M4RT, M5 or M6, Becton Dickinson UVT, or Copan UTM).

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 $\leq -70^{\circ}\text{C}$.

Storing Purified Nucleic Acid

Store purified nucleic acids at $\leq -70^{\circ}\text{C}$.



Note

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



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

Recommendation

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 Internal Control, use the expiration date of the originating stock control tube.
- ❖ Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.
- ❖ It is required to include the positive control in each run.

Positive Control (PC)

1. Thaw Positive Control on ice.
2. Make 15 aliquots of 20 µL, label and store at ≤ -70°C. **Ensure that aliquots do not undergo more than one (1) freeze-thaw cycle.**
3. Dilute the Positive Control just prior to setup of the RT-PCR reaction (see [Step 4](#) of the [Assay Procedure](#)).



Note

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

Internal Control (IC)

1. Thaw Internal Control (the yellow cap tube) on ice.
2. Create Intermediate stock tubes of the Internal Control using the following dilution scheme:

$$26 \mu\text{L Internal Control} + 65 \mu\text{L RNase Inhibitor} + 2509 \mu\text{L molecular grade water} = 2600 \mu\text{L total volume}$$

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 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](#) 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 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 seasonal influenza A (H1 or H3) sample, positive 2009 H1N1 influenza sample, or a negative sample spiked with a well characterized seasonal influenza A (H1 or H3) strain or 2009 H1N1 influenza strain) in each nucleic acid isolation run. The extraction control should be treated like a sample during assay performance and analysis.

Assay Procedure

Assay Overview:

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

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

OR

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



Note

- ❖ **When starting from purified nucleic acid begin at Step 4 of the Assay Procedure.**
- ❖ Instructions provided for the Cepheid SmartCycler II Real Time Instrument with Dx Software version 1.7b (Instructions for version 3.0 (a or b) noted).
- ❖ Do NOT deviate from the protocol settings defined in this section.

Get Ready: Create the Research Assay Protocol for the Cepheid SmartCycler 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** Research 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 Control and Extraction Control as if they were samples.**
- ❖ Interpret the control results and determine if the run is VALID or INVALID. All Control criteria must be met in order for the run to be VALID (see [Interpretation of Control Results](#) section).

1. Create the ProFlu-ST Assay protocol:

- a. Launch the Cepheid Dx software application.
- b. Click on the **Define Assay** box at the top of the screen.
- c. Click on the **New Assay** box at the bottom of the screen.
- d. Enter **ProFlu SUBTYPING Assay** for the assay protocol in the window that opens.
- e. Click **OK**.
- f. In the middle of the **Define Assay** screen, select **FTTC25** for the **Dye Set**.
- g. Select **NC fails if: Any target criterion is positive** (Dx 3.0: this is default setting in **Control Settings** Tab).
- h. Check the box to **Require Lot Number** (Dx 3.0: click on **Advanced** Tab and check **Require Lot Number** box).
- i. Deselect the box to **Use Patient IDs** (Dx 3.0: click on **Advanced** Tab, **Use Patient IDs** box is deselected by default).

j. 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 35 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 **Analysis Settings** tab as follows:



Note

❖ Gray boxes are default settings.

| Channel | Dye Name | Target | 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 | Bkgnd Max. Cycle | Boxcar Avg | EndPt Thresh | NC IC % | IC Delta |
|---------|----------|------------------|------------------|----------------|----------------|---------------|-------------|-----------------|-----------------|------------------|------------------|-----------|------------------|------------------|------------|--------------|---------|----------|
| 1 | FAM | Seasonal H1 | Assay** | Primary | Manual | 50.0 | NA | 5 | 10 | 13.0 | 40.0 | ON | 5 | 40 | 0 | 50 | 10.0† | NA |
| 2 | TET | Seasonal H3 | Assay** | Primary | Manual | 40.0 | NA | 5 | 10 | 13.0 | 40.0 | ON | 5 | 40 | 0 | 40 | 10.0† | NA |
| 3 | TxR | 2009 H1N1 | Assay** | Primary | Manual | 25.0 | NA | 5 | 10 | 13.0 | 40.0 | ON | 5 | 40 | 0 | 25 | 10.0† | NA |
| 4 | Cy5 | Internal Control | Internal Control | Primary | Manual | 25.0 | NA | 5 | 10 | 13.0 | 40.0 | ON | 5 | 40 | 0 | 25 | 10.0† | NA |

* If the Dye Names are incorrect, check that FTTC25 Dye Set is being used.

