IntelligentMDx
IMDx 2009 Influenza A H1N1
Real-Time RT-PCR Assay
Package Insert

A real-time reverse transcriptase polymerase chain reaction for the in vitro qualitative detection and differentiation 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) from patients with signs and symptoms of respiratory infection. Pre-mixed primers and probes and master mix for the detection and differentiation of 2009 H1N1 Influenza using the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems, and the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument.

Emergency Use Authorization
For *in vitro* Diagnostic Use

Kit for 92 patient sample tests

Table of Contents

1. **INTENDED USE**

   The IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay is intended for use in CLIA High Complexity Laboratories with the ability to perform RNA extraction with the manual Qiagen QIAamp Viral RNA Mini Kit and reverse transcription and polymerase chain reaction (RT-PCR) on the Applied Biosystems 7500 and 7500 FAST Real-Time PCR Systems, and the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument. The assay is designed for the *in vitro* qualitative detection and differentiation 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)) from patients with signs and symptoms of respiratory infection.

   Testing with the IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay 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 made in conjunction 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 management decisions. It is recommended that negative results be confirmed by culture.

   The IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay is for use under the Food and Drug Administration's Emergency Use Authorization only.

2. **EXPLANATION OF THE TEST**

   Influenza viruses are enveloped, single stranded, negative-sense segmented genome RNA viruses of the family *Orthomyxoviridae*. Influenza viruses are divided into three distinct types: A, B, and C. The major groups are determined by the sequence and relatedness of the core proteins. Of the three distinct influenza types only A and B have been identified as concern in human pathogenicity. Influenza C rarely produces clinical disease and has not been associated with either human pathogenicity or epidemics.[1]

   Influenza A viruses are subtyped based upon antigenicity and genetics of their surface proteins, hemagglutinin and neuraminidase. Currently, sixteen serologically distinct hemagglutinins (H1 – H16) and nine neuraminidases (N1-N9) have been identified. Contemporary circulating seasonal influenza A viruses are classified as H1N1 or H3N2. In March and April 2009, a novel 2009 influenza A/H1N1 (2009 H1N1 influenza) resulting from antigenic shift was identified in humans in Mexico and the United States. This virus was originally referred to as "swine flu" because laboratory testing showed that many of the genes in this new virus were very similar to influenza viruses that normally occur in pigs (swine) in North America. Further study, however, has shown that this new virus is very different from what normally circulates in North American pigs.[2]

   Human influenza is found worldwide. Seasonal influenza exhibits seasonal periodicity, with increased incidence of infection and morbidity during October to March/April in the Northern hemisphere, and April to October in the Southern hemisphere. In equatorial, temperate climates, seasonal influenza exists at a somewhat steady incidence. In March and April 2009, 2009 H1N1

influenza emerged and resulted in significant illness and distribution during the summer months in the Northern hemisphere, opposite of normal seasonal influenza. Due to widespread transmission, the 2009 H1N1 influenza was co-distributed with seasonal influenza in the Southern hemisphere. Early indications suggest dominance of 2009 H1N1 influenza over normal seasonal influenza A[3].

The influenza virus has been shown to be transmitted by aerosols created during coughing and sneezing. Transmission also occurs due to contact with nasal discharge either directly or on fomites. Close contact and closed environments favor transmission. Though the virus is fairly labile, it has been shown to persist for several hours in dried mucus. Although not confirmed, it is believed that 2009 H1N1 influenza is transmitted by the same mechanisms as traditional influenzas.

The incubation period in humans is thought to be short, since most infections usually exhibit clinical symptoms between 1 to 4 days post-exposure. Infections in humans with influenza A are characterized by upper respiratory symptoms, which may include: fever, chills, anorexia, headache, myalgia, weakness, sneezing, rhinitis, sore throat, and/or a nonproductive cough. More severe complications can include pneumonia, which is usually associated with patients with chronic respiratory or heart disease, and secondary bacterial or viral infections. In approximately 50% of cases, nausea and vomiting can occur. The human influenza viruses are easily transmitted from human to human. Studies have shown that in adults the virus usually begins to be shed a day before symptoms appear and the person continues to be infectious for an additional 3 to 5 days after initial symptoms manifest.[4]

3. **PRINCIPLES OF THE TEST:**
The IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay is a qualitative *in vitro* diagnostic assay consisting of reagents for RT-PCR amplification, detection, and differentiation of nucleic acids from influenza A, 2009 H1N1 influenza, and process control in clinical samples. The assay is intended to be used with RNA that has been extracted from 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) samples obtained from patients exhibiting symptoms suggestive of Influenza infection. The assay is composed of two principal steps: (1) extraction of RNA from patient specimens, (2) one-step reverse transcription, amplification and detection utilizing fluorogenic DNA hybridization probes for the specific detection of amplified target sequences.

The assay consists of reagents to detect matrix RNA from influenza A virus, hemagglutinin RNA from 2009 H1N1 influenza virus, and the extraction/process control. An extraction/process control is added to each sample prior to extraction to monitor the extraction procedure, overall reagent integrity, and presence of PCR-inhibitory substances. Positive controls for each target are also provided and are to be included in each assay run. The assay run time from PCR setup to result is approximately two hours or less.

An overview of the procedure is as follows:

1) Collect appropriate sample from symptomatic patients using accepted sample collection methods. All respiratory swab specimens should then be placed into viral collection medium. Respiratory wash specimens should be combined with viral collection media

following manufacturer's instructions. Refer to The Center for Disease Control Interim Guidance on Specimen Collection, Processing, and Testing for Patients with Suspected Novel Influenza A H1N1 Infection; (http://www.cdc.gov/h1n1flu/specimencollection.htm).

2) Add the extraction/process control to each sample prior to nucleic acid isolation to monitor for the extraction efficiency, reagent integrity, and presence of inhibitors in the specimens.

3) Perform isolation and purification of nucleic acids using the Qiagen QIAamp Viral RNA Mini Kit, following the manufacturer's instructions.

4) Add the eluted and enriched nucleic acids to the Influenza A/2009 H1N1 Influenza Mix along with Master Mix. The Influenza A/2009 H1N1 Influenza mix contains oligonucleotide primers and target-specific oligonucleotide probes. The primers are complementary to highly conserved regions of genetic sequences for these viruses. The probes are dual-labeled with a reporter dye attached to the 5'-end and a quencher attached to the 3'-end.

5) Perform reverse transcription of RNA into complementary DNA (cDNA) and subsequent amplification of DNA using either the Applied Biosystems (ABI) 7500 Real-Time PCR System, the ABI 7500 FAST Real-Time PCR System, or the ABI 7500 Fast Dx Real-Time PCR Instrument. In this process, the probe anneals specifically to the template followed by primer extension and amplification. The IMDx 2009 Influenza A H1N1 Real-Time RT-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 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 and is monitored by the real-time instrument. The following table provides a more detail outlining of the assay modules, the targets and the associated fluorophores used for detection.

Table 1: Basic Detection Scheme

Analyte	Gene Target	Probe Fluorophore	Absorbance Peak	Emission Peak	Equivalent Dye
Influenza A	Matrix	FAM	495 nm	520 nm	FAM
Novel 2009 H1N1 INFLUENZA	Hemagglutinin	CAL Fluor Red 610 (CFR)	590 nm	610 nm	Texas Red
MS2 Extraction/Process Control	NA	CAL Fluor Orange 560 (CFO)	540 nm	561 nm	HEX

4. ASSAY MATERIALS PROVIDED:
4.1 IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay Components Provided:
1) Oligonucleotide Reagent Mix containing Primers and Probes

        a) INF A (primers and probe for matrix gene of influenza A virus)

        b) 2009 H1N1 (Influenza HA primers and probe for hemagglutinin gene of 2009 H1N1 virus)

        c) MS2 Extraction/Process Control primers and probe

2) iScript ™ One-Step RT-PCR Reagents

        a) 2x RT-PCR Reaction Mix

        b) iScript Reverse Transcriptase Enzyme

        c) Nuclease Free Water

3) Controls

        a) MS2 RNA Phage Extraction/Process Control

        b) Assay Positive Controls (*In vitro* transcribed synthetic viral RNA targets)

           i) Positive Control 1 - Influenza A matrix gene target region

           ii) Positive Control 2 - 2009 H1N1 Influenza hemagglutinin target region

Table 2.   IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay Contents

Box 1 – Store at -20°C

Component	Label	Cap Color	Number of Vials per Kit	Volume per Vial
Oligonucleotide Reagent Mix	Oligo Reagent Mix	Black	2	110µL
Master Mix	2X RT-PCR Reaction Mix	Blue	2	1.25mL
RT Enzyme	iScript Reverse Transcriptase	Yellow	1	200µL
Nuclease Free Water	Nuclease Free Water	Clear	1	1.5mL
Extraction/Process Control	Process Control	Yellow	2	275µL

Box 2 – Store at -80°C

Component	Label	Cap Color	Number of Vials per Kit	Volume per Vial
Positive Control 1	Positive Control-1	Green	2	25µL
Positive Control 2	Positive Control-2	Red	2	25µL

4.2 Additional Materials Required but not Provided:

Equipment

1) The Applied Biosystems 7500 or 7500 Fast Real-Time PCR System, or the ABI 7500 Fast Dx Real-Time PCR Instrument. All systems or instruments should be capable of detecting fluorescent dyes FAM, CAL Fluor Orange 560 and CAL Fluor Red 610. The systems or instrument must be within manufacturing specifications for the ABI 7500 or 7500 Fast Real-Time PCR System, or the ABI 7500 Fast Dx Real-Time PCR Instrument.

2) ABI 7500 Software v2.01 or ABI SDS Software v1.4 for use with the ABI 7500 or 7500 Fast Real-Time PCR System. Note: The ABI 7500 Fast Dx Real-Time PCR

Instrument included SDS Software v1.4. ABI 7500 Software v2.01 cannot be used with the ABI 7500 Fast Dx Real-Time PCR Instrument.
3) Vortex Mixer
4) Pipettes with an accuracy range between 1-10 µL, 10-100 µL, and 100-1000 µL
5) Microcentrifuge for 1.5 mL tubes
6) Centrifuge capable of spinning 96 well plates at 3,000 rpm
7) -80°C ± 15°C and -20°C ± 10°C Freezers; 2-8°C Refrigerator
8) Laminar flow hood for extractions
9) Disposable, powder-free gloves

Instrument Calibration Standards
1) ABI 7500 Fast Real Time PCR Systems Spectral Calibration Kit I (ABI Cat. #4360788) or ABI 7500 Real Time PCR Systems Spectral Calibration Kit I (ABI Cat. #4349180)
2) Cal Fluor Orange 560 Calibration Dye (Biosearch Cat. # RD-5081-5)
3) Cal Fluor Red 610 Calibration Dye (Biosearch Cat. # RD-5082-5)

Disposables/Reagents
1) Qiagen QIAamp Viral RNA Mini Kit (Qiagen Cat. #52904/50 tests, #52906/250 tests)
2) BD Universal Viral Transport Standard Kit containing 3 mL viral transport medium with 2 standard swabs (UTM; Becton, Dickinson, and Co. Cat. #220221)
3) MicroAmp® Fast Optical 96-well Reaction Plate (ABI Cat. #4346906) or MicroAmp® Optical 96-well Reaction Plate (ABI Cat. #4306737)
4) MicroAmp® Optical Adhesive Cover (ABI Cat. #4360954)
5) MicroAmp® Splash Free 96-well Base (ABI Cat. #4312063)
6) MicroAmp® Adhesive Film Applicator (ABI Cat. #4333183)
7) Microcentrifuge tubes, sterile, RNase/DNase-free, 1.5 ml
8) Pipette tips with aerosol resistant barriers, RNase/DNase-free

5. WARNINGS AND PRECAUTIONS:
1) Wear gloves when handling specimens or reagents.
2) Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and samples are handled.
3) Observe all "Universal Precautions" and use required laboratory protection while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
4) Do not pipette by mouth.
5) Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.
6) Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 0.5% sodium hypochlorite or other suitable disinfectant.
7) Amplification technologies such as PCR are sensitive to accidental introduction of product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the Real-Time reagents used in the amplification step become contaminated by accidental introduction of amplification product. Measures to reduce the risk of contamination in the laboratory include physically separating the

8) Avoid microbial and nuclease contamination of reagents when removing aliquots. Use sterile, disposable pipettes and aerosol barrier pipette tips.
9) Change aerosol barrier pipette tips between all manual liquid transfers.
10) When using repeat pipettors to add reagents, avoid touching the individual reaction tube with the pipette tip to minimize chance of carryover.
11) During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
12) Work area and instrument platforms must be considered potential sources of contamination. Change gloves after contact with potential contaminants (specimens, eluates, and/or amplified product) before handling unopened reagents, controls, calibrators, or specimens.
13) Bring all reagents except RT Enzyme to room temperature, then place on ice prior to use.
14) Add reagents to the bottom of a tube or well without touching the pipette tip to the rim or side of the tube or well.
15) Do not use assay components beyond their recommended storage dates.
16) Do not use reagents if they appear turbid or cloudy after bringing them to specified temperatures except as noted.
17) Run one plate at a time. Set up each plate separately.
18) Include both negative and positive controls on each plate.

