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 As of June 23, 2010, the EUAs have been terminated and this information is no longer current.

CDC Swine Influenza Virus Real-time RT-PCR Detection Panel with the Roche LightCycler® 2.0 Real-time PCR System (CDC rRT-PCR Swine Flu Panel)

Instructions for Use

Roche LightCycler® 2.0 Real-time PCR System

1000 reactions

Emergency Use Authorization Only

Centers for Disease Control and Prevention
Influenza Division
1600 Clifton Rd NE
Atlanta GA 30333

CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

Intended Use

The Swine Influenza Virus Real-time RT-PCR Detection Panel is intended for use in Real-time RT-PCR assays on an Applied Biosystems 7500 Fast Dx Real-Time PCR System and the Roche LightCycler® 2.0 Real-time PCR system in conjunction with clinical and epidemiological information:

- To identify patients who may be infected with 2009 H1N1 influenza virus to allow public health authorities to respond to and limit transmission of the virus during this public health emergency;
- For the qualitative detection of influenza virus type A in viral RNA in upper respiratory tract clinical 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)), and lower respiratory tract specimens (such as broncheoalveolar lavage (BAL), bronchial aspirate – BA; bronchial wash – BW; endotracheal aspirate – EA; endotracheal wash – EW; tracheal aspirate – TA, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture;
- For identification of virus in patients who may be infected with the 2009 H1N1 influenza virus from viral RNA in upper respiratory tract clinical specimens (NPS, NS, TS, NA, NW, and dual NPS/TS), and lower respiratory tract specimens (such as BAL, BA, BW, EA, EW, TA, and lung tissue) from human patients with signs and symptoms of respiratory infection and viral culture, in conjunction with clinical and epidemiological risk factors;
- To provide epidemiologic information for surveillance for the 2009 H1N1 influenza virus.

Testing with the rRT-PCR Swine Flu Panel should not be performed unless the patient meets clinical and/or epidemiologic criteria for testing suspect specimens. The identification of 2009 H1N1 influenza A should be performed along with clinical and epidemiological assessment.

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

Use is limited to designated public health laboratories and other laboratories qualified to receive and use the CDC rRT-PCR Flu Panel (IVD) K080570

Summary and Explanation

Infections with the 2009 H1N1 influenza virus continue to cause illness and death among persons worldwide. As of August 2009, confirmed cases of novel influenza A (H1N1) virus infections had been reported in all 50 states in the United States and territories and additional cases abroad (for more information, visit http://www.cdc.gov/h1n1flu/). The infections with the 2009 H1N1 influenza virus and with the seasonal influenza A and B viruses in humans, may caused upper and lower and respiratory tract illness.

The 2009 H1N1 influenza virus infection may cause complications and severe lower respiratory tract disease, in critically ill patients who are hospitalized and placed on mechanical ventilation for respiratory failure, high and prolonged viral shedding may be present in the lower respiratory tract but low or absent in the upper respiratory tract. Data from autopsy findings in lung tissues and other lower respiratory specimens from fatal human cases have confirmed the presence of 2009 H1N1 influenza virus in the lower respiratory tract.

Principles of the Procedure

The rRT-PCR Swine Flu Panel is based on real-time reverse transcriptase polymerase chain reaction (rRT-PCR) technology. The rRT-PCR assays are one-tube assays that first reverse-transcribe specific regions of RNA into cDNA copies. The cDNA then serves as a template for a polymerase chain reaction (PCR) that utilizes a thermocyclic heating and cooling of the reaction to logarithmically amplify a specific region of DNA. The probe anneals to a specific, internal target sequence located between the target loci of the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades any probe molecules hybridized to amplified target sequence, causing the reporter dye to separate from the quencher dye, and generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle. Amplification of targets is reflected by logarithmic increase in fluorescence over time in comparison to background signal.

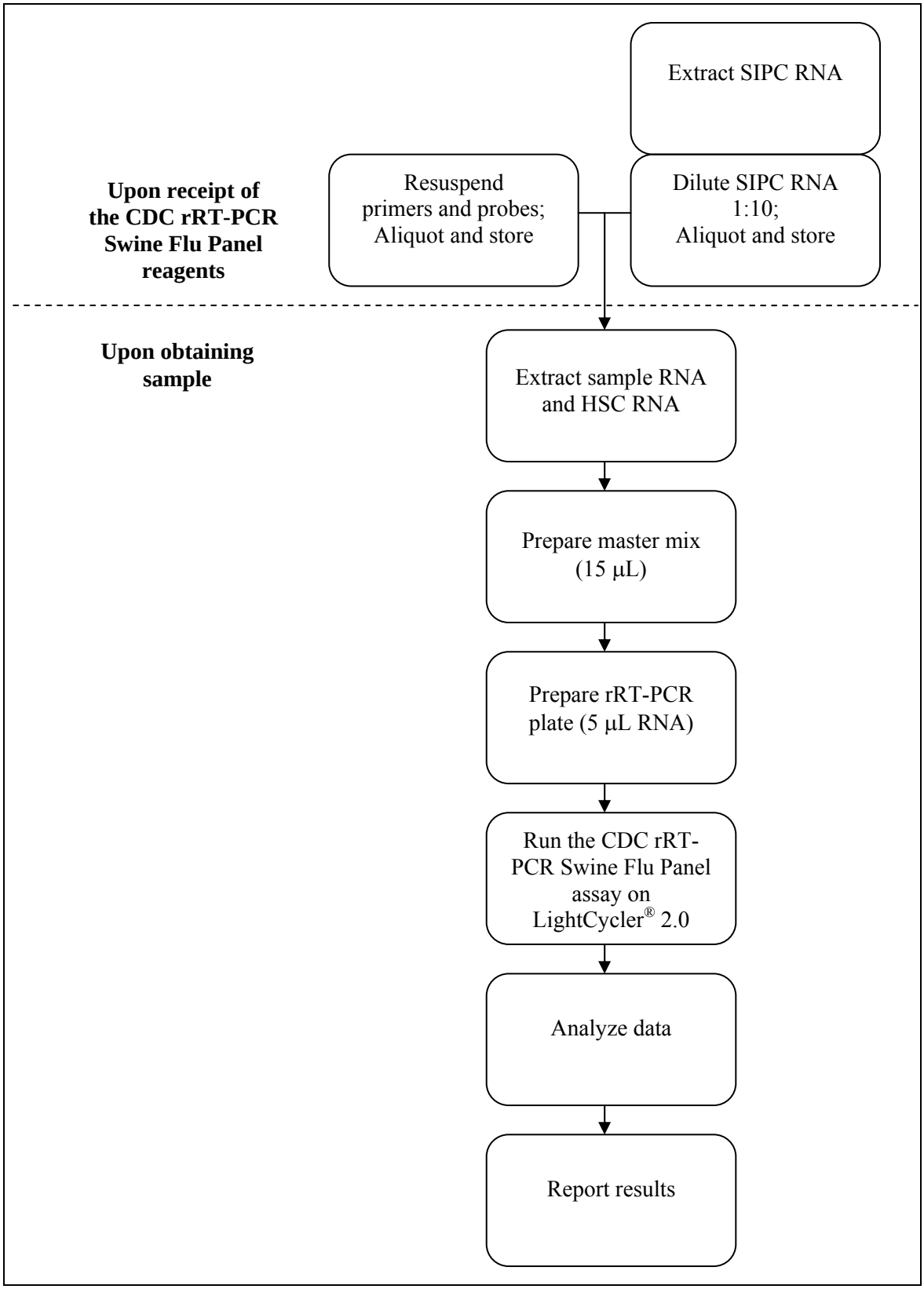
The rRT-PCR Swine Flu Panel includes four (4) sets of oligonucleotide primers and dual-labeled hydrolysis (Taqman®) probes to be used in real-time RT-PCR assays for the in vitro qualitative detection and characterization of 2009 H1N1 influenza virus in human respiratory specimens.

The influenza A (InfA) primer and probe sets are designed for universal detection of type A influenza viruses. Influenza A subtyping primer and probe sets swInfA and swH1 are designed to specifically detect the 2009 H1N1 influenza virus.

User Testing Options	Panel Sets to Use	Expected Outcome
#1. To detect influenza type A only in clinical specimens	InfA (Primer and Probe Set)	Influenza A (+ or -)
#2. To detect influenza 2009 A/H1N1 subtype in clinical specimens	InfA (Primer and Probe Set) swInfA (Primer and Probe Set) swH1 Primer and Probe Set)	Influenza 2009 A/H1N1 (+ or -)

Note: Options 1 and 2 can be performed at the user's discretion independently or simultaneously.

Summary of Influenza Testing Process



Materials Provided

Box 1: Detection Kit

(Store in PCR Reagent Preparation Area)

Label	*Description*	*Quantity / Tube*	*Reactions / Tube*
InfA-F	Influenza A Forward Primer	20 nmol	1000
InfA-R	Influenza A Reverse Primer	20 nmol	1000
swInfA-F	Swine Influenza A Forward Primer	20 nmol	1000
swInfA-R	Swine Influenza A Reverse Primer	20 nmol	1000
swH1-F	Swine Influenza H1 Forward Primer	20 nmol	1000
swH1-R	Swine Influenza H1 Reverse Primer	20 nmol	1000
RP-F	RNase P Forward Primer	20 nmol	1000
RP-R	RNase P Reverse Primer	20 nmol	1000
RP-P	RNase P Probe	5 nmol	1000
InfA-P	Influenza A Probe	5 nmol	1000
swInfA-P	Swine Influenza A Probe	5 nmol	1000
swH1-P	Swine Influenza H1 Probe	5 nmol	1000

Box 2: Positive Control

(Store in RNA Handling Area)

Reagent Label	*Description*	*Qty. / Tube*	*Reactions / Tube*
SIPC	**Swine Influenza Virus Positive Control (SIPC):** For use as a positive control with the CDC rRT-PCR Swine Flu Panel procedure to ensure the detection of the 2009 H1N1 influenza virus. Contains noninfectious (beta-propiolactone inactivated) positive control materials (influenza virus preparation) suspended in 0.01 M phosphate buffer saline (PBS) at pH 7.2–7.4. The SIPC consists of influenza virus representing 2009 H1N1 influenza and cultured human cells (A549). The SIPC will yield a positive result with the following primer and probe sets: InfA, swInfA, swH1, and RP.	1–500 µL tubes	1000

Box 3: Human Specimen Extraction Control Kit

(Store in Nucleic Acid Extraction Room)

Reagent Label	***Description***	***Qty. / Tube***	***Reactions / Tube***
HSC	**Human Specimen Control (HSC):** For use as a RNA extraction procedural control with the CDC rRT-PCR Flu Panel procedure to demonstrate successful recovery of RNA as well as extraction reagent integrity. Purified RNA from the Human Specimen Control material should yield a positive result with the RP primer and probe set and negative results with all influenza specific markers. The HSC consists of noninfectious (beta propiolactone inactivated) cultured human cell material supplied as a liquid suspended in 0.01 M PBS at pH 7.2–7.4.	17–500 µL tubes	5–1000 extractions

Materials Required But Not Provided

Ancillary Reagents Required BUT NOT Provided

Specific lots for the ancillary reagents listed below will be qualified for use with the CDC rRT-PCR Swine Flu Panel by CDC Influenza Division quality control testing and lot qualification program.

The rRT-PCR Swine Flu Panel test performance requires that only qualified ancillary reagent lots be used with the device. Any lots not specifically qualified by the CDC Influenza Division for use with the rRT-PCR Swine Flu Panel are not valid for use with this device, and may affect device performance.

A supplemental cumulative list of qualified ancillary reagents lots for use with the rRT-PCR Swine Flu Panel is provided with each shipment or can be requested by sending an email to FluSupport@cdc.gov

Use ancillary reagents only with the instructions for use contained within this package insert instructions for use. Discard any instructions for use that may be packaged with these ancillary reagents.

Any issues related to assay performance or test failure that are suspected to involve ancillary reagents should be reported to the CDC Influenza Division by emailing FluSupport@cdc.gov

	Reagent	Quantity	Catalog No.
rRT-PCR Enzyme Mastermix Options	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (without Rox)	100 reactions	11732-020
		500 reactions	11732-088
	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (with Rox)	100 reactions	11745-100
		500 reactions	11745-500
Nucleic Acid Purification Kit Options	Qiagen QIAamp® Viral RNA Mini Kit * (Qiagen Inc., Valencia, CA)	50 extractions	52904
		250 extractions	52906
	Qiagen RNeasy® Mini Kit * (Qiagen Inc., Valencia, CA)	50 extractions	74104
		250 extractions	74106
	Roche MagNA Pure LC Total Nucleic Acid Kit * (Roche Applied Science, Indianapolis, IN) **Protocol:** External Lysis Protocol	192 extractions	03 038 505 001
	Roche MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Applied Science, Indianapolis, IN) *** Protocol:** External Lysis Protocol	32 extractions	03 730 964 001
	Roche MagNA Pure Compact RNA Isolation Kit * (Roche Applied Science, Indianapolis, IN) **Protocol:** RNA _ Tissue_V3_1 Protocol	32 extractions	04 802 993 001

These products require reagents and consumables per the manufacturer's requirements.

Equipment and Consumables Required BUT NOT Provided

- Rnase/Dnase-free 1.5 ml polypropylene microcentrifuge tubes
- Molecular Grade Water (RNase/DNase Free)
- Sterile nuclease-free filtered pipette tips
  - Micropipettes (1-10 µL, 10-200 µL and 100-1000 µL)
  - Benchtop Microcentrifuge
  - Roche LightCycler 2.0 Carousel Centrifuge 2.0 (cat #03709507001 including rotor and bucket)
- Personnel Protective Equipment (PPE)
  - -70º C and -20º C Freezer(s)
  - +4º C Refrigerator
  - Roche MagNA Pure Compact System

- Required consumables specific for instrument defined by the manufacturer.
- Roche MagNA Pure LC System with software version 3.0.11
  - Required consumables specific for instrument defined by the manufacturer.
- Roche LightCycler® 2.0 consumables (Roche Applied Science, Indianapolis, IN)
- Roche LightCycler® 2.0 Capillaries (20 µl), cat. # 04929292001 including stoppers
- Roche LightCycler® 2.0 Real-Time PCR System with LightCycler version 4.1 Software, which includes:
  1. Roche LightCycler® 2.0 instrument
  2. Roche LightCycler® 2.0 Sample Carousel (20 µl)
  3. Roche LightCycler® 2.0 Software version 4.1
  4. Roche LightCycler® 2.0 Centrifuge Cooling Block/Adapters
  5. Roche LightCycler® 2.0 Capping Tool
  6. Roche LightCycler® 2.0 Capillary Releaser

Reagent Storage, Handling, and Stability

- Store all primers and probes at 2–8 ºC until re-hydrated for use; store all control materials (SIPC- Swine Influenza Positive Control) at ≤ 20 ºC.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic probes from light.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and kept on ice or cold block at all times during preparation and use.

Reagents and Controls Preparation

I. Primer and Probe Preparation:

A. Upon receipt, store primers and probes at 2–8°C.
B. Rehydration
   1. Remove primers and probes from 2–8°C.
   2. Pipet 0.5 ml (500 µL) of 10 mM Tris, pH 7.4–8.2 or PCR-grade water into each dried PCR primer or probe.
   3. Allow primers and probes to fully rehydrate for at least 15 minutes at room temperature.
   4. After primers and probes are fully rehydrated, pulse vortex to ensure a homogenous solution.
C. Aliquot
   1. Label one (1) new nuclease-free, sterile, tube for each primer and probe with the following information:
      a. Primer or Probe Name
      b. Kit Lot #

c. Expiration Date

2. Aliquot 100 µL of each primer and probe into respective labeled tubes and store at -20°C.

D. Storage

1. After rehydration

a. Primers

1. Aliquots of primers are stored at -20°C or below until expiration date as long as Quality Control (QC) requirements are met.

b. Probe

1. Aliquots of probes are stored at -20°C or below until expiration date as long as QC requirements are met.
2. Thawed aliquots of probes may be stored at 2–8°C in the dark for up to 3 months.

II. Swine Influenza Positive Control (SIPC) Preparation:

A. Reagent

1. Inactivated, noninfectious influenza virus preparation supplied as a liquid suspended in 0.01 M PBS.
2. Influenza viruses representing swine influenza A/H1N1 cultured human cells.
3. Volume: 0.5 ml yields approximately 5.0 ml of positive control RNA.

B. Storage

1.Store at -20°C or below upon receipt. Do not dilute.

C. Procedure

1. SIPC must be extracted prior to use. The final volume of eluted RNA should equal the volume of extracted control material. For example, 50µL of control material should result in 50µL of RNA extract (1:1).

a. Dilute the RNA 1:10 with nuclease-free water.

2. Label one (1) new nuclease-free, sterile, tube for each single-use aliquot with the following information:

a. Control RNA Name and 1:10 Dilution
b. Kit Lot #
c. Expiration Date

3. Dispense 1:10 diluted RNA into single-use aliquots and store at -20°C or below for up to 6 months.

a. Use one aliquot per run. Discard after use. Do not use residual RNA.

General Information

Equipment

Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used including 5% bleach, 70% ethanol, ***DNAzap***™ or ***RNase AWAY***® to minimize the risk of nucleic acid contamination.

Reagents

NOTE: All reagents should be kept on ice or cold rack during assay preparation.

Primers and Probes Reagents

- Thaw frozen aliquots of primer and probes. *Thawed aliquots of probes may be stored at 2-8°C in the dark for up to 3 months.* ***Do not re-freeze probes.***
- Vortex all primers and probes for 15 seconds.
- Briefly centrifuge all primers and probes.
- Place primers and probes on ice or in cold rack during master mix preparation.

Real-time RT-PCR Reagents

- Place Invitrogen 2X PCR Master Mix and Superscript III RT / Platinum Taq enzyme mix in a cold rack at 4-8 ºC.
- Completely thaw the 2X PCR Master Mix vial.
- Mix the 2X PCR Master Mix by inversion 10 times.
- Briefly centrifuge 2X PCR Master Mix and Superscript II RT / Platinum Taq enzyme mix then place in cold rack.

Warnings and Precautions

The use of sputum specimens has NOT been authorized under the Emergency Use Authorization.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Virus culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
Note: **Novel influenza A viruses are new or re-emergent human strains of influenza A that cause cases or clusters of human disease, as opposed to those strains commonly circulating in humans that cause seasonal epidemics and to which human populations have residual or limited immunity (either by vaccination or previous infection).**

Due to the sensitivity of Real-time RT-PCR assays, special precautions must be followed to avoid false positive amplifications. The following precautionary steps are recommended:

- Maintain separate areas for assay setup and handling of nucleic acids.
- Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.

- Work surfaces, pipets, and centrifuges should be cleaned and decontaminated with cleaning products such as 5% bleach, ***DNAzap***™ or ***RNase AWAY***® to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- Reagents, master mix, and RNA should be maintained on cold block during preparation and/or use to ensure stability.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic probes from light.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and maintained on cold block at all times during preparation and use.

Nucleic Acid Extraction

Performance of the CDC Swine Influenza Virus Real-time RT-PCR Detection Panel is dependent on the amount and quality of template RNA purified from human specimens. The following commercially available RNA extraction kits and procedures have been qualified and validated for recovery and purity of RNA for use with the panel:

- QIAamp® Viral RNA Mini Kit, or
- RNeasy® Mini Kit (QIAGEN), or
- Roche MagNA Pure LC Total Nucleic Acid Kit, **External Lysis Protocol**. Utilize 100.0 µl of sample and 300.0 µl of lysis buffer (total sample volume for input into LC is 400.0 µl). Elution volume is 100.0 µl. These volumes should be available on the LC software.
- Roche MagNA Pure Compact Nucleic Acid Isolation Kit I (catalog # 03 730 964 001), **External Lysis Protocol**. Utilize 100.0 µl of sample and 250.0 µl of lysis buffer (total sample volume for input into Compact is 350.0 µl). Elution is 100.0 µl.
- Roche MagNA Pure RNA Isolation Kit (catalog# 04 802 993 001), **RNA _ Tissue_V3_1 Protocol.** Utilize 100.0 µl of sample and 250.0 µl of lysis buffer (total sample volume for input into Compact is 350.0 µl). Elution is 100.0 µl.

Manufacturer's recommended procedures are to be used for sample extraction.

Disclaimer: Names of vendors or manufacturers are provided as examples of suitable product sources. Inclusion does not imply endorsement by the Centers for Disease Control and Prevention.

Assay Setup

Master Mix Preparation / Plate Setup

NOTE: All reagents should be kept on ice or cold rack during assay preparation.

1. In the assay preparation area, prepare primers/probes and Real-time RT-PCR Reagents.

Primers and Probes Reagents

- Thaw frozen aliquots of primer and probes. *Thawed aliquots of probes may be stored at 2–8°C in the dark for up to 3 months.* ***Do not re-freeze probes.***
- Vortex all primers and probes for 15 seconds.
- Briefly centrifuge all primers and probes.
- Place primers and probes on ice or in cold rack during master mix preparation.

Real-time RT-PCR Reagents

- Invitrogen SuperScript III Platinum One-Step Quantitative RT-PCR System (with Rox) Cat # 11745-100 or 11745-500 is recommended.
- Place Invitrogen 2X Reaction Mix with Rox and Superscript III RT / Platinum Taq enzyme mix in a cold rack at 4–8ºC.
- Completely thaw the 2X PCR Master Mix vial.
- Mix the 2X PCR Master Mix by inversion 10 times.
- Briefly centrifuge 2X PCR Master Mix and Superscript II RT / Platinum Taq enzyme mix then place in a cold rack.

2. Label a sterile, nuclease-free, 1.5 mL microcentrifuge tube for each of the following primer/probe sets:
   - Inf A
   - sw InfA
   - sw H1
   - RNase P

3. Determine the number of reactions (N) being prepared per assay.
NOTE*: It is necessary to make excess reaction cocktail to allow for the No Template Control (NTC), Swine Influenza Positive Control (SIPC), and Human Specimen Control (HSC) reactions and pipetting error.*

Example: If number of samples (n) including controls = 1 to 14, then N = n + 1
If number of samples (n) including controls > 15, then N = n + 2

4. Calculate the amount of each reagent to be added to the tube for each master mix according to Figure 1.

Figure 1. Calculations for Reagent Preparation

Reagent	Volume of Reagent Added per Reaction	Total Number of Reactions (N)	Total Volume of Reagent to Add to Master Mix
Molecular Grade (nuclease-free) Water	N x 1.4 µL	N= n+1 =8+1=9	12.6 µL
2.5 mg/mL Bovine Serum Albumin in Nuclease-free water	N x 2.0 µL	N= n+1 =8+1=9	18 µL
Forward Primer	N x 0.4 µL	N= n+1 =8+1=9	3.6 µL
Reverse Primer	N x 0.4 µL	N= n+1 =8+1=9	3.6 µL
Probe	N x 0.4 µL	N= n+1 =8+1=9	3.6 µL
SuperScript™ III RT/Platinum® Taq Mix	N x 0.4 µL	N= n+1 =8+1=9	3.6 µL
2 X PCR Master Mix	N x 10.0 µL	N= n+1 =8+1=9	90 µL
Total Volume	**N x 15.0 µL**		**135 µL**

- Add contents in the order provided in the table in order to minimize contamination.
- Due to the viscosity of the Taq Mix, pipette slowly and mix by pipetting up and down.