** Dx 3.0a or b = Target

† Dx3.0a or b = NA

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



Note

❖ Enter Positive Control and Extraction Control as samples. Do not use the Positive Control Settings. Enter 0 Replicates to inactivate Positive Controls PC1-3.

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

❖ Gray boxes are default settings.

| Control ID | Control Name | Replicate | H1 Valid Min Cycle | H1 Valid Max Cycle | H1 EndPt Thresh | H3 Valid Min Cycle | H3 Valid Max Cycle | H3 EndPt Thresh | 2009 H1N1 Valid Min Cycle | 2009 H1N1 Valid Max Cycle | 2009 H1N1 EndPt Thresh | IC +/- | IC Valid Min Cycle | IC Valid Max Cycle | IC 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 | 40.0 | 50 | 13.0 | 40.0 | 40 | 13.0 | 40.0 | 25 | + | 24.0 | 36.0 | 25 |
| 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. Confirm **Probe Check Settings** tab.



Note

The probe check is not used for the ProFlu-ST Assay protocol.

| Channel | Dye Name | Prb1 Min | Prb1 Max | Prb2 Min | Prb2 Max | Prb3 Min | Prb3 Max | Delta 12 Min | Delta 12 Max | Delta 23 Min | Delta 23 Max | Delta 13 Min | Delta 13 Max |
|---------|----------|----------|----------|----------|----------|----------|----------|--------------|--------------|--------------|--------------|--------------|--------------|
| 1 | FAM | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 2 | TET | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 3 | TxR | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |
| 4 | Cy5 | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |

5. Select **Save Assay**.

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

a. Add Internal Control to all samples and the Extraction Control.

- i. Thaw the appropriate number of aliquots of Intermediate stock of the Internal Control on ice (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.
- iii. Remove 180 µL of Extraction Control from the original sample tube and pipet into a labeled 1.5 mL microcentrifuge tube.
- iv. Add 20 µL of Intermediate stock of the 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 sample.**
- v. Keep tubes on ice.
- vi. Store any remaining sample at $\leq -70^{\circ}\text{C}$.

b. Add 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.
- iii. Add 20 µL of Intermediate Stock of 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 Internal Control – DO NOT reuse.



Do not reuse Internal Control.

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

a. Start the instrument and software.

- i. Turn power on to the MagNA Pure LC Instrument and then turn on 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 in **200 µL** for the sample volume and verify that **50 µL** elution volume is selected.

vii. Select **Stage Setup** and the software will automatically calculate 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 to correctly load reagents.

d. Load the 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 the 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 mouse-clicking the respective text boxes on the **Start Information** screen.
- ii. Select the **OK** button to start the automated isolation procedure. The instrument will automatically dispense all reagents and process the samples.

g. Store the eluted total nucleic acid.

After completing the run, place the Storage Cartridge containing the eluted nucleic acids immediately on ice or transfer eluted nucleic acid to 1.5 mL tubes and store for longer durations at $\leq -70^{\circ}\text{C}$.



Note

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

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

a. Start Instrument and Software.

Turn power on to 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 the “**Settings**” icon in the main toolbar which defaults to the “Application Settings” icon and choose the following run settings:

Default Request: 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 the “**Instrument**” icon to default to the “**Reagent Inventory**” icon 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 the “**Daily Use**” icon which will default to the “**Define Extraction Request**” icon and select the following settings:

Sample ID: Manually enter the sample name.

Matrix: Other

Request: 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)

Euate (µL): 55

Type: Primary

Priority: Normal

ii. Press **Enter** on the keyboard or touch the “**New Extraction Request**” icon after each manual sample addition. The settings above will 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.

