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 As of June 23, 2010, the EUAs have been terminated and this information is no longer current.

LONGHORN™
VACCINES & DIAGNOSTICS

Influenza A/H1N1-09 Prime RRT-PCR Assay

Instructions for Use

For detection of 2009 H1N1 Influenza Virus

Emergency Use Authorization
For *in vitro* Diagnostic Use

Table of Contents

Intended Use

The Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™ is an all inclusive RRT-PCR collection-to-detection assay intended for use in CLIA High Complexity Laboratories using the RNAqueous Micro Kit (Ambion) or Qiagen QIAamp Viral RNA Mini Kit (Qiagen) and Applied Biosystems' 7500 real-time RT-PCR detection instrument with Software version 1.4 for the *in-vitro* qualitative detection of 2009 H1N1 influenza viral RNA in upper respiratory tract specimens (such as nasopharyngeal swabs (NPS), nasal swabs (NS), throat swabs (TS), nasal aspirates (NA), nasal washes (NW), and dual nasopharyngeal/throat swabs (NPS/TS)) collected from human patients with signs and symptoms of respiratory infection in conjunction with clinical and epidemiological risk factors.

Testing with the Influenza A/H1N1-09 Prime RRT-PCR Assay should not be performed unless the patient meets clinical and epidemiologic criteria for testing suspect specimens. The identification of 2009 H1N1 Influenza should be performed along with a clinical and epidemiological assessment and should not be used as the sole basis for treatment or other patient management decisions.

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. There are two primary influenza types (A and B) with type A further categorized into one of 16 known subtypes based on the hemagglutinin (HA) and neuraminidase (NA) surface antigens. Human influenza epidemics occur yearly with both types A and B circulating, but type A is usually dominant. These yearly epidemics are partly due to antigenic variation in the HA and NA surface proteins of the virus. In March of 2009, a novel Influenza A virus (2009 H1N1) emerged in North America and subsequently spread worldwide. The 2009 H1N1 strain contains two genes from influenza viruses that normally circulate in swine throughout Europe and Asia in addition to avian and human genes.

Transmission of influenza is primarily via airborne droplets from coughing and sneezing. Symptoms of influenza arise 1-2 days after exposure and include a combination of upper and lower respiratory symptoms with fever, chills, headache, myalgia, and general malaise. Symptoms of the 2009 H1N1 virus are similar to those of seasonal influenza strains ranging from common cold-like illness to severe pneumonia requiring hospitalization. Higher risk subjects include children <5 years, pregnant women, older children, young adults, adults >65 years, and individuals with underlying medical conditions which could lead to severe complications related to infection with the 2009 H1N1 influenza virus.

Point mutations in the surface proteins of influenza virus (antigenic drift) allow the virus to evade immunity developed in prior seasons. A more significant change to the surface glycoproteins of the virus caused by recombination of genes leads to novel strains with pandemic potential, a process known as antigenic shift.

Principle of the Procedure

The Influenza A/H1N1-09 Prime RRT-PCR Assay enables detection of RNA from 2009 H1N1 Influenza Virus.

An overview of the procedure is as follows:

1. Upper respiratory tract specimens from symptomatic patients are collected and placed in PrimeStore™ MTM (included in the kit).

2. An Internal Positive Control (IPC) in the PrimeStore™ MTM solution is extracted along with the clinical specimen to monitor extraction efficiency and inhibitors present in the specimens.

   Due to microbial inactivating properties of PrimeStore MTM it is not recommended to attempt culture after storage or transport of samples in PrimeStore MTM.

3. Isolation and purification of nucleic acids is performed using a commercially available viral nucleic acid extraction kit (refer to **Materials required but not provided**).

4. Purified nucleic acids are added to the PrimeMix™ Influenza A/H1N1-09 detection assays (ready to use, all inclusive detection solutions to eliminate 'master mix' preparation).

5. Reverse transcription of RNA into complimentary DNA (cDNA) and subsequent amplification of DNA is performed in the AB 7500 Real Time PCR System. In this process, the probe anneals specifically to the template followed by primer extension and amplification. The Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay is based on Taqman chemistry, which utilizes the 5' – 3' exonuclease activity of the Taq polymerase to cleave the probe thus separating the reported 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 the time. Fluorescent intensity is monitored during each PCR cycle by the real-time instrument.

Materials Provided

Table 1. Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay Components

PrimeMix™ Assays	Description	Quantity Per Tube	Reactions Per Tube
Flu A	An all inclusive amplification solution with buffers, primers, probe and enzymes to detect Influenza A virus.	800 µL	40
H1-09	An all inclusive amplification solution with buffers, primers, probe, and enzymes to detect 2009 H1N1 influenza A virus.	800 µL	40
IPC	An all inclusive amplification solution with buffers, primers, probe, and enzymes for the PrimeStore™ MTM Internal Positive Control RNA	800 µL	40

Table 2. Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay Controls

Positive Controls	Description	Quantity Per Tube	Reactions Per Tube
Flu A	A positive amplification control for the PrimeMix™ Influenza A assay.	50 µL	20
H1-09	A positive amplification control for the PrimeMix™ Influenza H1-09 assay.	50 µL	20
IPC	A positive control for the extraction efficiency of samples collected in PrimeStore™ MTM.	50 µL	20

Table 3. PrimeStore Reagent

Sample Collection	Description	Quantity Per Tube	Number of Tubes
PrimeStore™ Molecular Transport Media	A blend of reagents for the collection, stabilization and storage of influenza viral RNA.	1.5 mL	42

Materials Required But Not Provided

Plasticware and Consumables

RNase/DNase-free 1.5 mL polypropylene microcentrifuge tubes

Sterile RNase/DNase-free filter or positive displacement micropipettor tips

96-well optical amplification reaction plate or tubes

Optical caps for 96-well plate or reaction tubes

Sterile, powder-free gloves

Reagents

Viral nucleic acid isolation; RNAqueous Micro Kit (*Ambion,Cat. # AM1931*), or Qiagen QIAamp Viral RNA Mini Kit *(Qiagen Cat. #52904)*

Molecular Grade Water (RNase/DNase-Free)

Other Materials and Instruments
Real-Time PCR System: Applied Biosystems 7500 Real-Time PCR System with SDS software v1.4

Single or multi-channel micropipette(s) with accuracy range between 1-10µL, 10-100µL, and 100-1000µL
(Note: Dedicated micropipettes are required for extraction, as well as for PrimeMix™ dispensing).

