



Lyra[®]
Influenza A+B ASSAY

For the qualitative detection and differentiation of influenza A and influenza B viral RNA extracted from nasal swab and nasopharyngeal swab specimens

For *in vitro* diagnostic use.

R_x ONLY

A symbols glossary can be found at quidel.com/glossary.

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INTENDED USE

The Lyra Influenza A+B Assay is a multiplex Real Time RT-PCR assay for the *in vitro* qualitative detection and differentiation of influenza A and influenza B viral RNA in nasal and nasopharyngeal swabs from patients with signs and symptoms of respiratory infection. This test is intended for use as an aid in the differential diagnosis of influenza A and influenza B viral infections in humans in conjunction with clinical and epidemiological risk factors. The assay does not detect the presence of influenza C virus.

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

Performance characteristics for influenza A were established during the 2011 and 2013 influenza seasons when influenza A/H3 and 2009 H1N1 influenza were the predominant influenza A viruses in circulation. When other influenza A viruses are emerging, performance characteristics may vary.

If infection with a novel influenza A virus is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions for novel virulent Influenza viruses and sent to state or local health department for testing. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.

The assay can be performed using either the Life Technologies QuantStudio™ Dx, the Applied Biosystems® 7500 Fast Dx (ABI 7500), and the Cepheid® SmartCycler® II.

SUMMARY AND EXPLANATION

Influenza viruses (family *Orthomyxoviridae*) contain a single-stranded RNA genome which is present in eight separate segments of ribonucleoprotein. This segmentation of the genome is rare among viruses and probably contributes to the rapid development of new influenza strains through interchange of gene segments if two different viruses infect the same cell. There are three types of influenza – A, B and C. Type A has counterparts in birds and pigs as well as humans, while types B and C are known only in humans.¹ Due to the possibility of another pandemic caused by influenza A, as occurred in 1918 when 30 to 50 million people worldwide died,² the Centers for Disease Control (CDC) and the World Health Organization (WHO) maintain surveillance of influenza strains and make predictions of suitable strains for vaccine production.

It is estimated that in the U.S. there are 30,000-49,000 deaths annually caused by flu-associated illnesses.³ Worldwide, annual epidemics of influenza result in about three to five million cases of severe illness, and about 250,000-500,000 deaths.⁴ Pandemics of influenza A occur about every 10 to 30 years and epidemics of influenza A or B occur annually. Infections are seasonal, typically extending from November to April in the northern hemisphere. Complications tend to occur in the young, elderly and people with chronic cardio-pulmonary diseases.

Incubation time is 1 to 3 days with rapid spread by inhalation via aerial droplets and fomites. It is characterized by fever, cough, sore throat, runny or stuffy nose, muscle or body aches, headache, fatigue, and in some cases vomiting and diarrhea (though this is more common in children than adults).⁵

PRINCIPLE OF THE PROCEDURE

The assay detects viral nucleic acids that have been extracted from a patient sample. A multiplex RT-PCR reaction is carried out under optimized conditions in a single tube generating amplicons for each of the target viruses present in the sample. Identification of influenza A occurs by the use of target specific primers and a fluorescent-labeled probe that hybridizes to a conserved region within the matrix protein gene. Identification of influenza B occurs by the use of target specific primers and fluorescent-labeled probes that hybridize to a conserved influenza B sequence within the neuraminidase gene.

Lyra Probe Labels	
Target	Dye
Influenza A	FAM
Influenza B	CAL Fluor® Orange 560
Process Control (PRC)	Quasar® 670

The following is a summary of the procedure:

- Sample Collection:** Obtain nasal swabs or nasopharyngeal swabs specimens using standard techniques from symptomatic patients. These specimens are transported, stored, and processed according to established laboratory procedures.⁶
- Nucleic Acid Extraction:** Extract nucleic acids from the specimens with the NucliSENS® easyMAG® or EMAG® System following the manufacturer's instructions and using the appropriate reagents (See **Materials Required but Not Provided**).
Prior to the extraction procedure, add 20 µL of the Process Control (PRC) to each 180 µL aliquot of specimen. The PRC serves to monitor inhibitors in the extracted specimen, assures that adequate amplification has taken place, and confirms that the nucleic acid extraction was sufficient.
- Rehydration of Master Mix:** Rehydrate the lyophilized Master Mix using the Rehydration Solution. The Master Mix contains oligonucleotide primers, fluorophore and quencher-labeled probes targeting highly conserved regions of the influenza A and influenza B viruses as well as the PRC sequence. The primers are complementary to highly specific and conserved regions in the genome of these viruses. The probes are dual labeled with a reporter dye attached to the 5' end and a quencher attached to the 3' end.
- Nucleic Acid Amplification and Detection:** Add 15 µL of the rehydrated Master Mix to each reaction tube or plate well. Then add 5 µL of extracted nucleic acids (specimen with PRC) to the reaction tube or plate well. Place the tube