Touch the “**Organize Runs**” icon and then the “**Create Run**” icon which will bring up the *New Run Window*. In this screen, name the run appropriately and verify that the **auto-number** box is left unchecked (NOT SELECTED) and that **Yes** is selected for both the On-Board Lysis Dispensing and On-Board Lysis Incubation options. Touch **OK** and the *New Run Window* will close and you will be in the “**Organize Runs**” screen. Assign samples to run with the positioning (arrow) icons. Touch the “**Load Run**” icon and select the run. Print the worklist with the “**Print worklist**” icon. This worklist will aid in keeping track of the order of the samples to be loaded into the sample vessel wells.

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

- g.** Add all of the 200 μ L from each sample into the proper wells of the sample vessel(s) as noted in the worklist. Insert tips and sample vessel(s) in the correct order as noted in the worklist and scan the sample vessel(s) position on the instrument and then the sample vessel itself. For example, scan position A and then the sample vessel in that position, then B and then C, if necessary. After scanning the sample vessel(s), the indicator will change from red to green on the screen.
- h. On-Board Lysis Dispensing.**
Once the samples and tips are loaded and the vessel(s) scanned, close the lid and touch the “**Dispense Lysis**” icon. The instrument will dispense 2 mL of Lysis Buffer and incubate for 10 minutes.
- i. Prepare the magnetic silica to add to the sample vessel wells.**
During the 10-minute lysis incubation, use the Biohit multi-channel pipettor to prepare the magnetic silica. This procedure will need to be performed 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 μ L 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 μ L 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 μ L 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 will transfer 8 volumes of the previous mix to the 8 wells 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 wells of an ELISA plate strip and eject the tip.
 - v.** After the 10 minute lysis incubation is done, 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 vessel). 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 to be delivered to the wells of the sample vessel where it will be mixed. Press the **start** button once and the pipette will mix the silica in the ELISA plate and then aspirate 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, which will then aspirate 800 μ L out of the sample vessel strip well and perform 3 mix cycles with 1000 μ L. At this point be sure to hold the pipette steady below the liquid/air interface as it is mixing so as not to introduce bubbles to the sample.
 - vi.** Repeat for each sample vessel in the run.
- j. Start the run.**
Touch the “**Start**” icon to begin the run. The instrument will perform 5 washes and heat and elute. The purified nucleic acids need to be transferred to appropriate storage tubes (1.5 mL microcentrifuge tubes) on ice within 30 minutes of extraction completion 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 Area)



Start the SmartCycler ProFlu-ST run within 1 hour of making the RT-PCR Mastermix.

Note

a. Dilute the Positive Control.

- i.* Thaw one (1) aliquot of the Positive Control on ice.
- ii.* Add 45 μL of molecular grade water to a 1.5 mL microcentrifuge tube.
- iii.* Transfer 5 μL from the Positive Control aliquot to the appropriate tube. **Pipet up and down a minimum of 5 times to mix.**
- iv.* Keep tube on ice.
- v.* Discard remaining volume of Positive Control – DO NOT reuse.



Do not reuse Positive Control aliquot.

b. Prepare the RT-PCR Mastermix.



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

Note

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

| | |
|---------------------|-----------------------------|
| 19.45 μL | ProFlu-ST Supermix |
| +0.30 μL | M-MLV Reverse Transcriptase |
| +0.25 μL | RNase Inhibitor |
| 20.00 μL | per reaction |
 - ii.* Thaw the ProFlu-ST Supermix 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.
 - iv.* Prepare the RT-PCR Mastermix 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 Mastermix on ice and protected from light before adding to SmartCycler tubes.
- c. Add 20 μL of RT-PCR Mastermix to the SmartCycler tubes.**
- i.* Load the required number of tubes into the Cepheid Cooling Block.
 - ii.* Pipet the RT-PCR Mastermix into the upper part of the SmartCycler tubes. Discard any unused RT-PCR Mastermix.
- d. Add 5 μL of each sample's nucleic acid to individual SmartCycler tubes containing RT-PCR Mastermix.**
- 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 the diluted Positive Control to a separate SmartCycler tube containing RT-PCR Mastermix.**
- i.* After adding the diluted Positive Control to the SmartCycler tube, pipet up and down 2 to 3 times in the upper part of the tube.
 - ii.* Close the tube.

iii. Discard remaining volume of diluted Positive Control – DO NOT reuse.