Freezer (manual defrost) at -20ºC (for kit components stored frozen)

Freezer (manual defrost) at -70 to -80ºC (for storage of extracted viral RNA)

Refrigerator at 2-8ºC

Microcentrifuge

Vortex Mixer

Cooler racks for 1.5 mL microcentrifuge tubes

Warning and Precautions

For *in-vitro* diagnostic use only.

Follow universal precautions for all patient specimens. All patient clinical samples should be handled as though infectious.

Do not insert swab into PrimeStore™ Molecular Transport Medium solution before collecting patient specimen.

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

Diagnostic laboratory work on clinical samples should be conducted in a BSL2+ laboratory. Clinical respiratory samples collected in PrimeStore™ MTM should be handled according to good laboratory practices.

Wear personal protective equipment, such as (but not limited to) gloves and lab coats when handling kit assays. Wash hands when testing is completed.

Use micropipettes with aerosol barrier or positive displacement tips for all procedures. Workflow in the laboratory should proceed in a unidirectional manner beginning with the pre-amplification areas and moving to the amplification/detection area. Below is the sequence of events that takes place from specimen collection to PCR set up.

- Begin pre-amplification by collecting sample from patient and placing in PrimeStore™ MTM (area I), followed by specimen extraction (area II), addition of PrimeMix™ Flu A/ H1-09 assays to reaction plate (area III), PCR set-up (area IV), and finally instrument set-up (Amplification/Detection).
- Do not use supplies and equipment across the dedicated areas of specimen extraction and assay set-up in the reaction plate (Do not cross nucleic acid areas with nucleic acid –free areas).
- All amplification supplies and equipment should be kept in the Amplification/Detection Area at all times.
- Personal protective equipment, disposable gloves and laboratory coats, should be area specific.

Contamination of patient samples or detection assays can produce erroneous results. Always use sterile technique.

Pipette and handle nucleic acid samples and controls carefully to prevent contamination of samples from adjacent wells.

Use proper pipetting techniques and maintain the same pipetting pattern throughout the procedure to ensure optimal and reproducible values.

Do not substitute or mix reagents from different kit lots or from other manufacturers.

Do not interchange the assay or control tube caps. This will cause contamination and compromise the test results.

Only use the protocol described in this insert. Deviations from the protocol or the use of times or temperatures other than those specified may give erroneous results.

Assay set-up should be performed at room temperature (approximate range of 18 to 25 ºC).

Do not re-use wells that have been exposed to patient samples or reagents.

Dispose of amplified samples without opening the reaction wells.

PrimeStore™ MTM contains chemicals that may be harmful if ingested. Contact with the skin may cause irritation. Upon ingestion or contact, first aid measures should be taken.

Reagent Storage, Handling and Stability

Store all Influenza A/H1N1-09 Prime RRT-PCR Assay reagents (opened and unopened) at -20ºC until the expiration date listed on the tube.
Always check the expiration date on the assay tubes.

Do not expose controls to more than 2 freeze-thaw cycles.

Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay reagents are shipped frozen, should arrive frozen, and should be stored frozen after receipt until ready for use. If the reagents are not frozen, contact Longhorn Vaccines and Diagnostics for assistance.

PrimeStore™ MTM is shipped at ambient temperature and should be stored at 2-8 ºC until ready for use. After specimen collection, PrimeStore™ MTM samples can be stored at ambient temperature for 14 days. For long-term, specimens should be stored a -20 ºC.

Visually inspect all reagent volumes prior to beginning any test procedures.

Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay reagents are supplied in amber tubes and should be protected from prolonged exposure to light.

Controls and aliquots of controls should be thawed and kept on ice or in a cold block at all times during use.*

Recommendation: Aliquoting of controls is recommended when running smaller sample runs.

Specimen Handling and Storage

Specimen Collection

Acceptable specimen types include, nasal swabs (NS), nasopharyngeal swabs (NP), nasal washes (NW), and throat swabs (TS).

Swabs are placed directly into PrimeStore™ Molecular Transport Medium tube for specimen storage and shipping.

Nasal Washes are added directly to the PrimeStore™ Molecular Transport Medium tube in volumes from 100 to 500 µL.

Transporting Specimens

Specimens placed in PrimeStore™ MTM are lysed and the nucleic acid is stabilized for transport at ambient temperature without the use of cold packs or dry ice.

Storing Specimens

Specimens placed in PrimeStore™ MTM can be held at ambient temperature for 14 days. For longer periods, store specimens in PrimeStore™ MTM at 2-8 ºC or -20 ºC.

Storing Purified Nucleic Acid
Store purified nucleic acids at ≤ -70 ºC.

Note: Inadequate or inappropriate specimen collection, storage and transport are likely to cause deterioration of the samples which may lead to decreased isolation yields and false negative results. The IPC present in PrimeStore™ will allow detection of RNA degradation during transport and extraction.

Detection Assay and Control Preparation

Influenza A/H1N1-09 PrimeMix Reagents
Assay reagents do not require preparation. Remove assay tubes from freezer and allow assay solutions to come to room temperature. Vortex briefly 2-5 seconds and quick spin to remove solution from cap prior to use.

Positive Controls
Remove controls from freezer and thaw on ice or in cold box. Aliquot controls into smaller volumes if fewer sample runs are performed.

Negative Control
Use an aliquot of PrimeStore™ MTM without patient specimen as the Negative Control. The negative aliquot of PrimeStore™ MTM is subjected to the same nucleic acid isolation protocol as used for the patient specimens.

Extraction Control
PrimeStore™ MTM contains a known amount of internal positive control (IPC) which is extracted along with the viral nucleic acid from the patient sample collected and stored in the tube.

This separate extraction control can be run for each sample by setting up the IPC reaction assay for each sample extracted.

Procedure

A. **Collection of Patient Specimen into PrimeStore™ MTM**

WARNING: DO NOT insert swab into PrimeStore™ Molecular Transport Medium solution before collecting patient specimen.

1. Collect nasal wash (NW), nasopharyngeal swab (NP), nasal swab (NS), or throat swab (TS) from symptomatic patient.
    - If NW is collected, add 100 – 500 µL of wash to the PrimeStore™ Molecular Transport Medium tube. Cap the tube.
    - If a swab is collected, insert the swab directly into the PrimeStore™ Molecular Transport Medium tube. Break off the excess swab handle at score mark. Cap the tube with swab inside.