5. After addition of the components, mix reaction mixtures by pipetting up and down. Do not vortex.

6. Centrifuge at full speed for 5 seconds to collect contents at bottom of the tube, and then place the tube in cold rack (2–8°C).

7. Set up capillaries or PCR reaction tubes as appropriate in a cooler rack.

8. Dispense 15 µL of each master mix into each capillary as indicated in Fig. 2 below. Dispense one master mix into all of the required positions before moving on to the next master mix. For example, dispense 15 µL Influenza A (Inf A) master mix into the appropriate capillaries before beginning dispense of the swFlu A master mix.

Figure 2. Example of Reaction Master Mix Plate Setup

Shows the positions for all four of the master mixes: For example, the influenza A (InfA) master mix should be dispensed into positions 1, 5, 9, 13, 17, 21, 25, & 29.

Position		Position		Position		Position	
1	InfA	**9**	InfA	**17**	InfA	**25**	InfA
2	swInfA	**10**	swInfA	**18**	swInfA	**26**	swInfA
3	swH1	**11**	swH1	**19**	swH1	**27**	swH1
4	RP	**12**	RP	**20**	RP	**28**	RP
5	InfA	**13**	InfA	**21**	InfA	**29**	InfA
6	swInfA	**14**	swInfA	**22**	swInfA	**30**	swInfA
7	swH1	**15**	swH1	**23**	swH1	**31**	swH1
8	RP	**16**	RP	**24**	RP	**32**	RP

Assuming singlet testing, up to 5 specimens can be extracted and batched on 1 rotor. If the user is testing 1 to 5 samples, then only 1 rotor set up would apply.

Sample Addition:

Figure 3. Example of Sample and Control Setup

Shows the positions for all specimens and controls: for example, the No Template Control (NTC) should be dispensed into positions 1, 2, 3 & 4.

Position		Position		Position		Position	
1	NTC	**9**	S2	**17**	S4	**25**	HSC
2	NTC	**10**	S2	**18**	S4	**26**	HSC
3	NTC	**11**	S2	**19**	S4	**27**	HSC
4	NTC	**12**	S2	**20**	S4	**28**	HSC
5	S1	**13**	S3	**21**	S5	**29**	SIPC
6	S1	**14**	S3	**22**	S5	**30**	SIPC
7	S1	**15**	S3	**23**	S5	**31**	SIPC
8	S1	**16**	S3	**24**	S5	**32**	SIPC

Note: No template controls (NTC) should be added first in the reagent preparation room before any of the samples are added to check for contamination in the master mix. HSC should be added after the samples have been added to check for cross-contamination during sample preparation or addition. Positive template controls (SIPC) should be added last after all samples and NTCs are sealed.

1) NTC addition

a) Before moving the capillaries to the nucleic acid handling area, set up the NTC reactions in the assay set-up area. As shown above, samples can be added by column.

b) Pipette 5 µL of nuclease free water into the NTC capillaries. Cap the NTC capillaries.

c) Close the lid on the capillary box and move the capillaries to the nucleic acid handling area.

d) Change Personal Protective Equipment (PPE) such as gloves and lab coat when moving from the reagent area to the nucleic acid handling area.

2) Specimen RNA addition

a) Set up the extracted nucleic acid samples in the cold rack (2–8°C).

b) Pipette 5 µL of the first sample into all the capillaries labeled for that sample (for example, Sample "S1" as shown in Figure. 3). Change tip after each addition.

c) Cap the capillaries to which the sample has been added to prevent cross contamination and ensure sample tracking.

d) Change gloves when necessary to avoid contamination, especially between samples.

e) Repeat previous steps for the remaining samples.

f) Add 5 µL of HSC extracted sample RNA to the HSC capillaries. Cap the HSC tubes.

g) Close the lid on the capillary box and move the capillaries to the Positive Template Control nucleic acid handling area.

3) Positive Template Control (SIPC) addition

a) Finally, pipette 5 µL of SIPC RNA into all SIPC tubes. Cap the SIPC tubes. All capillaries should now be capped.

b) Place capillaries into LightCycler sample carousel per product manual.

c) Briefly centrifuge capillaries for 10–15 seconds in the LightCycler Carousel Centrifuge (refer to the operator's manual) to ensure that the complete reaction mixture is in the bottom of the capillary. Place the sample carousel in the LightCycler® 2.0 instrument.

Create a Template on the Roche LightCycler® 2.0

1. Double Click **LightCycler Software 4.1** 

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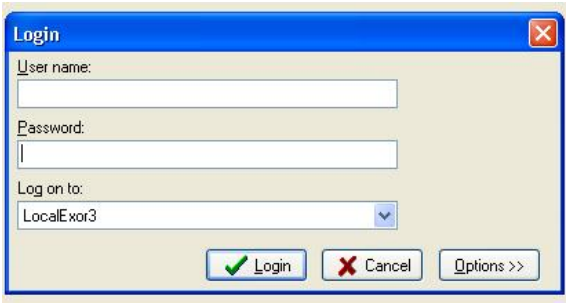
2. On the Login page, put info for “User name” and “Password”, click **Login**.\



3. Click 

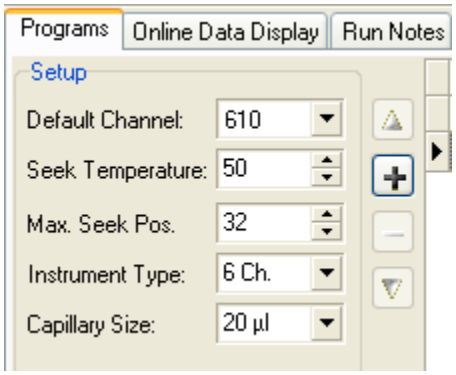
on top left corner; highlight **LightCycler Experiment** under **Create New Object** page.

4. Click **OK**.

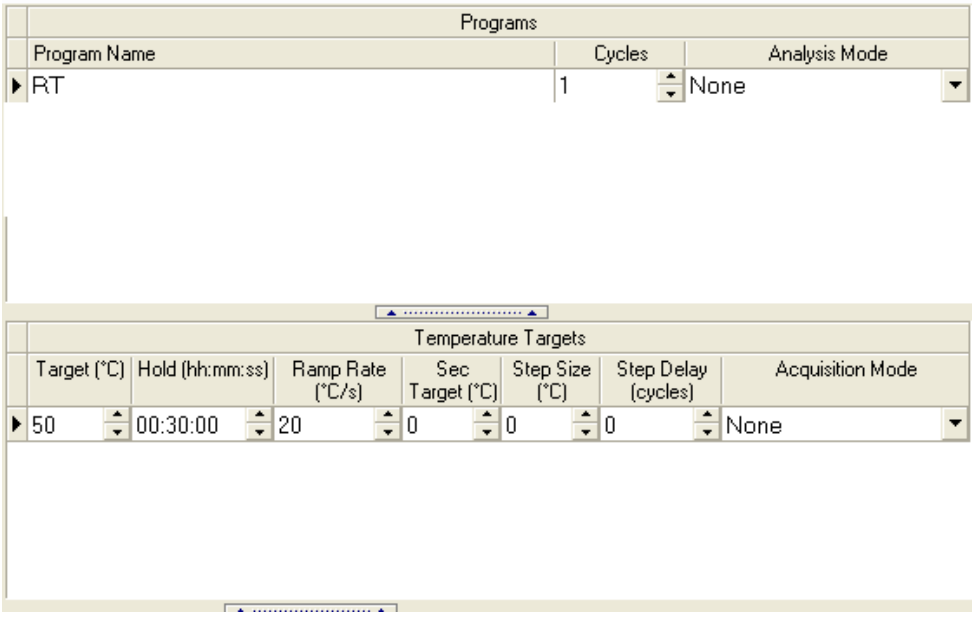
5. A new experiment Run profile appears on the screen.

6. In **Setup** profile:
   a. Under **Run** mode, select **610** for Default Channel.
   b. Enter **50** as Seek Temperature.

c. Enter **32** as Max. Seek Pos.
d. Select **6 Ch** as Instrument Type.
e. Select **20 µL** for Capillary Size.

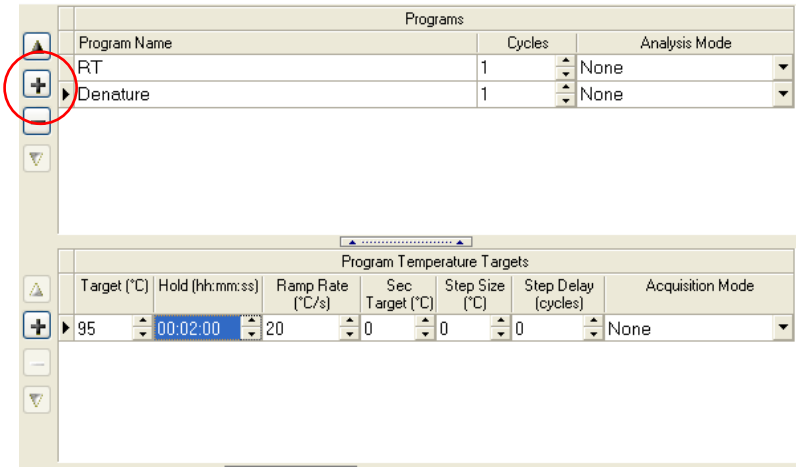


7. In **Programs** profile:
   a. Highlight **Program**, type **RT** as program name.
   b. Enter **1** for cycle.
   c. Select **None** for Analysis Mode.



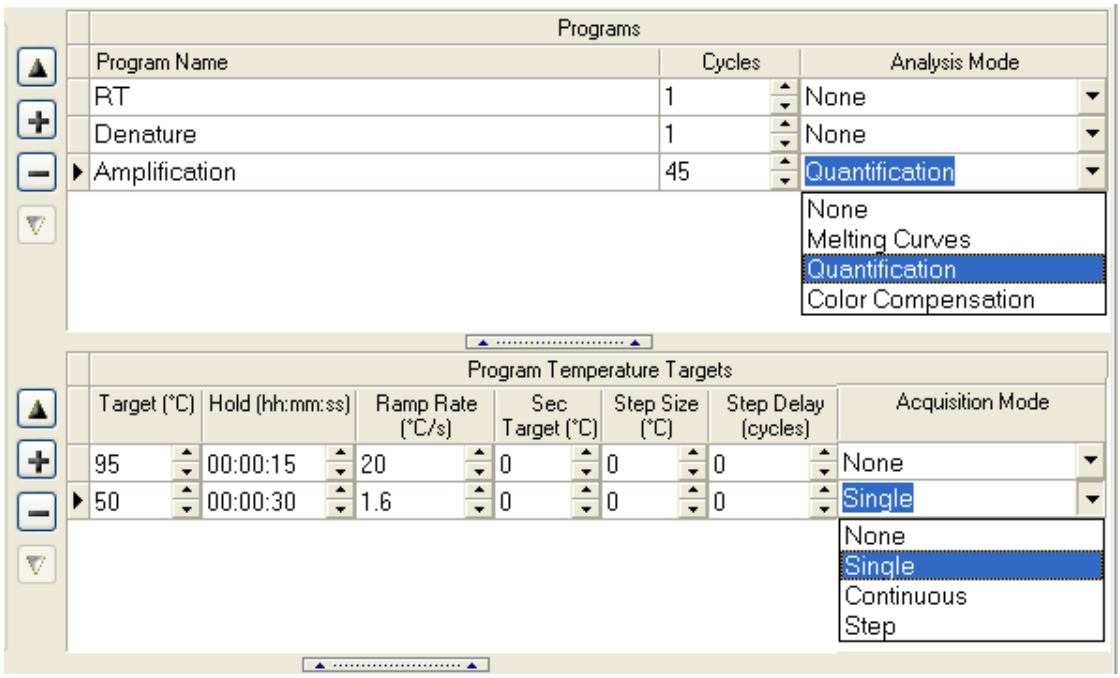
8. In **Program Temperature Targets** profile:
   a. Enter **50** for Target (◦C).
   b. Enter **00:30:00** for Hold (hh:mm:ss).
   c. Enter **20** for Ramp Rate (◦C/s).
   d. Enter **0** for Sec Target (◦C), Step Size (◦C), and Step Delay (cycles).
   e. Select **None** for Acquisition Mode.

9. Go back on top in **Programs** profile, Click "+" sign by **Programs**, a new Program option appears.
    a. Highlight **Program**, enter **Denature** as program name.
    b. Enter **1** for cycle.
    c. Select **None** for Analysis Mode.



10. In **Program Temperature Targets** profile:
    a. Enter **95** for Target (◦C).
    b. Enter **00:02:00** for Hold (hh:mm:ss).
    c. Enter **20** for Ramp Rate (◦C/s).
    d. Enter **0** for Sec Target (◦C), Step Size (◦C), and Step Delay (cycles).
    e. Select **None** for Acquisition Mode.

11. Go back on top in **Programs** profile, Click "+" sign by **Programs**, a new Program option appears.
    a. Highlight **Program**, enter **Amplification** as program name.
    b. Enter **45** for cycle.
    c. Select **Quantification** for Analysis Mode.

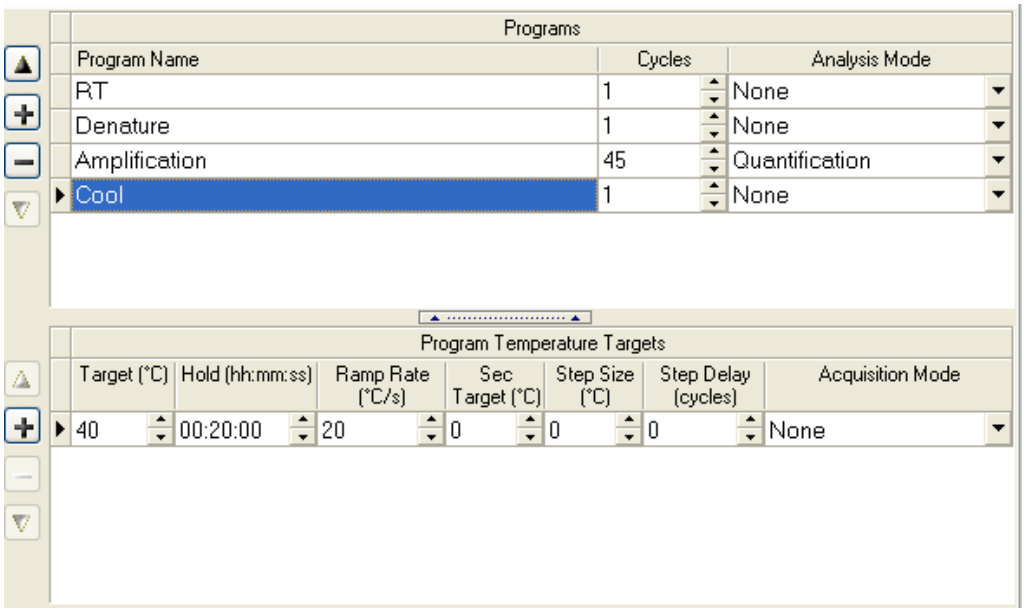


12. In **Program Temperature Targets** profile:
    a. Enter **95** for Target (◦C).
    b. Enter **00:00:15** for Hold (hh:mm:ss).
    c. Enter **20** for Ramp Rate (◦C/s).
    d. Enter **0** for Sec Target (◦C), Step Size (◦C), and Step Delay (cycles).
    e. Select **None** for Acquisition Mode.

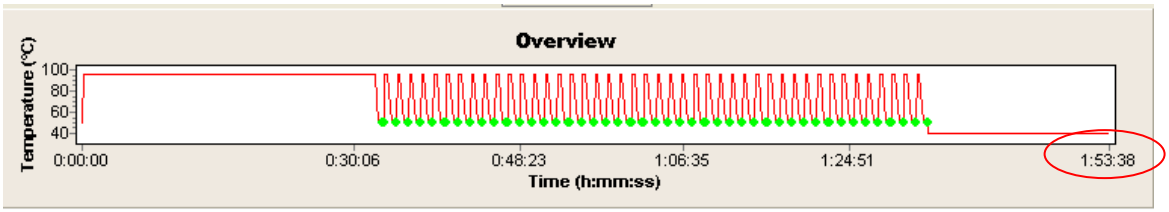
13. Click "+" sign by **Program Temperature Targets** profile, a new temperature option appears on the screen.
    a. Enter **55** for Target (◦C).
    b. Enter **00:00:30** for Hold (hh:mm:ss).
    c. Enter **1.6** for Ramp Rate (◦C/s).
    d. Enter **0** for Sec Target (◦C), Step Size (◦C), and Step Delay (cycles).
    e. Select **Single** for Acquisition Mode.

14. Go back on top in **Programs** profile, Click "+" sign by **Programs**, a new Program option appears.
    a. Highlight **Program**, enter "Cool" as program name.
    b. Enter **1** for cycle.
    c. Select **None** for Analysis Mode.

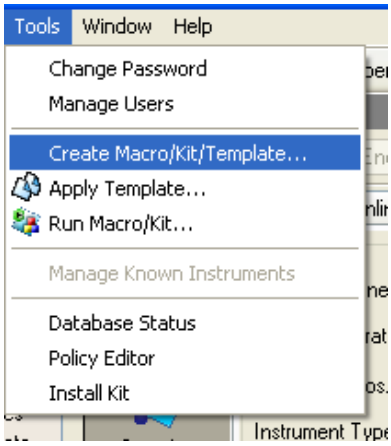
15. In **Program Temperature Targets** profile:
    a. Enter **40** for Target (◦C).
    b. Enter **00:20:00** for Hold (hh:mm:ss).
    c. Enter **20** for Ramp Rate (◦C/s).
    d. Enter **0** for Sec Target (◦C), Step Size (◦C), and Step Delay (cycles).
    e. Select **None** for Acquisition Mode.

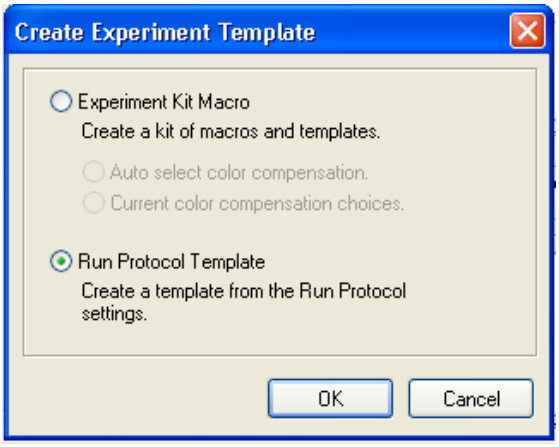


16. The total Time for experiment under Overview is about 1:53:38.

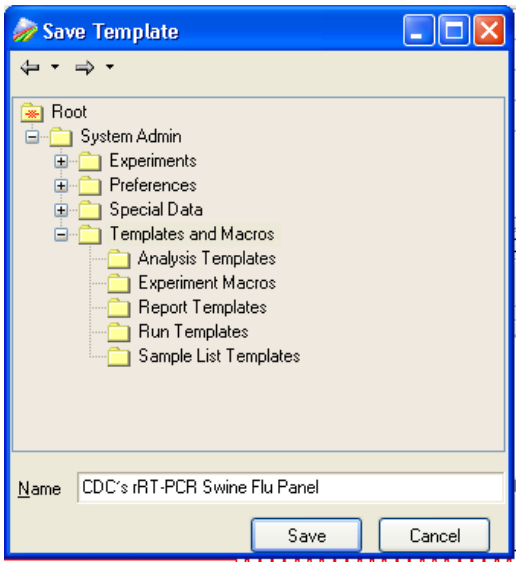


17. Go to **Tools**, select **Create Macro/Kit/Template**; a new window **Create Experiment Template** appears, highlight **Run Protocol Template** and click **OK**.





18. **Save Template** screen appears, highlight **Run Template** icon then enter **CDC rRT-PCR Swine Flu Panel** in the **Name** area, then click the **Save** icon.



19. The template is created and saved for future usage.

Running a Test on the LightCycler® 2.0

1. After login to the front page of LightCycler, click **New**, a new window **Create New Object** appears.

2. Highlight **LightCycler Experiment**, click **OK**.

3. A new Run window appears.

4. Click **Template** on top, a new window **Apply Template** appears.

5. Double click **Run templates** icon under **Template and Macros** to expand; click **Flu Protocol**; click **Open** to open the program.

6. Click **Samples**; a new window **Sample data** appears.

7. Under **Capillary View**, put in Numbers of the specimens for the run at **Sample Count**.

8. At Position 1, highlight Sample 1, put in specimen ID for Sample Name.

9. Repeat step 8 until all the specimen information have been put in.

10. Select **File** and **Save** from the drop down menu.

11. A new window **Save LightCycler Experiment** appears.

12. Highlight **Experiment**; enter run file name in the **Name** area, then click the **Save** icon.

13. **STOP!** Add carousel with samples to LightCycler® 2.0 and close cover.