Do not reuse diluted Positive Control.

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

- 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 Mastermix.

- 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-ST Assay (Amplification/Detection Area)

- a. Create a new run by clicking on the **Create Run** icon at the top of the screen. This will open the *Create Run* screen.
- b. Under **Run Name** in the left panel of the *Create Run* screen, enter a unique run identifier.
- c. Click on the **Assay** arrow in the left panel of the *Create Run* screen and select the **ProFlu SUBTYPING Assay** protocol from the drop-down menu.



Note

Double-check that the correct Assay has been chosen prior to starting the run.

- d. Under **Assay Information** in the left panel of the *Create Run* screen, enter the **Lot Number** and **Expiration Date** of the ProFlu-ST Supermix.
- e. In the left panel of the *Create Run* screen, enter the number of specimens (including the Positive Control but excluding Negative Control.) and click on **Apply**. This will display the **Site Table** and the SmartCycler Dx Software will automatically select 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 on **Report** at bottom of screen to open the **Report Preview** screen.
- b. Click on the **Print Icon** at the top of the screen.

Interpretation of Control Results

Validation of Run



Note

The user must interpret the Positive Control results and the Extraction Control (if included) results to determine whether the RT-PCR run and/or the extraction run are VALID; the SmartCycler Dx software will automatically interpret the Negative Control result.

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

| Sample ID ¹ | Assay Result | IC Result | Warning / Error Code | Sample Type | IC Ct | H1 or H3 or 2009 H1N1 result | H1 or H3 or 2009 H1N1 Ct |
|------------------------|--------------------|-----------|----------------------|-------------|-------|------------------------------|--------------------------|
| Extraction Control | Positive | 0 | | SPEC | NA | POS | 13-40 |
| Neg Control | Valid ² | Pass | | NC1 | 24-36 | Valid | 0 |

¹ Columns and data not used for interpretation are not included.

² (Typical) An Invalid assay will display Error Code 4098.

Invalid Extraction Run

If the conditions for a Valid Extraction run are not met (i.e., the Extraction Control is not positive within the specified Ct range 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](#)).

For a VALID RT-PCR run, the conditions in the table below must be met:

| Sample ID ¹ | Assay Result | IC Result | Warning / Error Code | Sample Type | IC Ct | H1 Result | H1 Ct | H3 Result | H3 Ct | 2009 H1N1 Result | 2009 H1N1 Ct |
|------------------------|--------------------|-----------|----------------------|-------------|-------|-----------|-------|-----------|-------|------------------|--------------|
| PF-ST PC | Positive | NA | | SPEC | 0 | POS | 24-36 | POS | 23-36 | POS | 22-36 |
| Neg Control | Valid ² | Pass | | NC1 | 24-36 | 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.

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](#)).

If the Negative Control is invalid, prepare all new extractions starting from original sample(s) using a new Extraction Control and a new Negative Control (starting at [Step 1](#) of the [Assay Procedure](#)).

Interpretation of Individual Specimen Results

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

| Sample ID ¹ | Assay Result | IC Result | Warning / Error Code | H1 Result | H3 Result | 2009 H1N1 Result | Interpretation of Results |
|------------------------|--------------|-----------|----------------------|------------|------------|------------------|---|
| Sample ID | Negative | Pass | | NEG | NEG | NEG | Seasonal influenza A/ H1, Seasonal influenza A/H3 and 2009 H1N1 influenza (S-OIV) RNA not detected |
| Sample ID | Positive | NA* | | POS | NEG | NEG | Seasonal influenza A/ H1 RNA detected |
| Sample ID | Positive | NA* | | NEG | POS | NEG | Seasonal influenza A/ H3 RNA detected |
| Sample ID | Positive | NA* | | NEG | NEG | POS | 2009 H1N1 influenza (S-OIV) RNA detected |
| 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 | Invalid | | 4098 ² | ND | ND | ND | Not Determined – error code 4098 |

¹ Columns and data not used for interpretation are not included

² 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.