2. Vortex the tube with swab inside for 5-10 seconds.
3. Store at ambient temperature for isolation of nucleic acid. (Store refrigerated, 2-8 ºC, or frozen, -20 ºC, for longer periods of time.) [Note: PrimeStore™ MTM tubes stored refrigerated or frozen should be brought to room temperture prior to start of the extraction procedure. Vortex each tube for 5-10 seconds and allow tubes to acclimate on the benchtop to ambient temperature.

B. Isolation of Nucleic Acid

1. Isolation of nucleic acid is performed using the RNAqueous Micro Kit (Ambion, Cat. #AM1931) or Qiagen QIAamp Viral RNA Mini Kit (Qiagen Cat. #52904).
2. Follow the manufacturer's recommended instructions and volumes for isolating the nucleic acid from an aliquot of the PrimeStore™ MTM containing the patient's specimen.
3. Elute the purified RNA in 20 µL per manufacturer recommendation (enough to run 8-10 2 µl reactions).
4. Store purified nucleic acid samples refrigerated, on ice, or in a cold box while preparing the 96 well amplification plate.

C. Set up the RRT-PCR Reaction (Pre-Amplification Area III)

1. Remove amber PrimeMix™ Flu A, H1-09, and IPC assay vials from the -20°C freezer and place in a nucleic acid-free area such as a clean room or PCR hood. Allow solutions to equilibrate to ambient temperature (18-25°C).
2. Vortex thawed assays briefly 2-3 seconds. Quick spin to collect liquid from cap of each vial.
3. Dispense 18 µL of the PrimeMix™ Flu A assay for each sample to be tested into the designated wells of an optical 96-well reaction plate. Dispense 2 additional wells for the Positive and Negative Control reactions.
4. After dispensing PrimeMix™ Flu A assay into each well, immediately cap the well designated as the No Template Control (NTC) or Negative Control to avoid any contamination.
5. Loosely place 8 or 12-well caps over the remaining assay wells on the plate.
6. Continue Steps 3-5 for PrimeMix™ H1-09 and IPC assays to be run on the reaction plate.
7. Remove the 96-well optical reaction plate from the nucleic acid-free area or PCR hood to the PCR set-up area where the sample RNA will be added to the plate.
8. Remove aliquots of Positive Controls from -20°C freezer and thaw controls on ice or in a cold block.
9. Carefully pipette 2 µL of sample RNA into the appropriate wells containing PrimeMix™ detection solution. **(NOTE: Change pipette tip after each sample addition to prevent cross-contamination of samples and PrimeMix™ assay solutions.)** Pipette technique is important to ensure that the 2 µL of sample RNA

is slowly added to the 18 µL of PrimeMix™ in the bottom of the well. Do not simply pipette RNA over the well or to the side of the well.

10. After the addition of RNA to the appropriate assay wells on the plate, fully seat the caps on each well of the plate. Make sure all caps are flat on the plate.
11. Continue adding RNA samples to the plate until all assays have had RNA template added and all sample wells have been capped.
12. Add 2 µL of the appropriate positive control to the appropriate assay well (Flu A Positive control into Flu A PrimeMix™ assay well, etc.) Seat caps on all positive control wells.
13. 96-well plate is ready to run on the AB 7500 Real Time PCR Instrument.

D. Instrument Set-Up and Run

Perform the following steps to configure the AB 7500 with SDS software version 1.4:

1. Refer to AB 7500 *Instructions for Use* for details on how to run the plate on the AB 7500 Real Time PCR System.

2. Launch AB 7500 Real Time PCR System software from icon on the computer desktop screen.

3. Click **Create New Document** in the **Quick Startup document** window.

4. Begin **to configure the t**emplate **by verifying the defau**lt settings appear as follows:
   - **Assay:** Standard Curve (Absolute Quantitation)
   - **Container:** 96-well Clear
   - **Template:** Blank Document
   - **Run Mode:** Standard 7500

5. Click **Next** and continue to configure the template in the **Quick Startup document** window as follows:
   - Define the **Detectors** and **Reporters** as indicated below. (NOTE: Click **New Detector** button if necessary).

Detector Name	Reporter Dye	Quencher
Influenza A	FAM	None
Influenza H1-09	FAM	None
IPC	FAM	None

   - Select **Passive Reference** dye to **ROX**.
   - Click **Next**.
   - Configure the plate according to the way the detection solutions and sample RNAs were added to the plate.
   - Click **Finish.**

6. Continue template configuration in the AB 7500 Software version 1.4 main screen by selecting the **Instrument** tab and entering the following thermal cycling settings:

Stage1, Reps 1	Stage 2, Reps 1	Stage 3, Reps 40
50.0 °C – 20.0 minutes	95.0 °C – 5.0 minutes	95.0 °C – 15 seconds 60.0 °C – 32 seconds

Sample Volume: 20 µL
Run Mode: Standard 7500
Data Collection: Stage 3, Step 2 (60.0 @ 0:32)

7. Save file by selecting **Save As** in the **File** dropdown menu, name appropriately, and ensure the file format is **.sds.**

8. Open the AB 7500 plate drawer and carefully place the test plate in the tray. Position the plate so the A1 well is located in the upper left corner of the plate holder. Carefully close the plate drawer.

9. Click **Start** in the **Instrument** tab.

   ***Note:** This plate document can be used for additional runs by Clicking on the **Plate Set Up** tab and modifying the samples names on the plate schematic. Click **Save As** in the **File** dropdown menu and giving the file a different name (example: Influenza Sample Run1 12.09.09 and Influenza Sample Run2 12.09.09). Ensure the file format is **.sds.**

E. Data Analysis

1. When the run finishes, click **OK** from the window.
2. Click on **Analysis** menu and choose **Analysis settings.**
3. Detectors should be set to **All Detectors**.
4. **Manual CT** should be selected.
5. **Manual Baseline** should be selected (Start at 3, Stop at 15).
6. Set the **Threshold** for all detectors to 0.1.
7. Click the **Analyze** icon (►) from the **toolbar. (Note:** Wait for a few seconds to allow the analysis process to complete.)
8. Click the **Results** tab. Click the **Amplification Plot** tab.
9. Select one sample well at a time and look at the Amplification plot and Component plots to check for the accuracy of the result.
10. Select all of the sample amplification plots for the IPC detector, one at a time, to check the extraction of each sample. All IPC amplifications plot results should be $\leq$ 32 CTs.
11. Choose **Save** option from the File menu after analyzing all the wells.
12. Select the **Report** tab and if desired, print a copy of the results for laboratory records.