6. STORAGE AND HANDLING CONDITIONS:

1) Store Oligonucleotide Reagent Mix reagents at -20°C ± 10°C.
2) Store all Positive Controls at -80°C ± 15°C.
3) Store MS2 Extraction/Process Control at -20°C ± 10°C.
4) Store PCR Master Mix and RT Enzyme at -20°C ± 10°C.
5) Thaw each component ONCE immediately prior to use.
6) Do not refreeze any reagents or targets.
7) Do not store any reagents in a frost-free freezer.
8) Do NOT use kits or reagents beyond their expiration dates.

PREVENTION OF NUCLEIC ACID CONTAMINATION
The possibility of nucleic acid contamination is minimized when:
1) PCR amplification, and oligonucleotide hybridization occur in a sealed 96-Well Optical Reaction Plate.
2) Detection is carried out automatically without the need to open the 96-Well Optical Reaction Plate.
3) Aerosol barrier pipette tips are used for all pipetting. The pipette tips are discarded after use.
4) Unidirectional work flow practices are followed, such that PCR master mix reagents are brought to the target preparation location for mixing, then the complete reaction plate to the instrument location.

SAMPLE COLLECTION, HANDLING AND STORAGE:

Collecting the Specimen

To obtain nasopharyngeal, nasal or throat swab samples:

1) Insert a flexible-shaft polyester, rayon, or nylon tipped swab containing a dry tip into one nostril or into the nasopharyngeal area or into the throat. Note: Do not use calcium alginate swabs as they may contain substances that inhibit PCR testing.

2) Press the swab gently against the nasal, nasopharyngeal or throat wall to allow the swab to absorb secretions.

3) Rotate the swab two to three times and withdraw it.

4) Place the swab into a tube containing 3 mL of viral transport medium (Becton Dickinson UTM).

5) Break off the shaft of the swab and cap the tube.

Transporting Specimens

Ensure all regulations for the transport of etiologic agents are met when transporting human specimens. 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 -80°C ± 15°C.

Refer to CDC:http://www.cdc.gov/H1N1 Influenzaflu/specimencollection.htm for suggested storage conditions.

Storing Purified Nucleic Acids

It is recommended to aliquot and store purified nucleic acids at -80°C ± 15°C.

Note: Inadequate or inappropriate specimen collection, storage, and transport can yield false negative results.

7. EQUIPMENT PREPARATION:

The following protocol is applicable to the ABI 7500 Real-Time PCR System, the ABI 7500 Fast Real-Time PCR System, and the ABI 7500 Fast Dx Real-Time PCR Instrument. The assay has undergone clinical performance evaluation on the ABI 7500 and 7500 Fast Real-Time PCR Systems in external site studies utilizing both version 1.4 and version 2.01 operating and analysis software. IntelligentMDx recommends instrument calibration for each dye before starting the assay protocol. Refer to manufacturer's documentation for detailed operating instructions for the ABI 7500 Real-Time PCR System, the ABI 7500 Fast Real-Time PCR System, and the ABI 7500 Fast Dx Real-Time PCR Instrument, including recommended procedures for performing dye calibration.

Note: If utilizing the ABI 7500 Fast Real-Time PCR System or the ABI 7500 Fast Dx Real-Time PCR Instrument, ensure that the instrument is configured to run in Standard mode, not Fast cycling mode. This applied to both v2.01 and v1.4 software. Refer to manufacturer's documentation for detailed operating instructions for the ABI 7500 Fast Real-Time PCR System or the ABI 7500 Fast Dx Real-Time PCR Instrument.

8. **REAGENT PREPARATION:**

   Batch Size

   The reagents included with the IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay Kit are sufficient for the testing of 92 patient samples. In each batch, at least one replicate of a Negative Control (NC) and one replicate of a No Template Control (NTC) must be included in addition to the two Positive Controls provided.

   Note: Prior to use, thaw each component (except mixes containing Enzymes) completely at room temperature, vortex briefly (2 to 3 sec) and microcentrifuge (10 sec) to ensure all liquid is brought to the bottom of the tube, then place on ice. Do not use heat to accelerate the thawing process. Remove Enzymes from the freezer just prior to use and keep on ice. Do not re-freeze reagents.

   Primer/Probe Mix

   Prepare the Primer/Probe Mix first in a separate room from the Final Reaction Mix to prevent contamination of primer probe aliquots. Each tube of Oligonucleotide Reagent Mix contains sufficient reagent to prepare Primer/Probe Mix for the testing of 46 patient samples, one Negative Control, one No Template Control, and the two Positive Controls. Once thawed, the Oligonucleotide Reagent Mix may be stored at 2-8°C for up to one month. Do not refreeze the Oligonucleotide Reagent Mix. Once prepared, the Primer/Probe Mix should be kept on ice, and can be used for multiple runs on the same day. Discard the unused portion of each aliquot at the end of the day. Keep fluorogenic TaqMan® probes in an opaque tube (or use aluminum foil to cover tube) to protect from light**.** Refer to the table below in the 'Assay Procedure' section for instructions on preparing the Primer/Probe Mix.

   Master Mix

   These assay reagents must be prepared using the 2X RT-PCR Reaction Mix (master mix) provided. The master mix must be supplemented with RT enzyme before use.

9. **IMDx 2009 H1N1 INFLUENZA EXTRACTION AND RUN CONTROLS:**

   MS2 RNA phage Extraction/Process Control: A buffered solution of MS2 RNA phage used to prepare the Negative Control. When used as instructed, the MS2 RNA phage Extraction/Process Control produces a Crossing Threshold (Ct) value typically greater than 25 cycles. The MS2 RNA phage Extraction/Process Control is added to every sample prior to extraction and isolation of nucleic acids. The MS2 RNA phage Extraction/Process Control serves the purpose of monitoring the extraction efficiency as well as the performance of the amplification/detection reagents.

   Negative Control: A negative control (NC), prepared using the MS2 RNA phage Extraction/Process Control described above must be included on every plate run and is necessary for meeting plate run validity and acceptance criteria. The Negative Control is prepared by spiking viral transport media with MS2 RNA phage Extraction/Process Control, and then subjecting the spiked viral transport media to the entire nucleic acid isolation procedure. Valid assay results for NC must be negative for the detection of all influenza analytes, and positive for MS2 RNA signal.

No Template Control (NTC): The NTC must be included on every plate run and is necessary for meeting plate validity and acceptance criteria. The NTC is prepared by pipetting nuclease-free water into the assay reaction mixture within a given well on the plate in place of sample. Valid assay result for NTC must be negative for the detection of all influenza and MS2 RNA targets.

Positive Control 1: The Positive Control 1 contains *in vitro* transcribed RNA produced from a synthetic plasmid containing the Influenza A matrix gene target region

Positive Control 2: The Positive Control 2 contains *in vitro* transcribed RNA produced from a synthetic plasmid the target region of 2009 H1N1 Influenza hemagglutinin.

10. SAMPLE PREPARATION:

The IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay does not include reagents for isolating nucleic acids from samples. The assay has been validated for use with the Qiagen QIAamp Viral RNA Mini Kit. The user is responsible for following all procedures recommended by the manufacturer of the sample preparation method.

The MS2 RNA Phage Extraction/Process control must be added to the sample prior to extraction. Add 5 μL of the MS2 RNA Phage Extraction/Process Control to each sample to monitor extraction procedure, reagent integrity, and presence of inhibitors in the specimens.

11. ASSAY PROCEDURE:

Note: The IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay has been validated on the ABI 7500 and 7500 Fast Real-Time PCR Systems operating in Standard Emulation mode. Although the ABI 7500 and ABI 7500 Fast Real-Time PCR Systems use different optical reaction plates, no changes to Assay Procedures are required when using either instrument platform. Note that the ABI 7500 Fast Real-Time PCR System and the ABI 7500 Fast Dx Real-Time PCR Instrument use the same optical reaction plate.

NOTE: The instrument should be calibrated for each dye following the manufacturer's protocol, as described in section 7.

1) Prepare the Primer/Probe Mix and Final Reaction Mix as follows (See Table 3). Keep all reagents on ice. In the context of this table, each single sample (one replicate) will provide for a final volume of 25 μL. If replicates for each sample are desired, the volume of reagents should be adjusted accordingly.

2) Make sure to include both positive controls (PC), a No Template Control (NTC) and a Negative Control (NC) when calculating the Primer/Probe Mix volumes and when assigning wells on the thermal cycler. When preparing the Primer/Probe Mix, it is recommended to factor in additional 1-2 sample volumes to cover any pipetting loss that may occur.

Table 3: Primer/ Probe Mix Preparation

Reagent	Each sample
Nuclease Free H$_2$O	5.0 μL
Oligonucleotide Reagent Mix	2.0 μL
2X RT-PCR Reaction Mix	12.5 μL
iScript Reverse Transcriptase Enzyme	0.5μL
Total Volume	20.0 μL

Final Reaction Mix

Reagent	Each sample
Primer/Probe Mix (above)	20.0 μL
Sample, PC, NTC or NC	5.0 μL
Total Volume	25.0 μL

3) Prepare both external **Positive** control final reaction mixes **first**, followed by the **Negative** control, the **No Template control**, and the clinical samples **last**, to ensure clinical samples and No Template and Negative controls are not contaminated with the positive control.

4) Once the final reaction mix has been vortexed and centrifuged, pipette 25 μL of this mixture into the appropriate wells of a 96-well optical reaction plate. Note that optical plates are different for the ABI 7500 Real-Time PCR System and 7500 Fast Real-Time PCR System and ABI 7500 Fast Dx Real-Time PCR Instrument. The **positive** control final reaction mix must be pipetted **first** onto the plate, followed by the **negative** control, the **no template control**, and the clinical samples **last**.

5) Cover the optical reaction plate with an optical adhesive cover and seal using a plate scraper following manufacturer's instructions. Verify sealed plate has no bubbles or open edges.

6) Spin optical reaction plate at 3,000 rpm for one minute to remove any bubbles and remove droplets from the well walls.

7) Program the real time PCR instrument using setting shown in Table 4. Follow manufacturer's instructions for specific instructions for software versions v1.4 or v2.01.

Table 4: Thermal Profile

Cycle Stage	Action	Temperature	Time	Cycles
Stage 1	Hold	50 °C	20 min	1
Stage 2	Hold	95 °C	5 min	1
Stage 3	Cycle	95 °C	15 sec	40
		58 °C	60 sec	

8) Place the loaded optical reaction plate into the PCR instrument and run the protocol using the appropriate detector channels described in Table 1.

12. SETTING THE THRESHOLD AND BASELINE FOR ABI 7500 SOFTWARE

ABI 7500 Software v2.0.1:

Note that each section number below corresponds to the numbered region of the images.

1) In the Analysis Window (Figure 1), set "Plot Type" to "ΔRn vs Cycle" and "Graph Type" to "Log" (#1).
2) Click on the "Analysis Settings'" box (#2; top right). This will open the "Analysis Settings" window (Figure 2). Highlight all of the Targets under "Select a Target" (#3) by dragging the cursor across all target boxes.
3) In the window "Ct Settings for the 3 Selected Targets" (#4), un-check all of the boxes after all targets have been highlighted. Set all baselines from 3 to 15 by clicking on the up/down arrow for "Start Cycle" and "End Cycle" to adjust values.
4) Click "Apply Analysis Settings" (#5; bottom middle button). This will close the "Analysis Settings" window and return the "Analysis" window to active status.
5) In the Analysis Window (Figure 1) identify the "Options" tab (#6), located below the Amplification Plot. Select each "Target" individually then highlight all control wells – i.e. Positive Controls (PC's) and Negative Controls (NC's) on the plate by clicking on each well. Select multiple wells by holding down the "Ctrl" key while clicking on each well.
6) In "Options" (#6), set the "Threshold" for each target above all background noise, including that observed within the earliest cycles (cycles 1~4). This is accomplished by dragging the Threshold line (arrow; in Amplification Plot window) up or down, to the appropriate setting.
7) Click the green "Analyze" button (#2) to apply the changes and analyze the run data.
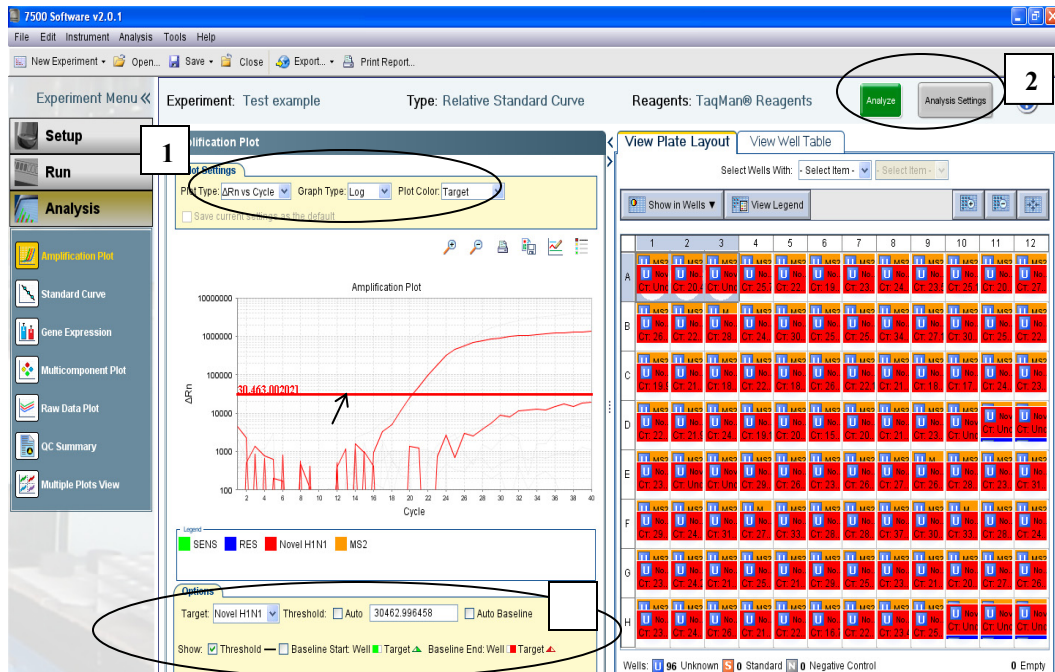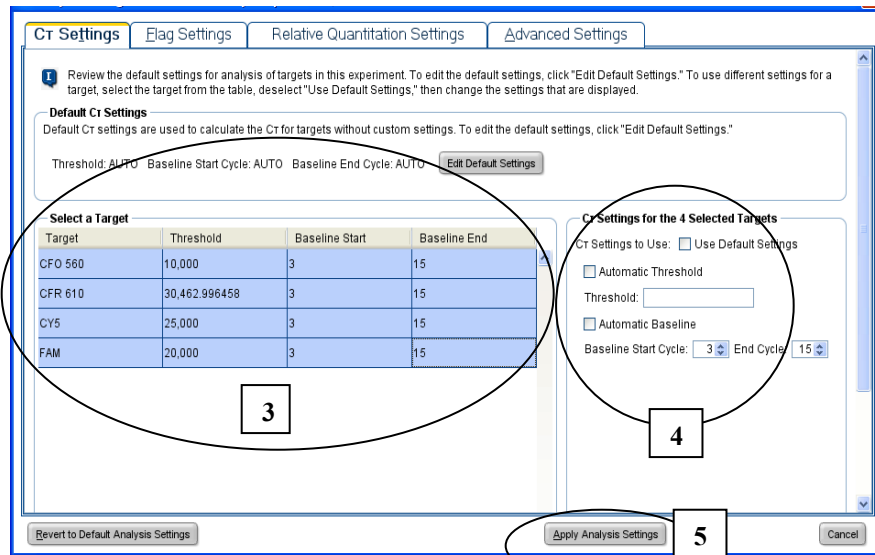
Figure 2. ABI 7500 Software v2.01 Analysis Settings Window