14. Click **Start Run** on top left corner, the LightCycler® 2.0 should start running.

Data analysis using the LightCycler Data Analysis (LCDA)

1. Click **Analysis** on the main toolbar.

2. Select **Absolute Quantification**, then click **OK**.

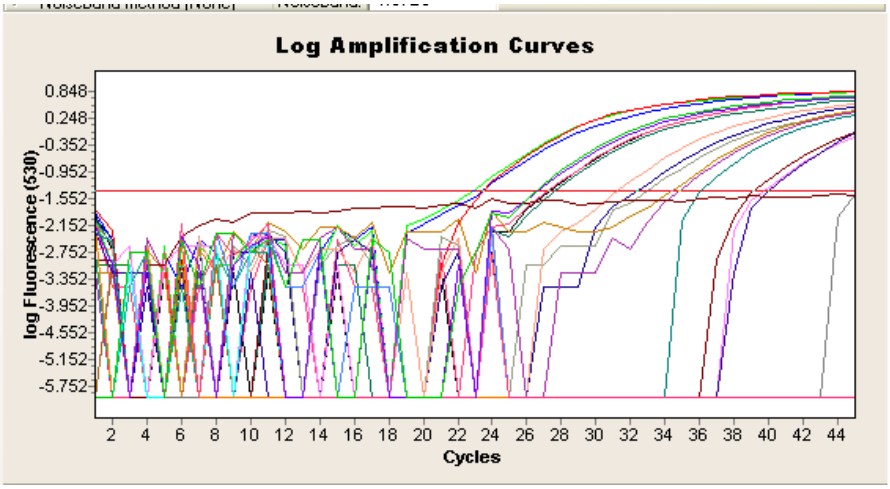
3. From the Channel (610) menu, select **530**.

4. From the Method (Auto) menu, select **Fit Points**.

5. Select the samples names to highlight the samples you want to analyze.

6. Select the **Step 1: Background** tab to view the amplification curves for the selected samples. By default, the background is set at **Arithmetic**. The curves are corrected so that the background fluorescence is the same for all the samples.

7. Select the **Step 2: Noise Band** tab. The jagged lines at the beginning of the curves represent noise. The Step2 Noise Band tab displays two graphs. The upper graph shows Fluorescence vs. Cycle Number. The graph contains a red horizontal line (the noise band) to delineate the background noise. The lower graph shows the same curves with the background noise eliminated.



8. To eliminate the noise from the samples, adjust the noise band to a position, as low as possible, but as high as necessary, where it clearly crosses all sample curves in the lower part of the log-linear phase. Only data points above the Noise Band are considered for analysis. Data that fall below the noise band are excluded from analysis.

9. Select the **Step 3: Analysis** tab.

10. From the Fit points menu, select the number of data points (the "fit points") used to generate the log-linear curves for the samples. By default, the fit points is set at "2".

11. To determine the crossing threshold for the samples, adjust the crossing line by dragging the red horizontal crossing line up or down

12. Click and drag the left border of the chart section to the right to display all the analysis results. The Ct is under CP column.

Interpretation and Reporting of Results

Extraction and Positive Control Results and Interpretation

No Template Control (NTC)
The NTC consists of using nuclease-free water in the rRT-PCR reactions instead of RNA. The NTC reactions for all primer and probe sets should not exhibit fluorescence growth curves that cross the threshold line. If any of the NTC reactions exhibit a growth curve that crosses the cycle threshold, sample contamination may have occurred. Invalidate the run and repeat the assay with stricter adherence to the guidelines.

Swine Influenza Positive Control (SIPC)
The SIPC consists of a swine influenza virus representing novel influenza A (H1N1) and cultured human cells (A549). Purified RNA from the SIPC will yield a positive result with the following primer and probe sets: InfA, swInfA, swH1, and RP.

Human Specimen Control (HSC) (Extraction Control)
The HSC control consists of noninfectious cultured human cell (A549) material. The HSC is used as a RNA extraction procedural control to demonstrate successful recovery of RNA as well as extraction reagent integrity. Purified RNA from the HSC should yield a positive result with the RP primer and probe set and negative results with all influenza specific markers.

Figure 4. Expected Performance of Controls Included in the CDC rRT-PCR Flu Panel

Control Type	Internal Control Name	Used to Monitor	InfA	swInfA	swH1	RP	Expected Ct Values
Positive	SIPC	Substantial reagent failure including primer and probe integrity	+	+	+	+	≤ 37 Ct
Negative	NTC	Reagent and/or environmental contamination	-	-	-	-	None detected
Extraction	HSC	Failure in lysis and extraction procedure	-	-	-	+	≤ 37 Ct

If the controls in assay do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

Specimen Results and Interpretation

RNase P (Extraction Control)

- All clinical samples should exhibit fluorescence growth curves in the RNase P (RP) reaction that cross the threshold line within 37 cycles, thus indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimens may indicate:
  - Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation
  - Absence of sufficient human cellular material due to poor collection or loss of specimen integrity
  - Improper assay set up and execution
  - Reagent or equipment malfunction
- If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:
  - If the InfA, swInfA, swH1 are positive even in the presence of a negative RP, the influenza result should be considered valid. It is possible, that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of influenza virus RNA in a clinical specimen.
  - If all influenza markers AND RNase P are all negative for the specimen, the assay is "inconclusive" for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as "inconclusive" and a new specimen should be collected if possible.
- The RP assay may be negative when testing virus culture samples.

Influenza Markers (InfA, swInfA, and swH1)

- When all controls exhibit the expected performance, a specimen is considered negative if influenza marker growth curves DO NOT cross the threshold line within 37 cycles and RNase P growth curve does cross the threshold line within 37 cycles.
- When all controls exhibit the expected performance and none of the growth curves for the influenza markers or RP marker cross the threshold line within 37 cycles, the result is "Inconclusive". The extracted RNA from the specimen should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the re-tested sample is negative for all markers and all controls exhibit the expected performance, the result is "Inconclusive."
- When all controls exhibit the expected performance, and growth curve for InfA influenza marker and one of the sw Inf markers (swInfA or swH1) cross the threshold line within 37 cycles but one of the growth curves for the sw Inf markers (swInfA or swH1) markers do not cross the threshold line within 37 cycles, the result is "Inconclusive". RP may be positive or negative as described above. The extracted RNA from the specimen should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test.

➢ When all controls exhibit the expected performance, and growth curve for InfA influenza marker does not cross the threshold line within 37 cycles but the growth curves for either or both sw Inf markers (swInfA and swH1) cross the threshold line within 37 cycles, the result is "Inconclusive". RP may be positive or negative as described above. The extracted RNA from the specimen should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test.

➢ When all controls exhibit expected performance, and when growth curve for InfA, marker crosses the threshold line within 37 cycles but the growth curves for the swInfA, and swH1 markers do not cross the threshold line within 37 cycles :

- Report the specimen to be "Positive Influenza A and negative for 2009 H1N1 influenza."

➢ When all controls exhibit expected performance, a specimen is considered positive for influenza 2009 H1N1 when growth curves for all InfA, swInfA, and swH1 markers cross the threshold line within 37 cycles:

- Report the specimen to be "Positive for 2009 H1N1 Influenza."

CDC rRT-PCR Swine Flu Panel Users Guide for Interpretation of Results - Quick Reference and Reporting

InfA	swInfA	swH1	RP	Interpretation	Report
-	-	-	+	No influenza virus detected	Influenza Not Detected (negative)
+	-	+	±	2009 Influenza H1	Inconclusive for 2009 H1N1 Influenza
+	+	-	±	2009 Influenza A	Inconclusive for 2009 H1N1 Influenza
-	±	±	±	2009 Influenza A and H1	Inconclusive for 2009 H1N1 Influenza
+	-	-	±	Influenza A	Positive for Influenza A, 2009 H1N1 Influenza not detected
+	+	+	±	2009 A/H1N1	Positive for 2009 H1N1 Influenza

Please follow most current recommendations for reporting novel influenza A (H1N1) diagnostic results to state/local authorities.

To refer a specimen to the CDC, the following shipping instructions should be followed:

- Ship all specimens and related RNA overnight to CDC.
- Ship frozen specimens on dry ice and non-frozen specimens on cold packs. Ship extracted RNA on dry ice.
- Refer to the International Air Transport Association (IATA - www.iata.org) for requirements for shipment of human or potentially infectious biological specimens.
- Prior to shipping, notify CDC Influenza Division (see contact information below) that you are sending specimens.
- Send all samples to the following recipient:

Alexander Klimov
Chief, Virus Surveillance and Diagnosis Branch Influenza Division
Centers for Disease Control and Prevention
c/o DASH, MS G-16
Attention: Dr. Stephen Lindstrom
1600 Clifton Rd., Atlanta, GA 30333
Phone: (404) 639-3387 or (404) 639-3591
Fax: (404) 639-2334

The emergency contact number for CDC is 770-488-7100.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity
Note: It has been demonstrated that the CDC rRT-PCR Swine Flu Panel has similar analytical performance to the CDC rRT-PCR Flu Panel (IVD, K080570). In side-by-side testing, the primer and probe sets have equivalent limit of detection ranges.

Limit of Detection Study (Upper Respiratory Specimens)

Influenza virus was grown and harvested from tissue culture virus preparations. at the CDC Influenza Division. Fifty percent (50%) infectious dose (TCID50 /mL) virus titer determinations were performed following procedures utilized by the CDC Influenza Division Virus Surveillance and Diagnosis Branch.

The following influenza strains were characterized and used for the limit of detection study:

- A/Iowa/1/2006 Swine H1N1.

LoD Study for the rRT-PCR Swine Flu Panel along with the IVD InfA Markers on the ABI 7500 Fast Dx PCR System

Virus Strain Tested	Analyte Tested	Stock Virus Titer	Serial 10-Fold Dilution Factor	TCID 50/mL Dilution Tested	Call Rate	Run 1 Ct	Run 2 Ct	Run 3 Ct	Run 4 Ct	Run 5 Ct	Avg. Ct (n=5)	Lowest Conc. with Uniform Positivity per Analyte	Limit of Detection (LoD) per Virus Strain
A/Iowa/1/2006 (swine A/H1N1)	IVD Inf A	$10^{9.0}$ $TCID_{50}$/mL	10e3	$10^{6.0}$	5/5	26.50	26.89	27.03	26.22	26.13	26.55	$10^{3.0}$ $TCID_{50}$/mL (36.19 Ct)	$10^{3.0}$ $TCID_{50}$/mL
			10e4	$10^{5.0}$	5/5	30.40	31.04	31.65	30.07	30.32	30.70		
			10e5	$10^{4.0}$	5/5	34.21	33.24	33.22	33.62	33.06	33.47		
			10e6	$10^{3.0}$	5/5	36.70	35.53	36.21	36.07	36.46	36.19		
			10e7	$10^{2.0}$	4/5	0.00	39.00	40.47	38.69	37.61	na		
			10e8	$10^{1.0}$	1/5	0.00	0.00	0.00	0.00	39.62	na		
			10e9	10^{0}	0/5	0.00	0.00	0.00	0.00	0.00	na		
	Sw InfA	$10^{9.0}$ $TCID_{50}$/mL	10e3	$10^{6.0}$	55	23.01	23.07	22.87	23.05	23.19	23.04	$10^{2.0}$ $TCID_{50}$/mL (35.48 Ct)	
			10e4	$10^{5.0}$	5/5	27.26	26.84	26.72	26.77	26.78	26.87		
			10e5	$10^{4.0}$	5/5	30.16	30.85	29.87	29.96	30.01	30.17		
			10e6	$10^{3.0}$	5/5	33.19	33.37	33.63	33.25	33.35	33.36		
			10e7	$10^{2.0}$	5/5	38.32	37.10	37.56	36.67	37.28	37.39		
			10e8	$10^{1.0}$	1/5	0.00	0.00	0.00	0.00	42.77	Na		
			10e9	10^{0}	0/5	0.00	0.00	0.00	0.00	0.00	Na		
	Sw H1	$10^{9.0}$ $TCID_{50}$/mL	10e3	$10^{6.0}$	5/5	27.07	27.56	27.35	27.35	27.17	27.30	$10^{3.0}$ $TCID_{50}$/mL (35.48 Ct)	
			10e4	$10^{5.0}$	5/5	31.02	31.06	30.98	30.75	30.48	30.86		
			10e5	$10^{4.0}$	5/5	34.03	33.33	33.43	34.01	33.69	33.70		
			10e6	$10^{3.0}$	5/5	37.24	36.66	36.29	36.62	36.81	36.72		

			10e7	$10^{2.0}$	2/5	0.00	38.40	40.37	0.00	0.00	Na	
			10e8	$10^{1.0}$	0/5	0.00	0.00	0.00	0.00	0.00	Na	
			10e9	10^{0}	0/5	0.00	0.00	0.00	0.00	0.00	Na	

RNA was extracted with the Qiagen QIAamp® Viral RNA Purification kit from each of the above characterized viruses. Ten-fold serial dilutions of the characterized influenza viruses were tested on the ABI 7500 Fast Dx PCR System to identify an end-point for detection with each primer and probe set included in the rRT-PCR Swine Flu Panel.

The rRT-PCR Swine Flu Panel demonstrated limits of detection similar to that of the InfA primer and probe set in the CDC rRT-PCR Flu Panel (IVD). Please see performance data submitted in 510(k) 080705 cleared by FDA Sept. 30, 2008.

Limit of Detection using the Roche LightCycler® 2.0 Real-Time PCR system

Ten fold serial dilutions of the characterized swine-like influenza A/H1N1 A/California/04/2009 virus were tested to identify an end point for detection with each primer and probe set included in the CDC rRT-PCR Swine Flu Panel. A preliminary range-finding experiment was performed to determine the approximate end point dilution. Subsequently, this dilution and two (2) ten-fold dilutions above and below were tested in triplicate (n=3) for each primer and probe set on both the Applied Biosystems 7500 Fast Dx Real-Time PCR and the Roche LightCycler® 2.0 Real-Time PCR systems. The LoD for each primer and probe set (InfA, swInfA, and swH1) was calculated to indicate the range of lowest detectable concentration of influenza virus ($TCID_{50}$/mL).

Side-by-Side LoD Study for the CDC rRT-PCR Swine Flu Panel on the Applied Biosystems 7500 Fast Dx Real-Time PCR System and Roche LightCycler® 2.0 Systems

Virus Strain Tested	Analyte Tested	Stock Virus Titer	Serial 10-Fold Dilution Factor	TCID 50/mL Dilution Tested	ABI 7500 Fast Run 1 Ct	ABI 7500 Fast Run 2 Ct	ABI 7500 Fast Run 3 Ct	Light Cycler® 2.0 Run 1 Ct	Light Cycler® 2.0 Run 2 Ct	Light Cycler® 2.0 Run 3 Ct	Lowest Conc. with Uniform Positivity per Analyte	Limit of Detection (LoD)
A/CA/04/09 swine Cx/C2 passage 4/27/09 batch (swine A/H1N1)	IVD Inf A	$10^{7.6}$ $TCID_{50}$/mL	10e3	$10^{4.6}$	26.4	26.16	26.08	24.39	23.84	24.22	$10^{1.6}$ $TCID_{50}$/mL	$10^{1.6}$ $TCID_{50}$/mL
			10e4	$10^{3.6}$	30.16	30.01	29.59	28.31	28.18	28.58		
			10e5	$10^{2.6}$	33.5	33.38	33.09	33.44	33.98	32.23		
			10e6	$10^{1.6}$	35.9	36.9	35.9	35.77	35.43	36.78		
			10e7	$10^{0.6}$	Undet	40.88	37.85	40.1	Undet	40.73		
			10e8		Undet	Undet	Undet	Undet	40.8	Undet		
			10e9		Undet	Undet	Undet	Undet	Undet	Undet		
	Sw InfA	$10^{7.6}$ $TCID_{50}$/mL	10e3	$10^{4.6}$	29.68	29.76	28.93	23.92	24.48	23.60	$10^{1.6}$ $TCID_{50}$/mL	
			10e4	$10^{3.6}$	33.95	34.49	34.10	28.50	29.13	28.27		
			10e5	$10^{2.6}$	37.66	39.67	33.95	33.72	33.35	32.24		

			10e6	$10^{1.6}$	41.11	41.71	39.47	35.37	36.9	37.42	
			10e7	$10^{0.6}$	42.55	44.63	43.00	Undet	Undet	Undet	
			10e8		Undet	Undet	Undet	Undet	Undet	Undet	
			10e9		Undet	Undet	Undet	Undet	Undet	Undet	
	Sw H1	$10^{7.6}$ $TCID_{50}/mL$	10e3	$10^{4.6}$	27.39	28.31	28.05	26.37	26.02	26.33	$10^{0.6}$ $TCID_{50}/mL$
			10e4	$10^{3.6}$	32.06	31.68	31.81	29.63	29.77	29.75	
			10e5	$10^{2.6}$	35.13	34.75	34.94	33.14	33.23	33.09	
			10e6	$10^{1.6}$	38.17	38.41	37.45	36.74	36.33	36.41	
			10e7	$10^{0.6}$	39.86	39.26	41.89	39.16	38.88	40.77	
			10e8		Undet	Undet	Undet	Undet	Undet	41.39	
			10e9		Undet	Undet	Undet	Undet	Undet	Undet	

The IVD Inf A and the Sw H1 primers and probes included in the CDC rRT-PCR Swine Flu Panel demonstrated equivalent limits of detection on both the Applied Biosystems 7500 Fast Dx Real-Time PCR System and the Roche LightCycler® 2.0 Real-Time PCR systems

Limit of Detection (Lower Respiratory Specimens)

The following influenza strain was characterized and used for the limit of detection study:

A/California/04/2009 – swine-like influenza A/H1N1 strain

Analytical sensitivity was demonstrated by determining the limit of detection (LoD) of each primer and probe set in the rRT-PCR Swine Flu Panel in a side by study on both the ABI 7500 Fast Dx and the Roche LightCycler® 2.0 Real-Time PCR systems. Ten-fold serial dilutions of the 2009 A/H1N1 influenza virus strain was tested to identify an end-point for detection of each primer and probe set included in the rRT-PCR Swine Flu Panel on both instruments. RNA was extracted with the Qiagen QIAamp® Viral RNA Purification kit from each of the characterized viruses. The limit of detection for each primer and probe set (InfA, swInfA, and swH1) was calculated to indicate the range of lowest detectable concentration of influenza virus (EID_{50}/ml) at which ≥95% of all replicates tested positive. The lowest concentration of influenza virus detected was determined to be the end-point concentration where the type and subtype primer and probe sets had uniform detection on both instruments. If the two end-points differed in concentration the higher (or limiting) point was used.

Table 1. Summary of the results from the limit of detection study for each primer and probe set utilizing the characterized reference influenza novel H1N1 virus on both the ABI 7500 Fast Dx and the Roche LightCycler® 2.0 Real-Time PCR systems.

Influenza Virus Tested	Influenza Strain Designation	LIMIT OF DETECTION (EID_{50}/ML)
2009 A/H1N1	A/California/04/2009 Swine-like influenza A/H1N1 strain	$10^{2.4}$ EID_{50}/mL

Analytical specificity

Analytical Specificity was demonstrated by testing characterized human A/H1 (10) and human A/H3 (10) influenza virus with both the rRT-PCR Flu Panel (IVD) and the rRT-PCR Swine Flu Panel (IUO-EUA). The purified viral RNA was analyzed with each marker (analyte) in the each panel using the Applied Biosystems 7500 Fast Dx Real-Time PCR System.

Analytical Specificity of the rRT-PCR Swine Flu Panel with Human Influenza A/H1 Virus (Cross Reactivity)

Influenza Strain Tested	$TCID_{50}$/ml	ABI 7500 Fast Real time RT-PCR				Expected Result	Actual Result
		IVD Inf A	IVD H1	Sw InfA	Sw H1		
A/BANGLADESH/7286/2007	6.1	13.53	14.04	neg	neg	Human A/H1	Human A/H1
A/BEIJING/262/95	6.0	11.71	12.08	neg	neg	Human A/H1	Human A/H1
A/FUKUSHIMA/141/06	5.7	17.49	16.65	neg	neg	Human A/H1	Human A/H1
A/HAWAII/15/2001*	6.6 EID_{50}	15.67	16.02	neg	neg	Human A/H1	Human A/H1
A/JIANGXI/160/05	5.6	13.32	13.98	neg	neg	Human A/H1	Human A/H1
A/South Dakota/6/2007	8.2 EID_{50}	13.73	13.47	neg	neg	Human A/H1	Human A/H1
A/MEXICO/1729/2007	4.8	13.67	14.18	neg	neg	Human A/H1	Human A/H1
A/NEW CALEDONIA/20/1999*	6.6 EID_{50}	12.73	13.42	neg	neg	Human A/H1	Human A/H1
A/SOLOMON ISLANDS/3/06	6.2	15.12	14.3	neg	neg	Human A/H1	Human A/H1
A/Brisbane/59/2007	8.4 EID_{50}	12.83	13.15	neg	neg	Human A/H1	Human A/H1
Seasonal Positive Control		24.99	29.89	neg	neg	Human A/H1	Human A/H1
Swine Positive Control		neg	neg	20.46	25.04	Sw A/H1N1	Sw A/H1N1

Analytical Specificity of the rRT-PCR Swine Flu Panel with Human Influenza A/H3 Virus (Cross Reactivity)

Influenza Strain Tested	$TCID_{50}$/ml	ABI 7500 Fast Real time RT-PCR				Expected Result	Actual Result
		IVD Inf A	IVD H3	Sw InfA	Sw H3		
A/ANHUI/1239/2005	8.1	11.94	11.45	neg	neg	Human A/H3	Human A/H3
A/AFGHANISTAN/2903/2008	5.0	14.79	15.73	neg	neg	Human A/H3	Human A/H3
A/BRISBANE/10/2007	6.8	11.72	12.58	neg	neg	Human A/H3	Human A/H3
A/HAWAII/08/2006	7.8	11.6	11.88	neg	neg	Human A/H3	Human A/H3

A/MEXICO/1842/2007	6.1	13.37	14.36	neg	neg	Human A/H3	Human A/H3
A/NEW YORK/55/2004*	6.4 (EID_{50})	12.8	13.00	neg	neg	Human A/H3	Human A/H3
A/WISCONSIN/67/2005*	6.5 (EID_{50})	14.51	14.79	neg	neg	Human A/H3	Human A/H3
A/Uruguay/716/2007	8.2 (EID_{50})	11.78	11.69	neg	neg	Human A/H3	Human A/H3
A/Taiwan/760/2007	5.5	10.99	11.06	neg	neg	Human A/H3	Human A/H3
A/British Columbia/RV1287/2007	6.0	12.77	12.89	neg	neg	Human A/H3	Human A/H3
Seasonal Positive Control		23.29	23.36	neg	neg	Human A/H3	Human A/H3
Swine Positive Control		neg	neg	20.26	24.72	Sw A/H1N1	Sw A/H1N1

Clinical Seasonal Specimens Tested with rRT-PCR Flu Panel (IVD) and rRT-PCR Swine Flu Panel

CDC Sample ID#	Clinical Specimen	Real-time RT-PCR (ABI 7500 Fast)						Result
		IVD InfA	IVD H1	IVD H3	RNP	sw InfA	sw H1	
2008726969	Original	24.04	25.57	Neg	28.02	Neg	Neg	human AH1
2008726970	Original	24.00	26.05	Neg	23.65	Neg	Neg	human AH1
2008726971	Original	17.55	19.65	Neg	23.64	Neg	Neg	human AH1
2008726972	Original	18.19	29.84	Neg	20.68	Neg	Neg	human AH1
2008726973	Original	19.98	22.80	Neg	19.68	Neg	Neg	human AH1
2008726974	Original	21.84	24.00	Neg	21.79	Neg	Neg	human AH1
2008726975	Original	30.42	30.04	Neg	22.76	Neg	Neg	human AH1
2008726976	Original	15.99	17.97	Neg	23.00	Neg	Neg	human AH1
2008726977	Original	26.18	27.71	Neg	26.10	Neg	Neg	human AH1
2008726978	Original	21.90	23.74	Neg	23.34	Neg	Neg	human AH1
2008726979	Original	18.27	20.43	Neg	20.75	Neg	Neg	human AH1
2008726980	Original	29.62	29.70	Neg	25.31	Neg	Neg	human AH1
2008726981	Original	25.92	26.69	Neg	21.86	Neg	Neg	human AH1
2008726982	Original	29.12	31.80	Neg	22.89	Neg	Neg	human AH1
2008726983	Original	17.50	18.86	Neg	20.90	Neg	Neg	human AH1
2008726984	Original	29.35	29.94	Neg	28.55	Neg	Neg	human AH1
2008726985	Original	25.94	26.80	Neg	21.66	Neg	Neg	human AH1
2008726986	Original	Neg	Neg	Neg	19.97	Neg	Neg	negative
2008726987	Original	17.44	18.08	Neg	21.83	Neg	Neg	human AH1
20087260988	Original	16.88	18.71	Neg	22.94	Neg	Neg	human AH1
2008726989	Original	27.35	28.62	Neg	23.88	Neg	Neg	human AH1
2008726990	Original	21.50	22.55	Neg	25.29	Neg	Neg	human AH1

The analytical specificity (reactivity) testing showed that the primer and probe sets within the rRT-PCR Swine Flu Panel performed as expected with seasonal human influenza type A/H1 and A/H3 viruses with no cross detection or non-specific binding to those targets. No cross reactivity was observed when human seasonal influenza A/H1 and A/H3 clinical specimens and cultured viruses were tested.