Interpretation of Control Results

The user must interpret the Positive Control results and the Extraction Control (IPC) results to determine whether the RRT-PCR runs are VALID; the AB software will automatically interpret the Negative Control result. A negative control extraction should be performed for each run. The negative control extraction includes performing a 'blank PrimeStore™' extraction. This ensures that all of the extraction reagents are free of nucleic acid contamination.

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

1. The IPC PrimeMix™ reaction should yield a result for each unknown sample.
2. The IPC PrimeMix™ reaction should yield a CT score $\leq$ 32 for each extraction assuming the RNaqueous Micro Kit (Ambion) or Qiagen QIAamp Viral RNA Mini Kit (Qiagen) was used.
3. The IPC PrimeMix™ positive reaction should be positive.

INVALID Extraction Run:

If the conditions for a VALID Extraction run are not met, repeat the entire extraction run starting from the original sample(s) preserved in PrimeStore™ and perform a new Negative Control.

For a VALID RRT-PCR run the following conditions must be met:

1. The PrimeMix™ IPC assay for each sample reaction should have a CT score of $\leq$ 32 for each extraction
2. The PrimeMix™ Flu A, and H1-09 Positive Control reactions should be positive for each run analyzed and should yield CT $\leq$32.
3. The PrimeMix™ Flu A, and H1-09 Negative Control reactions should be negative with CT scores >40.

INVALID RRT-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 nucleic acids from the Positive Control reagents supplied in the kit.

If the Negative Control is invalid, prepare all new extractions starting from original sample(s) using a new PrimeStore™ (*No Template*) Extraction Control for Flu A and H1-09 assays and a new *No Template* (water blank) control for the IPC.

1. All assay controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

2. Examine the: 1) PrimeMix™ Flu A, 2) PrimeMix™ H1-09 and 3) PrimeMix™ IPC Positive and Negative Controls.

    a. If the Negative Control is:

        i. Positive (CT value less than 40 for the Flu A, H1-09 or IPC, then this control is invalid. This indicates possible contamination of prepared samples. Positive patient results cannot be reported. Positive specimens on this run must be repeated. Negative specimens may be reported given that all other assay run criteria are met.

        ii. Negative (CT value of 40 or listed by the system as "Undetermined"), then this control is valid and acceptable.

    b. If the Positive Control is:

        i. Above the upper value of the acceptance criteria (CT ≤32) for Flu A, H1-09 or IPC, the assay run is considered invalid and unacceptable. All patient specimens must be re-assayed.

        ii. Within the respective acceptable criteria, the assay run for Flu A, H1-09 and IPC is considered valid and acceptable.

Individual Specimen Results

a. Examination of clinical specimen results should be performed after the Positive and Negative Controls have been examined and determined to be valid and acceptable. Flu A, H1-09 or IPC results must be examined for each patient specimen.
b. Amplification plots should be examined for every positive sample (those with CT value below 40). If the amplification plot shows an exponential increase, the amplification curve is valid.

INTERPRETATION OF RESULTS

1. All influenza assay controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted and the test must be repeated.

2. Examine the PrimeMix™ 1) Flu A, 2) H1-09 and 3) IPC Positive and Negative Controls.

    a. If the Negative Controls are:

        i. Positive (CT value less than 40 for the Flu A, H1-09 or IPC), then this control is invalid. This indicates possible contamination of prepared

samples. Positive patient results cannot be reported. Positive specimens on this run must be repeated.

ii. Negative (CT value of 40 or listed as “Undetermined”), then the control is valid and acceptable.

b. If the Positive Controls are:

i. Negative (CTs above the upper value of the predetermined acceptance criteria for Flu A, H1-09 or IPC (e.g. **Table 4**)), then the assay run is considered invalid and unacceptable. All patient specimens must be re-assayed.

ii. Positive (CT values for Flu A, H1-09 or IPC are within their respective acceptable criteria), then the assay run is considered valid and acceptable.

3. Examination of Patient Specimen Results

a. Examination of clinical specimen results should be performed after the Positive and Negative Controls have been examined and determined to be valid and acceptable. Flu A, H1-09 or IPC results must be examined for each patient specimen.

b. Amplification plots should be examined for every positive sample (those with CT value below 40). If the amplification plot shows an exponential increase, the amplification curve is valid.

4. Interpretation of Results

a. A specimen that does not contain any influenza A (pan A, seasonal flu A, or 2009 H1N1 influenza viral RNA) will be negative for the Flu A and H1-09 reactions, the IPC reaction should still be positive.

b. A specimen positive for seasonal influenza A virus, i.e., H3N2 or seasonal H1N1 (i.e. non-2009) will have a positive result for the Flu A reaction and will be negative for H1-09 reaction. A specimen positive for 2009 H1N1 should be positive for the Flu A detector and the H1-09 reaction.

c. A specimen with a positive result for FluA and negative result for H1-09 does not necessarily mean it is positive for seasonal flu A.

d. Validation studies have shown that specimens containing influenza A viruses (seasonal or 2009 H1N1 influenza) will most likely have CT values below 38 for the Flu A reaction. Specimens with CT values in the range of 38 – 40 for any of these detectors most likely represent specimens with very low influenza A virus concentrations. Repeat testing on these specimens must be performed to confirm positive results.

e. If the Flu A CT value of a patient sample is ≥ 40 or is listed as "Undetermined" and the IPC CT value falls within or below the acceptable range, the "Influenza A" result is reported as "Not Detected".

f. If the Flu A CT value of a patient specimen is less than 38 and an amplification curve is observed for the well, the "Influenza A RNA" result is reported as "Detected".

g. If the H1-09 CT value of a patient sample is ≥ 40 or is listed as "Undetermined" and the IPC CT value falls within or below the acceptable range, the "2009 H1N1 Influenza H1" result is reported as "Not Detected".

h. If the H1-09 CT value of a patient specimen is less than 38 and an amplification curve is observed for each well, the "2009 H1N1 Influenza" result is reported as "Detected".

i. If the Flu A and H1-09 CT value of a patient specimen is in the range of 38–40 and an amplification plot is observed for the well, the specimen must be retested to verify the positive result.

j. If upon retesting, the Flu A CT value is < 40 for the Flu A and an amplification plot is observed for the well, the "Influenza A" result is reported out as "Detected".