ABI 7500 SDS Software v1.4:

Note that each section number below corresponds to the numbered region of the images unless otherwise indicated.

1) The "Threshold" settings are adjusted based on the fluorescence values observed for the amplification curve to be analyzed. A representative amplification curve is shown (Fig. 3).

2) In the Amplification Plot Tab (#1, Fig. 4) set "Data" field to "Delta Rn vs. Cycle" and "Detector" to dye of interest (#2, Fig. 4). Verify under "Analysis Settings" that "Manual Ct" and "Manual Baseline" are selected and baseline is set from 3 'Start (cycle)" to 15 "End (cycle)", unless a Ct value of less than 15 is observed (#2).

Note: The Baseline must be set ensuring that the earliest amplification curve growth begins at a cycle after the "End (cycle)" value.

3) Select the positive control with the target corresponding to the detector selected and a negative control simultaneously on the plate and set "Line Color" to "Detector Color" in the drop down menu (See #2, Fig 4).
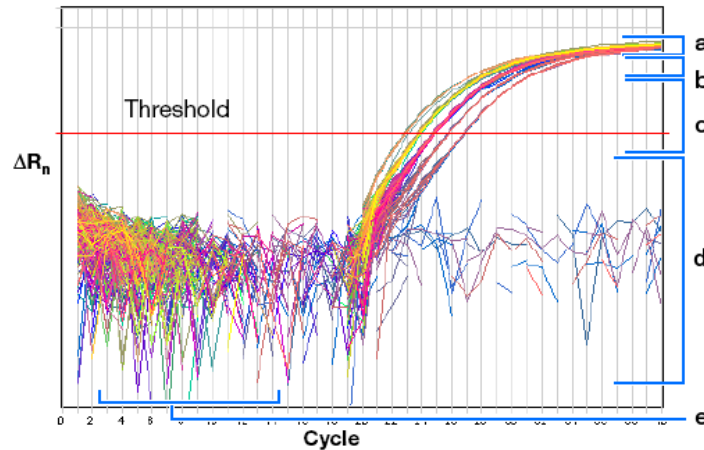
A typical amplification curve has a:

- Plateau phase (a)
- Linear phase (b)
- Exponential (geometric phase) (c)
- Background (d)
- Baseline (e)

Figure 3: Acceptable Position of Threshold Setting on Amplification Curve

4) Set the "Threshold" by clicking and dragging the red line or by entering an appropriate value in the "Threshold" box, following these criteria (Fig. 3):

- Above the background (d)
- Below the plateau and linear regions of the amplification curve (a, b)
- Within the exponential phase of the amplification curve (c)

Note: It is common to find that the precision amongst the replicates increases as the amplification progresses further into the exponential phase of the reaction. Therefore, to assure maximal precision, the threshold value must be set within the exponential range above any background noise within the assay.

5) Click the "Analyze" button (see # 4, Fig. 4) to apply the changes and analyze the run data.

6) Repeat steps 1 – 5 for each detector.

Figure 4. ABI 7500 Software Version 1.4 Amplification Tab Window.

13. **INTERPRETATION OF RESULTS:**

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted or reported and testing of patient samples should be repeated.

A positive signal is defined as a Ct value of less than or equal to 38 cycles (Ct ≤ 38 cycles).

A negative signal is defined as a Ct value of greater than 38 cycles (Ct > 38 cycles).

1) **Validity Criteria**
   a) **Valid Assay Run:** An assay run is determined to be <u>valid</u> when all of the following criteria are met:
      i) Positive Control returns a positive signal for the target sequence present in the Control.
         (1) Positive Control-1 (INF A: FAM channel)
         (2) Positive Control-2 (2009 H1N1: CFR channel)
      ii) Negative Control is negative for all targets except MS2 RNA signal (MS2: CFO channel).
      iii) No Template Control is negative for all targets, including MS2 RNA.

b) **Invalid Assay Run:** An assay run is determined to be <u>invalid</u> when any of the following criteria are met:

    i) Positive Control returns a negative signal for the target sequence present in the control.
        (1) Positive Control-1 negative in FAM channel
        (2) Positive Control-2 negative in CFR channel
    ii) Positive Control returns a positive signal in the incorrect channel.
        (1) Positive Control-1 signal in CFR and/or CFO channel
        (2) Positive Control-2 signal in FAM and/or CFO channel
    iii) Negative control returns a positive signal in the incorrect channel.
        (1) Positive signal in FAM and/or CFR channel
    iv) Negative control is negative for all target signals, including MS2 RNA signal
    v) No Template Control (NTC) is positive for any target signal (FAM, CFO or CFR channel).

2) **Acceptance Criteria**
    a) **Required acceptance criteria for each sample are as follows:**
        i) A sample that does not return a positive signal for any influenza RNA target (INF A or 2009 H1N1) must return a positive signal for MS2 RNA (CFO channel).
        ii) If the strongest positive signal (lowest Ct value) observed for any of the influenza RNA targets (INF A or 2009 H1N1) is greater than 32 cycles then a positive signal for MS2 RNA must also be observed (MS2: CFO channel).
        iii) If the positive signal for any influenza RNA target (INF A or 2009 H1N1) is $\leq$ 32 cycles, the MS2 RNA signal may be negative. This is due to the design of the MS2 extraction/process control so as to not affect the sensitivity of the assay for a specific target (Figure 5).
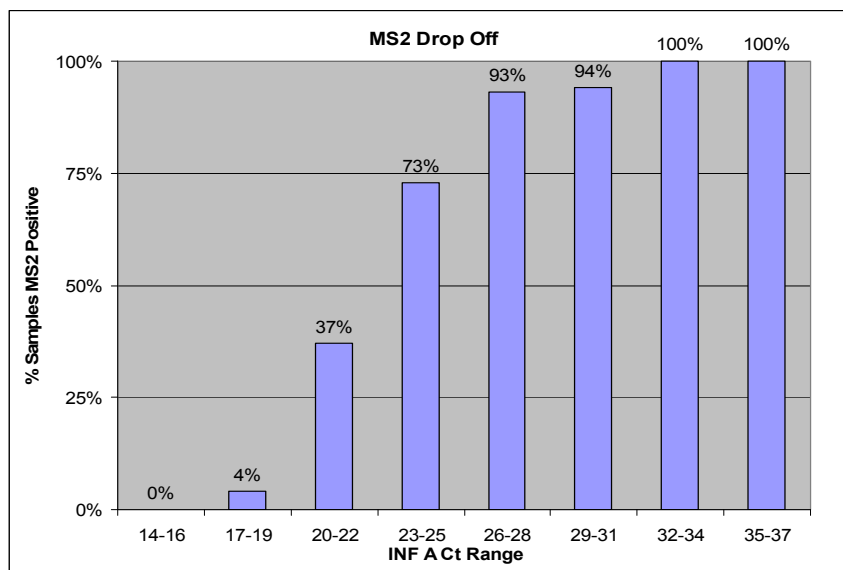
Figure 5: Analysis of Extraction/Process Control Drop Off

3) Positive and Negative Controls
   a) Use of Controls
      i) All Positive Controls and the MS2 RNA phage Extraction/Process Control should be used in accordance with accepted laboratory procedures. All controls should be tested prior to running patient samples and with each new kit lot to ensure proper functioning of all reagents and kit components.
      ii) MS2 RNA phage Extraction/Process Control must be added to each sample prior to nucleic acid isolation.
      iii) The Negative Control, which is prepared using the MS2 RNA phage Extraction/Process Control, must be subjected to all assay procedures including nucleic acid isolation and RT-PCR for each run.
      iv) Positive Controls-1 and Positive Control-2 must not be subjected to nucleic acid isolation procedures. Positive Controls are only to be added directly to an assay well containing the assay reaction mixture.
      v) Positive Controls-1 and Positive Control-2 are isolated *in vitro* transcribed RNAs. They must not be subjected to more than two freeze-thaw cycles, and must be stored at -80°C (±10°C).
      vi) A Negative Control, No Template Control reaction and both Positive Control reaction must be run on each plate.
      vii) Table 5 describes the function of each assay control.

Table 5: Use of Controls

Control Type	Used to Monitor
Positive	Substantial reagent failure, including primer and probe integrity
Extraction/Process	Sample extraction efficiency; PCR inhibition; Process error
Negative	Reagent and/or environmental contamination
No Template Control	Contamination of assay reagents or optical plate

   b) Control Failure
      i) Failure of a Negative Control (signal for any Influenza target ≤ 38 cycles) invalidates the run. Results should not be reported as repeat testing must be performed for all samples on the invalid plate. Based upon the result obtained, either:
        (1) Contamination of common assay reagents with Influenza target may have occurred
        (2) IMDx 2009 Influenza A H1N1 Real-Time RT-PCR reagents may have degraded
        (3) Failure to follow correct assay procedure
      ii) Failure of the MS2 extraction/process control signal in the Negative Control invalidates the run. Results should not be reported as repeat testing must be performed for all samples on the invalid plate. Based upon the result obtained, either:
        (1) MS2 extraction/process control is degraded
        (2) Nucleic acid isolation procedure has failed to provide high quality nucleic acids for analysis
        (3) Nucleic acid isolation reagents may have degraded
        (4) Inhibitory or contaminating substance has co-isolated with nucleic acids
        (5) IMDx 2009 Influenza A H1N1 Real-Time RT-PCR reagents may have degraded

(6) Failure to follow correct assay procedure

iii) Failure of Positive Control (negative signal (Ct>38 cycles) for either Positive Control targets) invalidates the run. Results cannot not be reported. Repeat testing must be performed for all samples on the invalid plate. Based upon the results, either:

(1) Degradation of the Positive Control has occurred

(2) IMDx 2009 Influenza A H1N1 Real-Time RT-PCR reagents may have degraded

(3) Failure to follow correct assay procedure

iv) Failure of Positive Control (signal is positive (Ct≤38 cycles in an incorrect channel) invalidates the run. Results should not be reported as repeat testing must be performed for all samples on the invalid plate. Based upon the results, either:

(1) Positive Control reaction well may have been contaminated

(2) Failure to follow correct assay procedure

v) Failure of the No Template Control (NTC; signal is positive (Ct≤38 cycles) in any channel including MS2 RNA signal) invalidates the run. Results should not be reported as repeat testing of controls must be performed. Base on results, either:

(1) Assay reagents may have become contaminated

(2) Contamination of the optical reaction plate may have occurred

4) **Examination of Patient Specimen Results**

All assay controls must be examined and must pass validity criteria prior to examination of individual patient results. If the assay controls are not valid, the run is invalid and must be repeated. Each individual patient result must pass acceptance criteria prior to being interpreted. If an individual patient sample does not pass acceptance criteria, that sample is invalid and must be repeated. Amplification plots should be examined for every positive result (those with a Ct value ≤ 38). If the amplification plot shows an exponential increase, the amplification curve is valid. Results with a Ct>38 cycles are considered negative. If a sample is re-run and remains invalid with non-amplification of the MS2 RNA control, this suggests the presence of PCR inhibitors in the patient sample. The results should be reported as 'Indeterminate due to Inhibition'. An additional sample should be submitted for testing if clinically warranted.