Influenza A Positive (as determined by a currently available FDA-cleared or authorized Influenza A device), but Seasonal Influenza A/H1, Seasonal Influenza A/H3, and 2009 H1N1 Influenza (S-OIV) Negative Samples

Due to possible differences in assay sensitivity, an influenza A positive sample as determined by a currently available FDA-cleared or authorized influenza A device may test negative for seasonal influenza A/H1, seasonal influenza A/H3, and 2009 H1N1 influenza virus (S-OIV) by the Prodesse ProFlu-ST Assay. Such a sample should be further confirmed by other FDA cleared influenza subtyping tests and other 2009 H1N1 influenza tests authorized under EUAs.

Dual Positive Samples

Dual positive results can occur when multiple Influenza A subtypes are co-circulating. Repeat testing of dual positives is required. Repeated dual positives should be further confirmed by other FDA cleared influenza subtyping tests and other 2009 H1N1 influenza tests authorized under EUAs.

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. It is recommended that the user refer to CLSI document C24-A3, *Statistical Quality Control for Quantitative Measurement Procedures: 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 493.1202(c).

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, environmental, or carry-over contamination |
| Extraction | Failure in lysis and extraction procedure |
| Internal | PCR inhibition in individual samples and Reagent failure or process error |

Dilute and test Positive Control and the 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 and a Negative Control in each nucleic acid isolation run. The Extraction Control should be treated as a sample.

Never run the Positive Control through nucleic acid isolation.

Always include one Negative Control (containing Internal Control) and 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 should be done starting from the purified nucleic acid and using a new aliquot of the Positive Control. If repeat results are still invalid, results should not be reported and testing should be repeated from the original sample or a new sample should be collected and tested.

If the Extraction Control is not positive within the specified Ct range or the Negative Control is invalid, repeat testing should be done starting from the original sample and using a new Extraction Control and a new Negative Control. If repeat results are still invalid, results should not be reported and a new sample should be collected and tested.

Limitations

Detection of 2009 H1N1 Influenza virus targets the nucleoprotein (NP) gene which may react with other swine origin Influenza A strains.

Once the RT-PCR Mastermix has been made, the run must be started within one hour.

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.

Negative results do not preclude infection and should not be used as the sole basis for diagnosis, treatment or other management decisions.

A specimen yielding a negative result may contain respiratory viruses other than Influenza A.

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 loss of nucleic acid. The Internal Control is included in the ProFlu-ST Assay to aid in the identification of specimens that contain inhibitors to PCR amplification. The Internal Control does not indicate whether or not nucleic acid has been lost due to inadequate collection, transport or storage of specimens.

There is a risk of false positive values resulting from cross-contamination by target organisms or their nucleic acids.

The performance of the ProFlu-ST Assay has not been established in immunocompromised patients.

Performance Characteristics

Clinical Performance

Retrospective nasopharyngeal (NP) swab samples that were collected from symptomatic individuals suspected of Influenza infection were included in a study to evaluate the clinical performance of the ProFlu-ST Assay. These samples had been previously tested using the CDC rRT-PCR Flu Panel (IVD) to detect seasonal influenza A/H1 and A/H3 or the CDC rRT-PCR Swine Flu Panel (EUA) to detect 2009 H1N1 influenza virus.

A total of 24 seasonal A/H1 positive, 20 seasonal A/H3 positive, 52 2009 H1N1 Influenza A positive and 72 Influenza A negative NP swab samples were included in the study.