Please see performance data submitted in 510(k) 080705 cleared by FDA Sept. 30, 2008 for InfA primer and probe set specificity.

Clinical Performance

Retrospective testing was performed on 70 specimens (20 positive for 2009 H1N1 influenza and 50 negative for 2009 H1N1 influenza) representing nasopharyngeal swabs, nasal swabs, throat swabs, and throat wash specimens). Fourteen specimens of the 50 specimens negative for 2009 H1N1 influenza were positive for either seasonal influenza A/H1 or A/H3. Thirty-six of the 50 2009 H1N1 influenza negative specimens were also negative for seasonal A/H1 and A/H3.

Results from Retrospective Testing of Clinical 2009 H1N1 Influenza Specimens

Table 2. Results from Retrospective Testing of 2009 H1N1 Influenza Specimens-InfA

		ABI 7500 Fast Dx CDC rRT-PCR Swine Flu Panel			
		Influenza A Positive	Influenza A Negative	Total	
Light Cycler® 2.0 CDC rRT-PCR Swine Flu Panel	Influenza A Positive	34	0	34	100 % Positive Agreement (89.72%-100.0%) 95% CI
	Influenza A Negative	0	36	36	100 % Negative Agreement (90.36%- 100.0%) 95% CI
	Total	34	36	70	

Table 3. Results from Retrospective Testing of 2009 H1N1 Influenza Specimens-swInfA

		ABI 7500 Fast Dx CDC rRT-PCR Swine Flu Panel			
		Swine Influenza A Positive	Swine Influenza A Negative	Total	
Light Cycler® 2.0 CDC rRT-PCR Swine Flu Panel	Swine Influenza A Positive	20	0	20	100 % Positive Agreement (83.16%-100.0%) 95% CI
	Swine Influenza A Negative	0	50	50	100 % Negative Agreement (92.89%- 100.0%) 95% CI
	Total	20	50	70	

Table 4. Results from Retrospective Testing of Swine-like A/H1N1 Influenza Specimens-swH1

		ABI 7500 Fast Dx CDC rRT-PCR Swine Flu Panel			
		Swine Influenza H1 Positive	Swine Influenza H1 Negative	Total	
Light Cycler® 2.0 CDC rRT-PCR Swine Flu Panel	Swine Influenza H1 Positive	20	0	20	100.0 % Positive Agreement (83.16%-100.0%) 95% CI
	Swine Influenza H1 Negative	0	50	50	100.0 % Negative Agreement (92.89%-100.0%) 95% CI
	Total	20	50	70	

Clinical sensitivity for the swine influenza swInfA and swH1 primer and probe sets was 100% for the A/H1N1 swine influenza virus when present in clinical specimens.

Clinical Performance of the CDC rRT-PCR Swine Flu Panel with Lower Respiratory Specimens

Performance characteristics of the CDC rRT-PCR Swine Flu Panel with human lower respiratory tract specimens were established during surveillance testing at the CDC Influenza Division laboratory. Specimens used for this study were collected for influenza surveillance testing at U.S. public health laboratories and referred to CDC for confirmational testing during the 2009 H1N1 influenza pandemic. Specimens were tested at the CDC with the CDC rRT-PCR Swine Flu Panel on the ABI 7500 Fast Dx Real-Time PCR system followed by virus culture. The specimens have since been retrospectively tested with the CDC rRT-PCR Swine Flu Panel with the Roche LightCycler® 2.0 system. Each lower respiratory specimen was tested with the CDC rRT-PCR Swine Flu Panel (InfA, swInfA, and swH1) markers since the onset of the 2009 H1N1 influenza pandemic.

Retrospective testing was performed on 20 lower respiratory tract specimens from hospitalized patients referred to CDC by U.S. public health laboratories for testing during the 2009 H1N1 influenza pandemic. The specimens tested represent the following: 12 bronchoalveolar lavage (BAL), 2 bronchial washes (BW), 2 endotracheal aspirates (EA), 2 lung tissues, and 2 endotracheal swabs (ES). Sixteen (16) specimens of the lower respiratory tractwere positive for 2009 H1N1 influenza and four specimens were negative for seasonal or 2009 H1N1 influenza.

Shown below is performance data generated on both the ABI 7500 Fast Dx (prospective) and the Roche LightCycler® 2.0 (retrospective) Real-Time PCR systems using the CDC rRT-PCR Swine Flu panel.

Table 5. Results from Prospective Testing of Lower Respiratory Clinical Swine Influenza Specimens with the ABI 7500 Fast Dx Real-Time PCR System and the CDC rRT-PCR Swine Flu Panel with Virus Culture Reference Method - InfA

		Virus Culture Reference Method			
		Influenza A Positive	Influenza A Negative	Total	
ABI 7500 Fast Dx Real-time RT-PCR Results	Influenza A Positive	9	2	11	81.8 % Positive Agreement (48.2%-97.7%) 95% CI
	Influenza A Negative	0	0	0	
	Total	9	2	11	

Table 6. Results from Prospective Testing of Lower Respiratory Clinical Swine Influenza Specimens with the ABI 7500 Fast Dx Real-Time PCR System and the CDC rRT-PCR Swine Flu Panel with Virus Culture Reference Method - swInfA

		Virus Culture Reference Method			
		Swine Influenza A Positive	Swine Influenza A Negative	Total	
ABI 7500 Fast Dx Real-time RT-PCR Results	Swine Influenza A Positive	9	2	11	81.8 % Positive Agreement (48.2%-97.7%) 95% CI
	Swine Influenza A Negative	0	0	0	
	Total	9	2	11	

Table 7. Results from Prospective Testing of Lower Respiratory Clinical Swine Influenza Specimens with the ABI 7500 Fast Dx Real-Time PCR System and the CDC rRT-PCR Swine Flu Panel with Virus Culture Reference Method-swH1

		Virus Culture Reference Method			
		Swine Influenza H1 Positive	Swine Influenza H1 Negative	Total	
ABI 7500 Fast Dx Real-time RT-PCR Results	Swine Influenza H1 Positive	9	2	11	81.8 % Positive Agreement (48.2%-97.7%) 95% CI
	Swine Influenza H1 Negative	0	0	0	
	Total	9	2	11	

Table 8. Results from Retrospective Testing of Lower Respiratory Clinical Swine Influenza Specimens with the Roche LightCycler® 2.0 and the CDC rRT-PCR Swine Flu Panel with the Applied Biosystems 7500 Fast Dx Real-Time PCR System Comparator Only - InfA

		ABI 7500 Fast Dx CDC rRT-PCR Swine Flu Panel			
		Influenza A Positive	Influenza A Negative	Total	
Light Cycler® 2.0 CDC rRT-PCR Swine Flu Panel	Influenza A Positive	15	1	16	93.75 % Positive Agreement (69.8%-99.8%) 95% CI
	Influenza A Negative	0	4	4	100 % Negative Agreement (39.76%- 100.0%) 95% CI
	Total	15	5	20	

Table 9. Results from Retrospective Testing of Lower Respiratory Clinical Swine Influenza Specimens with the Roche LightCycler® 2.0 and the CDC rRT-PCR Swine Flu Panel with the ABI 7500 Fast Dx Real-Time PCR System Comparator Only - swInfA

		ABI 7500 Fast Dx CDC rRT-PCR Swine Flu Panel			
		Swine Influenza A Positive	Swine Influenza A Negative	Total	
Light Cycler® 2.0 CDC rRT-PCR Swine Flu Panel	Swine Influenza A Positive	15	1	16	93.75 % Positive Agreement (69.8%-99.8%) 95% CI
	Swine Influenza A Negative	0	4	4	100 % Negative Agreement (39.76%- 100.0%) 95% CI
	Total	15	5	20	

Table 10. Results from Retrospective Testing of Lower Respiratory Clinical Swine Influenza Specimens with the Roche LightCycler® 2.0 and the CDC rRT-PCR Swine Flu Panel with the ABI 7500 Fast Dx Real-Time PCR System Comparator Only - swH1

		ABI 7500 Fast Dx CDC rRT-PCR Swine Flu Panel			
		Swine Influenza H1 Positive	Swine Influenza H1 Negative	Total	
Light Cycler® 2.0 CDC rRT-PCR Swine Flu Panel	Swine Influenza H1 Positive	15	0	15	100.0 % Positive Agreement (78.2%-100.0%) 95% CI
	Swine Influenza H1 Negative	0	5	5	100.0 % Negative Agreement (47.8%-100.0%) 95% CI
	Total	15	5	20	

Technical Support

For technical and product support, contact the CDC Influenza Division Support team directly.

Send email to: FluSupport@cdc.gov

Ordering

Send email request to: FluOrder@cdc.gov

Include the following information in the message:

- Laboratory Name and Address
- CDC Qualified Contact Person, Phone Number, Email Address, and Shipping Address
- Qualification Date

CDC Swine Influenza Virus Real-time RT-PCR Detection Panel (rRT-PCR Swine Flu Panel)

Instructions for Use

Applied Biosystems 7500 Fast Dx Real-time PCR Instrument
with the SDS Software version 1.4

1000 reactions

Emergency Use Authorization Only

Centers for Disease Control and Prevention
Influenza Division
1600 Clifton Rd NE
Atlanta GA 30333

Intended Use

The Swine Influenza Virus Real-time RT-PCR Detection Panel is intended for use in Real-time RT-PCR assays on an Applied Biosystems 7500 Fast Dx Real-Time PCR System and the Roche LightCycler® 2.0 Real-time PCR system in conjunction with clinical and epidemiological information:

- To identify patients who may be infected with 2009 H1N1 influenza virus to allow public health authorities to respond to and limit transmission of the virus during this public health emergency;
- For the qualitative detection of influenza virus type A in viral RNA in upper respiratory tract clinical 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)), and lower respiratory tract specimens (such as broncheoalveolar lavage (BAL), bronchial aspirate – BA; bronchial wash – BW; endotracheal aspirate – EA; endotracheal wash – EW; tracheal aspirate – TA, and lung tissue) from human patients with signs and symptoms of respiratory infection and/or from viral culture;
- For identification of virus in patients who may be infected with the 2009 H1N1 influenza virus from viral RNA in upper respiratory tract clinical specimens (NPS, NS, TS, NA, NW, and dual NPS/TS), and lower respiratory tract specimens (such as BAL, BA, BW, EA, EW, TA, and lung tissue) from human patients with signs and symptoms of respiratory infection and viral culture, in conjunction with clinical and epidemiological risk factors;
- To provide epidemiologic information for surveillance for the 2009 H1N1 influenza virus.

Testing with the rRT-PCR Swine Flu Panel should not be performed unless the patient meets clinical and/or epidemiologic criteria for testing suspect specimens.
The identification of 2009 H1N1 influenza A should be performed along with clinical and epidemiological assessment.

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

Use is limited to designated public health laboratories and other laboratories qualified to receive and use the CDC rRT-PCR Flu Panel (IVD) K080570

Summary and Explanation

Infections with the 2009 H1N1 influenza virus continue to cause illness and death among persons worldwide. As of August 2009, confirmed cases of novel influenza A (H1N1) virus infections had been reported in all 50 states in the United States and territories and additional cases abroad (for more information, visit http://www.cdc.gov/h1n1flu/). The infections with the 2009 H1N1 influenza virus and with the seasonal influenza A and B viruses in humans, may caused upper and lower and respiratory tract illness.

The 2009 H1N1 influenza virus infection may cause complications and severe lower respiratory tract disease in critically ill patients who are hospitalized and placed on mechanical ventilation for respiratory failure, high and prolonged viral shedding may be present in the lower respiratory tract but low or absent in the upper respiratory tract. Data from autopsy findings in lung tissues and other lower respiratory specimens from fatal human cases have confirmed the presence of 2009 H1N1 influenza virus in the lower respiratory tract.

Principles of the Procedure

The rRT-PCR Swine Flu Panel is based on real-time reverse transcriptase polymerase chain reaction (rRT-PCR) technology. The rRT-PCR assays are one-tube assays that first reverse-transcribe specific regions of RNA into cDNA copies. The cDNA then serves as a template for a polymerase chain reaction (PCR) that utilizes a thermocyclic heating and cooling of the reaction to logarithmically amplify a specific region of DNA. The probe anneals to a specific, internal target sequence located between the target loci of the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades any probe molecules hybridized to amplified target sequence, causing the reporter dye to separate from the quencher dye, and generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle. Amplification of targets is reflected by logarithmic increase in fluorescence over time in comparison to background signal.

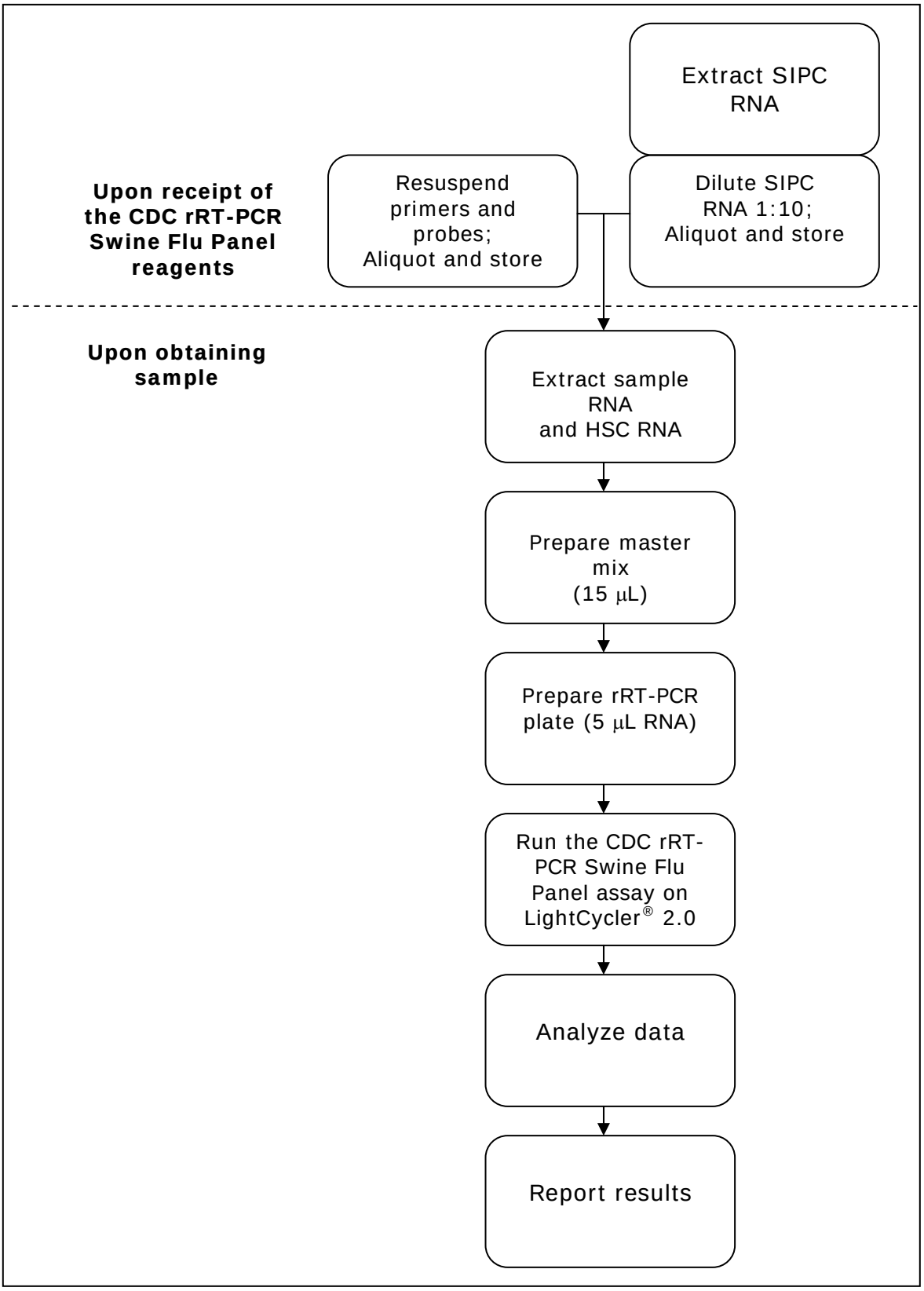
The rRT-PCR Swine Flu Panel includes four (4) sets of oligonucleotide primers and dual-labeled hydrolysis (Taqman®) probes to be used in real-time RT-PCR assays for the in vitro qualitative detection and characterization of 2009 H1N1 influenza virus in human respiratory specimens.

The influenza A (InfA) primer and probe sets are designed for universal detection of type A influenza viruses. Influenza A subtyping primer and probe sets swInfA and swH1 are designed to specifically detect the 2009 H1N1 influenza virus.

User Testing Options	Panel Sets to Use	Expected Outcome
#1. To detect influenza type A only in clinical specimens	InfA (Primer and Probe Set)	Influenza A (+ or -)
#2. To detect influenza 2009 A/H1N1 subtype in clinical specimens	InfA (Primer and Probe Set) swInfA (Primer and Probe Set) swH1 Primer and Probe Set)	Influenza 2009 A/H1N1 (+ or -)

Note: Options 1 and 2 can be performed at the user's discretion independently or simultaneously.

Summary of Influenza Testing Process



Materials Provided

Box 1: Detection Kit

(Store in PCR Reagent Preparation Area)

Label	*Description*	*Quantity / Tube*	*Reactions / Tube*
InfA-F	Influenza A Forward Primer	20 nmol	1000
InfA-R	Influenza A Reverse Primer	20 nmol	1000
swInfA-F	Swine Influenza A Forward Primer	20 nmol	1000
swInfA-R	Swine Influenza A Reverse Primer	20 nmol	1000
swH1-F	Swine Influenza H1 Forward Primer	20 nmol	1000
swH1-R	Swine Influenza H1 Reverse Primer	20 nmol	1000
RP-F	RNase P Forward Primer	20 nmol	1000
RP-R	RNase P Reverse Primer	20 nmol	1000
RP-P	RNase P Probe	5 nmol	1000
InfA-P	Influenza A Probe	5 nmol	1000
swInfA-P	Swine Influenza A Probe	5 nmol	1000
swH1-P	Swine Influenza H1 Probe	5 nmol	1000

Box 2: Positive Control

(Store in RNA Handling Area)

Reagent Label	*Description*	*Qty. / Tube*	*Reactions / Tube*
SIPC	**Swine Influenza Virus Positive Control (SIPC):** For use as a positive control with the CDC rRT-PCR Swine Flu Panel procedure to ensure the detection of the 2009 H1N1 influenza virus. Contains noninfectious (beta-propiolactone inactivated) positive control materials (influenza virus preparation) suspended in 0.01 M phosphate buffer saline (PBS) at pH 7.2–7.4. The SIPC consists of influenza virus representing 2009 H1N1 influenza and cultured human cells (A549). The SIPC will yield a positive result with the following primer and probe sets: InfA, swInfA, swH1, and RP.	1–500 µL tubes	1000

Box 3: Human Specimen Extraction Control Kit

(Store in Nucleic Acid Extraction Room)

Reagent Label	*Description*	*Qty. / Tube*	*Reactions / Tube*
HSC	**Human Specimen Control (HSC):** For use as a RNA extraction procedural control with the CDC rRT-PCR Flu Panel procedure to demonstrate successful recovery of RNA as well as extraction reagent integrity. Purified RNA from the Human Specimen Control material should yield a positive result with the RP primer and probe set and negative results with all influenza specific markers. The HSC consists of noninfectious (beta propiolactone inactivated) cultured human cell material supplied as a liquid suspended in 0.01 M PBS at pH 7.2–7.4.	17–500 µL tubes	5–1000 extractions

Materials Required But Not Provided

Ancillary Reagents Required BUT NOT Provided

Specific lots for the ancillary reagents listed below will be qualified for use with the CDC rRT-PCR Swine Flu Panel by CDC Influenza Division quality control testing and lot qualification program.

The rRT-PCR Swine Flu Panel test performance requires that only qualified ancillary reagent lots be used with the device. Any lots not specifically qualified by the CDC Influenza Division for use with the rRT-PCR Swine Flu Panel are not valid for use with this device, and may affect device performance.