5) **Explanation and Resolution of Invalid Results**

a) To resolve instances where a sample result is invalid, in order:

i) Rerun the isolated nucleic acid sample

ii) Re-isolate, then rerun nucleic acid from the same sample

iii) Obtain a new sample

Table 6: Results Interpretation

Result Number	Result			Interpretation
	INF A	2009 H1N1	Extraction/ Process Control	
1	+	+	+	**Influenza A RNA: detected; 2009 H1N1 RNA: detected.**
2	+	ND[1]	+	**Influenza A RNA: detected; 2009 H1N1 RNA: not detected.**
3	+	+	ND	**Influenza A RNA: detected; 2009 H1N1 RNA: detected.**
4	ND	ND	+	**Influenza A RNA: not detected; 2009 H1N1 RNA: not detected.**
5	ND	+	+	Rerun sample. If both results are in agreement, Report as **Overall Result Indeterminate: Influenza A RNA: not detected; 2009 H1N1 RNA: detected** A repeat specimen should be obtained for testing. If results are not in agreement, test the sample a third time, and report the consensus result.
6	ND	+	ND	Rerun sample. If both results are in agreement, Report as **Overall Result Indeterminate: Influenza A RNA: not detected; 2009 H1N1 RNA: detected.** A repeat specimen should be obtained for testing. If results are not in agreement, test the sample a third time, and report the consensus result.
7	+	ND	ND	If INF A Ct < 32 cycles, report sample as **Influenza A RNA: detected; 2009 H1N1 RNA: not detected**. If INF A Ct $\geq$ 32 rerun the sample.[2] If both results are in agreement report the sample as **Influenza A RNA: detected; 2009 H1N1: not detected.** If results are not in agreement, test the sample a third time, and report the consensus result.
8	ND	ND	ND	Rerun sample; if both results are in agreement; Report sample result as **Indeterminate due to Inhibition**
[1]ND = Not Detected				
[3]2009 H1N1 is an Influenza A-subtype. In validation testing performed, no examples were observed where a 2009 H1N1 specimen was negative for INF A signal and positive for 2009 H1N1 signal.				

14. QUALITY CONTROL:

1) Quality control requirements should be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard quality control procedures. For reference, please use CLSI document C24-A2, *Statistical Quality Control for Quantitative Measurements: Principles and Definitions*:[Approved Guideline – Second Edition] or other published guidelines.

2) Quality control procedures are intended to monitor reagent and assay performance.

3) All positive controls and the MS2 RNA phage Extraction/Process control should be tested prior to running samples using a new kit lot to ensure proper functioning of all reagents and kit components.

4) It is recommended to include an MS2 RNA phage Extraction/Process control in each nucleic acid isolation run. The MS2 RNA phage Extraction/Process control should be treated as a sample.

5) Never run the positive controls through nucleic acid isolation.

6) Always include one Negative Control, one No Template control, and both Positive Control-1 and Positive Control-2 in each run performed.

7) Failure of the controls (Positive, Negative, or No Template) invalidates the run. Results should not be reported as repeat testing should be done starting from purified nucleic acid, using a new aliquot of 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.

15. LIMITATIONS:

1) Training and familiarity with testing procedures and interpretation of results is necessary prior to performing the assay.

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

3) Once RT-PCR reaction Master Mix including primer and probe preparations has been prepared, the reaction must be run within 1 hour.

4) This test does not differentiate Influenza A viruses other than 2009 Influenza A/H1N1 Influenza from Influenza A.

5) A trained health profession should interpret test results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.

6) Analyte targets (viral nucleic acids) may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) is infectious, or is the causative agent for clinical symptoms.

7) 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.

8) There is a risk of false negative results due to the presence of sequence variants in viral targets of the assay, procedural errors, amplification inhibitors in specimens, or inadequate numbers of organisms present in a clinical sample for amplification.

9) A sample yielding negative results can contain pathogens other than influenza virus.

10) There is a risk of false positive results resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.

11) Interference by substances other than those described below can lead to erroneous results.

12) Cross-reactivity with respiratory tract organisms others that those listed below can lead to erroneous results.

13) This assay has not been evaluated for patients receiving intranasal administered influenza vaccine.

14) This assay has not been evaluated for immunocompromised individuals.

16. PERFORMANCE CHARACTERISTICS:

Limit of Detection (LoD)

Limit of Detection studies were performed using six H1N1 influenza virus strains: Two influenza A/H1N1 (A/Taiwan/42/06 and Brisbane/59/07), one influenza A/H3N2 (A/Brisbane/10/07) and three 2009 H1N1 strains (cultured clinical isolates) designated HFD-2, HFD-6 and VAN-1. Dilutions of characterized influenza viruses were tested in replicates of 6. The lowest concentration at which all of the replicates were positive was treated as the tentative LoD for each primer and probe set. The LoD of each primer and probe set was then confirmed by testing a minimum of 60 replicates with concentrations at the tentative LoD. The final LoD of each primer and probe set was determined to be the lowest concentration resulting in positive detection of minimally 95% of replicates tested with 95% confidence (Table 7).

In addition, the data sets were subjected to probit analysis for determination of LoD. Results were considered to be "positive" if the Ct was less than or equal to 38 cycles.

Using pooled data from all sites, the proportion "p" of the replicates that were positive was calculated for each sample and target. The LoD was estimated for each target in each study by fitting the probit model:
$$\text{probit(p)} = \alpha + \beta\log_{10}(\text{Concentration})$$

The $\log_{10}$ (LoD) was estimated as:

$$\text{Log}_{10}(\text{LoD}) = (\text{probit}(0.95) - \alpha)/\beta = (1.645 - \alpha)/\beta$$

The 95% confidence interval for the log LoD was estimated as log LoD ± 1.96 times the asymptotic standard error. The LoD with 95% confidence interval were then obtained by taking the antilog.

For influenza A/H1N1, strain Taiwan/42/06, LoD measured using the INF A target is 5.42 $\text{TCID}_{50}/\text{mL}$. For influenza A/H1N1, strain Brisbane/59/07, LoD measured using the INF A target is 0.95 $\text{TCID}_{50}/\text{mL}$. For influenza A/H3N2, strain Brisbane/10/07, LoD measured using the INF A target is 0.66 $\text{TCID}_{50}/\text{mL}$.

For 2009 H1N1 influenza, which is detected by both INF A and 2009 H1N1 assay components, LoD is considered to be highest $\text{TCID}_{50}/\text{mL}$ value returned, irrespective of target. For 2009 H1N1 strains tested here, all limits of detection are based on 2009 H1N1 target, not INF A target. For isolate HFD-2, LoD is 4.48 $\text{TCID}_{50}/\text{mL}$. For isolate HFD-2, LoD is 16.78 $\text{TCID}_{50}/\text{mL}$. For isolate VAN-1, LoD is 88.99 $\text{TCID}_{50}/\text{mL}$.

Table 7: Limit of Detection

Virus Strain	Target	Virus Titer (TCID$_{50}$/mL)		Call Rate	% Pos	Ct Ave± SD	Probit LoD (TCID$_{50}$/mL)	95% CI (TCID$_{50}$/mL)
		Stock	Test					
Influenza A Taiwan/42/06 (H1N1)*	INF A	5.0x10^4	16.0	36/36	100.0%	31.60 ± 1.77	5.42	2.45 - 12
			5.0	64/66	97.0%	33.40 ± 1.65		
			2.0	55/66	83.3%	35.13 ± 1.92		
Influenza A Brisbane/59/07 (H1N1)*	INF A	5.0x10^4	16.0	36/36	100.0%	30.94 ± 1.73	0.95	0.85 - 1.06
			5.0	36/36	100.0%	32.57 ± 1.37		
			2.0	66/66	100.0%	34.10 ± 1.46		
			0.6	55/66	83.3%	35.34 ± 1.28		
Influenza A Brisbane/10/07 (H3N2)*	INF A	1.6x10^5	6.3	60/60	100.0%	31.7 ± 0.69	0.66	0.59 – 0.72
			2.0	60/60	100.0%	33.4 ± 0.65		
			0.6	55/60	92.0%	35.4 ± 1.03		
			0.2	24/60	40.0%	37.3 ± 1.04		
2009 H1N1 isolate (HFD-2)	INF A	2.0x10^4	6.3	66/66	100.0%	31.22 ± 0.35	0.61	0.56 - 0.66
			2.0	59/59	100.0%	32.93 ± 1.21		
			0.8	58/60	96.7%	34.53 ± 0.84		
			0.3	44/60	73.3%	34.76 ± 2.22		
	2009 H1N1	2.0x10^4	6.3	65/66	98.5%	34.42 ± 1.05	4.48	1.5 - 13.38
			2.0	54/59	91.5%	34.58 ± 1.73		
			0.8	34/60	56.7%	36.20 ± 1.28		
			0.3	29/60	48.3%	36.47 ± 1.09		

Virus Strain	Target	Virus Titer (TCID$_{50}$/mL)		Call Rate	% Pos	Ct Ave± SD	Probit LoD (TCID$_{50}$ /mL)	95% CI (TCID$_{50}$/mL)
		Stock	Test					
2009 H1N1 isolate (HFD-6)	INF A	1.0x10^4	3.2	35/35	100.0%	31.50 ± 0.90	0.33	0.24 - 0.45
			1	81/81	100.0%	34.25 ± 2.03		
			0.4	64/66	97.0%	34.09± 1.08		
			0.1	43/66	65.2%	36.05 ±0.93		
	2009 H1N1	1.0x10^4	31.6	60/60	100.0%	29.53 ± 0.38	16.78	10.14 - 27.76
			10	80/90	88.9%	32.72 ± 1.82		
			3.2	25/36	69.4%	34.19 ± 1.66		
2009 H1N1 isolate (VAN-1)	INF A	5.0x10^5	158.5	64/64	100.0%	30.52 ± 0.86	13.36	7.81 - 22.86
			50.1	60/60	100.0%	33.23 ± 1.24		
			20.0	65/70	92.9%	34.50 ± 1.56		
			6.3	32/35	91.4%	35.20 ± 1.02		
	2009 H1N1	5.0x10^5	158.5	62/64	96.9%	31.82 ± 0.96	88.99	46.24 - 171.24
			50.1	56/60	93.3%	35.08 ± 1.81		
			20.0	29/70	41.4%	35.22 ± 2.40		
			6.3	8/35	22.9%	36.68 ± 1.74		

* The following strains did not generate a 2009 H1N1-positive signal: A/Taiwan/42/06, Brisbane/59/07 and Brisbane/10/07.

Analytical Precision Studies

Analytical precision studies were performed for each of the assay components of the IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay. Studies performed included intra-assay precision, inter-assay (day to day) precision, lot to lot, and instrument to instrument. Studies were performed using one influenza A H1N1 (A/Taiwan/42/06) and two 2009 H1N1 influenza strains (cultured clinical isolates; designated HFD-2 and VAN-1). Test samples were prepared for each strain at three concentrations by diluting viral stocks in universal transport medium and freezing single use aliquots. Nucleic acids were extracted using the Qiagen QIAamp® Viral RNA Mini Kit and tested in triplicate with the IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay. The viral titers ($\log_{10}$ $TCID_{50}$/mL) for dilutions of each strain are as follows, listed in descending order from High to Low titer: Influenza A H1N1; 3.7, 2.7, 1.7. 2009 H1N1 HFD-2; 3.6, 2.9, 2.2. 2009 H1N1 VAN-1; 5.0, 4.6, 3.6. Crossing threshold (Ct) average, standard deviation and %CV (sd/ave*100) was calculated for each data set as shown.

Intra-Assay Precision

Three nucleic acid extractions were performed for each virus type dilution and tested in triplicate. Analyses of assay precision data for detection of INF A target show a high of 4.0% CV for the highest titer influenza A specimen tested. All other %CV values ranged from 3.3% down to 0.6% (Table 8). For detection of 2009 H1N1 target, %CV values ranged from 2.6% to 4.2% (Table 9).