k. If upon retesting, the H1-09 CT values are < 40 and the amplification plot is observed for the well and the Flu A result is reported as "Detected", then the "2009 H1N1 Influenza" result is reported as "Detected".

l. If upon retesting the Flu A and H1-09 CT value of a patient specimen is ≥ 40 or is listed as "Undetermined" and the IPC CT value falls within the acceptable range, the respective result is interpreted as "Not Detected".

m. If upon repeat testing, the interpretation of the result for Flu A or H1-09 changes, a third replicate should be tested, and the consensus of the three replicates should be reported.

n. If the Flu A or H1-09 CT value of a patient specimen is ≥ 40 or is listed as "Undetermined" but the IPC CT value falls above the upper value of the acceptable range, the specimen must be re-extracted and retested. If upon repeat testing the same situation occurs the patient result is reported as "Indeterminate due to inhibition" with the additional comment: "After repeat analysis, non-amplification of the internal control suggests the presence of PCR inhibitors in the patient sample or sample RNA degradation. An additional sample should be submitted for testing if clinically warranted."

Table 4B: Interpretation of PrimeMix™ RRT-PCR Result

Example	Flu A CT value	H1-09 CT value	IPC CT value	Interpretation
1	≥40	≥40	≤ 32	Influenza A RNA: Not detected Influenza H1-09 RNA: Not detected
2	<38	≥40	≤ 32	Influenza A RNA: Detected Influenza H1-09 RNA: Not detected
3	≥40	<38	≤ 32	Indeterminate – repeat test
4	<38	<38	≤ 32	Influenza A RNA: Detected Influenza H1-09 RNA: Detected
5	≥40	≥40	>32	Invalid - repeat testing with new extraction
6	If Flu A or H1-09 CT value is 38-40, repeat testing to confirm			
Below are interpretations of specimens upon repeat testing:				
7	≥40	≥40	≤ 32	Influenza A RNA: Not detected Influenza H1-09 RNA: Not detected
8	<40	≥40	≤ 32	Influenza A RNA: Detected Influenza H1-09 RNA: Not detected
9	≥40	<40	≤ 32	Influenza A RNA: Not detected Influenza H1-09 RNA: Detected
10	<40	<40	≤ 32	Influenza A RNA: Detected Influenza H1-09 RNA: Detected
11	If Flu A and H1-09 interpretation changes upon repeat testing, a third replicate should be tested, and the consensus of the three replicates should be reported.			

Assay Performance

Analytical Sensitivity/Limit of Detection (LoD). Analytical sensitivity (LoD) studies determine the lowest detectable concentration of influenza virus at which approximately 95% of all (true positive) replicates test positive. The LoD was determined for the **Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™** by dilution studies using characterized reference samples. The LoD of the IPC was determined by serial dilutions of the IPC RNA template.

Viral Strains and Titers. Viral stocks were obtained by inoculation of 0.5 mL of influenza-positive nasal wash specimen into MDCK according the traditional culturing technique described in Daum *et al* (reference 15). Two contemporary influenza strains designated as A/Texas/78209/2008 (H3N2) and A/New Caledonia/20/1999 were used for Limits of Detection for the **Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™**. Also included as a stock strain was A/Wuhan/395/1995, a human H3N2 reference strain. Two 2009 H1 strains were prepared and quantified (**Table 5**). All reference strains were grown and quantified at Virion Systems Incorporated and Battelle Memorial Institute.

Table 5: Influenza Virus Standard Strains

Reference Strain	Type	Subtype	Stock Concentration
A/Texas/78209/2008	Flu A	H3N2	6.07 x 10^6 $TCID_{50}$/mL
A/New Caledonia/20/1999	Flu A	H1N1	6.5 x 10^6 $TCID_{50}$/mL
A/California/04/2009	Flu A	H1N1-09	4.3x 10^5 $TCID_{50}$/mL
A/Mexico/4108/2009	Flu A	H1N1-09	5.3 x 10^4 $TCID_{50}$/mL
A/Wuhan/395/1995	Flu A	H3N2	1.8 x 10^7 TCID50/mL

A 10-fold serial dilution for each stock strain was prepared to identify an end-point for detection for each Longhorn Influenza A/H1N1-09 PrimeMix™ Assay. Viral RNA was extracted with the RNAqueous Micro (Ambion, Cat#1931) from each serial dilution.

Serial dilution of characterized influenza viruses were analyzed in triplicate. The lowest concentration at which all triplicates were positive was treated as the tentative LoD for each test. The LoD of each test was then confirmed by testing 20 replicates with concentrations at the tentative limit of detection. The final LoD of each test was determined to be the lowest concentration resulting in positive detection of at least 19 out of 20 replicates.

Serial dilutions of IPC RNA template were analyzed in triplicate. The lowest concentration at which all triplicates were positive was treated as the tentative LoD for each test. The LoD of each test was then confirmed by testing 20 replicates with concentrations at the tentative limit of detection. The final LoD of each test was determined to be the lowest concentration resulting in positive detection of at least 19 out of 20 replicates.

Table 5 A and B: Limit of Detection of PrimeMix™ Influenza A

Virus Strain Tested	Analyte Tested	Stock Virus Titer	Serial 10-Fold Dilution Factor	TCID 50/mL Dilution Tested	Call Rate	C_T (Triplicate) Avg	20-Hit Call Rate	C_T (20 Hit-Avg)	Lowest Concentration with Uniform Positivity per Analyte	Limit of Detection (LoD) per Virus Strain
A/Texas/78209/2008 (H3N2)	PrimeMix™ Flu A	6.07 x10^6 $TCID_{50}$/mL	10e3	$10^{3.0}$	3/3	25.9			10^0 $TCID_{50}$/mL (34.0 Avg C_T)	10^0 $TCID_{50}$/mL
			10e4	$10^{2.0}$	3/3	28.6				
			10e5	$10^{1.0}$	3/3	33.5				
			10e6	10^0	3/3	35.6	20/20	34.0		
			10e7	10^{-1}	2/3	-				