A supplemental cumulative list of qualified ancillary reagents lots for use with the rRT-PCR Swine Flu Panel is provided with each shipment or can be requested by sending an email to FluSupport@cdc.gov

Use ancillary reagents only with the instructions for use contained within this package insert instructions for use. Discard any instructions for use that may be packaged with these ancillary reagents.

Any issues related to assay performance or test failure that are suspected to involve ancillary reagents should be reported to the CDC Influenza Division by emailing FluSupport@cdc.gov

	Reagent	Quantity	Catalog No.
rRT-PCR Enzyme Mastermix Options	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (without Rox)	100 reactions	11732-020
		500 reactions	11732-088
	Invitrogen SuperScript™ III Platinum® One-Step Quantitative RT-PCR System (with Rox)	100 reactions	11745-100
		500 reactions	11745-500
Nucleic Acid Purification Kit Options	Qiagen QIAamp® Viral RNA Mini Kit * (Qiagen Inc., Valencia, CA)	50 extractions	52904
		250 extractions	52906
	Qiagen RNeasy® Mini Kit * (Qiagen Inc., Valencia, CA)	50 extractions	74104
		250 extractions	74106
	Roche MagNA Pure LC Total Nucleic Acid Kit * (Roche Applied Science, Indianapolis, IN) **Protocol:** External Lysis Protocol	192 extractions	03 038 505 001
	Roche MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche Applied Science, Indianapolis, IN) *** Protocol:** External Lysis Protocol	32 extractions	03 730 964 001
	Roche MagNA Pure Compact RNA Isolation Kit * (Roche Applied Science, Indianapolis, IN) **Protocol:** RNA _ Tissue_V3_1 Protocol	32 extractions	04 802 993 001

These products require reagents and consumables per the manufacturer's requirements.

Equipment and Consumables Required BUT NOT Provided

- Rnase/Dnase-free 1.5 ml polypropylene microcentrifuge tubes
- Molecular Grade Water (RNase/DNase Free)
- Sterile nuclease-free filtered pipette tips
- Micropipettors (1-10 µL, 10-200 µL and 100-1000 µL)
- 96-well cold block
- Benchtop Microcentrifuge
- Personnel Protective Equipment (PPE)
- -70º C and -20º C Freezer(s)
- +4º C Refrigerator
- Roche MagNA Pure Compact System
  - Required consumables specific for instrument defined by the manufacturerer.
- Roche MagNA Pure LC System with software version 3.0.11

- Required consumables specific for instrument defined by the manufacturer.

- Applied Biosystems 7500 Fast Dx Real-time PCR instrument with SDS Software version 1.4 (Applied Biosystems, Foster City, CA).
- Applied Biosystems 7500 Fast Sequence Detection Consumables (Applied Biosystems, Foster City, CA).
    - ABI MicroAmp™ Fast 8-tube strip 0.1 ml, cat #4358293 (required), or
    - ABI MicroAmp™ Optical 8-cap strip, cat #4323032 (required)
    - ABI MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 ml, part #4346906 or part #4366932 (alternate to 8-strip tubes)

Reagent Storage, Handling, and Stability

- Store all primers and probes at 2-8 ºC until re-hydrated for use; Store all control materials (SIPC – swine influenza positive control) at ≤ -20 ºC.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic probes from light.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and kept on ice or cold block at all times during preparation and use.

Reagents and Controls Preparation

1. Primer and Probe Preparation:

1. Upon receipt store primers and probes at 2-8°C.
2. Rehydration
    a. Remove primers and probes from 2-8°C.
    b. Pipet 0.5 ml (500 µL) of 10 mM Tris, pH 7.4-8.2 or PCR-grade water into each dried PCR primer or probe.
    c. Allow primers and probes to fully rehydrate for at least 15 minutes at room temperature.
    d. After primers and probes are fully rehydrated, pulse vortex to ensure a homogenous solution.
3. Aliquot
    a. Label one (1) new nuclease-free, sterile, tube for each primer and probe with the following information:
        - Primer or Probe Name
        - Kit Lot #
        - Expiration Date
    b. Aliquot 100 µL of each primer and probe into respective labeled tubes and store at -20°C.
4. Storage
    a. After rehydration
        i. Primers
            1. Aliquots of primers are stored at -20°C or below until expiration date as long as QC requirements are met.
        ii. Probe
            1. Aliquots of probes are stored at -20°C or below until expiration date as long as QC requirements are met.
            2. Thawed aliquots of probes may be stored at 2-8°C in the dark for up to 3 months.

2. Swine Influenza Positive Control (SIPC) Preparation:

1. Reagent
    a. Inactivated, noninfectious influenza virus preparation supplied as a liquid suspended in 0.01 M PBS.
    b. Influenza virus representing swine influenza A/H1N1 and cultured human cells.
    c. Volume: 0.5 ml yields approximately 5.0 ml of positive control RNA.

2. Storage
    a. Store at -20°C or below upon receipt. Do not dilute.

3. Procedure
    a. SIPC must be extracted prior to use. The final volume of eluted RNA should equal the volume of extracted control material. For example, 50µL of control material should result in 50µL of RNA extract (1:1).
    b. Dilute the RNA 1:10 with nuclease free water.
    b. Label one (1) new nuclease-free, sterile, tube for each single-use aliquot with the following information:
        - Control RNA Name and 1:10 Dilution
        - Kit Lot #
        - Expiration Date
    c. Dispense 1:10 diluted RNA into single-use aliquots and store at -20°C or below for up to 6 months.
    d. Use one aliquot per run. Discard after use. Do not use residual RNA.

General Information

Equipment

Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used including 5% bleach, 70% ethanol, ***DNAzap***™ or ***RNase AWAY***® to minimize the risk of nucleic acid contamination.

Reagents

NOTE: All reagents should be kept on ice or cold rack during assay preparation.

Primers and Probes Reagents

- Thaw frozen aliquots of primer and probes. *Thawed aliquots of probes may be stored at 2-8°C in the dark for up to 3 months.* ***Do not re-freeze probes.***
- Vortex all primers and probes for 15 seconds.
- Briefly centrifuge all primers and probes.
- Place primers and probes on ice or in cold rack during master mix preparation.

Real-time RT-PCR Reagents

- Place Invitrogen 2X PCR Master Mix and Superscript III RT / Platinum Taq enzyme mix in a cold rack at 4-8 ºC.
- Completely thaw the 2X PCR Master Mix vial.
- Mix the 2X PCR Master Mix by inversion 10 times.
- Briefly centrifuge 2X PCR Master Mix and Superscript II RT / Platinum Taq enzyme mix then place in cold rack.

Warnings and Precautions

The use of sputum specimens has NOT been authorized under the Emergency Use Authorization

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent influenza viruses and sent to state or local health departments for testing. Virus culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
Note: **Novel influenza A viruses are new or re-emergent human strains of influenza A that cause cases or clusters of human disease, as opposed to those strains commonly circulating in humans that cause seasonal epidemics and to which human populations have residual or limited immunity (either by vaccination or previous infection).**

Due to the sensitivity of Real-time RT-PCR assays, special precautions must be followed to avoid false positive amplifications. The following precautionary steps are recommended:

- Maintain separate areas for assay setup and handling of nucleic acids.
- Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.
- Work surfaces, pipets, and centrifuges should be cleaned and decontaminated with cleaning products such as 5% bleach, ***DNAzap***™ or ***RNase AWAY***® to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- Reagents, master mix, and RNA should be maintained on cold block during preparation and/or use to ensure stability.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect fluorogenic probes from light.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and maintained on cold block at all times during preparation and use.

Nucleic Acid Extraction

Performance of the CDC Swine Influenza Virus Real-time RT-PCR Detection Panel is dependent on the amount and quality of template RNA purified from human specimens. The following commercially available RNA extraction kits and procedures have been qualified and validated for recovery and purity of RNA for use with the panel;

- QIAamp® Viral RNA Mini Kit, or
- RNeasy® Mini Kit (QIAGEN), or
- Roche MagNA Pure LC Total Nucleic Acid Kit. Protocol: **External Lysis Protocol**. Utilize 100.0 µl of sample and 300.0 µl of lysis buffer (total sample volume for input into LC is 400.0 µl). Elution volume is 100.0 µl. These volumes should be available on the LC software.
- Roche MagNA Pure Compact Nucleic Acid Isolation Kit I (catalog # 03 730 964 001). **Protocol: External Lysis Protocol**. Utilize 100.0 µl of sample and 300.0 µl of lysis buffer (total sample volume for input into Compact is 400.0 µl). Elution is 100.0 µl.

- Roche MagNA Pure RNA Isolation Kit (catalog# 04 802 993 001). **Protocol: RNA _ Tissue_V3_1 Protocol.** Utilize 100.0 µl of sample and 250.0 µl of lysis buffer (total sample volume for input into Compact is 350.0 µl). Elution is 100.0 µl.

Manufacturer's recommended procedures are to be used for sample extraction.

Disclaimer: Names of vendors or manufacturers are provided as examples of suitable product sources. Inclusion does not imply endorsement by the Centers for Disease Control and Prevention.

ASSAY SETUP

Master Mix Preparation / Plate Setup

1. In the assay preparation area, label a sterile, nuclease-free, 1.5 ml tube for each reaction master mix to be prepared (i.e. InfA, swInfA, swH1, and RP).
2. Determine the number of reactions (N) being prepared per assay
3. Calculate the amount of each reagent to be added to the tube for each master mix (**Figure 1**).

NOTE: *It is necessary to make excess reaction master mix to allow for the No Template Control (NTC), Swine Influenza Positive Control (SIPC), and Human Specimen Control (HSC) reactions and pipetting error.*

Example: If number of samples (n) including controls = 1 to 14, then N = n + 1
If number of samples (n) including controls > 15, then N = n + 2

Figure 1. Example Calculation for Master Mix Preparation

Step #	Reagent	Volume of Reagent Added per Reaction	Total Number Reactions (N)	Total Volume of Reagent to Add to Master Mix
1	Nuclease-free Water	N x 5.5 µL	N = n +1 = 12 + 1 = 13	71.5 µL
2	Forward Primer	N x 0.5 µL	N = n +1 = 12 + 1 = 13	6.5 µL
3	Reverse Primer	N x 0.5 µL	N = n +1 = 12 + 1 = 13	6.5 µL
4	Probe	N x 0.5 µL	N = n +1 = 12 + 1 = 13	6.5 µL
5	SuperScript™ III RT/Platinum® *Taq* Mix	N x 0.5 µL	N = n +1 = 12 + 1 = 13	6.5 µL
6	2X PCR Master Mix	N x 12.5 µL	N = n +1 = 12 + 1 = 13	162.5 µL
	Total Volume	**N x 20.0 µL**		**260.0 µL**

4. In a sterile labeled 1.5 ml tube, prepare master mix for each marker set tested (i.e. InfA, swInfA, swH1, and RP) by first calculating the amount of each reagent to be added for each primer/probe set reaction master mix. *Use **Figure 1** to determine reagent volumes required for a full reaction plate (full reaction plate = 19 SAMPLES + CONTROLS).*

5. In the assay preparation area, dispense reagents into labeled 1.5 ml microcentrifuge tubes. After addition of the water, mix reaction mixtures by pipetting up and down. ***Do not vortex***.

6. Centrifuge for 5 sec to collect contents at bottom of the tube, and then place the tube in cold rack.

7. Set up reaction strip tubes or plates in 96-well cooler rack.

8. Dispense 20µL of each master mix into each well going across the row as shown below (**Figure 2**):

Figure 2. Example of Reaction Master Mix Plate Set-up

	1	2	3	4	5	6	7	8	9	10	11	12
A	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA
B	swInfA	swInfA	swInfA	swInfA	swInfA	swInfA	swInfA	swInfA	swInfA	swInfA	swInfA	swInfA
C	swH1	swH1	swH1	swH1	swH1	swH1	swH1	swH1	swH1	swH1	swH1	swH1
D	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP
E	blank	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	InfA	blank
F	blank	swInfA	swInfA	swInfA	swInfA	swInfA	swInfA	swInfA	swInfA	swInfA	swInfA	blank
G	blank	swH1	swH1	swH1	swH1	swH1	swH1	swH1	swH1	swH1	swH1	blank
H	blank	RP	RP	RP	RP	RP	RP	RP	RP	RP	RP	blank

9. Prior to moving to the nucleic acid handling area, prepare the NTC reactions for column #1 in the assay preparation area. Add samples to columns as illustrated in **Figure 3 (see below)**.

Figure 3. Example of Sample and Control Set-up

	1	2	3	4	5	6	7	8	9	10	11	12
A	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	SIPC
B	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	SIPC
C	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	SIPC
D	NTC	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19	SIPC
E	blank	S2	S4	S6	S8	S10	S12	S14	S16	S18	HSC	blank
F	blank	S2	S4	S6	S8	S10	S12	S14	S16	S18	HSC	blank
G	blank	S2	S4	S6	S8	S10	S12	S14	S16	S18	HSC	blank
H	blank	S2	S4	S6	S8	S10	S12	S14	S16	S18	HSC	blank

10. Pipette 5.0 µL of nuclease-free water into the NTC sample wells. Securely cap NTC wells before proceeding.

11. Cover the entire reaction plate and move the reaction plate to the specimen nucleic acid handling area.

Template Addition

12. Gently vortex nucleic acid sample tubes for approximately 5 seconds. Centrifuge tubes for approximately 5 seconds.

13. After centrifugation, place extracted nucleic acid sample tubes in the cold rack.

14. Samples should be added by column as illustrated in **Figure 3**. Carefully pipette 5.0 µL of the first sample into all the wells labeled for that sample. *Keep other sample wells covered during addition. Change tips after each addition.*

15. Securely cap the column to which the sample has been added to prevent cross contamination and to ensure sample tracking.

16. Change gloves often and when necessary to avoid contamination.

17. Repeat steps #14 through #16 for the remaining samples.

18. Cover the entire reaction plate and move the reaction plate to the positive template control handling area.

Assay Control Addition

19. Add 5.0 µL of Human Specimen Control (HSC) extracted sample to the HSC wells (column 11). Securely cap wells after addition.

20. Pipette 5.0 µL of SIPC RNA to the appropriate sample wells (**Figure 3**). Securely cap wells after addition of the control RNA.

NOTE: *If using 8-tube strips, label the TAB of each strip to indicate sample position.* ***DO NOT LABEL THE TOPS OF THE REACTION TUBES!).***

21. Briefly centrifuge reaction tube strips for 10-15 seconds. After centrifugation return to cold rack.
NOTE*: If using 96-well plates, centrifuge plates for 30 seconds at 500 x g, 4°C.*

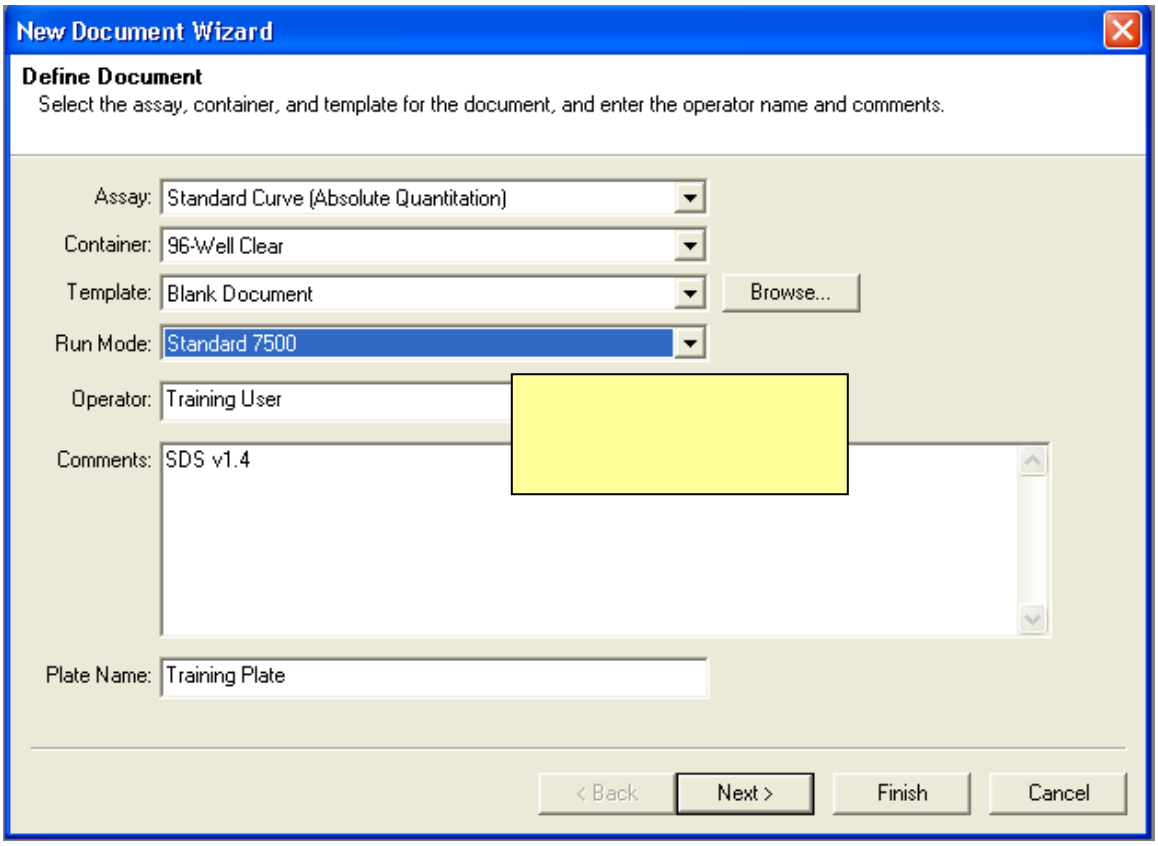
CREATE A RUN TEMPLATE ON THE AB 7500 FAST Dx Real-time PCR Instrument

(REQUIRED IF NO TEMPLATE EXISTS)

If the template already exists on your instrument please proceed to the **RUNNING A TEST** section.

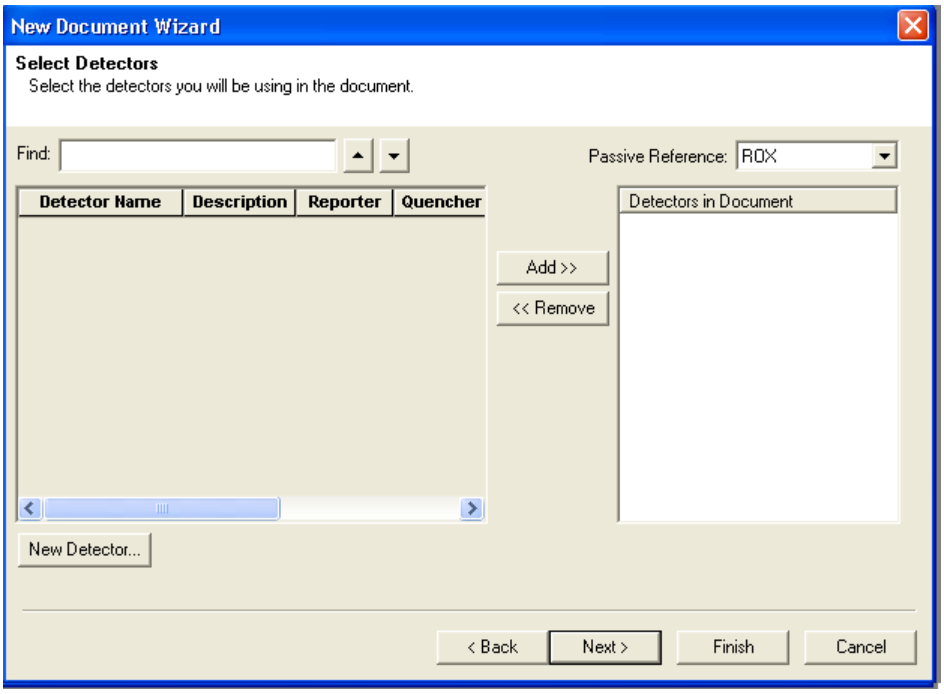
1. Launch the Applied Biosystems 7500 Fast Dx Real-time PCR System by double clicking on the Applied Biosystems 7500 Fast Dx System icon on the desktop.
2. A new window should appear, select **Create New Document** from the menu.

Figure 4. New Document Wizard Window



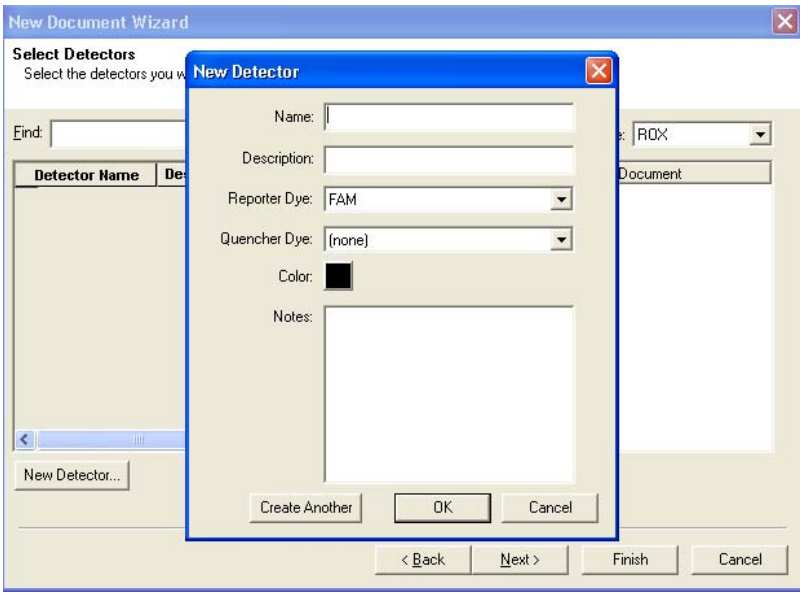
3. The **New Document Wizard** screen in **Figure 4** will appear. Select:
    a. Assay: **Standard Curve (Absolute Quantitation)**
    b. Container: **96-Well Clear**
    c. Template: **Blank Document**
    d. Run Mode: **Standard 7500**
    e. Operator: ***Your Name***
    f. Comments: **SDS v1.4**
    g. Plate Name: ***Your Choice***
4. After making selections click **Next** at the bottom of the window.

Figure 5. Creating New Detectors



5. After selecting next, the ***Select Detectors*** screen (**Figure 5**) will appear.
6. Click the **New Detector** button (see **Figure 5**).
7. The **New Detector** window will appear (**Figure 6**). A new detector will need to be defined for each influenza primer and probe set. Creating these detectors will enable you to analyze each primer and probe set individually at the end of the reaction.