into the SmartCycler II instrument, or place the plate into either the 7500 Fast Dx instrument (ABI 7500), and the QuantStudio Dx.

Once the reaction tube or plate is added to the instrument, the assay protocol is initiated. This protocol initiates reverse transcription of the RNA targets generating complementary DNA, and the subsequent amplification of the target sequences occurs. The Lyra Influenza A+B Assay is based on TaqMan® chemistry, and uses an enzyme with reverse transcriptase, DNA polymerase, and 5'-3' exonuclease activities. During DNA amplification, this enzyme cleaves the probe bound to the complementary DNA sequence, separating the quencher dye from the reporter dye. This step generates an increase in fluorescent signal upon excitation by a light source of the appropriate wavelength. With each cycle, additional dye molecules are separated from their quenchers resulting in additional signal. If sufficient fluorescence is achieved the sample is reported as positive for the detected target sequence.

MATERIALS PROVIDED

Cat. #M100

Detection Kit (96 Reactions) – Store at 2°C to 8°C

Component	Quantity
Rehydration Solution Part M5003	1 vial/kit 1.9 mL
Lyra Influenza A+B Master Mix Part M5004 Lyophilized Contents: DNA polymerase enzyme with reverse transcriptase activity Primers Oligonucleotide primer pairs; Oligonucleotide probes dNTPs (dATP, dCTP, dGTP, dUTP, dTTP) Stabilizers	12 vials/kit, 8 reactions/vial
Process Control Part M5005	1 vial/kit 2.0 mL

OPTIONAL MATERIALS

- Positive controls for influenza A and influenza B (i.e., Quidel Molecular A+B Control Set, Cat. #M106 which serves as an external processing and extraction control).

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettors (range between 1 to 10 µL and 100 to 1000 µL)
- Non-aerosol pipette tips
- SmartCycler II software version 3.0b
- SmartCycler tubes
- SmartCycler centrifuge
- 7500 Fast Dx software version 1.4.1
- 7500 Fast Dx 96 well PCR plate
- Applied Biosystems optical plate films.
- Plate centrifuge for Applied Biosystems 96 well plate.
- QuantStudio Dx software version 1.0 or higher
- NucliSENS easyMAG software (version 2.0 or later) or EMAG software
- NucliSENS easyMAG or EMAG Buffers 1, 2, 3
- NucliSENS easyMAG or EMAG Lysis Buffer
- NucliSENS easyMAG or EMAG Silica Magnetic Beads
- NucliSENS easyMAG or EMAG disposables
- Biohit pipettor
- Biohit Tips for use with EasyMAG
- EMAG Tips 1000µL for use with EMAG

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use

- The assay has been validated using bioMérieux NucliSENS easyMAG software version 2.0-2.1, and EMAG software 1.3.2-9, Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated with a custom extraction program defined in the EMAG procedures below. All other extraction programs used on this instrument must be validated by the user.
- The assay has been validated using SmartCycler II software version 3.0b. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using 7500 Fast Dx software version 1.4.1. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using QuantStudio Dx software version 1.0. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- 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. Viral culture should not be attempted in these cases unless a BSL 3+ facility is available to receive and culture specimens.
- Performance characteristics of this test have been established with the specimen types listed in the **Intended Use Section** only. The performance of this assay with other specimen types or samples has not been evaluated.
- The use of this product should be limited to personnel with sufficient training in PCR and RT-PCR techniques.
- Treat all specimen/samples as potentially infectious. Follow universal precautions when handling samples, this kit and its contents.
- Proper sample collection, storage and transport are essential for correct results.
- Store assay reagents as indicated on their individual labels.
- For accurate results, pipette carefully using only calibrated equipment.
- Thoroughly clean and disinfect all surfaces with a 10% bleach solution followed by molecular grade water.
- Use micropipettes with an aerosol barrier or positive displacement tips for all procedures.
- Avoid microbial and cross contamination of the kit reagents. Follow Good Laboratory Procedures.
- Do not mix reagents from kits with different lot numbers.
- Do not use reagents from other manufacturers with this kit.
- Do not use product after its expiration date.
- Proper workflow planning is essential to minimize contamination risk. Always plan laboratory workflow in a uni-directional manner, beginning with pre-amplification and moving through amplification and detection.
- Use dedicated supplies and equipment in pre-amplification and amplification areas.
- Do not allow cross movement of personnel or equipment between areas.
- Keep amplification supplies separate from pre-amplification supplies at all times.
- Do not open sample tubes or unseal plates post amplification.
- Do not use supplies dedicated for reagent or sample preparation for processing target nucleic acid.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