Table 8. Intra-Assay Precision; Detection of INF A Target

	Replicate	Influenza A/Taiwan/42/06			2009 H1N1 (HFD-2)			2009 H1N1 (VAN-1)		
		High	Medium	Low	High	Medium	Low	High	Medium	Low
Extract#1	1	22.74	27.9	31.31	23.11	23.6	28.03	22.21	24.29	26.71
	2	24.79	27.98	31.47	23.05	25.37	28.32	22.28	24.55	26.58
	3	24.97	27.93	31.06	21.27	25.27	26.82	22.65	24.26	26.67
Extract#2	1	24.38	27.8	31.48	23.22	26.04	27.8	22.02	24.54	26.78
	2	23.49	28.06	31.78	22.11	25.38	27.78	20.77	24.59	26.96
	3	23.50	26.54	31.29	23.16	24.31	27.66	20.81	24.55	26.74
Extract#3	1	25.25	27.91	31.62	23.35	25.49	27.58	20.96	24.39	26.93
	2	25.45	27.93	30.17	21.85	24.37	27.66	21.94	24.43	27.00
	3	25.34	27.83	31.86	22.15	25.34	27.76	22.04	23.35	27.01
	Ave	24.43	27.76	31.34	22.59	25.02	27.71	21.74	24.33	26.82
	SD	0.97	0.47	0.50	0.75	0.76	0.40	0.70	0.39	0.16
	% CV	4.0%	1.7%	1.6%	3.3%	3.0%	1.5%	3.2%	1.6%	0.6%

Table 9. Intra-Assay Precision; Detection of 2009 H1N1 Target

	Replicate	2009 H1N1 (HFD-2)			2009 H1N1 (VAN-1)		
		High	Medium	Low	High	Medium	Low
Extract#1	1	26.92	27.24	31.43	23.42	26.3	29.12
	2	26.93	29.94	32.24	23.16	26.49	29.02
	3	24.75	27.65	32.12	24.25	26.54	28.84
Extract#2	1	27.22	30.14	31.35	22.77	25.43	27.79
	2	25.39	29.89	31.54	24.32	25.35	29.21
	3	25.51	29.80	32.34	22.97	26.75	27.46

Table 9. Intra-Assay Precision; Detection of 2009 H1N1 Target

	Replicate	2009 H1N1 (HFD-2)			2009 H1N1 (VAN-1)		
		High	**Medium**	**Low**	**High**	**Medium**	**Low**
Extract#3	**1**	26.86	30.09	29.84	22.91	25.15	27.75
	2	25.21	29.21	29.94	22.73	25.09	27.51
	3	25.31	27.58	30.64	24.53	25.29	27.75
	Ave	**26.01**	**29.06**	**31.27**	**23.45**	**25.82**	**28.27**
	SD	**0.95**	**1.21**	**0.94**	**0.72**	**0.68**	**0.75**
	% CV	**3.7%**	**4.2%**	**3.0%**	**3.1%**	**2.6%**	**2.7%**

Inter-Assay Precision (Day-to-Day Precision)

Testing was performed over the course of 6 days within a 30-day period, with each sample tested in triplicate twice within a given day. For influenza A H1N1, %CV ranged from 0.6% to 1.3% for INF A target (Table 10). For 2009 H1N1 HFD-2, %CV for INF A target ranged from 0.5% to 1.1%, and for 2009 H1N1 target, 0.8% to 1.0% (Table 11). For 2009 H1N1 VAN-1, %CV for INF A target ranged from 0.9% to 1.8%, and for 2009 H1N1 target, 0.6% to 1.5% (Table 12).

Table 10: Inter-Assay (Day to Day) Precision for Influenza A/Taiwan/42/06

Target	INFA A								
Viral Titer	**High**			**Medium**			**Low**		
Run # (n=3)	**Ct Mean**	**Ct SD**	**%CV**	**Ct Mean**	**Ct SD**	**%CV**	**Ct Mean**	**Ct SD**	**%CV**
1	21.71	0.49	2.3%	25.81	0.16	0.6%	29.64	0.21	0.7%
2	22.07	0.1	0.5%	25.81	0.12	0.5%	29.65	0.16	0.5%
3	22.97	0.06	0.3%	25.9	0.08	0.3%	26.26	0.13	0.5%
4	21.21	0.74	3.5%	26.62	0.08	0.3%	29.53	0.12	0.4%
5	21.38	0.21	1.0%	26.14	0.61	2.3%	28.61	0.63	2.2%
6	22.57	0.08	0.4%	25.04	0.12	0.5%	30.09	0.21	0.7%
7	22.15	0.17	0.8%	30.5	0.12	0.4%	29.45	0.78	2.6%
8	22.71	0.98	4.3%	26.74	0.26	1.0%	30.42	0.27	0.9%
9	21.41	0.64	3.0%	25.58	0.08	0.3%	28.98	0.2	0.7%
10	22.04	0.31	1.4%	25.79	0.12	0.5%	29.49	0.46	1.6%
11	22.03	0.2	0.9%	25.66	0.21	0.8%	29.48	0.26	0.9%
12	22.27	0.21	0.9%	26.04	0.19	0.7%	29.68	0.09	0.3%
Average	**22.04**	**0.30**	**1.3%**	**26.30**	**0.15**	**0.6%**	**29.27**	**0.22**	**0.7%**

Table 11: Inter-Assay (Day to Day) Precision for 2009 H1N1 HFD-2

Target	2009 H1N1									INFA A								
Viral Titer	High			Medium			Low			High			Medium			Low		
Run # (n=3)	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV
1	23.78	0.81	3.4%	25.56	0.16	0.6%	28.16	0.43	1.5%	21.82	0.48	2.2%	23.69	0.17	0.7%	26.11	0.09	0.3%
2	23.39	0.34	1.5%	26.14	0.64	2.4%	28.19	0.19	0.7%	20.99	0.34	1.6%	23.87	0.37	1.6%	26.01	0.15	0.6%
3	23.29	0.72	3.1%	24.85	0.08	0.3%	27.55	0.17	0.6%	20.97	0.24	1.1%	23.21	0.03	0.1%	25.53	0.07	0.3%
4	23.84	0.54	2.3%	25.96	0.67	2.6%	27.89	0.18	0.6%	21.12	0.59	2.8%	23.44	0.43	1.8%	25.57	0.13	0.5%
5	24.18	0.3	1.2%	26.66	0.27	1.0%	30	0.55	1.8%	22.23	0.12	0.5%	24.9	0.16	0.6%	27.76	0.35	1.3%
6	23.31	0.15	0.6%	26.18	0.25	1.0%	27.76	0.88	3.2%	21.2	0.19	0.9%	23.81	0.09	0.4%	25.36	0.51	2.0%
7	22.93	0.36	1.6%	25.48	0.42	1.6%	28.45	0.11	0.4%	20.93	0.26	1.2%	23.45	0.12	0.5%	26.21	0.21	0.8%
8	25.39	0.03	0.1%	27.72	0.42	1.5%	30.79	0.36	1.2%	22.24	0.02	0.1%	24.82	0.23	0.9%	27.61	0.09	0.3%
9	23.51	0.61	2.6%	25.4	0.09	0.4%	28.45	0.25	0.9%	21.09	0.29	1.4%	23.09	0.06	0.3%	25.83	0.11	0.4%
10	23.85	0.27	1.1%	27.11	0.98	3.6%	29.44	0.32	1.1%	21.16	0.22	1.0%	24.4	0.99	4.1%	26.23	0.24	0.9%
11	23.89	0.16	0.7%	26.01	0.27	1.0%	28.59	0.28	1.0%	21.16	0.2	0.9%	23.43	0.15	0.6%	25.68	0.08	0.3%
12	23.97	0.13	0.5%	26.46	0.24	0.9%	29.07	0.05	0.2%	21.41	0.08	0.4%	23.61	0.18	0.8%	26.3	0.04	0.2%
Average	**23.78**	**0.25**	**1.0%**	**26.13**	**0.27**	**1.0%**	**28.70**	**0.23**	**0.8%**	**21.36**	**0.16**	**0.8%**	**23.81**	**0.26**	**1.1%**	**26.18**	**0.14**	**0.5%**

Table 12: Inter-Assay (Day to Day) Precision for 2009 H1N1 VAN-1

Target	2009 H1N1									INF A								
Viral Titer	High			Medium			Low			High			Medium			Low		
Run # (n=3)	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV
1	19.92	0.34	1.7%	22.2	0.24	1.1%	24.38	0.1	0.4%	19.19	0.16	0.8%	21.54	0.07	0.3%	23.89	0.02	0.1%
2	19.1	0.04	0.2%	22.28	1.06	4.8%	24.33	0.74	3.0%	19.1	0.04	0.2%	21.89	0.52	2.4%	24.2	0.33	1.4%
3	19.94	0.17	0.9%	22.13	0.05	0.2%	23.19	0.05	0.2%	19.59	0.21	1.1%	21.95	0.05	0.2%	23.49	0.06	0.3%
4	18.55	0.92	5.0%	21.97	0.3	1.4%	24.2	0.36	1.5%	18.28	0.49	2.7%	21.08	0.32	1.5%	23.53	0.07	0.3%
5	20.25	0.37	1.8%	23.21	1.34	5.8%	25.33	0.1	0.4%	18.63	0.04	0.2%	21.53	1.2	5.6%	23.64	0.15	0.6%
6	19.48	0.51	2.6%	22.49	0.05	0.2%	24.75	0.28	1.1%	19.36	0.06	0.3%	22.03	0.1	0.5%	24.25	0.31	1.3%

Table 12: Inter-Assay (Day to Day) Precision for 2009 H1N1 VAN-1

										INF A								
																Low		
																		% CV
7	19.8	0.1	0.5%	22.21	0.2	0.9%	24.81	0.36	1.5%	19.32	0.04	0.2%	21.85	0.09	0.4%	24.4	0.42	1.7%
8	21.08	0.17	0.8%	23.59	0.42	1.8%	26.06	0.03	0.1%	20.13	0.08	0.4%	22.91	0.21	0.9%	25.18	0.02	0.1%
9	19.3	0.16	0.8%	21.92	0.2	0.9%	24.44	0.09	0.4%	18.87	0.21	1.1%	21.24	0.09	0.4%	23.51	0.04	0.2%
10	20.26	0.06	0.3%	23.03	0.18	0.8%	25.2	0.1	0.4%	18.93	0.48	2.5%	21.5	0.07	0.3%	23.86	0.05	0.2%
11	19.84	0.12	0.6%	22.48	0.18	0.8%	25.33	0.05	0.2%	18.87	0.03	0.2%	21.45	0.08	0.4%	23.94	0.06	0.3%
12	19.69	0.19	1.0%	22.17	0.02	0.1%	24.99	0.38	1.5%	18.77	0.19	1.0%	21.19	0.24	1.1%	23.98	0.34	1.4%
Average	19.77	0.25	1.3%	22.47	0.42	1.8%	24.75	0.21	0.9%	19.09	0.16	0.9%	21.68	0.33	1.5%	23.99	0.15	0.6%

Lot to Lot Variation

The performance of two different lots of IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay reagents was compared. Testing was performed over the course of up to 7 days within a 30-day period, with each sample tested in triplicate. For each lot, %CV for each dilution of each virus target was determined, then %CV for all runs within a lot. Lot 1 and Lot 2 %CV values are then compared. For influenza A H1N1, high target concentration %CV for Lot 1 is 1.3%, and for Lot 2, 1.5%. For medium target concentration, %CV for Lot 1 is 0.9%, and Lot 2, 0.2%. For low concentration target, %CV for Lot 1 is 0.7%, and Lot 2, 0.8% (Table 13).

For 2009 H1N1 HFD-2, INF A target, high concentration %CV is 0.9% for Lot 1, and 0.5% for Lot 2. Medium target level %CV is 0.7% for Lot 1, and 1.4% for Lot 2. Low concentration target %CV is 0.4% for Lot 1 and 0.6% for Lot 2. For 2009 H1N1 target, high concentration %CV is 1.0% for Lot 1, and 0.8% for Lot 2. Medium target level %CV is 1.1% for both lots. Low concentration target %CV is 0.6% for Lot 1 and 0.9% for Lot 2 (Table 14).

For 2009 H1N1 VAN-1, INF A target, high concentration %CV is 1.0% for Lot 1, and 0.8% for Lot 2. Medium target level %CV is 2.2% for Lot 1, and 0.3% for Lot 2. Low concentration target %CV is 0.5% for Lot 1 and 0.7% for Lot 2. For 2009 H1N1 target, high concentration %CV is 1.7% for Lot 1, and 0.7% for Lot 2. Medium target level %CV for Lot 1 is 2.5%, and Lot 2, 0.6%. Low concentration target %CV is 1.2% for Lot 1 and 0.6% for Lot 2 (Table 15).