Figure 6. Creating New Detector

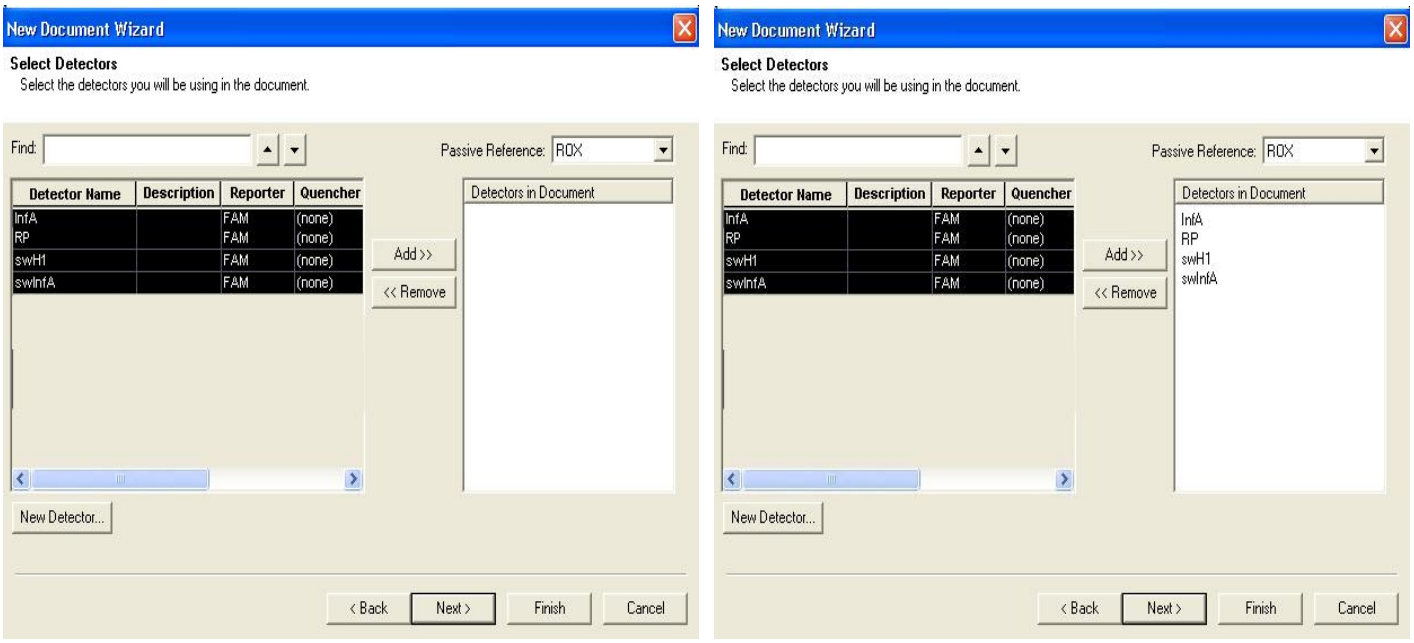


8. Start by creating the InfA Detector. Include the following:
   a. Name: **InfA**
   b. Description: *leave blank*
   c. Reporter Dye: **FAM**
   d. Quencher Dye: **(none)**
   e. Color: *to change the color of the detector indicator do the following:*
      ⇒ Click on the color square to reveal the color chart
      ⇒ Select orange as the color by clicking on the orange square
      ⇒ After selecting color click **OK** to return to the New Detector screen
   f. Click the **OK** button of the New Detector screen to return to the screen shown in **Figure 5**.
9. Repeat step 6-8 for each influenza target in the panel. Please select the colors as indicated in the chart below.

Name	Reporter Dye	Quencher Dye	Color
InfA	FAM	(none)	Your choice
swInfA	FAM	(none)	Your choice
swH1	FAM	(none)	Your choice
RP	FAM	(none)	Your choice

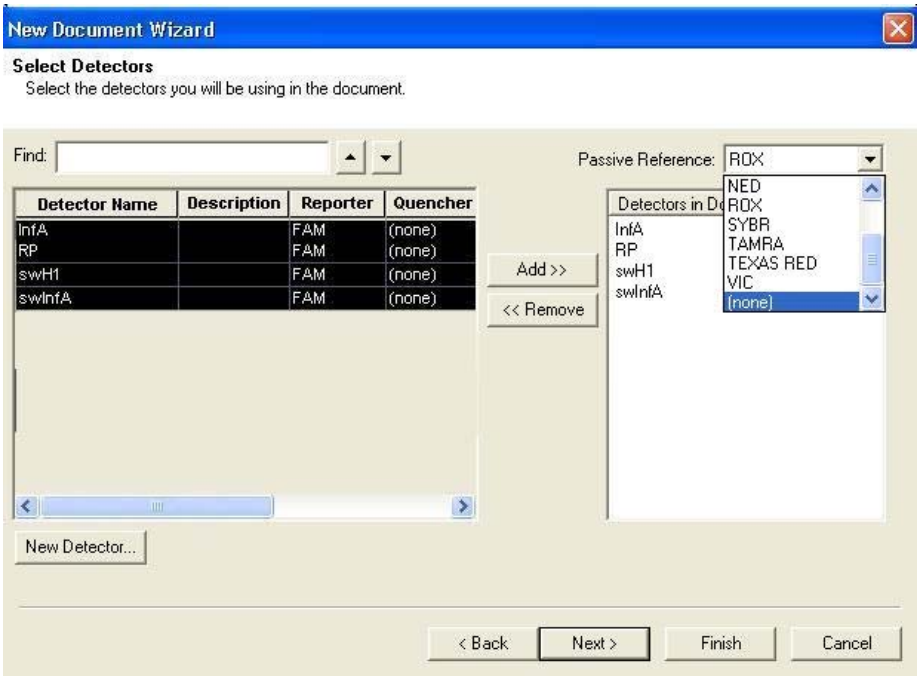
10. After each Detector is added, the **Detector Name**, **Description**, **Reporter** and **Quencher** fields will become populated in the **Select Detectors** screen (**Figure 7**).
11. Before proceeding, the newly created detectors must be added to the document. To add the new detectors to the document, click **ADD** (see **Figure 7**). Detector names will appear on the right hand side of the **Select Detectors** window (**Figure 7**).

Figure 7. Adding New Detectors to Document



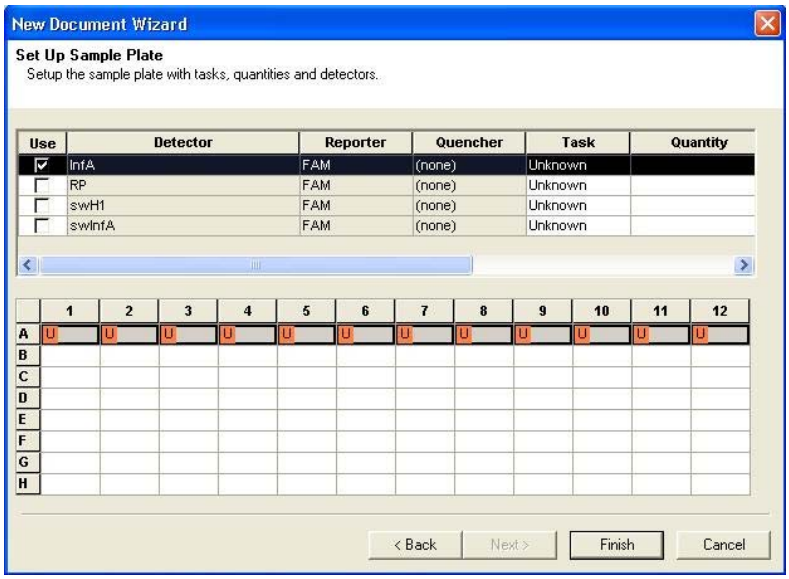
12. Once all detectors have been added, select **(none)** for **Passive Reference** at the top right hand drop down menu (**Figure 8**)

Figure 8. Select Passive Reference



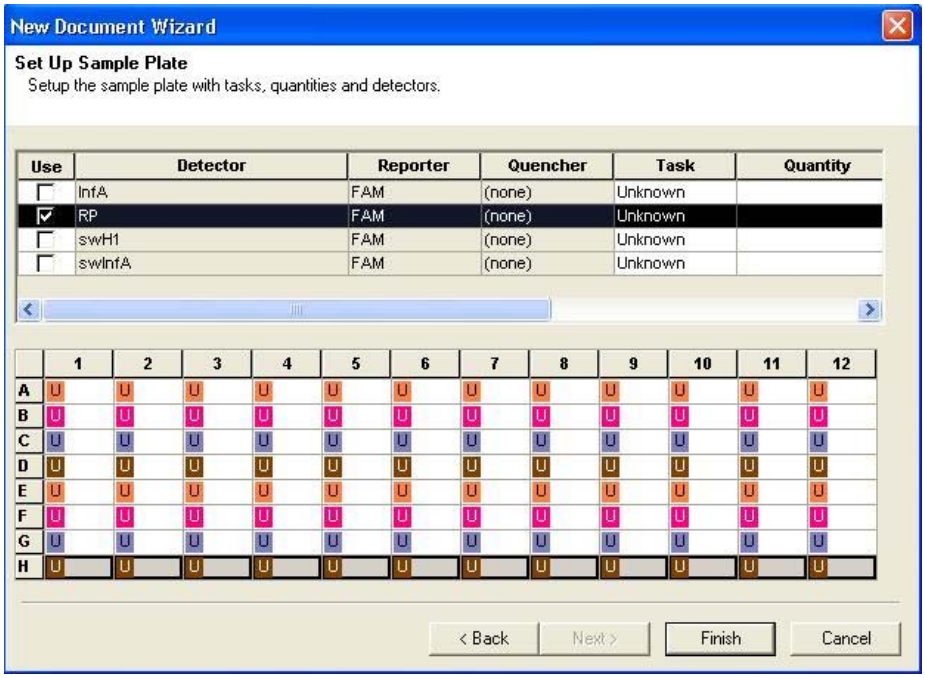
13. Click **Next** at the bottom of the **Select Detectors** window to proceed to the **Set Up Sample Plate** window (**Figure 9**).
14. In the **Set Up Sample Plate** window (**Figure 9**), use your mouse to select row A from the lower portion of the window, in the spreadsheet (see **Figure 9**).
15. Then in the top portion of the window, select detector **InfA**. A check will appear next to the detector you have selected (**Figure 9**). You will also notice the column in the spreadsheet will be populated with a colored "U" icon to indicate which detector you've selected.
16. Repeat step 14-15 for each detector that will be used in the assay.

Figure 9. Sample Plate Set-up



17. Select **Finish** after detectors have been assigned to their respective columns. (**Figure 10**).

Figure 10. Sample Plate Set-up

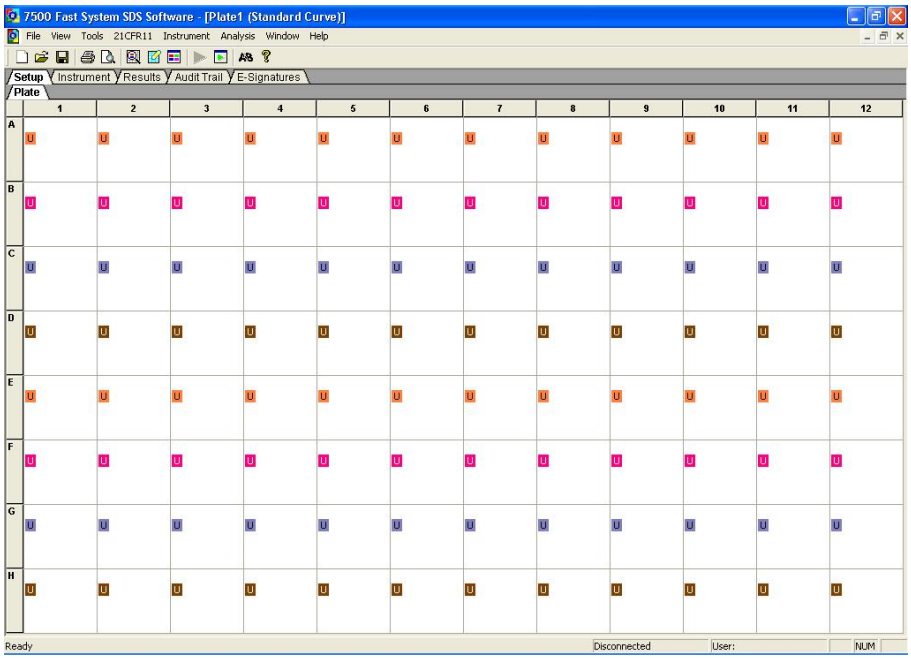


18. After clicking "Finish", there will be a brief pause allowing the Applied Biosystems 7500 Fast Dx to initialize. This initialization is followed by a clicking noise. ***Note: The machine must be turned on for initialization.***

19. After initialization, the **Plate** tab of the Setup (**Figure 11)** will appear.

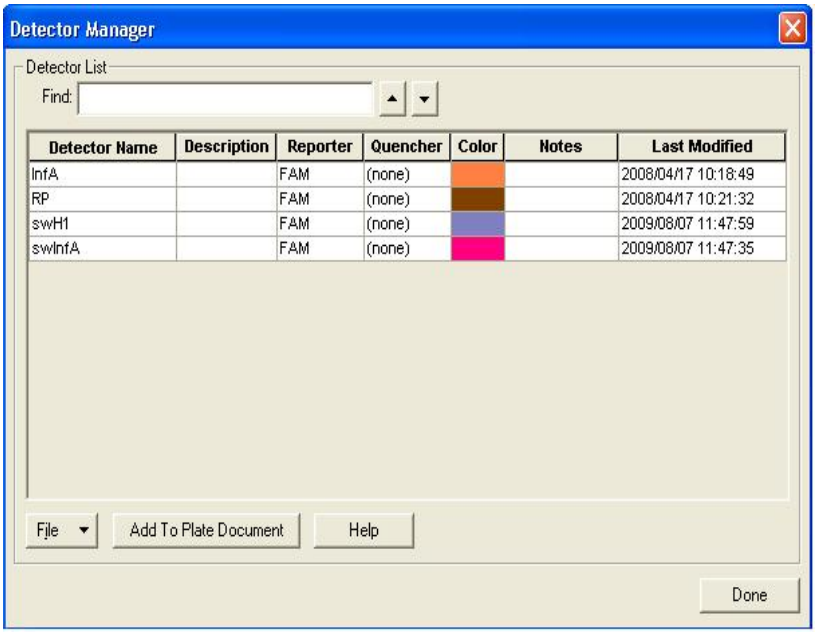
20. Each well of the plate should contain colored U icons that correspond with the detector labels that were previously chosen. To confirm detector assignments, select **Tools** from the file menu, then select **Detector Manager.**

Figure 11. Plate Set-up Window



21. The Detector Manager window will appear (**Figure 12**).

Figure 12. Detector Manager Window



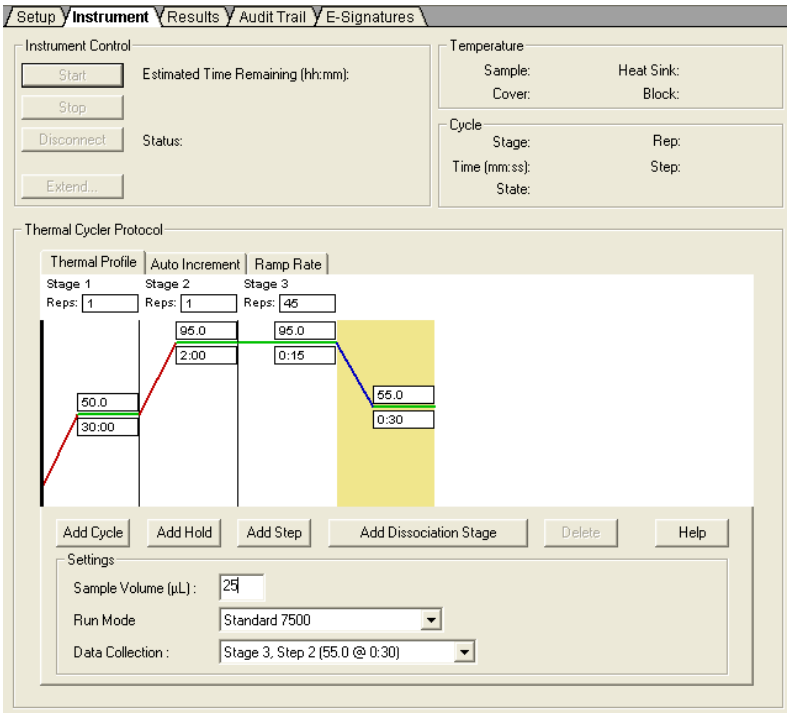
22. Confirm all influenza detectors are included and that each influenza target has a **Reporter** set to **FAM** and the **Quencher** is set to **(none)**.

23. If all detectors are present, select **Done**. The detector information has been created and assigned to wells on the plate.

DEFINING THE INSTRUMENT SETTINGS

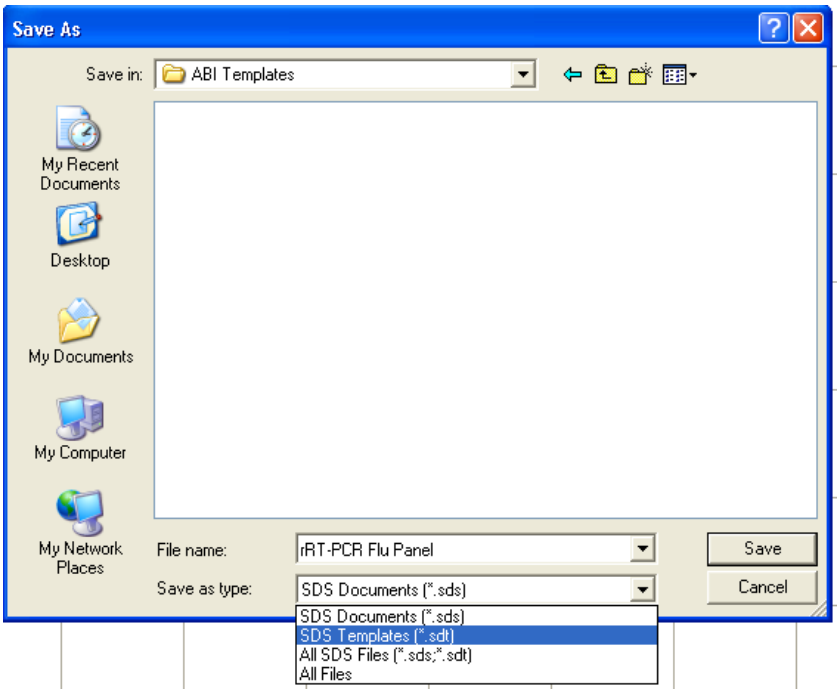
1. After detectors have been created and assigned, proceed to instrument set up.
2. Select the **Instrument** tab to define thermal cycling conditions.
3. Modify the thermal cycling conditions as follows (**Figure 13**):
   a. In Stage 1, Set to **30 min** at **50°C**; **1 Rep**.
   b. In Stage 2, Set to **2.0 min** at **95°C**; **1 Rep**.
   c. In Stage 3, Step 1 set to **15 sec** at **95°C.**
   d. In Stage 3, Step 2 set to **30 sec** at **55.0°C**
   e. In Stage 3, Reps should be changed to **45**
   f. Under **Settings** (**Figure 13**), bottom left-hand box, change volume to 25 µL.
   g. Under **Settings**, **Run Mode** selection should be **Standard 7500**.
   h. Step 2 of Stage 3 should be highlighted in yellow to indicate data collection (see **Figure 13**).