STORAGE AND HANDLING OF KIT REAGENTS

- Store the unopened kit at 2°C to 8°C until the expiration date listed on the outer kit box.
- The rehydrated Master Mix may be stored at room temperature (20°C to 25°C) for up to 24 hours. For longer storage, the rehydrated Master Mix should be recapped, sealed with parafilm, and stored in an upright position at ≤-20°C for up to 14 days. Protect the Master Mix from light during storage.

Indications of Instability or Deterioration of Reagents: Cloudiness of the Rehydration Solution, when within expiration, may indicate deterioration of this reagent. Contact Quidel Technical Support for a replacement.

SPECIMEN COLLECTION, STORAGE AND HANDLING

Nasal and nasopharyngeal specimens should be collected, transported, stored, and processed according to CLSI M41-A. Specimens should be stored at 2°C to 8°C until tested. If specimens cannot be tested within 72 hours of collection, they should be frozen at -70°C or colder until tested.

The following viral transport media: M4, M4-RT, M5, M6, and UTM (1 mL and 3 mL) are compatible with the Lyra Influenza A+B Assay.














NUCLEIC ACID EXTRACTS STORAGE



Eluates from the easyMAG and EMAG can be stored at room temperature (20°C to 25°C) for 2 hours, at 2°C to 8°C for 8 hours and 1 month at -20° to -70°C.

EXTRACTION – INSTRUMENT SETUP

NucliSENS easyMAG Nucleic Acid Extraction Programming Instructions


Note: An influenza A/influenza B positive processing/extraction control (i.e. Quidel Molecular A+B Control Set, Cat. #M106 or previously characterized positive influenza A or influenza B specimen) and a negative process control (i.e. viral transport media or previously characterized influenza A and influenza B negative specimen) should be included in each extraction run. Turn on the instrument and wait for the instrument light to appear orange. Then switch on the computer/launch easyMAG software. Do not log into software until the light on the instrument has turned green.

1. Barcode reagents after pressing the 'Instrument'  and 'Reagent Inventory'  buttons.
2. To enter samples, press the 'Daily Use'  button, which will default to the 'Define Request'  screen. Select the following settings:
 - a. Sample ID: Enter the sample name using the keyboard.
 - b. Matrix: Select **Other** from the drop-down menu
 - c. Request: Select **Generic** from the drop-down menu
 - d. Volume (mL): Select **0.200** from the drop-down menu.
 - e. Eluate (µL): Select **50** from the drop-down menu.
 - f. Type: Primary
 - g. Priority: Normal
3. Upon pressing the 'Save'  button, the sample will appear in the 'Unassigned Sample' window on the left side of the screen. Press the 'Enter New Extraction Request'  button, and repeat the process for additional samples. 
Alternatively multiple samples can be entered by pressing the 'Auto Create New Extraction Requests'  button.
4. Once all samples are created, go to 'Organize Runs' by clicking the  icon near the top of the page. Create a run by pressing the 'Create Run'  button. Enter a run name or use the default.
5. Add samples to the run by using the 'Auto Fill Run'  button (auto fills up the 24 samples from the 'Unassigned Sample list' on the left hand side of the screen). Alternatively, individual samples can be moved into and out of the run by using the left and right 'Positioning icons'  after selection the appropriate sample. The sample order within the run can be changed using the 'Move Extraction Request Up/Down' buttons .