Seasonal Influenza A/H1 Results

| | | Reference Method | | Total | Comments |
|-----------------|----------|------------------|----------|-------|--|
| | | Positive | Negative | | |
| ProFlu-ST Assay | Positive | 23 | 0 | 23 | Positive Percent Agreement = 95.8% (79.8% - 99.3%) 95% CI |
| | Negative | 1 ^a | 144 | 145 | Negative Percent Agreement = 100% (97.4% - 100%) 95% CI |
| | Total | 24 | 144 | 168 | |

^a 1 sample tested negative by the ProFlu+ Assay (IVD) and was negative by bi-directional sequencing.

Seasonal Influenza A/H3 Results

| | | Reference Method | | Total | Comments |
|-----------------|----------|------------------|----------|-------|--|
| | | Positive | Negative | | |
| ProFlu-ST Assay | Positive | 20 | 0 | 20 | Positive Percent Agreement = 100% (83.9% - 100%) 95% CI |
| | Negative | 0 | 148 | 148 | Negative Percent Agreement = 100% (97.5% - 100%) 95% CI |
| | Total | 20 | 148 | 168 | |

2009 H1N1 Influenza Virus Results

| | | Reference Method | | Total | Comments |
|-----------------|----------|------------------|----------|-------|--|
| | | Positive | Negative | | |
| ProFlu-ST Assay | Positive | 50 | 0 | 50 | Positive Percent Agreement = 96.2% (87.0% - 98.9%) 95% CI |
| | Negative | 2 ^a | 116 | 118 | Negative Percent Agreement = 100% (96.8% - 100%) 95% CI |
| | Total | 52 | 116 | 168 | |

^a Two samples tested positive for Influenza A by the ProFlu+ Assay (IVD). One sample was positive for 2009 H1N1 by bi-directional sequencing and one sample was inconclusive by sequencing.

Analytical Sensitivity

The analytical sensitivity (limit of detection or LoD) of the ProFlu-ST Assay was determined using quantified (TCID₅₀/mL) cultures of 6 Influenza A (2 seasonal H1N1, 2 seasonal H3N2, and 2 2009 H1N1 influenza) 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) was defined as the lowest concentration at which ≥ 95% of all replicates tested positive.

| Viral Strain | LoD Concentration |
|--------------------------------|--|
| H1N1 A/Virginia/1/06 | 10 ⁰ TCID ₅₀ /mL |
| H1N1 A/Hong Kong/2506/06 | 10 ⁰ TCID ₅₀ /mL |
| H3N2 A/Anhui/1239/05 | 10 ¹ TCID ₅₀ /mL |
| H3N2 A/California/07/04 | 10 ¹ TCID ₅₀ /mL |
| 2009 H1N1 Clinical Isolate #1* | 10 ³ TCID ₅₀ /mL |
| 2009 H1N1 Clinical Isolate #5* | 10 ³ TCID ₅₀ /mL |

* Clinical isolate cultured and titered. Culture confirmed positive for 2009 H1N1 Influenza A strain using the CDC rRT-PCR Swine Flu Panel (EUA).

Analytical Reactivity

The analytical reactivity of the ProFlu-ST Assay was evaluated against multiple strains of Influenza A tested near the Limit of Detection. The reactivity panel consisted of 9 seasonal influenza A subtype H1N1, 11 seasonal influenza A subtype H3N2, and 8 Swine-Origin Influenza A strains (including 5 2009 H1N1 influenza isolates). 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-ST Assay.