Table 13: Lot to Lot Variation for Influenza A/Taiwan/42/06

Lot	Target Titer	Influenza A/Taiwan/42/06 INF A								
		High			Medium			Low		
		Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV
LOT 1	1	21.71	0.49	2.3%	25.81	0.16	0.6%	29.64	0.21	0.7%
	2	22.07	0.1	0.5%	25.81	0.12	0.5%	29.65	0.16	0.5%
	3	22.97	0.06	0.3%	25.9	0.08	0.3%	26.26	0.13	0.5%
	4	21.21	0.74	3.5%	26.62	0.08	0.3%	29.53	0.12	0.4%
	5	21.38	0.21	1.0%	26.14	0.61	2.3%	28.61	0.63	2.2%
LOT 2	1	22.57	0.08	0.4%	25.04	0.12	0.5%	30.09	0.21	0.7%
		22.15	0.17	0.8%	30.5	0.12	0.4%	29.45	0.78	2.6%
	3	22.71	0.98	4.3%	26.74	0.26	1.0%	30.42	0.27	0.9%
	4	21.41	0.64	3.0%	25.58	0.08	0.3%	28.98	0.2	0.7%
	5	22.04	0.31	1.4%	25.79	0.12	0.5%	29.49	0.46	1.6%
	6	22.03	0.2	0.9%	25.66	0.21	0.8%	29.48	0.26	0.9%
	7	22.27	0.21	0.9%	26.04	0.19	0.7%	29.68	0.09	0.3%
Lot 1 Ave		**21.87**	**0.29**	**1.3%**	**26.06**	**0.23**	**0.9%**	**28.74**	**0.22**	**0.7%**
Lot 2 Ave		**22.17**	**0.32**	**1.5%**	**26.48**	**0.06**	**0.2%**	**29.66**	**0.23**	**0.8%**

Table 14: Lot to Lot Variation for 2009 H1N1 HFD-2

Lot	Target Titer Run # (n=3)	Novel H1N1 (HFD-2) INF A									2009 H1N1								
		High			Medium			Low			High			Medium			Low		
		Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV
LOT 1	1	21.82	0.48	2.2%	23.69	0.17	0.7%	26.11	0.09	0.3%	23.78	0.81	3.4%	25.56	0.16	0.6%	28.16	0.43	1.5%
	2	20.99	0.34	1.6%	23.87	0.37	1.6%	26.01	0.15	0.6%	23.39	0.34	1.5%	26.14	0.64	2.4%	28.19	0.19	0.7%
	3	20.97	0.24	1.1%	23.21	0.03	0.1%	25.53	0.07	0.3%	23.29	0.72	3.1%	24.85	0.08	0.3%	27.55	0.17	0.6%
	4	21.12	0.59	2.8%	23.44	0.43	1.8%	25.57	0.13	0.5%	23.84	0.54	2.3%	25.96	0.67	2.6%	27.89	0.18	0.6%
	5	22.23	0.12	0.5%	24.9	0.16	0.6%	27.76	0.35	1.3%	24.18	0.3	1.2%	26.66	0.27	1.0%	30	0.55	1.8%
LOT 2	1	21.2	0.19	0.9%	23.81	0.09	0.4%	25.36	0.51	2.0%	23.31	0.15	0.6%	26.18	0.25	1.0%	27.76	0.88	3.2%
	2	20.93	0.26	1.2%	23.45	0.12	0.5%	26.21	0.21	0.8%	22.93	0.36	1.6%	25.48	0.42	1.6%	28.45	0.11	0.4%
	3	22.24	0.02	0.1%	24.82	0.23	0.9%	27.61	0.09	0.3%	25.39	0.03	0.1%	27.72	0.42	1.5%	30.79	0.36	1.2%

Table 14: Lot to Lot Variation for 2009 H1N1 HFD-2

Lot	Target	Novel H1N1 (HFD-2)																
		INF A									2009 H1N1							
	Titer	High			Medium			Low			High			Medium			Low		
	Run # (n=3)	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV
	4	21.09	0.29	1.4%	23.09	0.06	0.3%	25.83	0.11	0.4%	23.51	0.61	2.6%	25.4	0.09	0.4%	28.45	0.25	0.9%
	5	21.16	0.22	1.0%	24.4	0.99	4.1%	26.23	0.24	0.9%	23.85	0.27	1.1%	27.11	0.98	3.6%	29.44	0.32	1.1%
	6	21.16	0.2	0.9%	23.43	0.15	0.6%	25.68	0.08	0.3%	23.89	0.16	0.7%	26.01	0.27	1.0%	28.59	0.28	1.0%
	7	21.41	0.08	0.4%	23.61	0.18	0.8%	26.3	0.04	0.2%	23.97	0.13	0.5%	26.46	0.24	0.9%	29.07	0.05	0.2%
Lot 1 Average		21.43	0.19	0.9%	23.82	0.16	0.7%	26.20	0.11	0.4%	23.70	0.23	1.0%	25.83	0.27	1.1%	28.36	0.18	0.6%
Lot 2 Average		21.31	0.10	0.5%	23.80	0.33	1.4%	26.17	0.16	0.6%	23.84	0.19	0.8%	26.34	0.29	1.1%	28.94	0.27	0.9%

Table 15: Lot to Lot Variation for 2009 H1N1 VAN-1

Lot	Target	2009 H1N1 (VAN-1)																
		INF A									2009 H1N1							
	Titer	High			Medium			Low			High			Medium			Low		
	Run # (n=3)	Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV
LOT 1	1	19.19	0.16	0.8%	21.54	0.07	0.3%	23.89	0.02	0.1%	19.92	0.34	1.7%	22.2	0.24	1.1%	24.38	0.1	0.4%
	2	19.1	0.04	0.2%	21.89	0.52	2.4%	24.2	0.33	1.4%	19.1	0.04	0.2%	22.28	1.06	4.8%	24.33	0.74	3.0%
	3	19.59	0.21	1.1%	21.95	0.05	0.2%	23.49	0.06	0.3%	19.94	0.17	0.9%	22.13	0.05	0.2%	23.19	0.05	0.2%
	4	18.28	0.49	2.7%	21.08	0.32	1.5%	23.53	0.07	0.3%	18.55	0.92	5.0%	21.97	0.3	1.4%	24.2	0.36	1.5%
	5	18.63	0.04	0.2%	21.53	1.2	5.6%	23.64	0.15	0.6%	20.25	0.37	1.8%	23.21	1.34	5.8%	25.33	0.1	0.4%
LOT 2	1	19.36	0.06	0.3%	22.03	0.1	0.5%	24.25	0.31	1.3%	19.48	0.51	2.6%	22.49	0.05	0.2%	24.75	0.28	1.1%
	2	19.32	0.04	0.2%	21.85	0.09	0.4%	24.4	0.42	1.7%	19.8	0.1	0.5%	22.21	0.2	0.9%	24.81	0.36	1.5%
	3	20.13	0.08	0.4%	22.91	0.21	0.9%	25.18	0.02	0.1%	21.08	0.17	0.8%	23.59	0.42	1.8%	26.06	0.03	0.1%
	4	18.87	0.21	1.1%	21.24	0.09	0.4%	23.51	0.04	0.2%	19.3	0.16	0.8%	21.92	0.2	0.9%	24.44	0.09	0.4%
	5	18.93	0.48	2.5%	21.5	0.07	0.3%	23.86	0.05	0.2%	20.26	0.06	0.3%	23.03	0.18	0.8%	25.2	0.1	0.4%
	6	18.87	0.03	0.2%	21.45	0.08	0.4%	23.94	0.06	0.3%	19.84	0.12	0.6%	22.48	0.18	0.8%	25.33	0.05	0.2%
	7	18.77	0.19	1.0%	21.19	0.24	1.1%	23.98	0.34	1.4%	19.69	0.19	1.0%	22.17	0.02	0.1%	24.99	0.38	1.5%
Lot 1 Ave		18.96	0.18	1.0%	21.60	0.47	2.2%	23.75	0.12	0.5%	19.55	0.34	1.7%	22.36	0.57	2.5%	24.29	0.29	1.2%
Lot 2 Ave		19.18	0.16	0.8%	21.74	0.07	0.3%	24.16	0.17	0.7%	19.92	0.15	0.7%	22.56	0.13	0.6%	25.08	0.15	0.6%

Instrument to Instrument Variation
Assay results for two different ABI 7500 Real-Time PCR Systems, running software v2.01 were obtained. Testing was performed over the course of up to 8 days within a 30-day period on one instrument, then compared with results from testing performed on the other instrument over the course of 2 days within the same 30 day period. All samples were tested in triplicate. For each instrument, %CV for each dilution of each virus target was determined, then %CV for all runs on that instrument. Instrument 1 and Instrument 2 %CV values are then compared. Testing was performed using influenza A H1N1 and 2009 H1N1 HFD-2 strain. For influenza A H1N1, high target concentration %CV for Instrument 1 is 1.4%, and for Instrument 2, 1.8%. For medium target concentration, %CV for Instrument 1 is 0.2%, and Instrument 2, 1.4%. For low concentration target, %CV for Instrument 1 is 0.4%, and Instrument 2, 1.2% (Table 16).

For 2009 H1N1 HFD-2, INF A target, high concentration %CV is 0.7% for Instrument 1, and 1.5% for Instrument 2. Medium target level %CV is 1.3% for Instrument 1, and 0.8% for Instrument 2. Low concentration target %CV is 0.2% for Instrument 1 and 0.6% for Instrument 2. For 2009 H1N1 target, high concentration %CV is 1.2% for Instrument 1, and 0.7% for Instrument 2. Medium target level %CV for Instrument 1 is 1.2%, and Instrument 2, 1.1%. Low concentration target %CV is 0.4% for Instrument 1 and 0.9% for Instrument 2 (Table 17).

Table 16: Instrument to Instrument Variation for Influenza A/Taiwan/42/06

ABI 7500 Standard Instrument No.	Influenza A/Taiwan/42/06									
	Target	INF A								
	Viral Titer	High			Medium			Low		
	Run # (n=3)	Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV	Ct Mean	Ct SD	%CV
1	1	21.71	0.49	2.3%	25.81	0.16	0.6%	29.64	0.21	0.7%
	2	22.07	0.1	0.5%	25.81	0.12	0.5%	29.65	0.16	0.5%
	3	22.97	0.06	0.3%	25.9	0.08	0.3%	26.26	0.13	0.5%
	4	22.71	0.98	4.3%	26.74	0.26	1.0%	30.42	0.27	0.9%
	5	21.41	0.64	3.0%	25.58	0.08	0.3%	28.98	0.2	0.7%
	6	22.04	0.31	1.4%	25.79	0.12	0.5%	29.49	0.46	1.6%
	7	22.03	0.2	0.9%	25.66	0.21	0.8%	29.48	0.26	0.9%
	8	22.27	0.21	0.9%	26.04	0.19	0.7%	29.68	0.09	0.3%
2	1	21.21	0.74	3.5%	26.62	0.08	0.3%	29.53	0.12	0.4%
	2	21.38	0.21	1.0%	26.14	0.61	2.3%	28.61	0.63	2.2%
No. 1 Ave		22.15	0.31	1.4%	25.92	0.06	0.2%	29.20	0.11	0.4%
No. 2 Ave		21.30	0.37	1.8%	26.38	0.37	1.4%	29.07	0.36	1.2%

Table 17: Instrument to Instrument Variation for 2009 H1N1 HFD-2

ABI 7500 Standard	2009 H1N1 HFD-2 Strain																	
	Target	INF A								2009 H1N1								
	Viral Titer	High			Medium			Low			High			Medium			Low		
	Run # (n=3)	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	% CV	Ct Mean	Ct SD	%CV
No. 1	1	21.82	0.48	2.2%	23.69	0.17	0.7%	26.11	0.09	0.3%	23.78	0.81	3.4%	25.56	0.16	0.6%	28.16	0.43	1.5%
	2	20.99	0.34	1.6%	23.87	0.37	1.6%	26.01	0.15	0.6%	23.39	0.34	1.5%	26.14	0.64	2.4%	28.19	0.19	0.7%
	3	20.97	0.24	1.1%	23.21	0.03	0.1%	25.53	0.07	0.3%	23.29	0.72	3.1%	24.85	0.08	0.3%	27.55	0.17	0.6%
	4	22.24	0.02	0.1%	24.82	0.23	0.9%	27.61	0.09	0.3%	25.39	0.03	0.1%	27.72	0.42	1.5%	30.79	0.36	1.2%
	5	21.09	0.29	1.4%	23.09	0.06	0.3%	25.83	0.11	0.4%	23.51	0.61	2.6%	25.4	0.09	0.4%	28.45	0.25	0.9%
	6	21.16	0.22	1.0%	24.4	0.99	4.1%	26.23	0.24	0.9%	23.85	0.27	1.1%	27.11	0.98	3.6%	29.44	0.32	1.1%
	7	21.16	0.2	0.9%	23.43	0.15	0.6%	25.68	0.08	0.3%	23.89	0.16	0.7%	26.01	0.27	1.0%	28.59	0.28	1.0%
	8	21.41	0.08	0.4%	23.61	0.18	0.8%	26.3	0.04	0.2%	23.97	0.13	0.5%	26.46	0.24	0.9%	29.07	0.05	0.2%
No. 2	1	21.12	0.59	2.8%	23.44	0.43	1.8%	25.57	0.13	0.5%	23.84	0.54	2.3%	25.96	0.67	2.6%	27.89	0.18	0.6%
	2	22.23	0.12	0.5%	24.9	0.16	0.6%	27.76	0.35	1.3%	24.18	0.3	1.2%	26.66	0.27	1.0%	30	0.55	1.8%
No. 1 Ave		**21.36**	**0.14**	**0.7%**	**23.77**	**0.31**	**1.3%**	**26.16**	**0.06**	**0.2%**	**23.88**	**0.29**	**1.2%**	**26.16**	**0.31**	**1.2%**	**28.78**	**0.12**	**0.4%**
No. 2 Ave		**21.68**	**0.33**	**1.5%**	**24.17**	**0.19**	**0.8%**	**26.67**	**0.16**	**0.6%**	**24.01**	**0.17**	**0.7%**	**26.31**	**0.28**	**1.1%**	**28.95**	**0.26**	**0.9%**

Analytical Specificity
Analytical specificity of the IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay was evaluated with respect to Analytical Reactivity (Inclusivity), Cross Reactivity, Interference and Interfering Substances.

Analytical Reactivity
Reactivity (inclusivity) was tested using a total of thirty-one (31) 2009 H1N1 and other influenza A strains of the virus. The IMDx 2009 Influenza A H1N1 Real-Time RT-PCR was able to detect all samples tested. The INF A primer and probe set are specific for the strains tested. The 2009 H1N1 hemagglutinin primer and probe set are specific for the strains tested. Table 18 represents the data collected.