6. Obtain 1 to 3 (for 8 to 24 samples, respectively) sample vessel(s), and add 20 µL of Process Control to each sample well used.
7. Add 180 µL of each sample to the appropriate well as designated.
8. Go to 'Load Run' by pressing the  button near the top of the screen. Insert tops and sample vessel(s) into the instrument.
9. Enter the barcode(s) of the sample vessel(s).
10. Enter the barcode(s) of silica beads to be used.
11. Close the instrument lid.
12. Assign silica beads to samples as follows:
 - a. Click the reagents symbol below number 1 in the picture below. The lot number of the silica beads should appear below the Silica tab at number 2 in the picture below.
 - b. Highlight and select the samples in the run for which beads need to be assigned (in the box containing number 3 in the picture below).
 - c. Click the  positioning icon (below number 4 in the picture below) to assign the silica lot number to the selected samples.
 - d. If the bead symbol to the right of number 5 in the picture below is selected, the silica bead lot number should be displayed for each sample.



13. Print work list by touching 'Load Run' icon followed by pressing the 'Print Work List' icon .

14. Press the 'Dispense Lysis'  button. The on-board lysis will take approximately 12 minutes to complete.
15. For each sample vessel, prepare magnetic particles using the Biohit pipettors and tips for up to eight reactions as follows:
 - a. Using 1 tip and Program 1, aspirate 550 µL nuclease-free water and dispense into a 1.5 mL DNase / RNase free microfuge tube.
 - b. Vortex the magnetic silica. Using 1 tip and Program 1, aspirate 550 µL of magnetic silica, dispense into the water and mix by vortexing.

- c. Using 1 tip and Program 2, aspirate 1050 μL of the magnetic silica mixture and dispense 25 μL back into the same tube.
- d. Dispense 125 μL magnetic silica mixture each into 8 wells of an ELISA strip plate. Discard tip.
- e. After Lysis is complete (**Note:** the 'Instrument Status' at the bottom of the screen must be 'IDLE!'), using 8 tips and Program 3, aspirate 100 μL of magnetic silica mixture in strip wells, dispense 100 μL of magnetic silica mixture in strip wells, and aspirate 100 μL of magnetic silica mixture in strip wells.
- f. Insert tips into liquid within the sample vessels. Aspirate 800 μL then dispense 900 μL of magnetic silica mixture back into vessel. Aspirate 1000 μL of magnetic silica mixture from vessel and dispense 1000 μL of magnetic silica back into vessel. Repeat aspiration / dispensing of 1000 μL two more times.



16. Close the instrument and press the 'Start' button to begin the run. Upon completion of run, transfer purified nucleic acid to nuclease-free tubes. Eluates from the easyMAG can be stored at room temperature (20°C to 25°C) for 2 hours, at 2°C to 8°C for 8 hours and 1 month at -20°C to -70°C.

EMAG Nucleic Acid Extraction Programming Instructions

Note: An influenza A/influenza B positive processing/extraction control (i.e. Quidel Molecular A+B Control Set, Cat. #M106 or previously characterized positive influenza A or influenza B specimen) and a negative process control (i.e. viral transport media or previously characterized influenza A and influenza B negative specimen) should be included in each extraction run.

The assay has been validated with a custom extraction program defined in the EMAG procedures below. All other extraction programs used on this instrument must be validated by the user.

Section 1: EMAG SYSTEM STARTUP

1. Please complete the following steps in order. Turn on the EMAG instrument screen and the EMAG computer. Turn on the power switch to the EMAG instrument. This switch is located on the back of the instrument above the main power supply cord. Close all the instrument doors and make sure all racks are in place. Turn on the EMAG instrument. After powering on the instrument, wait for the LED status indicator light to come on, then log in to the EMAG software.



2. Click the "Load and Run" icon.
3. Click the "Load" button on the screen under Reagents Display. Scan in the reagent bottles for either the left, right, or both sections depending on your use.
4. Verify that the tips are loaded and there are enough tips for the extraction run. If tips need to be loaded, press Load button on the screen under the Tips display. If tips need loaded, ensure that the barcode is facing the corresponding barcode readers for the instrument side.



- Once all the reagents, tips, and waste containers have been verified and scanned in, each display will appear in green.