| Viral Strain | Target | Concentration | Seasonal IA/H1 | Seasonal IA/H3 | 2009 H1N1 |
|--|-------------|--|----------------|----------------|-----------|
| A/Taiwan/42/06 | Seasonal H1 | 2x10 ¹ TCID ₅₀ /mL | + | - | - |
| A/Henan/8/05 | Seasonal H1 | 2x10 ¹ TCID ₅₀ /mL | + | - | - |
| A/Fujian/156/00 | Seasonal H1 | 2x10 ¹ TCID ₅₀ /mL | + | - | - |
| Brazil/1137/99 | Seasonal H1 | 2x10 ⁵ TCID ₅₀ /mL | + | - | - |
| A/Kentucky/2/06 | Seasonal H1 | 2x10 ² TCID ₅₀ /mL | + | - | - |
| A/Hawaii/15/01 | Seasonal H1 | 2x10 ² TCID ₅₀ /mL | + | - | - |
| A/New Caledonia/12/99 | Seasonal H1 | 2x10 ³ TCID ₅₀ /mL | + | - | - |
| A/Brisbane/59/2007 CDC#2008704388 | Seasonal H1 | 2x10 ² TCID ₅₀ /mL | + | - | - |
| A/Solomon Islands/03/06 CDC#2007725989 E2/E2 (7/06/2007)** | Seasonal H1 | 2x10 ⁻⁶ of HA: 16 | + | - | - |
| A/Hiroshima/52/05 | Seasonal H3 | 2x10 ⁰ TCID ₅₀ /mL | - | + | - |
| A/Victoria/512/05 | Seasonal H3 | 2x10 ¹ TCID ₅₀ /mL | - | + | - |
| VR 822 A/Victoria/3/75 | Seasonal H3 | 2x10 ⁴ TCID ₅₀ /mL | - | + | - |
| A/Brazil/02/99 | Seasonal H3 | 2x10 ³ TCID ₅₀ /mL | - | + | - |
| A/New York/55/2004 | Seasonal H3 | 2x10 ¹ TCID ₅₀ /mL | - | + | - |
| A/Hong Kong/2831/05 | Seasonal H3 | 2x10 ² TCID ₅₀ /mL | - | + | - |
| A/Port Chalmers/1/73 | Seasonal H3 | 2x10 ² TCID ₅₀ /mL | - | + | - |
| A/Bahamas/2686/99 | Seasonal H3 | 2x10 ² TCID ₅₀ /mL | - | + | - |

| Viral Strain | Target | Concentration | Seasonal IA/H1 | Seasonal IA/H3 | 2009 H1N1 |
|--|--------------------------------------|--|----------------|----------------|-----------|
| A/Fujian/411/02 | Seasonal H3 | 2×10^2 TCID ₅₀ /mL | - | + | - |
| A/Kentucky/03/06 | Seasonal H3 | 2×10^1 TCID ₅₀ /mL | - | + | - |
| A/Costa Rica/07/99 | Seasonal H3 | 2×10^3 TCID ₅₀ /mL | - | + | - |
| 2009 H1N1 Clinical Isolate #2* | Swine-Origin Influenza A (2009 H1N1) | 2×10^2 TCID ₅₀ /mL | - | - | + |
| 2009 H1N1 Clinical Isolate #3* | Swine-Origin Influenza A (2009 H1N1) | 2×10^2 TCID ₅₀ /mL | - | - | + |
| 2009 H1N1 Clinical Isolate #4* | Swine-Origin Influenza A (2009 H1N1) | 2×10^2 TCID ₅₀ /mL | - | - | + |
| VR 897 A/New Jersey/8/76 | Swine-Origin Influenza A | 2×10^3 TCID ₅₀ /mL | - | - | + |
| 2009 H1N1 A/California/04/2009 CDC# 2009712047, C3 (4/27/09)** | Swine-Origin Influenza A (2009 H1N1) | 2×10^{-5} of HA: 16 | - | - | + |
| A/South Dakota/02/3008 CDC#2009713788, E2 (1/29/09)** | Swine-Origin Influenza A | 2×10^{-4} of HA: 16 | - | - | + |
| A/Wisconsin/10/2009 CDC#99006509, C3E2 (4/24/09)** | Swine-Origin Influenza A (2009 H1N1) | 2×10^{-3} of HA: 64 | - | - | + |
| A/Iowa/2006 CDC#2007700833, E4 (3/12/07)** | Swine-Origin Influenza A | 2×10^{-4} of HA: 24 | - | - | + |

* Clinical isolate cultured and titered. Culture confirmed positive for 2009 H1N1 Influenza A strain using CDC rRT-PCR Swine Flu Panel.

** Some of the strains received from CDC were quantified by Hemagglutination Assay titer and a TCID₅₀/mL titer was not available.