Table 18: Analytical Reactivity

Specimen Tested	Viral Classification	TCID$_{50}$ Log$_{10}$/mL	No. Tests	Number Detected by IMDx 2009 Influenza A H1N1 Assay	
				INF A (Ct)	2009 H1N1 (Ct)
A/New Caledonia/20/99	H1N1	7.06	1	1 (14.7)	0
		2.06	3	3 (31.6)	0
A/Taiwan/42/06	H1N1	-0.2	6	6 (34.2)	0
A/Brisbane/59/07	H1N1	4.70	1	1 (19.3)	0
A/Brisbane/10/07	H3N2	5.39	1	1 (16.1)	0
		0.39	3	3 (30.6)	0
A/Wisconsin/67/05	H3N2	7.06	1	1 (15.1)	0
		2.06	3	3 (23.6)	0
A/Solomon Islands/3/2006	H1N1	5.15	1	1 (18.8)	0
		0.15	3	3 (30.8)	0
A/Hong Kong/8/68	H3N2	7.50	1	1 (14.8)	0
		2.5	3	3 (32.5)	0
Clinical isolate, 2009 H1N1[1]	2009 H1N1	4.30	6	6 (29.4)	6 (30.8)
Clinical isolate, 2009 H1N1[1]	2009 H1N1	1.7	6	6 (32.1)	6 (30.1)
Clinical sample, 2009 H1N1 (CDC MMWR specimen)[3]	2009 H1N1	NA	2	1 (14.4)	2 (15.2)
Clinical sample, 2009 H1N1 (CDC MMWR specimen)[3]	2009 H1N1	NA	2	1 (14.2)	2 (15.0)
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (24.0)	0
Clinical specimen, H1N1[2]	Seasonal	NA	1	1 (29.3)	0
Clinical specimen, H1N1[2]	Seasonal	NA	1	1 (31.8)	0

Table 18: Analytical Reactivity

Specimen Tested			Tests	Number Detected by IMDx 2009 Influenza A H1N1Assay	
					2009 H1N1 (Ct)
Clinical specimen, H1N1[2]	Seasonal	NA	1	1 (27.6)	0
Clinical specimen, H1N1[2]	Seasonal	NA	1	1 (27.0)	0
Clinical specimen, H1N1[2]	Seasonal	NA	1	1 (22.0)	0
Clinical specimen, H1N1[2]	Seasonal	NA	1	1 (33.8)	0
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (25.8)	0
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (27.1)	0
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (25.0)	0
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (25.8)	0
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (20.6)	0
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (26.3)	0
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (28.5)	0
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (27.5)	0
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (29.8)	0
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (24.8)	0
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (24.0)	0
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (19.1)	0
Clinical specimen, H3N2[2]	Seasonal	NA	1	1 (24.3)	0
NA: not available					
[1] Clinical isolates cultured and titered. Culture confirmed positive for 2009 H1N1 Influenza A using Focus Diagnostics and Prodesse ProFluST 2009 H1N1 assays					
[2] Clinical specimens collected during 2009 and identified as seasonal influenza A and subtyped H1N1 or H3N2 using CDC rRT-PCR Flu Panel IVD Seasonal Influenza Typing and Subtyping assay					
[3] Clinical specimens confirmed as 2009 H1N1 by pyrosequencing at CDC as referenced in MMWR Morb Mortal Rep 2009 Aug 21; 58(32):893-6					

Cross-reactivity

Potential cross-reactivity with other respiratory pathogens was tested. Cross-reactivity of the IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay was evaluated using other human respiratory pathogens and influenza B strains at $\geq 2\times10^6$ genome equivalents per mL. The results are described in Table 19.

Table 19: Cross-reactivity Panel Results

Target			2009 H1N1 (Ct)	INF A (Ct)
Organism	Strain	Source		
Adenovirus 1	Adenoid 71	ATCC	ND	ND
Adenovirus 7	Subgrp. B, serotype 7A	ATCC	ND	ND
B. pertussis	ATCC® BAA-589	ATCC	ND	ND
C. trachomatis	UW-3/Cx	ATCC	ND	ND
C.diptheria	NCTC 13129	ATCC	ND	ND
Coronavirus 229	229E	Zeptometrix	ND	ND
Coronavirus OCH3	OC43	Zeptometrix	ND	ND
Coronavirus SARS	2003-00592	Zeptometrix	ND	ND
Cytomegalovirus	Merlin	ATCC	ND	ND
E. coli	ATCC® 25922 Serotype O6	ATCC	ND	ND
Enterovirus	Coxsackievirus A9	Zeptometrix	ND	ND
Epstein-Barr Virus	B95-8	ATCC	ND	ND
H. influenzae	ATCC® 51907	ATCC	ND	ND
Influenza B	Yamanashi/166/98	Zeptometrix	ND	ND
L. acidophilus	ATCC® 4356	ATCC	ND	ND
L.pneumophila	*pneumophila Philadelphia-1*	ATCC	ND	ND
M. pneumoniae	M129-B7	ATCC	ND	ND
M.avium	*paratuberculosis K-10*	ATCC	ND	ND
Human metapneumovirus	Clinical source	Zeptometrix	ND	ND
N. gonorrhoeae	ATCC® 700825	ATCC	ND	ND
P. aeruginosa	ATCC® 29260	ATCC	ND	ND
Human parainfluenza virus 1	Type 1	Zeptometrix	ND	ND
Human parainfluenza virus 2	Type 2	Zeptometrix	ND	ND
Human parainfluenza virus 3	Type 3	Zeptometrix	ND	ND
Respiratory Syncytial Virus A	A2	Zeptometrix	ND	ND
Respiratory Syncytial Virus B	CH93-18(18)	Zeptometrix	ND	ND
Rhinovirus	Clinical source	Zeptometrix	ND	ND
S. aureus	MRSA ATCC® 700699	ATCC	ND	ND
S. epidermidis	ATCC® 12228	ATCC	ND	ND
S. pyogenes	ATCC® 700294	ATCC	ND	ND
S.pneumoniae	R6	ATCC	ND	ND
S.salivarius	ATCC® BAA-1024	ATCC	ND	ND

ND: Not detected

Interference

The IMDx 2009 Influenza A H1N1Assay was tested with the same panel of respiratory pathogens to determine if any of the pathogens would interfere with the detection of a defined amount of an admixture of 2009 H1N1 HFD-2 and VAN1 strains of influenza A virus. Genomic DNA or RNA from the test organism was added to a reaction mix that included a 1:1 mixture of the 2009 H1N1 HFD-2 and VAN1 strains and tested in duplicate. Ct value averages and standard deviations were calculated and are reported in Table 20 below. Results show no interference with the primer probe sets for the detection of the 2009 H1N1 HFD-2 and VAN1 virus mixture.

Interfering Substances

A panel of interfering substances was tested to determine if commonly available and utilized substances interfere with the detection of 2009 H1N1 Influenza virus using the IMDx 2009 Influenza A H1N1 Real-Time RT-PCR. Interfering substances were diluted to the listed concentrations using universal transport medium (UTM). A swab was then dipped into the interfering substance and transferred to a new tube containing 3 mL of transport medium. A 1:1 mixture of 2009 H1N1 HFD-2 and 2009 H1N1 VAN1 strains of influenza virus was spiked into each tube containing the interfering substance prior to nucleic acid purification. Extractions of each interfering substance (with and without virus present) were performed using the Qiagen Viral RNA isolation kit following the manufacturer's instructions. Table 21 provides the data set for the tested substances. Interference was not observed for any of the potential interferants tested, for any of the primer-probe sets.

Table 20: Inhibition of Targeted Signals Due to Competitive Organisms

Target			2009 H1N1 (Ct)		INF A (Ct)	
Organism	Strain	Source	Mean	SD	Mean	SD
Reference (Novel H1N1 Mix)	VAN-1/HFD-2	Clinical source	23.36	0.21	21.73	0.41
Adenovirus 1	Adenoid 71	ATCC	23.48	0.21	21.5	0.21
Adenovirus 7A	Subgrp. B, serotype 7A	ATCC	22.68	0.08	21.06	0.08
B. pertussis	ATCC® BAA-589	ATCC	22.96	0.51	21.51	0.31
C. trachomatis	UW-3/Cx	ATCC	22.83	0.03	20.77	0.059
C.diptheria	NCTC 13129	ATCC	22.86	0.11	21.14	0.04
Coronavirus 229	229E	Zeptometrix	23.00	0.13	21.36	0.01
Coronavirus OCH3	OC43	Zeptometrix	22.47	0.18	20.84	0.10
Coronavirus SARS	2003-00592	Zeptometrix	23.46	0.22	21.50	0.24
Cytomegalovirus	Merlin	ATCC	22.45	0.31	20.85	0.03
E. coli	ATCC® 25922 Serotype O6	ATCC	22.64	0.07	21.11	0.19
Enterovirus	Coxsackievirus A9	Zeptometrix	22.89	0.93	21.14	0.49
Epstein-Barr Virus	B95-8	ATCC	23.09	0.31	21.43	0.28
H. influenzae	ATCC® 51907	ATCC	22.87	0.08	21.13	0.28
Influenza B	Yamanashi/166/98	Zeptometrix	22.73	0.04	21.10	0.22
L. acidophilus	ATCC® 4356	ATCC	22.90	0.30	21.10	0.27
L.pneumophila	*pneumophila* Philadelphia-1	ATCC	22.99	0.14	21.32	0.24
M. pneumoniae	M129-B7	ATCC	22.48	0.23	21.13	0.14
M.avium	*paratuberculosis* K-10	ATCC	23.41	0.28	21.74	0.06
Human metapneumovirus	Clinical source	Zeptometrix	23.38	0.06	21.42	0.11
N. gonorrhoeae	ATCC® 700825	ATCC	22.78	0.00	21.18	0.22
P. aeruginosa	ATCC® 29260	ATCC	22.78	0.19	21.22	0.06

Table 20: Inhibition of Targeted Signals Due to Competitive Organisms

					INF A (Ct)	
						SD
Human parainfluenza virus 1	Type 1	Zeptometrix	22.98	0.17	21.16	0.23
Human parainfluenza virus 2	Type 2	Zeptometrix	23.16	0.39	21.34	0.16
Human parainfluenza virus 3	Type 3	Zeptometrix	23.29	0.54	21.48	0.28
Respiratory Syncytial Virus A	A2	Zeptometrix	22.90	0.11	21.13	0.33
Respiratory Syncytial Virus B	CH93-18(18)	Zeptometrix	23.00	0.04	21.16	0.14
Rhinovirus	Clinical source	Zeptometrix	22.91	0.05	21.12	0.15
S. aureus	MRSA ATCC® 700699	ATCC	22.77	0.10	20.90	0.14
S. epidermidis	ATCC® 12228	ATCC	22.52	0.30	20.86	0.01
S. pyogenes	ATCC® 700294	ATCC	23.00	0.30	21.37	0.06
S.pneumoniae	R6	ATCC	23.04	0.10	21.41	0.11
S.salivarius	ATCC® BAA-1024	ATCC	22.58	0.90	21.51	0.32

Table 21: Interfering Substances Panel Results

Target		2009 H1N1		INF A	
	2009 H1N1 HFD-2 /VAN-1 Admixture	-	+	-	+
Substance	Concentration	Ct	Ct	Ct	Ct
Transport Medium	-	ND	27.48	ND	25.96
Acetominophen	0.2 mg/mL	ND	27.74	ND	26.24
Aspirin	32.4 mg/mL	ND	28.07	ND	26.49
Beclomethasone	80 mg/mL	ND	27.32	ND	25.70
Benzocaine	5% solution	ND	27.52	ND	25.99
Blood	whole; undiluted	ND	27.38	ND	25.76
Dexamethasone	6 mg/mL	ND	27.86	ND	26.15
Fluticasone	100 mg/mL	ND	27.42	ND	25.86
Histaminum Hydrochloricum 30C	1 mg/mL	ND	27.20	ND	25.62
Ibuprofen	80 mg/mL	ND	27.48	ND	26.01
Menthol	250 mg/mL	ND	27.59	ND	26.37
Mucin: bovine submaxillary gland, type1-S	19 mg/mL	ND	28.17	ND	26.50
Mupirocin	0.3 mg/mL	ND	27.40	ND	25.76
Oseltamivir	16 mg/mL	ND	26.55	ND	25.34
Oxymetozaline	0.05%	ND	27.91	ND	25.86
Phenylephrine	1%	ND	27.15	ND	25.20
Saline	0.65% NaCl	ND	27.12	ND	25.53
Streptomycin	200 mg/ml	ND	27.25	ND	25.50
Zanamivir	2 mg/mL	ND	27.79	ND	26.09

ND: Not detected

Clinical Performance

Clinical Specimens Tested
A total of 160 clinical specimens were tested using the comparator assays described below as well as a third EUA-cleared 2009 H1N1 assay not used as a reference method. Upper respiratory tract sample matrices tested included nasopharyngeal swab, nasal swab and throat swab

Clinical Reactivity
Clinical reactivity of the IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay was assessed in a multi-center clinical study. Comparator assays used for detection of 2009 H1N1 influenza virus are the EUA-cleared Prodesse pro-FluST assay, and the CDC 2009 H1N1 assay. A total of 160 Clinical Reactivity specimens including a mix of nasal and nasopharyngeal swabs were analyzed, consisting of forty (40) 2009 H1N1 specimens, 100 influenza-negative, and twenty (20) seasonal influenza A, 2009 H1N1-negative specimens (Table 22). Seasonal influenza A viruses were confirmed negative for 2009 H1N1 using the CDC 2009 H1N1 assay, and confirmed for influenza A H1 or H3 subtype using the CDC Influenza A Typing assay. Twenty of the 2009 H1N1 specimens, and fifty of the influenza-negative specimens were tested using the ABI 7500 Fast Real-Time PCR System running software version 2.01. The remaining twenty 2009 H1N1 specimens, fifty influenza-negative specimens, and twenty seasonal influenza A specimens were tested using the ABI 7500 Fast Real-Time PCR System running software version 1.4.