Section 2: Creating a Custom Vessel to Vessel Method for Respiratory Samples

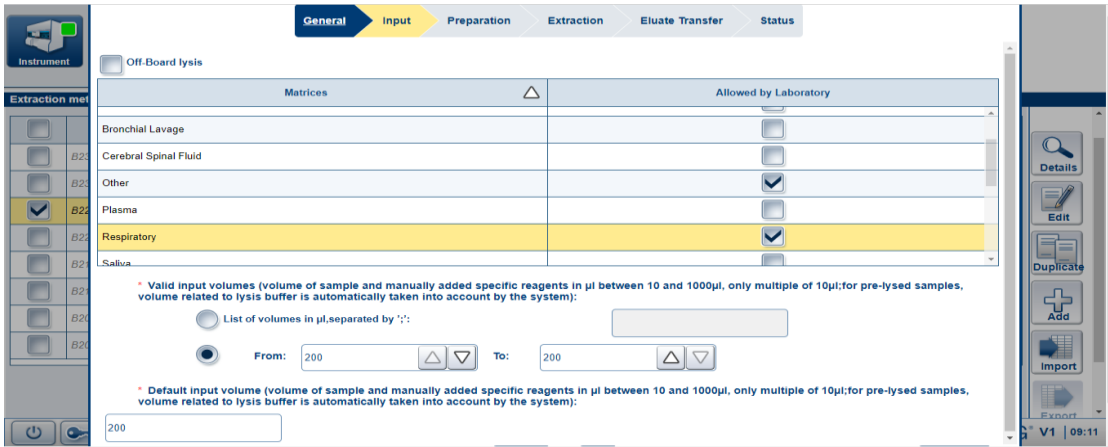
- Navigate to the Configuration screen.
- Within Extraction Methods select **B22V_On_Ves_GE_IC1**.

Note: If the program is inactivated (greyed out), select the Inactivate Tab and check the box to make it active.

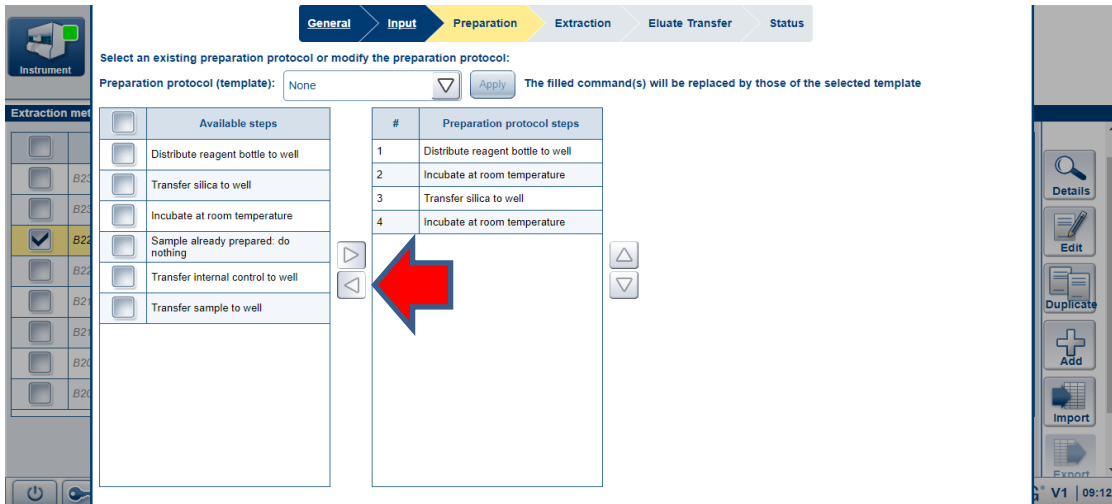
- Click the “Duplicate” button to create a copy of this extraction method and a **General tab** window will pop up.

	Extraction method name	Version	Extraction protocol	Lab. Status	Bmx. Status	Author	Creation date	Description
<input type="checkbox"/>	B23v_On_Ves_GE_IC2	1.1	Generic_3.0.4	Inactivated	Validated	bMx	2023-01-05 15:03	Description for B23v_On_Ve...
<input type="checkbox"/>	B23t_On_Ves_GE_IC2	1.1	Generic_3.0.4	Inactivated	Validated	bMx	2023-01-05 15:03	Description for B23t_On_Ves...
<input checked="" type="checkbox"/>	B22v_On_Ves_GE_IC1	1.1	Generic_3.0.4	Inactivated	Validated	bMx	2023-01-05 15:03	Description for B22v_On_Ve...
<input type="checkbox"/>	B22t_On_Ves_GE_IC1	1.1	Generic_3.0.4	Inactivated	Validated	bMx	2023-01-05 15:03	Description for B22t_On_Ves...
<input type="checkbox"/>	B21v_On_Ves_SB_IC2	1.2	Specific_B_3.0.5	Inactivated	Validated	bMx	2023-01-05 15:03	Description for B21v_On_Ve...
<input type="checkbox"/>	B21t_On_Ves_SB_IC2	1.2	Specific_B_3.0.5	Inactivated	Validated	bMx	2023-01-05 15:03	Description for B21t_On_Ves...
<input type="checkbox"/>	B20v_On_Ves_SB_IC1	1.2	Specific_B_3.0.5	Inactivated	Validated	bMx	2023-01-05 15:03	Description for B20v_On_Ve...
<input type="checkbox"/>	B20t_On_Ves_SB_IC1	1.2	Specific_B_3.0.5	Inactivated	Validated	bMx	2023-01-05 15:03	Description for B20t_On_Ves...