Analytical Specificity

The analytical specificity of the ProFlu-ST Assay was evaluated by testing a panel of 53 cultures consisting of 29 viral, 23 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 or 10^4 TCID₅₀/mL (Chlamydia). 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-ST Assay was 100%.

| Strains | Concentration | Seasonal IA/H1 | Seasonal IA/H3 | 2009 H1N1 |
|--|-------------------------------|----------------|----------------|-----------|
| Influenza A/Henan/8/05 (seasonal H1N1) | 10^3 TCID ₅₀ /mL | + | - | - |
| Influenza A/California/07/04 (seasonal H3N2) | 10^3 TCID ₅₀ /mL | - | + | - |
| 2009 H1N1 Influenza A | 10^4 TCID ₅₀ /mL | - | - | + |
| Influenza A/Port Chalmers (seasonal H3N2) | 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 | - | - | - |
| Coxsackie B4 | 10^4 TCID ₅₀ /mL | - | - | - |
| Coxsackie B5/10/2006 | 10^5 TCID ₅₀ /mL | - | - | - |
| Cytomegalovirus | 10^4 TCID ₅₀ /mL | - | - | - |
| Echovirus 2 | 10^6 TCID ₅₀ /mL | - | - | - |
| Echovirus 3 | 10^4 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 MacIntyre 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 | - | - | - |

| Strains | Concentration | Seasonal IA/H1 | Seasonal IA/H3 | 2009 H1N1 |
|-------------------------------------|--|----------------|----------------|-----------|
| 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 Virus | 10 ⁴ TCID ₅₀ /mL | - | - | - |
| <i>Bordetella pertussis</i> | 10 ⁶ CFU/mL | - | - | - |
| <i>Bordetella bronchiseptica</i> | 10 ⁷ CFU/mL | - | - | - |
| <i>Chlamydia 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>Legionella micdadei</i> | 10 ⁻¹ dilution | - | - | - |
| <i>Mycobacterium intracellulare</i> | 10 ⁷ CFU/mL | - | - | - |
| <i>Mycoplasma pneumoniae</i> | 10 ⁻¹ dilution | - | - | - |
| <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>Candida albicans</i> | 10 ⁶ CFU/mL | - | - | - |

Extraction Equivalency

Extraction equivalency of the bioMérieux NucliSENS easyMAG and Roche MagNA Pure LC was evaluated by performing a limit of detection study, confirming ≥ 95% detection of two strains each of seasonal H1N1 Influenza A, seasonal H3N2 Influenza A, and 2009 H1N1 Influenza Virus in 20 individual replicates extracted on each instrument.

The bioMérieux NucliSENS easyMAG and the Roche MagNA Pure LC performed equivalently with respect to limit of detection.

Disposal

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

References

1. Couch, R.B. and Kasel, J.A. 1995. *Influenza in Diagnostic Procedures for Viral, Rickettsial, and Chlamydial Infections*. 7th Edition. 431-446.
2. Harper, S.A., Fukida, K., Uyeki, T.M., Cox, N.J., and Bridges, C.B. 2005. Prevention and Control of Influenza. *MMWR*. 54(RR08):1-40.
3. Centers for Disease Control and Prevention. 2009. Swine Influenza A (H1N1) Infection in Two Children—Southern California, March–April 2009. *MMWR* 58 (Dispatch);1-3.
4. <http://www.cdc.gov/h1n1flu/qa.htm>
5. <http://www.cdc.gov/h1n1flu/antiviral.htm>
6. http://www.cdc.gov/h1n1flu/guidelines_labworkers.htm

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Understanding the Symbols

The following symbols may appear on the packaging and labeling:

| Symbol | Definition | Symbol | Definition |
|---|--------------------------------------|--|-------------------------------------|
| REF | Reference Number or Catalog number |  | Contains sufficient for < n > tests |
|  | Batch code or Lot Number |  | Do not reuse |
|  | Use By Date or Expiration Date |  | Manufacturer |
|  | Upper Storage Temperature Limitation |  | Authorized Representative |

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