Virus Strain Tested	Analyte Tested	Stock Virus Titer	Serial 10-Fold Dilution Factor	TCID 50/mL Dilution Tested	Call Rate	C_T (Triplicate) Avg	20-Hit Call Rate	C_T (20 Hit-Avg)	Lowest Concentration with Uniform Positivity per Analyte	Limit of Detection (LoD) per Virus Strain
A/New Caledonia/20/ 1999 (H1N1)	PrimeMix™ Flu A	6.5 x10^6 $TCID_{50}$/ mL	10e3	$10^{3.0}$	3/3	24.2			$10^{-1.0}$ $TCID_{50}$/mL (35.5 C_T)	$10^{-1.0}$ $TCID_{50}$/mL
			10e4	$10^{2.0}$	3/3	28.3				
			10e5	$10^{1.0}$	3/3	31.9				
			10e6	10^{0}	3/3	33.9				
			10e7	10^{-1}	3/3	35.5	19/20	37.1		

Table 6 A and B: Limit of Detection of PrimeMix™ Influenza H1-09

Virus Strain Tested	Analyte Tested	Stock Virus Titer	Serial 10-Fold Dilution Factor	TCID 50/mL Dilution Tested	Call Rate	C_T (Triplicate) Avg	20-Hit Call Rate	C_T (20 Hit-Avg)	Lowest Concentration with Uniform Positivity per Analyte	Limit of Detection (LoD) per Virus Strain
A/Mexico/4108/2009 (H1N1)	PrimeMix™ H1 2009	5.3 x 10^4 $TCID_{50}$/mL	10e2	$10^{2.0}$	3/3	21.1			$10^{-1.0}$ $TCID_{50}$/mL (31.8 Avg C_T)	$10^{-1.0}$ $TCID_{50}$/mL
			10e3	$10^{1.0}$	3/3	24.4				
			10e4	10^{0}	3/3	29.9				
			10e5	10^{-1}	3/3	33.4	20/20	31.8		

Virus Strain Tested	Analyte Tested	Stock Virus Titer	Serial 10-Fold Dilution Factor	TCID 50/mL Dilution Tested	Call Rate	C_T (Triplicate) Avg	20-Hit Call Rate	C_T (20 Hit-Avg)	Lowest Concentration with Uniform Positivity per Analyte	Limit of Detection (LoD) per Virus Strain
A/California/04/ 2009 (H1N1)	PrimeMix™ H1 2009	4.3 x 10^5 $TCID_{50}$/mL	10e2	$10^{3.0}$	3/3	21.10			10^{0} $TCID_{50}$/mL (30.9 Avg C_T)	10^{0} $TCID_{50}$/mL
			10e3	$10^{2.0}$	3/3	24.43				
			10e4	$10^{1.0}$	3/3	28.10				
			10e5	10^{0}	3/3	29.34	20/20	30.9		

An additional study was conducted to determine the affect on limit of detection when a second extraction method (QIAamp viral RNA mini kit) was used. The analytical sensitivity of the assay was the same for samples extracted with RNAqueous (Ambion) or QIAamp viral RNA minikit (Qiagen). The data is summarized below in Table 7. The information presented is the average Ct value from the five replicates and the $TCID_{50}$/mL.

Table 7. Analytical Sensitivity Comparison of Extraction Methods

Standard Strain Tested	PrimeMix Influenza A		Prime Mix H1-09	
	QIAamp	RNAqueous	QIAamp	RNAqueous
A/New Caledonia/20/1999 (H1N1)	**35.3 / 10^{-1}**	**33.7 / 10^{-1}**	**NA**	**NA**
A/Texas/78209/2008 (H3N2)	**34.31 / 10^{-1}**	**31.81 / 10^{-1}**	**NA**	**NA**
A/Mexico/4108/2009 (H1-09)	**35.59 / 10^{-1}**	**33.39 / 10^{-1}**	**32.6 / 10^{0}**	**27.01 / 10^{1}**
A/California/04/2009 (H1-09)	**35.31 / 10^{-1}**	**33.7 / 10^{-1}**	**33.82 / 10^{0}**	**32.1 / 10^{0}**

Analytical Reactivity and Specificity. Six different strains of Influenza including two influenza A H3N2 strains, one influenza A H1N1 (seasonal), two influenza A 2009 H1N1 strains, and one influenza B strain were analyzed at a level much higher than the LOD (~3 logs greater) in triplicate with the **Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™**. The Flu A detector gave positive results for all five of the Flu A strains. The H1-09 detector gave negative results for four of the six strains and gave a positive result for the two H1N1 2009 stains tested. The results are summarized in Table 8A. Experiments were performed in triplicate with the results for all three measurements indicated by + for detection of viral RNA and – when viral RNA was not detected (+++ indicates detection in all three reactions).

Five of the six strains tested in table 8A (not including influenza B strain), were also analyzed at a concentration very close to the LOD of the assay. The FluA detector gave positive results for all five of the Flu A strains. The H1-09 detector gave negative results for three of the five strains and gave a positive result for the two H1N1 2009 stains tested. The results are summarized in Table 8B. Experiments were performed in triplicate with the results for all three measurements indicated by + for detection of viral RNA and – when viral RNA was not detected.

Table 8A. Evaluation of Analytical Reactivity with Target Concentrations Significantly above LOD

Standard Strain Tested*	Titer	# Tested	Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™		
			Flu A	H1-09	IPC
A/New Caledonia/20/1999 (H1N1)	10^3 $TCID_{50}$/mL	3	**+++**	**- - -**	**+++**
A/Wuhan/359/1995(H3N2)	10^3 $TCID_{50}$/mL	3	**+++**	**- - -**	**+++**
A/Texas/78209/2008 (H3N2)	10^3 $TCID_{50}$/mL	3	**+++**	**- - -**	**+++**
B/Texas/96a/2008 (B-Yam)	10^3 $TCID_{50}$/mL	3	**- - -**	**- - -**	**+++**
A/Mexico/4108/2009 (H1-09)	10^3 $TCID_{50}$/mL	3	**+++**	**+++**	**+++**
A/California/04/2009 (H1-09)	10^3 $TCID_{50}$/mL	3	**+++**	**+++**	**+++**

Table 8B. Evaluation of Analytical Reactivity with Target Concentrations near LOD

Standard Strain Tested*	Titer	# Tested	Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™		
			Flu A	H1-09	IPC
A/New Caledonia/20/1999 (H1N1)	10^0 $TCID_{50}$/mL	3	+++	- - -	+++
A/Wuhan/359/1995(H3N2)	10^0 $TCID_{50}$/mL	3	+++	- - -	+++
A/Texas/78209/2008 (H3N2)	10^0 $TCID_{50}$/mL	3	+++	- - -	+++
A/Mexico/4108/2009 (H1-09)	10^0 $TCID_{50}$/mL	3	+++	+++	+++
A/California/04/2009 (H1-09)	10^0 $TCID_{50}$/mL	3	+++	+++	+++