Figure 13. Instrument Window



4. After making changes to the **Instrument** tab, the template file is ready to be saved. To save the template, select **File** from the top menu, then select **Save As**.
5. Save the template as **rRT-PCR Swine Flu Panel** in desktop folder labeled "***ABI Run Templates***" (*you must create this folder*). Save as type should be SDS Templates (*.sdt). (**Figure 14**)

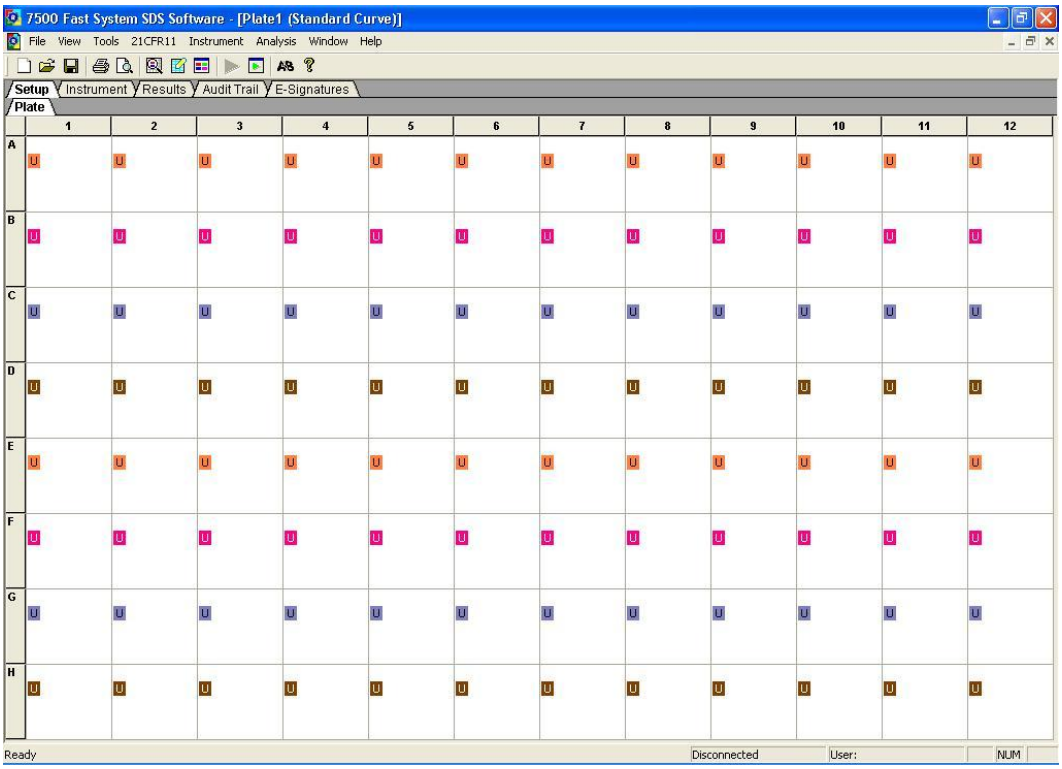
Figure 14. Saving Template



RUNNING A TEST

1. Turn on the ABI 7500 Fast Dx Real-time PCR instrument.
2. Launch the Applied Biosystems 7500 Fast Dx Real-time PCR System by double clicking on the 7500 Fast Dx System icon on the desktop.
3. A new window should appear, select **Open Existing Document** from the menu.
4. Navigate to select your ABI Run Template folder from the desktop.
5. Double click on the **rRT-PCR Swine Flu Panel** template file.
6. There will be a brief pause allowing the Applied Biosystems 7500 Fast Dx Real-time PCR system to initialize. This initialization is followed by a clicking noise. ***Note: The machine must be turned on for initialization.***

Figure 15. Plate Set-up Window



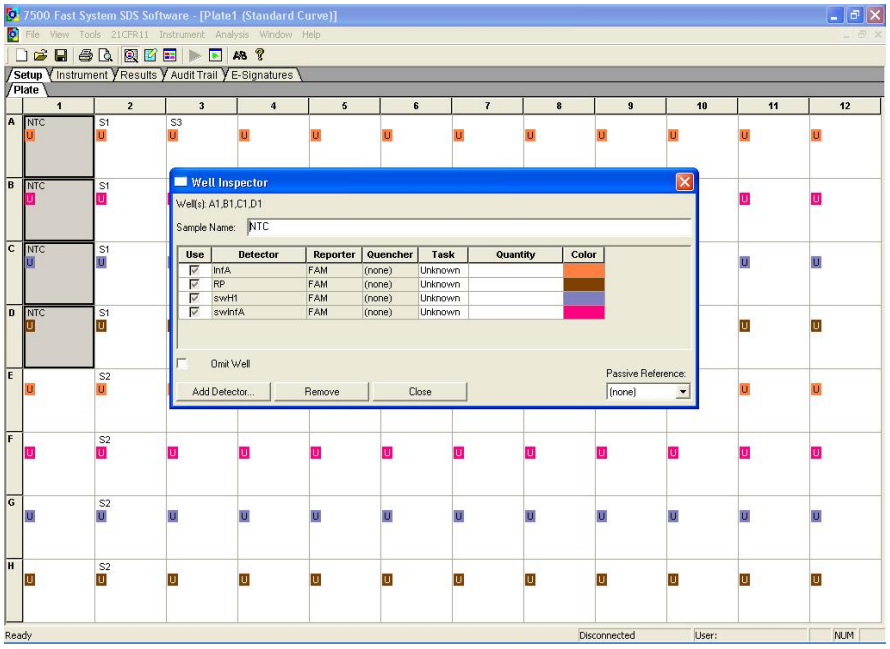
7. After the instrument initializes, a plate map will appear (**Figure 15**). The detectors and controls should already be labeled as they were assigned in the original template.

8. Click the **Well Inspector** icon  from the top menu.

9. Highlight specimen wells of interest on the plate map.

10. Type sample identifiers to **Sample Name** box in the **Well Inspector** window (see **Figure 16**).

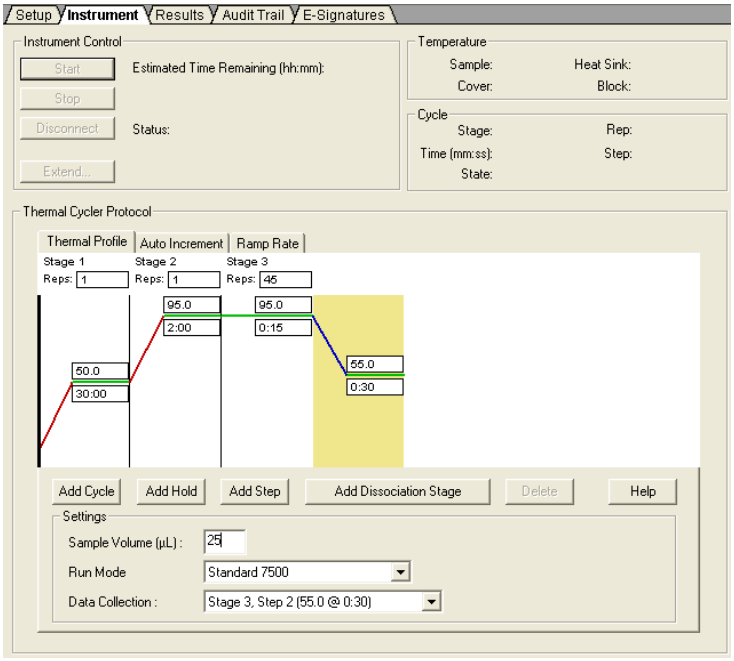
Figure 16. Labeling Wells



11. Repeat steps 9-10 until all sample identifiers are added to the plate setup.

12. Once all specimen and control identifiers are added click the **Close** button on the **Well Inspector** window to return to the **Plate** set up tab.
13. Click the **Instrument** tab at the upper left corner.
14. The reaction conditions, volumes, and type of 7500 reaction should already be loaded. (**Figure 17**)

Figure 17. Instrument Settings

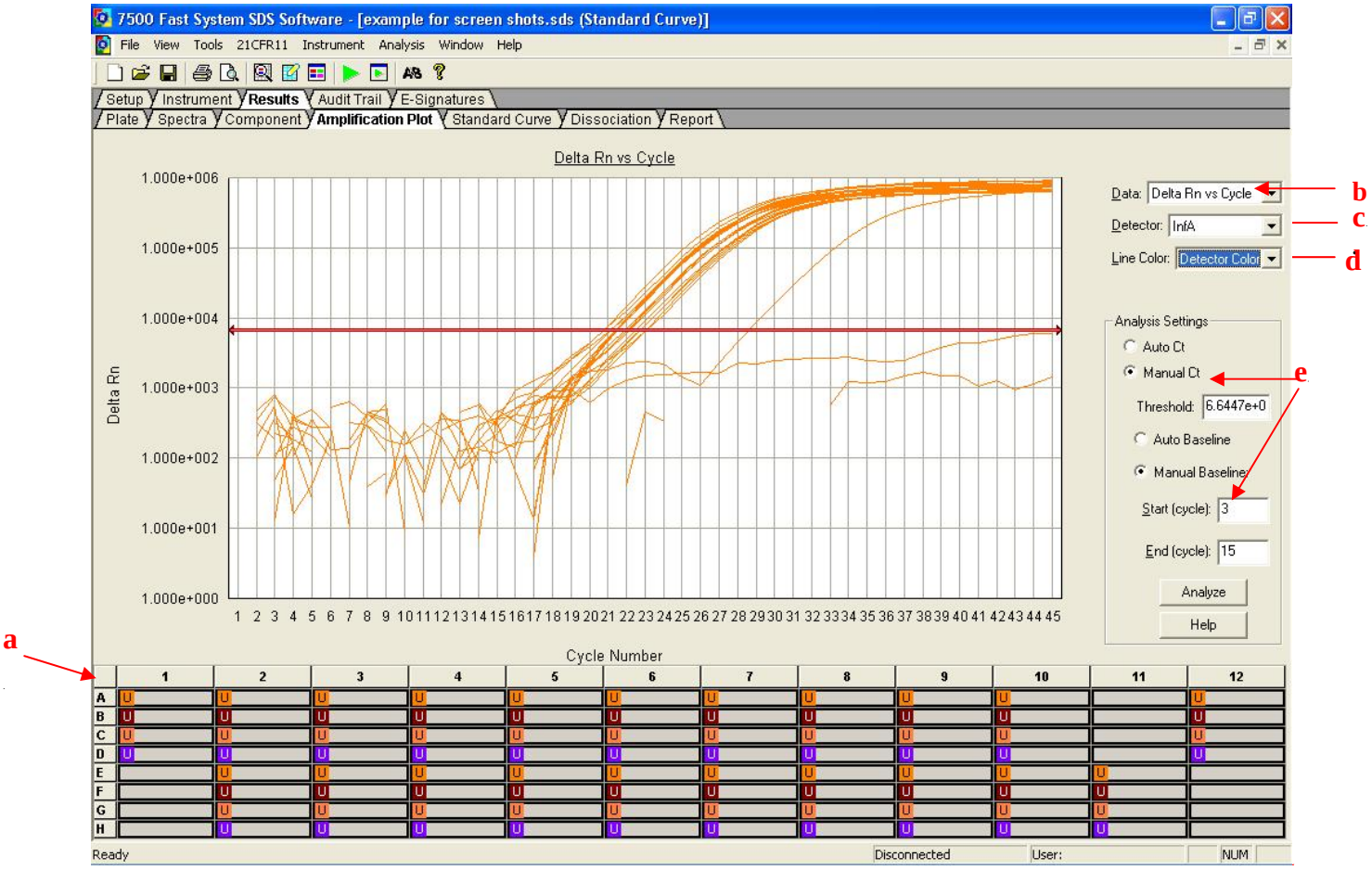


15. Ensure settings are correct (refer to the *Defining Instrument Settings*).
16. Before proceeding, the run file must be saved; from the main menu, select **File,** then **Save As**. Save in appropriate run folder designation.
17. Once run file is saved, add test reaction tube strips or plates to the instrument.
18. Click the **Start** button to begin the run. *Note: The run should take approximately 1hr and 45 minutes to complete.*

DATA ANALYSIS

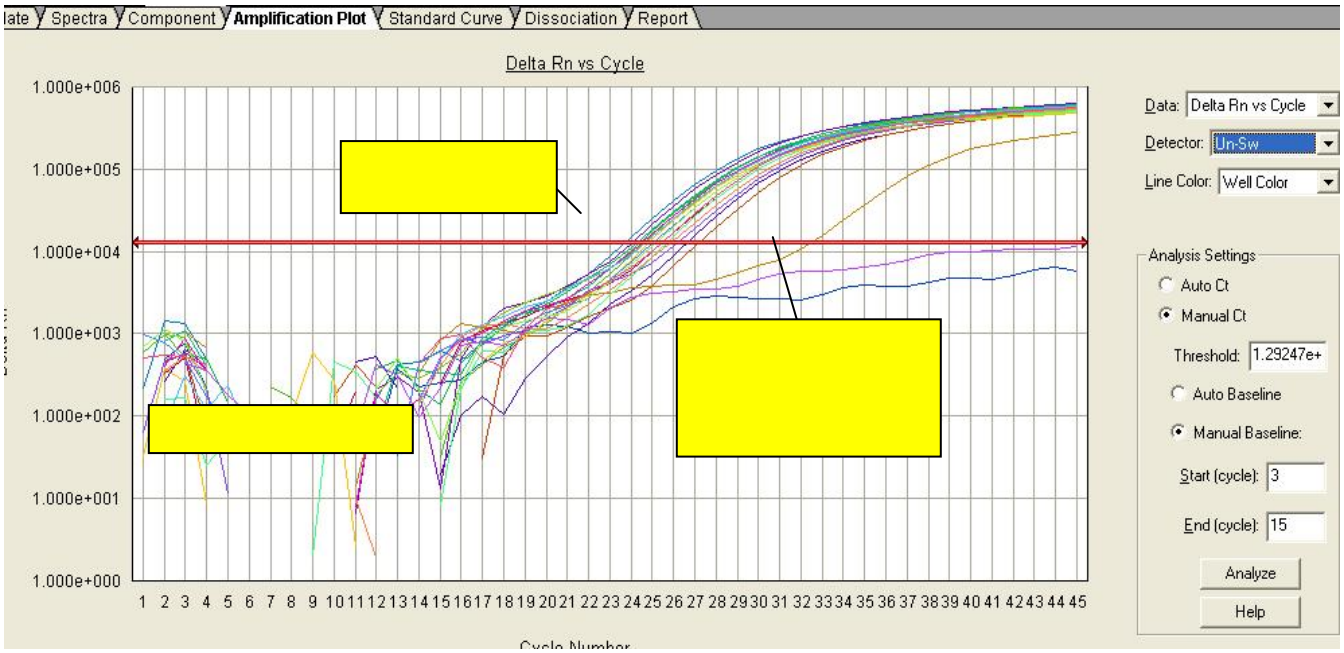
1. After the run has completed, select the **Results** tab at the upper left corner of the software.
2. Select the **Amplification Plot** tab to view the raw data (**Figure 18**).

Figure 18. Amplification Plot Window



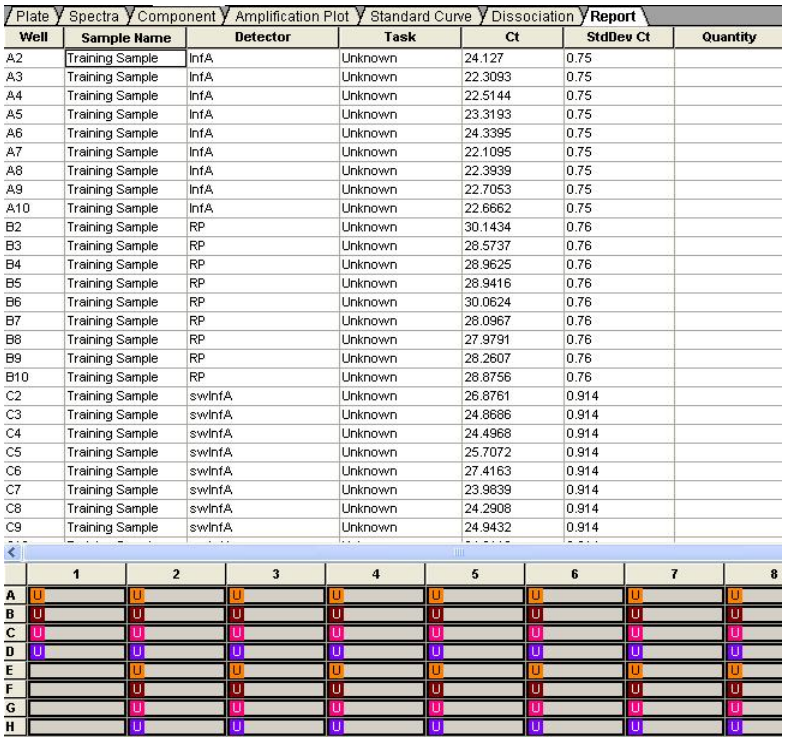
3. Start by highlighting all the samples from the run; to do this, click on the upper left hand box **(a)** of the sample wells (**Figure 18**). All the growth curves should appear on the graph.
4. On the right hand side of the window, **(b)** the **Data** drop down selection should be set to **Delta Rn vs. Cycle**.
5. Select **InfA** from **(c)** the **Detector** drop down menu using the downward arrow.
    a. Please note that each detector is analyzed individually to reflect different performance profiles of each primer and probe set.
6. In the **Line Color** drop down, **(d) Detector Color** should be selected.
7. Under **Analysis Settings** select **Manual Ct (e)**
    a. Do not change the **Manual Baseline** default numbers.
8. Using the mouse, click and drag the red threshold line until it lies within the exponential phase of the fluorescence curves and above any background signal (**Figure 19)**.

Figure 19. Amplification Plot



9. Click the **Analyze** button in the lower right corner of the window. The red threshold line will turn to green, indicating the data has been analyzed.
10. Repeat steps 5-9 to analyze results generated for each set of markers (i.e. InfA, swInfA, swH1, RP).
11. Save analysis file by selecting **File** then **Save As** from the main menu.
12. After completing analysis for each of the markers, select the **Report** tab above the graph to display the Ct values. To filter report by sample name in ascending or descending order, simply click on **Sample Name** on the table.

Figure 20. Report



Well	Sample Name	Detector	Task	Ct	StdDev Ct	Quantity
A2	Training Sample	InfA	Unknown	24.127	0.75	
A3	Training Sample	InfA	Unknown	22.3093	0.75	
A4	Training Sample	InfA	Unknown	22.5144	0.75	
A5	Training Sample	InfA	Unknown	23.3193	0.75	
A6	Training Sample	InfA	Unknown	24.3395	0.75	
A7	Training Sample	InfA	Unknown	22.1095	0.75	
A8	Training Sample	InfA	Unknown	22.3939	0.75	
A9	Training Sample	InfA	Unknown	22.7053	0.75	
A10	Training Sample	InfA	Unknown	22.6662	0.75	
B2	Training Sample	RP	Unknown	30.1434	0.76	
B3	Training Sample	RP	Unknown	28.5737	0.76	
B4	Training Sample	RP	Unknown	28.9625	0.76	
B5	Training Sample	RP	Unknown	28.9416	0.76	
B6	Training Sample	RP	Unknown	30.0624	0.76	
B7	Training Sample	RP	Unknown	28.0967	0.76	
B8	Training Sample	RP	Unknown	27.9791	0.76	
B9	Training Sample	RP	Unknown	28.2607	0.76	
B10	Training Sample	RP	Unknown	28.8756	0.76	
C2	Training Sample	swInfA	Unknown	26.8761	0.914	
C3	Training Sample	swInfA	Unknown	24.8686	0.914	
C4	Training Sample	swInfA	Unknown	24.4968	0.914	
C5	Training Sample	swInfA	Unknown	25.7072	0.914	
C6	Training Sample	swInfA	Unknown	27.4163	0.914	
C7	Training Sample	swInfA	Unknown	23.9839	0.914	
C8	Training Sample	swInfA	Unknown	24.2908	0.914	
C9	Training Sample	swInfA	Unknown	24.9432	0.914	

	1	2	3	4	5	6	7	8
A	U	U	U	U	U	U	U	U
B	U	U	U	U	U	U	U	U
C	U	U	U	U	U	U	U	U
D	U	U	U	U	U	U	U	U
E		U	U	U	U	U	U	U
F		U	U	U	U	U	U	U
G		U	U	U	U	U	U	U
H		U	U	U	U	U	U	U

Interpretation and Reporting of Results

Extraction and Positive Control Results and Interpretation

No Template Control (NTC)

The NTC consists of using nuclease-free water in the rRT-PCR reactions instead of RNA. The NTC reactions for all primer and probe sets should not exhibit fluorescence growth curves that cross the threshold line. If any of the NTC reactions exhibit a growth curve that crosses the cycle threshold, sample contamination may have occurred. Invalidate the run and repeat the assay with stricter adherence to the guidelines.

Swine Influenza Positive Control (SIPC)

The SIPC consists of a swine influenza virus representing novel influenza A (H1N1) and cultured human cells (A549). Purified RNA from the SIPC will yield a positive result with the following primer and probe sets: InfA, swInfA, swH1, and RP.

Human Specimen Control (HSC) (Extraction Control)

The HSC control consists of noninfectious cultured human cell (A549) material. The HSC is used as a RNA extraction procedural control to demonstrate successful recovery of RNA as well as extraction reagent integrity. Purified RNA from the HSC should yield a positive result with the RP primer and probe set and negative results with all influenza specific markers.

Figure 4. Expected Performance of Controls Included in the CDC rRT-PCR Flu Panel

Control Type	Internal Control Name	Used to Monitor	InfA	swInfA	swH1	RP	Expected Ct Values
Positive	SIPC	Substantial reagent failure including primer and probe integrity	+	+	+	+	≤ 37 Ct
Negative	NTC	Reagent and/or environmental contamination	-	-	-	-	None detected
Extraction	HSC	Failure in lysis and extraction procedure	-	-	-	+	≤ 37 Ct

If the controls in assay do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

Specimen Results and Interpretation

RNase P (Extraction Control)

- All clinical samples should exhibit fluorescence growth curves in the RNase P (RP) reaction that cross the threshold line within 37 cycles, thus indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimens may indicate:
  - Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation

- Absence of sufficient human cellular material due to poor collection or loss of specimen integrity
- Improper assay set up and execution
- Reagent or equipment malfunction

➢ If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:

- If the InfA, swInfA, swH1 are positive even in the presence of a negative RP, the influenza result should be considered valid. It is possible, that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of influenza virus RNA in a clinical specimen.
- If all influenza markers AND RNase P are all negative for the specimen, the assay is "inconclusive" for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as "inconclusive" and a new specimen should be collected if possible.

➢ The RP assay may be negative when testing virus culture samples.

Influenza Markers (InfA, swInfA, and swH1)

➢ When all controls exhibit the expected performance, a specimen is considered negative if influenza marker growth curves DO NOT cross the threshold line within 37 cycles and RNase P growth curve does cross the threshold line within 37 cycles.

➢ When all controls exhibit the expected performance and none of the growth curves for the influenza markers or RP marker cross the threshold line within 37 cycles, the result is "Inconclusive". The extracted RNA from the specimen should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test. If the re-tested sample is negative for all markers and all controls exhibit the expected performance, the result is "Inconclusive."

➢ When all controls exhibit the expected performance, and growth curve for InfA influenza marker and one of the sw Inf markers (swInfA or swH1) cross the threshold line within 37 cycles but one of the growth curves for the sw Inf markers (swInfA or swH1) markers do not cross the threshold line within 37 cycles, the result is "Inconclusive". RP may be positive or negative as described above. The extracted RNA from the specimen should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test.

➢ When all controls exhibit the expected performance, and growth curve for InfA influenza marker does not cross the threshold line within 37 cycles but the growth curves for either or both sw Inf markers (swInfA and swH1) cross the threshold line within 37 cycles, the result is "Inconclusive". RP may be positive or negative as described above. The extracted RNA from the specimen should be re-tested. If residual RNA is not available, re-extract RNA from residual specimen and re-test.

➢ When all controls exhibit expected performance, and when growth curve for InfA, marker crosses the threshold line within 37 cycles but the growth curves for the swInfA, and swH1 markers do not cross the threshold line within 37 cycles :

- Report the specimen to be "Positive Influenza A and negative for 2009 H1N1 influenza."

➢ When all controls exhibit expected performance, a specimen is considered positive for influenza 2009 H1N1 when growth curves for all InfA, swInfA, and swH1 markers cross the threshold line within 37 cycles:

- Report the specimen to be "Positive for 2009 H1N1 Influenza."

CDC rRT-PCR Swine Flu Panel Users Guide for Interpretation of Results - Quick Reference and Reporting

InfA	swInfA	swH1	RP	Interpretation	Report
-	-	-	+	No influenza virus detected	Influenza Not Detected (negative)
+	-	+	±	2009 Influenza H1	Inconclusive for 2009 H1N1 Influenza
+	+	-	±	2009 Influenza A	Inconclusive for 2009 H1N1 Influenza
-	±	±	±	2009 Influenza A and H1	Inconclusive for 2009 H1N1 Influenza
+	-	-	±	Influenza A	Positive for Influenza A, 2009 H1N1 Influenza not detected
+	+	+	±	2009 A/H1N1	Positive for 2009 H1N1 Influenza

Please follow most current recommendations for reporting novel influenza A (H1N1) diagnostic results to state/local authorities.