Table 22: Summary of Testing of Clinical Specimens

		Comparator			
		Seasonal Influenza A Positive	2009 H1N1 Positive	Negative	Total
IMDx 2009 Influenza A H1N1 Real-Time RT-PCR Assay	Influenza A Positive	20**	1	0	21
	2009 H1N1 Positive	0	39	1	40
	Indeterminate*	0	0	1	1
	Negative	0	0	98	98
	Total	20	40	100	160

2009 H1N1	Results	95% Confidence Interval
Positive Agreement	97.5% (39/40)	87.1% - 99.6%
Negative Agreement	98.3% (118/120)***	94.1% – 99.5%

* One Indeterminate result (retested as per the assay instructions) that did not resolve in a valid result was considered indeterminate and tallied against the performance of the *IMDx* test.
** Specimens corresponding to seasonal influenza A virus.
*** Seasonal influenza A positives, negative for 2009 H1N1, were included in the analysis.

Detection of 2009 H1N1

For detection of 2009 H1N1, positive result agreement is 97.5%, and negative result agreement 98.3% (Table 22). Specimens tested include 40 2009 H1N1 specimens, 20 2009 H1N1-negative, seasonal influenza A-positive specimens, and 100 influenza A-negative specimens. One 2009 H1N1 specimen was negative for 2009 H1N1 but positive for INF A in the IMDx 2009 Influenza A H1N1 Real-Time RT-PCR, hence discordant with reference method. The same result was obtained on repeat testing. For the 120 2009 H1N1-negative specimens, one influenza-negative specimen gave an Indeterminate result, confirmed on repeat testing., and one was positive for 2009 H1N1 using both the IMDx 2009 Influenza A H1N1 Real-Time RT-PCR and an additional EUA-cleared 2009 H1N1 assay. This specimen also was positive for INF A by the IMDx 2009 Influenza A H1N1 Real-Time RT-PCR and the other EUA-cleared 2009 H1N1 assay.

Instrument and Operating Software Comparison

Clinical specimen testing using the IMDx 2009 Influenza A H1N1 Real-Time RT-PCR was performed on the ABI 7500 Fast Real-Time PCR Instrument. Both versions of ABI 7500 software were utilized. A Methods Comparison was performed to compare assay results generated using the ABI 7500 and ABI 7500 Fast Instruments, and software versions v2.01 and v1.4. For assay analytical sensitivity, Limit of Detection was determined for one influenza A/H1N1, one influenza A/H3N2 and one 2009 H1N1 (Table 23). For Clinical Reactivity testing, forty 2009 H1N1 clinical specimens and fifty influenza-negative specimens were analyzed (Table 24, 25).

Limit of Detection Comparison between Instrument and Software Versions

Three different strains of influenza were tested to compare Limit of Detection (LoD) determinations between the ABI 7500 Fast Instrument running software v2.01 and v1.4 and between the ABI 7500 Fast and 7500 Standard instruments running software v2.01. For influenza A/H1N1 and A/H3N2, and the INF A and 2009 H1N1 targets of 2009 H1N1, the $\log_{10}$ $TCID_{50}$ levels representing the LoD for all targets are identical for the ABI 7500 Fast Instrument running v1.4 software (Table 23, Col. A) and v2.01 software (Table 23, Col B). When comparing the ABI 7500 Fast (Table 23, Col. B) to the ABI 7500 Standard (Table 23, Col. C), both running software v2.01, influenza A/H1N1 (Taiwan/42/06) LoD is 3x lower for the 7500 Standard compared to the ABI 7500 Fast, a level corresponding to approximately 1 amplification cycle. LoD level is identical for influenza A/H3N2 (Brisbane/10/07). For 2009 H1N1 (HFD-2), LoD for INF A target is 1.7x higher on the 7500 Standard compared to the 7500 Fast, a level corresponding to less than one amplification cycle. The LoD level is identical for the 2009 H1N1 target. For the number of replicates tested, the values are within statistical limits of being indistinguishable.

Clinical Reactivity Testing: Comparison between Instruments and Software Versions

For comparing Clinical Reactivity data between software (Table 24) and instrument (Table 25) versions, there were no discrepancies noted for the forty 2009 H1N1 specimens tested. One specimen (specimen #34, Table 24) returned 2009 H1N1 target Ct values above the assay cutoff level of 38 cycles. However, this result was concordant between software versions being compared. There were no discordant results between software or instrument version for the fifty influenza-negative specimens tested (data not shown).

Table 23. Limit of Detection Comparison

Virus Strain Tested	Analyte Tested	Stock Virus Titer (TCID 50/ml)	Dilution Tested (TCID 50/mL)	Column A ABI 7500 FAST (v1.4)				Column B ABI 7500 FAST (v2.01)				Column C ABI 7500 (v2.01)			
				Call Rate	% detected	Ct Ave	Ct SD	Call Rate	% detected	Ct Ave	Ct SD	Call Rate	% detected	Ct Ave	Ct SD
Taiwan /42/06 (H1N1)	INFA	5.0×10^4	16.0	20/20	100%	34.5	1.05	20/20	100%	34.7	1.03	20/20	100%	34.3	0.59
			5.0	18/20	90%	36.2	1.24	17/20	85%	36.4	1.21	20/20	100%	36.1	0.74
			2.0	6/20	30%	38.1	1.26	6/20	30%	38.2	1.21	10/20	50%	37.4	0.81
Brisbane /10/07 (H3N2)	INFA	1.6×10^5	2.0	20/20	100%	33.7	0.48	20/20	100%	33.9	0.45	20/20	100%	35.5	0.72
			0.6	20/20	100%	34.9	0.69	20/20	100%	35.1	0.67	19/20	95%	36.4	1.11
			0.2	2/20	10%	38.7	0.84	2/20	10%	38.7	0.89	12/20	60%	37.0	0.76
2009 H1N1 Influenza HFD-2	INFA	2.0×10^4	6.3	20/20	100%	31.9	0.49	20/20	100%	32.2	0.49	20/20	100%	31.5	0.72
			2.0	20/20	100%	33.2	0.50	20/20	100%	33.4	0.47	19/20	95%	32.5	0.73
			0.8	20/20	100%	34.7	0.88	20/20	100%	34.9	0.84	18/20	90%	32.8	0.72
			0.3	15/20	75%	37.0	1.04	15/20	75%	37.1	0.99	7/20	35%	36.1	1.06
	2009 H1N1	2.0×10^4	6.3	20/20	100%	33.7	0.91	20/20	100%	33.8	0.88	20/20	100%	35.0	1.63
			2.0	18/20	90%	35.5	1.69	18/20	90%	35.6	1.70	15/20	75%	35.4	1.16
			0.8	13/20	65%	35.8	1.39	13/20	65%	35.6	1.02	14/20	70%	35.6	1.27
			0.3	0/20	0%	ND	NA	1/20	5%	37.1	NA	2/20	10%	36.0	0.10

Table 24. Clinical Reactivity Software Comparison

Sample		v2.01	2009 H1N1 (Ct)	
			SDS v1.4	v2.01
1	21.3	21.7	23.4	23.7
2	20.6	21.0	23.3	23.4
3	21.7	22.2	24.2	24.6
4	26.2	26.6	29.3	29.5
5	24.1	24.6	27.0	27.2
6	29.3	29.7	30.5	30.8
7	22.6	23.0	26.1	26.3
8	24.0	24.4	26.4	26.7
9	26.2	26.6	28.1	28.4
10	29.1	29.6	32.5	32.7
11	20.3	20.7	21.1	21.2
12	19.2	19.5	22.1	22.3
13	25.1	25.5	27.9	28.2
14	23.3	23.7	26.1	26.3
15	23.2	23.6	25.7	25.9
16	20.4	20.8	22.7	23.0
17	21.7	22.2	24.5	24.7
18	22.6	23.0	25.4	25.6
19	17.3	17.6	19.6	19.9
20	22.8	23.2	26.3	26.4
21	26.4	26.9	28.9	29.2
22	20.5	20.9	23.0	23.2
23	21.6	22.0	24.4	24.6
24	26.9	27.2	29.4	29.5
25	25.4	25.9	28.2	28.5
26	26.3	26.7	28.2	28.6
27	19.5	19.8	22.1	22.2
28	30.1	30.5	33.5	33.6
29	26.0	26.4	28.2	28.4
30	30.1	30.6	33.1	33.2
31	26.1	26.6	27.4	27.6
32	32.5	32.9	35.0	35.2
33	18.5	18.9	20.4	20.7
34*	33.4	33.9	39.4	39.6
35	23.8	24.1	26.7	26.8
36	22.2	22.7	24.0	24.3
37	21.2	21.6	23.0	23.2
38	28.9	29.3	29.9	30.2
39	27.1	27.5	29.9	30.1
40	17.4	17.7	20.1	20.2

* Signal detected but classified negative (Ct>38 cycles).

Table 25. Clinical Reactivity Instrument Comparison

Sample	INFA (Ct)		2009 H1N1 (Ct)	
	7500 FAST	7500 STD	7500 FAST	7500 STD
1	20.5	20	21.6	21.9
2	19.9	20	21.7	22.5
3	20.9	21.2	23	23.4
4	25.6	25.8	27.8	28.5
5	22.7	23.8	24.4	26
6	29	29	30.1	30.5
7	22	21.8	24.6	25
8	22.9	23.1	24.8	25.5
9	25.9	26	27.6	28
10	29.2	29.9	30.5	32
11	19.9	20.2	19.4	19.7
12	18.3	18.7	20.4	21
13	24.4	24.9	26.5	27.3
14	22.5	23	24.5	25.1
15	22.4	22.8	24.2	24.9
16	19.8	19.6	21.4	21.9
17	21.4	21.4	23	23.4
18	21.8	22.4	23.7	24.9
19	16.6	16.5	18.3	18.3
20	21.9	23.1	24.4	25.5
21	25.8	26.4	27.5	28.5
22	19.8	19.8	21.2	21.4
23	21	20.9	22.9	23.2
24	26.5	26.7	27.4	28.3
25	24.9	24.9	26.4	26.7
26	25.5	25.8	26.7	27.5
27	18.5	18.8	20.3	20.8
28	30.2	30.3	31.3	33.4
29	25.4	25.7	27.2	27.7
30	29.1	29.9	28.2	31.5
31	24.4	24.7	24.9	26.1
32	31.5	31.3	32.9	33.9
33	17.6	17.3	18.6	18.8
34	33	35	36.2	36.6
35	22.8	23.6	24.5	26
36	21.7	21.7	23	23.1
37	20.6	21.4	21.7	22.6
38	26.7	26.8	27.5	28.1
39	26.1	26.2	27.4	28.7
40	16.4	16.6	18	18.6

17. DISPOSAL:

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

18. REFERENCES:

1. Lynch JP 3rd, Walsh EE. Influenza: evolving strategies in treatment and prevention. Semin Respir Crit Care Med. 2007 Apr;28(2):144-58.
2. Gallaher WR. Towards a sane and rational approach to management of Influenza H1N1 Influenza 2009. Virol J. 2009 May 7;6:51.
3. Novel H1N1 Influenza Flu: Background on the Situation. CDC: http://www.cdc.gov/H1N1 Influenzaflu/background.htm.
4. Seasonal Influenza: The Disease. CDC: http://www.cdc.gov/flu/about/disease/index.htm.
5. Burch J, Corbett M, Stock C, Nicholson K, Elliot AJ, Duffy S, Westwood M, Palmer
6. S, Stewart L. Prescription of anti-Influenza drugs for healthy adults: a systematic review and meta-analysis. Lancet Infect Dis. 2009 Sep;9(9):537-45.
7. Hersh AL, Maselli JH, Cabana MD. Changes in prescribing of antiviral medications for Influenza associated with new treatment guidelines. Am J Public Health. 2009 Oct;99 Suppl 2:S362-4.
8. Pandemic (H1N1 Influenza) 2009. WHO: http://www.who.int/csr/disease/swineflu/en/index.html
9. H1N1 Influenza Virus Biosafety Guidelines for Laboratory Workers. http://www.cdc.gov/h1n1flu/guidelines labworkers.htm

TaqMan® is a registered trademark of the Roche Group.

THE PURCHASE OF THIS PRODUCT GRANTS THE PURCHASER RIGHTS UNDER CERTAIN ROCHE PATENTS TO USE IT SOLELY FOR PROVIDING HUMAN IN VITRO DIAGNOSTIC SERVICES. NO GENERAL PATENT OR OTHER LICENSE OF ANY KIND OTHER THAN THIS SPECIFIC RIGHT FOR USE FROM PURCHASE IS GRANTED HEREBY.

19. CONTACT INFORMATION:

ORDERING INFORMATION:
IntelligentMDx, Inc
19 Blackstone Street
Cambridge, Massachusetts 02139
Phone: 617.871.6400
Fax: 617.871.6399
Email: infor@IntelligentMDx.com

GENERAL/TECHNICAL ASSISTANCE:
IntelligentMDx, Inc
19 Blackstone Street
Cambridge, Massachusetts 02139
Phone: 617.871.6400
Fax: 617.871.6399
Email: infor@IntelligentMDx.com