*****Viral strains courtesy of Virion Systems Inc. 9610 Medical Center Dr., Rockville, MD, 20850 and Battelle Memorial

Cross-Reactivity. Cross reactivity of the Longhorn Influenza PrimeMix™ Flu A and H1/09 Assays were evaluated using different human pathogens. Genomic nucleic acid from the various microorganisms was extracted using the Qiagen M48 system and tested with indicated Longhorn PrimeMix™ Assays. The results are summarized in Table 9A. The absence of IPC signal was observed due to the fact that the PrimeStore™ reagent was not tested in this study. In a smaller study, the cross reactivity of the **Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™** was evaluated using a subset of pathogens from table 9A**.** The results from this analysis demonstrated no cross reactivity with the tested pathogens. The CT values obtained for the reactions are tabulated. Together, tables 9A and 98B indicate that the Flu A and H1-09 assays do not cross react with nucleic acid extracted from the microorganisms listed.

Table 98A. Cross Reactivity of the Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™ with Other Upper Respiratory Pathogens

Specimen Tested	Titer	PrimeMix™ Influenza A/H1-09		
		Flu A	H1/09	IPC
Parainfluenza 1	10^5 $TCID_{50}$/mL	-	-	-
Parainfluenza 2	10^5 $TCID_{50}$/mL	-	-	-
Parainfluenza 3	10^5 $TCID_{50}$/mL	-	-	-
Parainfluenza 4	10^5 $TCID_{50}$/mL	-	-	-
CMV (HHV-5)	10^3 $TCID_{50}$/mL	-	-	-
Herpes-1 (HHV-1)	10^5 $TCID_{50}$/mL	-	-	-
Adenovirus Type 4	10^5 $TCID_{50}$/mL	-	-	-
Adenovirus Type 5	10^5 $TCID_{50}$/mL	-	-	-
Adenovirus Type 7	10^5 $TCID_{50}$/mL	-	-	-
Echovirus	10^4 $TCID_{50}$/mL	-	-	-
Respiratory syncytial virus	10^5 $TCID_{50}$/mL	-	-	-
Enterovirus	10^4 $TCID_{50}$/mL	-	-	-
S. pyogenes	10^6 CFU/mL	-	-	-

S. aureus	10^6 CFU/mL	-	-	-
B. pertussis	10^6 CFU/mL	-	-	-
S. pneumoniae	10^6 CFU/mL	-	-	-
N. gonorrhea	10^6 CFU/mL	-	-	-
H. influenzae (B)	10^6 CFU/mL	-	-	-
E. coli	10^6 CFU/mL	-	-	-
Total Human Genomic DNA	200 pg	-	-	-
Total Human Genomic RNA	200 pg	-	-	-
Human throat swab extraction	50 ng	-	-	-
Human nasal wash extraction	50 ng	-	-	-

Table 9B. Cross Reactivity of the Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™ with Other Upper Respiratory Pathogens

Specimen Tested	Titer	Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™		
		Flu A	H1/09	IPC
Respiratory syncytial virus	*10^4 TCID50/mL*	--	--	15.6291
Parainfluenza 3	*10^4 TCID50/mL*	--	--	16.518
Herpes-1 (HHV-1)	*10^6 TCID50/mL*	--	--	16.4966
Herpes-2 (HHV-2)	*10^5 TCID50/mL*	--	--	15.9971
Adenovirus Type 4	*10^5 TCID50/mL*	--	--	16.3457
Adenovirus Type 5	*10^5 TCID50/mL*	--	--	17.0341
Adenovirus Type 7	*10^5 TCID50/mL*	--	--	18.0586
E. coli	*10^6 CFU/mL*	--	--	16.0867
H. influenzae (B)	*10^6 CFU/mL*	--	--	16.4419
S.aureus	*10^6 CFU/mL*	--	--	16.1325
Total Human Genomic RNA	*200 pg*	--	--	15.9216
Total Human Genomic DNA	*200 pg*	--	--	16.0628
Human throat swab extraction	50 ng	--	--	16.2567
human nasal wash extraction	50 ng	--	--	16.3345

Clinical Studies:

A total of seventy nine (79) clinical samples were tested with the **Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™** using the CDC Swine Flu RRT-PCR Panel as the comparator method. Only upper respiratory (seventy eight (78)) nasal wash specimens and one (1) nasal swab were tested. Twenty one (21) Influenza H1-09 positive and fifty eight (58) H1-09 negative clinical samples were collected and obtained through Wilford Hall Medical Center (collected in 2009) and confirmed by the CDC Swine RRT-PCR Panel. Specimens were stored (1:3) in PrimeStore™ MTM and RNA extractions were performed using the RNAqueous Micro Kit (Ambion) according to the recommended instructions and tested with **Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™**

Of the total H1N1-09 positive samples evaluated (*N*=21), **Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™** correctly detected all of the samples according to comparison with results from the CDC Swine RRT-PCR Panel, with the exception of one swine H1-positive sample that tested negative using the **Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™**. Table 10 summarizes the performance comparison with the authorized CDC 2009 H1N1 assay.

Table 10: Summary of Clinical Performance Evaluation

		CDC assay			
		2009 H1N1 positive	2009 H1N1 negative	Total	
Longhorn Assay	2009 H1N1 positive	20	0	20	95% (20/21) positive agreement (95%CI 76.2% - 99.9%)
	2009 H1N1 negative	1*	58	59	100% (58/58) negative agreement (95%CI 93.7% - 100%)
	Total	21	58		

* After review of the single negative result, it was concluded that the clinical samples were archived original nasal wash specimens (i.e. not stored in PrimeStore™ MTM) and were later subjected to multiple freeze-thaws for aliquoting. The initial positive result obtained using the CDC Swine RRT-PCR panel was generated when the samples were fresh

An additional clinical study with a limited number of samples was conducted to determine the clinical performance of the **Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™** when the QIAamp viral RNA minikit was used for extraction of upper respiratory specimens. For this study a total of eleven (11) 2009 H1N1 positive upper respiratory specimens (combined NS/TS, NS, TS) and twenty one (21) 2009 H1N1 negative upper respiratory specimens (combined NS/TS, NS, TS) were analyzed in parallel using the **Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™** and RNAqueous (Ambion) and **Longhorn Influenza A/H1N1-09 Prime RRT-PCR Assay™** and QIAamp viral RNA minikt (Qiagen). Results between both methods were in agreement and are summarized below in Table 11.