To refer a specimen to the CDC, the following shipping instructions should be followed:

- Ship all specimens and related RNA overnight to CDC.
- Ship frozen specimens on dry ice and non-frozen specimens on cold packs. Ship extracted RNA on dry ice.
- Refer to the International Air Transport Association (IATA - www.iata.org) for requirements for shipment of human or potentially infectious biological specimens.
- Prior to shipping, notify CDC Influenza Division (see contact information below) that you are sending specimens.
- Send all samples to the following recipient:

Alexander Klimov
Chief, Virus Surveillance and Diagnosis Branch Influenza Division
Centers for Disease Control and Prevention
c/o DASH, MS G-16
Attention: Dr. Stephen Lindstrom
1600 Clifton Rd., Atlanta, GA 30333
Phone: (404) 639-3387 or (404) 639-3591
Fax: (404) 639-2334

The emergency contact number for CDC is 770-488-7100.

PERFORMANCE CHARACTERISTICS

Analytical Sensitivity

Note: It has been demonstrated that the CDC rRT-PCR Swine Flu Panel has similar analytical performance to the CDC rRT-PCR Flu Panel (IVD, K080570). In side-by-side testing, the primer and probe sets have equivalent limit of detection ranges.

Limit of Detection Study (Upper Respiratory Specimens)

Influenza virus was grown and harvested from tissue culture virus preparations. at the CDC Influenza Division. Fifty percent (50%) infectious dose (TCID50 /mL) virus titer determinations were performed following procedures utilized by the CDC Influenza Division Virus Surveillance and Diagnosis Branch.

The following influenza strains were characterized and used for the limit of detection study:

- A/Iowa/1/2006 Swine H1N1.

LoD Study for the rRT-PCR Swine Flu Panel along with the IVD InfA Markers on the ABI 7500 Fast Dx PCR System

Virus Strain Tested	Analyte Tested	Stock Virus Titer	Serial 10-Fold Dilution Factor	TCID 50/mL Dilution Tested	Call Rate	Run 1 Ct	Run 2 Ct	Run 3 Ct	Run 4 Ct	Run 5 Ct	Avg. Ct (n=5)	Lowest Conc. with Uniform Positivity per Analyte	Limit of Detection (LoD) per Virus Strain
A/Iowa/1/2006 (swine A/H1N1)	IVD Inf A	$10^{9.0}$ $TCID_{50}$/mL	10e3	$10^{6.0}$	5/5	26.50	26.89	27.03	26.22	26.13	26.55	$10^{3.0}$ $TCID_{50}$/mL (36.19 Ct)	$10^{3.0}$ $TCID_{50}$/mL
			10e4	$10^{5.0}$	5/5	30.40	31.04	31.65	30.07	30.32	30.70		
			10e5	$10^{4.0}$	5/5	34.21	33.24	33.22	33.62	33.06	33.47		
			10e6	$10^{3.0}$	5/5	36.70	35.53	36.21	36.07	36.46	36.19		
			10e7	$10^{2.0}$	4/5	0.00	39.00	40.47	38.69	37.61	na		
			10e8	$10^{1.0}$	1/5	0.00	0.00	0.00	0.00	39.62	na		
			10e9	10^{0}	0/5	0.00	0.00	0.00	0.00	0.00	na		
	Sw InfA	$10^{9.0}$ $TCID_{50}$/mL	10e3	$10^{6.0}$	55	23.01	23.07	22.87	23.05	23.19	23.04	$10^{2.0}$ $TCID_{50}$/mL (35.48 Ct)	
			10e4	$10^{5.0}$	5/5	27.26	26.84	26.72	26.77	26.78	26.87		
			10e5	$10^{4.0}$	5/5	30.16	30.85	29.87	29.96	30.01	30.17		
			10e6	$10^{3.0}$	5/5	33.19	33.37	33.63	33.25	33.35	33.36		
			10e7	$10^{2.0}$	5/5	38.32	37.10	37.56	36.67	37.28	37.39		
			10e8	$10^{1.0}$	1/5	0.00	0.00	0.00	0.00	42.77	Na		
			10e9	10^{0}	0/5	0.00	0.00	0.00	0.00	0.00	Na		
	Sw H1	$10^{9.0}$ $TCID_{50}$/mL	10e3	$10^{6.0}$	5/5	27.07	27.56	27.35	27.35	27.17	27.30	$10^{3.0}$ $TCID_{50}$/mL (35.48 Ct)	
			10e4	$10^{5.0}$	5/5	31.02	31.06	30.98	30.75	30.48	30.86		
			10e5	$10^{4.0}$	5/5	34.03	33.33	33.43	34.01	33.69	33.70		
			10e6	$10^{3.0}$	5/5	37.24	36.66	36.29	36.62	36.81	36.72		
			10e7	$10^{2.0}$	2/5	0.00	38.40	40.37	0.00	0.00	Na		
			10e8	$10^{1.0}$	0/5	0.00	0.00	0.00	0.00	0.00	Na		
			10e9	10^{0}	0/5	0.00	0.00	0.00	0.00	0.00	Na		

RNA was extracted with the Qiagen QIAamp® Viral RNA Purification kit from each of the above characterized viruses. Ten-fold serial dilutions of the characterized influenza viruses were tested on the ABI 7500 Fast Dx PCR System to identify an end-point for detection with each primer and probe set included in the rRT-PCR Swine Flu Panel.

The rRT-PCR Swine Flu Panel demonstrated limits of detection similar to that of the InfA primer and probe set in the CDC rRT-PCR Flu Panel (IVD). Please see performance data submitted in 510(k) 080705 cleared by FDA Sept. 30, 2008.

Limit of Detection (Lower Respiratory Specimens)

The following influenza strain was characterized and used for the limit of detection study:
A/California/04/2009 – swine-like influenza A/H1N1 strain

Analytical sensitivity was demonstrated by determining the limit of detection (LoD) of each primer and probe set in the rRT-PCR Swine Flu Panel in a side by study on both the ABI 7500 Fast Dx and the Roche LightCycler® 2.0 Real-Time PCR systems. Ten-fold serial dilutions of the 2009 A/H1N1 influenza virus strain was tested to identify an end-point for detection of each primer and probe set included in the rRT-PCR Swine Flu Panel on both instruments. RNA was extracted with the Qiagen QIAamp® Viral RNA Purification kit from each of the characterized viruses. The limit of detection for each primer and probe set (InfA, swInfA, and swH1) was calculated to indicate the range of lowest detectable concentration of influenza virus (EID_{50}/ml) at which ≥95% of all replicates tested positive. The lowest concentration of influenza virus detected was determined to be the end-point concentration where the type and subtype primer and probe sets had uniform detection on both instruments. If the two end-points differed in concentration the higher (or limiting) point was used.

Table 1. Summary of the results from the limit of detection study for each primer and probe set utilizing the characterized reference influenza novel H1N1 virus on both the ABI 7500 Fast Dx and the Roche LightCycler® 2.0 Real-Time PCR systems.

Influenza Virus Tested	Influenza Strain Designation	LIMIT OF DETECTION (EID_{50}/ML)
2009 A/H1N1	A/California/04/2009 Swine-like influenza A/H1N1 strain	$10^{2.4}$ EID_{50}/mL

Analytical specificity

Analytical Specificity was demonstrated by testing characterized human A/H1 (10) and human A/H3 (10) influenza virus with both the rRT-PCR Flu Panel (IVD) and the rRT-PCR Swine Flu Panel (IUO-EUA). The purified viral RNA was analyzed with each marker (analyte) in the each panel with on the Applied Biosystems 7500 Fast Dx Real-Time PCR System.

Analytical Specificity of the rRT-PCR Swine Flu Panel with Human Influenza A/H1 Virus (Cross Reactivity)

Influenza Strain Tested	$TCID_{50}$ /ml	ABI 7500 Fast Real time RT-PCR				Expected Result	Actual Result
		IVD Inf A	IVD H1	Sw InfA	Sw H1		
A/BANGLADESH/7286/2007	6.1	13.53	14.04	neg	neg	Human A/H1	Human A/H1
A/BEIJING/262/95	6.0	11.71	12.08	neg	neg	Human A/H1	Human A/H1
A/FUKUSHIMA/141/06	5.7	17.49	16.65	neg	neg	Human A/H1	Human A/H1
A/HAWAII/15/2001*	6.6 EID_{50}	15.67	16.02	neg	neg	Human A/H1	Human A/H1
A/JIANGXI/160/05	5.6	13.32	13.98	neg	neg	Human A/H1	Human A/H1
A/South Dakota/6/2007	8.2 EID_{50}	13.73	13.47	neg	neg	Human A/H1	Human A/H1
A/MEXICO/1729/2007	4.8	13.67	14.18	neg	neg	Human A/H1	Human A/H1
A/NEW CALEDONIA/20/1999*	6.6 EID_{50}	12.73	13.42	neg	neg	Human A/H1	Human A/H1
A/SOLOMON ISLANDS/3/06	6.2	15.12	14.3	neg	neg	Human A/H1	Human A/H1
A/Brisbane/59/2007	8.4 EID_{50}	12.83	13.15	neg	neg	Human A/H1	Human A/H1
Seasonal Positive Control		24.99	29.89	neg	neg	Human A/H1	Human A/H1
Swine Positive Control		neg	neg	20.46	25.04	Sw A/H1N1	Sw A/H1N1

Analytical Specificity of the rRT-PCR Swine Flu Panel with Human Influenza A/H3 Virus (Cross Reactivity)

Influenza Strain Tested	$TCID_{50}$/ml	ABI 7500 Fast Real time RT-PCR				Expected Result	Actual Result
		IVD Inf A	IVD H3	Sw InfA	Sw H3		
A/ANHUI/1239/2005	8.1	11.94	11.45	neg	neg	Human A/H3	Human A/H3
A/AFGHANISTAN/2903/2008	5.0	14.79	15.73	neg	neg	Human A/H3	Human A/H3
A/BRISBANE/10/2007	6.8	11.72	12.58	neg	neg	Human A/H3	Human A/H3
A/HAWAII/08/2006	7.8	11.6	11.88	neg	neg	Human A/H3	Human A/H3
A/MEXICO/1842/2007	6.1	13.37	14.36	neg	neg	Human A/H3	Human A/H3
A/NEW YORK/55/2004*	6.4 (EID_{50})	12.8	13.00	neg	neg	Human A/H3	Human A/H3
A/WISCONSIN/67/2005*	6.5 (EID_{50})	14.51	14.79	neg	neg	Human A/H3	Human A/H3
A/Uruguay/716/2007	8.2 (EID_{50})	11.78	11.69	neg	neg	Human A/H3	Human A/H3
A/Taiwan/760/2007	5.5	10.99	11.06	neg	neg	Human A/H3	Human A/H3
A/British Columbia/RV1287/2007	6.0	12.77	12.89	neg	neg	Human A/H3	Human A/H3
Seasonal Positive Control		23.29	23.36	neg	neg	Human A/H3	Human A/H3
Swine Positive Control		neg	neg	20.26	24.72	Sw A/H1N1	Sw A/H1N1

Clinical Seasonal Specimens Tested with rRT-PCR Flu Panel (IVD) and rRT-PCR Swine Flu Panel

CDC Sample ID#	Clinical Specimen	Real-time RT-PCR (ABI 7500 Fast)						Result
		IVD InfA	IVD H1	IVD H3	RNP	sw InfA	sw H1	
2008726969	Original	24.04	25.57	Neg	28.02	Neg	Neg	human AH1
2008726970	Original	24.00	26.05	Neg	23.65	Neg	Neg	human AH1
2008726971	Original	17.55	19.65	Neg	23.64	Neg	Neg	human AH1
2008726972	Original	18.19	29.84	Neg	20.68	Neg	Neg	human AH1
2008726973	Original	19.98	22.80	Neg	19.68	Neg	Neg	human AH1
2008726974	Original	21.84	24.00	Neg	21.79	Neg	Neg	human AH1
2008726975	Original	30.42	30.04	Neg	22.76	Neg	Neg	human AH1
2008726976	Original	15.99	17.97	Neg	23.00	Neg	Neg	human AH1
2008726977	Original	26.18	27.71	Neg	26.10	Neg	Neg	human AH1
2008726978	Original	21.90	23.74	Neg	23.34	Neg	Neg	human AH1
2008726979	Original	18.27	20.43	Neg	20.75	Neg	Neg	human AH1
2008726980	Original	29.62	29.70	Neg	25.31	Neg	Neg	human AH1
2008726981	Original	25.92	26.69	Neg	21.86	Neg	Neg	human AH1
2008726982	Original	29.12	31.80	Neg	22.89	Neg	Neg	human AH1
2008726983	Original	17.50	18.86	Neg	20.90	Neg	Neg	human AH1
2008726984	Original	29.35	29.94	Neg	28.55	Neg	Neg	human AH1
2008726985	Original	25.94	26.80	Neg	21.66	Neg	Neg	human AH1
2008726986	Original	Neg	Neg	Neg	19.97	Neg	Neg	negative
2008726987	Original	17.44	18.08	Neg	21.83	Neg	Neg	human AH1
20087260988	Original	16.88	18.71	Neg	22.94	Neg	Neg	human AH1
2008726989	Original	27.35	28.62	Neg	23.88	Neg	Neg	human AH1
2008726990	Original	21.50	22.55	Neg	25.29	Neg	Neg	human AH1

The analytical specificity (reactivity) testing showed that the primer and probe sets within the rRT-PCR Swine Flu Panel performed as expected with seasonal human influenza type A/H1 and A/H3 viruses with no cross detection or non-specific binding to those targets. No cross reactivity was observed when human seasonal influenza A/H1 and A/H3 clinical specimens and cultured viruses were tested.

Please see performance data submitted in 510(k) 080705 cleared by FDA Sept. 30, 2008 for InfA primer and probe set specificity.

Clinical Performance

Retrospective testing was performed on 70 specimens (20 2009 H1N1 influenza positive and 50 2009 H1N1 influenza negative) representing nasopharyngeal swabs, nasal swabs, throat swabs, and throat wash specimens). Fourteen specimens of the 50 2009 H1N1 influenza negative specimens were positive for either seasonal influenza A/H1 or A/H3. Thirty-six of the 50 2009 H1N1 influenza negative specimens were also negative for seasonal A/H1 and A/H3.

Results from Retrospective Testing of Clinical 2009 H1N1 Influenza Specimens

Table 2. Results from Retrospective Testing of 2009 H1N1 Influenza Specimens-InfA

		ABI 7500 Fast Dx CDC rRT-PCR Swine Flu Panel			
		Influenza A Positive	Influenza A Negative	Total	
Light Cycler® 2.0 CDC rRT-PCR Swine Flu Panel	Influenza A Positive	34	0	34	100 % Positive Agreement (89.72%-100.0%) 95% CI
	Influenza A Negative	0	36	36	100 % Negative Agreement (90.36%- 100.0%) 95% CI
	Total	34	36	70	

Table 3. Results from Retrospective Testing of 2009 H1N1 Influenza Specimens-swInfA

		ABI 7500 Fast Dx CDC rRT-PCR Swine Flu Panel			
		Swine Influenza A Positive	Swine Influenza A Negative	Total	
Light Cycler® 2.0 CDC rRT-PCR Swine Flu Panel	Swine Influenza A Positive	20	0	20	100 % Positive Agreement (83.16%-100.0%) 95% CI
	Swine Influenza A Negative	0	50	50	100 % Negative Agreement (92.89%- 100.0%) 95% CI
	Total	20	50	70	

Table 4. Results from Retrospective Testing of Swine-like A/H1N1 Influenza Specimens-swH1

		ABI 7500 Fast Dx CDC rRT-PCR Swine Flu Panel			
		Swine Influenza H1 Positive	Swine Influenza H1 Negative	Total	
Light Cycler® 2.0 CDC rRT-PCR Swine Flu Panel	Swine Influenza H1 Positive	20	0	20	100.0 % Positive Agreement (83.16%-100.0%) 95% CI
	Swine Influenza H1 Negative	0	50	50	100.0 % Negative Agreement (92.89%-100.0%) 95% CI
	Total	20	50	70	

Clinical sensitivity for the swine influenza swInfA and swH1 primer and probe sets was 100% for the A/H1N1 swine influenza virus when present in clinical specimens.

Clinical Performance of the CDC rRT-PCR Swine Flu Panel with Lower Respiratory Specimens

Performance characteristics of the CDC rRT-PCR Swine Flu Panel with human lower respiratory tract specimens were established during surveillance testing at the CDC Influenza Division laboratory. Specimens used for this study were collected for influenza surveillance testing at U.S. public health laboratories and referred to CDC for confirmational testing during the 2009 H1N1 influenza pandemic. Specimens were tested at the CDC with the CDC rRT-PCR Swine Flu Panel on the ABI 7500 Fast Dx Real-Time PCR system followed by virus culture. The specimens have since been retrospectively tested with the CDC rRT-PCR Swine Flu Panel with the Roche LightCycler® 2.0 system. Each lower respiratory specimen was tested with the CDC rRT-PCR Swine Flu Panel (InfA, swInfA, and swH1) markers since the onset of the 2009 H1N1 influenza pandemic.

Retrospective testing was performed on 20 lower respiratory tract specimens from hospitalized patients referred to CDC by U.S. public health laboratories for testing during the 2009 H1N1 influenza pandemic. The specimens tested represent the following: 12 bronchoalveolar lavage (BAL), 2 bronchial washes (BW), 2 endotracheal aspirates (EA), 2 lung tissues, and 2 endotracheal swabs (ES). Sixteen (16) specimens of the lower respiratory tract were positive for 2009 H1N1 influenza and four specimens were negative for seasonal or 2009 H1N1 influenza.

Shown below is performance data generated on both the ABI 7500 Fast Dx (prospective) and the Roche LightCycler® 2.0 (retrospective) Real-Time PCR systems using the CDC rRT-PCR Swine Flu panel.

Table 5. Results from Prospective Testing of Lower Respiratory Clinical Swine Influenza Specimens with the ABI 7500 Fast Dx Real-Time PCR System and the CDC rRT-PCR Swine Flu Panel with Virus Culture Reference Method – InfA

		Virus Culture Reference Method			
		Influenza A Positive	Influenza A Negative	Total	
ABI 7500 Fast Dx Real-time RT-PCR Results	Influenza A Positive	9	2	11	81.8 % Positive Agreement (48.2%-97.7%) 95% CI
	Influenza A Negative	0	0	0	
	Total	9	2	11	

Table 6. Results from Prospective Testing of Lower Respiratory Clinical Swine Influenza Specimens with the ABI 7500 Fast Dx Real-Time PCR System and the CDC rRT-PCR Swine Flu Panel with Virus Culture Reference Method – swInfA

		Virus Culture Reference Method			
		Swine Influenza A Positive	Swine Influenza A Negative	Total	
ABI 7500 Fast Dx Real-time RT-PCR Results	Swine Influenza A Positive	9	2	11	81.8 % Positive Agreement (48.2%-97.7%) 95% CI
	Swine Influenza A Negative	0	0	0	
	Total	9	2	11	

Table 7. Results from Prospective Testing of Lower Respiratory Clinical Swine Influenza Specimens with the ABI 7500 Fast Dx Real-Time PCR System and the CDC rRT-PCR Swine Flu Panel with Virus Culture Reference Method-swH1

		Virus Culture Reference Method			
		Swine Influenza H1 Positive	Swine Influenza H1 Negative	Total	
ABI 7500 Fast Dx Real-time RT-PCR Results	Swine Influenza H1 Positive	9	2	11	81.8 % Positive Agreement (48.2%-97.7%) 95% CI
	Swine Influenza H1 Negative	0	0	0	
	Total	9	2	11	

Table 8. Results from Retrospective Testing of Lower Respiratory Clinical Swine Influenza Specimens with the Roche LightCycler® 2.0 and the CDC rRT-PCR Swine Flu Panel with the Applied Biosystems 7500 Fast Dx Real-Time PCR System Comparator Only – InfA

		ABI 7500 Fast Dx CDC rRT-PCR Swine Flu Panel			
		Influenza A Positive	Influenza A Negative	Total	
Light Cycler® 2.0 CDC rRT-PCR Swine Flu Panel	Influenza A Positive	15	1	16	93.75 % Positive Agreement (69.8%-99.8%) 95% CI
	Influenza A Negative	0	4	4	100 % Negative Agreement (39.76%- 100.0%) 95% CI
	Total	15	5	20	

Table 9. Results from Retrospective Testing of Lower Respiratory Clinical Swine Influenza Specimens with the Roche LightCycler® 2.0 and the CDC rRT-PCR Swine Flu Panel with the ABI 7500 Fast Dx Real-Time PCR System Comparator Only – swInfA

		ABI 7500 Fast Dx CDC rRT-PCR Swine Flu Panel			
		Swine Influenza A Positive	Swine Influenza A Negative	Total	
Light Cycler® 2.0 CDC rRT-PCR Swine Flu Panel	Swine Influenza A Positive	15	1	16	93.75 % Positive Agreement (69.8%-99.8%) 95% CI
	Swine Influenza A Negative	0	4	4	100 % Negative Agreement (39.76%- 100.0%) 95% CI
	Total	15	5	20	

Table 10. Results from Retrospective Testing of Lower Respiratory Clinical Swine Influenza Specimens with the Roche LightCycler® 2.0 and the CDC rRT-PCR Swine Flu Panel with the ABI 7500 Fast Dx Real-Time PCR System Comparator Only -swH1

		ABI 7500 Fast Dx CDC rRT-PCR Swine Flu Panel			
		Swine Influenza H1 Positive	Swine Influenza H1 Negative	Total	
Light Cycler® 2.0 CDC rRT-PCR Swine Flu Panel	Swine Influenza H1 Positive	15	0	15	100.0 % Positive Agreement (78.2%-100.0%) 95% CI
	Swine Influenza H1 Negative	0	5	5	100.0 % Negative Agreement (47.8%-100.0%) 95% CI
	Total	15	5	20	

Technical Support

For technical and product support, contact the CDC Influenza Division Support team directly.

Send email to: FluSupport@cdc.gov

Ordering

Send email request to: FluOrder@cdc.gov

Include the following information in the message:

- Laboratory Name and Address
- CDC Qualified Contact Person, Phone Number, Email Address, and Shipping Address
- Qualification Date