Table 11: Summary of Extraction Method Comparison using Clinical Samples

		Longhorn Assay/ QIAamp Extraction			
		2009 H1N1 positive	2009 H1N1 negative	Total	
Longhorn Assay/ RNAqueous Extraction	2009 H1N1 positive	11	0	11	100% (21/21) positive agreement (95%CI 71.5% - 100%)
	2009 H1N1 negative	0	21	21	100% (58/58) negative agreement (95%CI 83.9% - 100%)
	Total	11	21		

LIMITATIONS

1. Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.

2. All results from this and other tests must be correlated with the clinical history, epidemiological data and other data available to the clinician evaluating the patient.

3. The prevalence of infection will affect the test's predictive value.

4. As with other tests, negative results do not rule out Influenza A or 2009 H1N1 influenza infections.

5. False negative results may occur when the infecting organism has genomic mutations, insertions, deletions, or rearrangements or when performed very early in the course of illness.

6. False negative results may occur if inadequate numbers of organisms are present in the specimen due to improper collection, transport or handling.

7. As with other tests, false positive results may occur. Repeat testing or testing with a different device may be indicated in some settings.

8. This test is a qualitative test and does not provide the quantitative value of detected organism present.

9. This test has not been evaluated for patients without symptoms of influenza infection.

10. This test has not been evaluated for monitoring treatment of Influenza A or 2009 H1N1 influenza infection.

11. This test has not been evaluated for screening of blood or blood product for the presence of Influenza A or 2009 H1N1 influenza.

12. This test cannot rule out diseases caused by other bacterial or viral pathogens.

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. Each laboratory should establish their own Quality Control ranges and frequency of QC testing based on these local laws and regulations. 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).

References

1. Clark ES, Daum LT, Alm EW. Development of Methods for Rapid Assessment of Influenza Virus in Avian Environmental Samples - ASM Mtg - May 2008 Abstract.
2. Daum LT, Chambers JP, Fischer GW. A Rapid, Simplified Collection-to-Detection System for Typing and Sub typing Influenza Viruses Using Real-Time RT-PCR, ASM Conferences, Emerging Technologies of Medical Importance for the Diagnosis of Infectious Diseases and Detection of Pathogenic Microbes, 1st International ASM Conference in Beijing - April 5-10, 2008 Abstract.
3. Daum LT, Yim K, Fischer GW. Real-Time RT-PCR Detection of Influenza A Virus in Asymptomatic Culture-Negative Cotton Rats, PAS Mtg - May 3-5, 2008 Abstract.
4. Daum LT, Chambers JP, Fischer GW. A Novel Specimen Collection Solution for Molecular Diagnostic Applications, PAS Mtg - May 3-5, 2008 Abstract.
5. Daum LT, Yim K, Fischer GW. Quantitation of Influenza A Virus From Nasal and Lung Tissue of Cotton Rats Using Real-Time RT-PCR and Culture, ESPID Mtg - May 13-17, 2008 Abstract.
6. Daum LT, Chambers JP, Fischer GW. Development and Clinical Evaluation of Rapid Real-Time RT-PCR Assays for Detection of Influenza A and B Viruses, ESPID Mtg - May 13-17, 2008 Abstract.
7. Daum LT, Sutter DE, Worthy SA, Dotiwala AK, Koenig JA, Salinas EA, Chambers JP, Elwood RL, Ottolini MG, and Fischer GW. Detection and Molecular Characterization of Clinical Influenza A and B Viruses from Original Nasal Wash Specimens Preserved in PrimeStore™, 3rd European Influenza Mtg, - Sep 13-17,

2008 Abstract.
8. Daum LT, Sutter DE, Ottolini MG, Elwood R and Fischer GW. Real-Time RT-PCR Detection of Influenza Virus Within Symptomatic and Asymptomatic Family Members, ICAAC-IDSA Mtg, Oct 25-28, 2008 Abstract.
9. Sutter DE, Daum LT, Myhre KE, Worthy SA and Fischer GW. Clinical and Molecular Evidence of Influenza Transmission Within Households, ESPID – June 9-13, 2009 Abstract.
10. Daum LT, Sutter DE, Worthy SA and Fischer GW. Genetic Analysis of Influenza A (H3N2) Viruses from Index Patients and Family Contacts, ESPID – June 9-13, 2009 Abstract.
11. Daum LT, Canas LC, Arulanandam BP, Niemeyer D, Valdes JJ and Chambers JP. Real-time RT-PCR assays for type and subtype detection of influenza A and B viruses, *Influenza and Other Respiratory Viruses*, 1, 167–175, 2007.
12. Final Report - PrimeStore Antimicrobial Activity AC28LK.510410.BSV, BioReliance
13. Final Report - Kill Rate of PrimeStore on Influenza H3N2 and Adenovirus Type 5, Virion Systems, Inc.
14. Final Report - PF004, Killing of MRSA 33592 by PrimeStore, Antibody and Immunoassay Consultants, LLC.
15. Daum LT, Canas LC, Smith CB, Klimov A, Huff W, Barnes W, Lohman KL. Genetic and antigenic analysis of the first A/New Caledonia/20/99-like H1N1 influenza isolates reported in the Americas. *Emerging Infectious Diseases*. 2002 Apr; 8(4):408-12.
16. Daum LT, Worthy S, Yim, K., Schuman R, Fischer GW. An Innovative Specimen Transport Solution for Molecular Diagnostics. Manuscript in preparation. 2010.
17. Daum LT, Worthy SA, Sutter DE and Fischer GW. Development of a Real Time Reverse-Transcription PCR (RRT-PCR) Assay for the Detection of Influenza A H1N1 2009 from Clinical Respiratory Specimens. Abstract submitted to ESPIDS for 2010 Meeting.

Contact Information

For customer support, contact Longhorn Vaccines & Diagnostics directly. Contact information may be found at www.lhnvd.com.

Longhorn Vaccines & Diagnostics
1747 Citadel Plaza, Ste. 206
San Antonio, TX 78209
Phone: 210-826-0910
E-mail: longhorn-vandd@sbcglobal.net

Notice to Purchaser

The procedure outlined in this protocol contains proprietary information. By purchasing this product, the user is granted a limited license by PanFlu, LLC to use this information as described. The user of this product agrees not to use any of these proprietary methods in any other application and agrees not to communicate (either orally or in writing) these proprietary methods to any other person or institution.