- Under the **General tab** Name this new method “Respy_Vessel_Vessel”.
- Select “Next” to move to the **Input tab**.
- Ensure only “Other” and “Respiratory” are selected in the Allowed in Laboratory Column.
- Ensure the valid input volumes are set to from 200 to 200 and the default input volume is set to 200 as well.

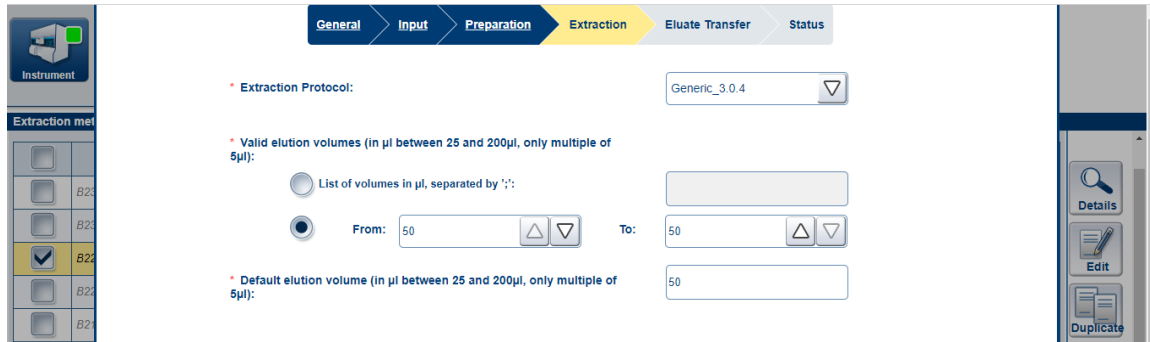


8. Select “Next” to move to the **Preparation tab**.
9. Under the Preparation Protocol Steps Column, select “Transfer internal control to well” and click the back arrow to remove this step. This step will be completed off-board during sample loading.
10. Ensure the following items remain under the Preparation Protocol Steps Column:
 - a. Distribute reagent bottle to well.
 - b. Incubate at room temperature.
 - c. Transfer silica to well
 - d. Incubate at room temperature



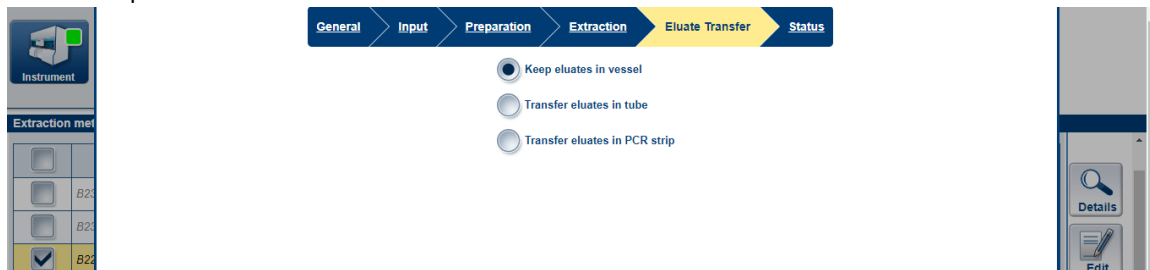
11. Select “Next” to move to the **Extraction tab**.

12. Ensure the Extraction Protocol is set to Generic_3.0.4 and elution volumes are set to 50.



13. Select next to move to the **Eluate Transfer** tab.

14. Select “Keep eluates in vessel”.



15. Select “Next” to move to **Status** tab.

16. Set Status to “Activated” and click Save.

Section 3: Loading an EMAG Extraction Run



1. To create a Request List, Navigate to the Request List screen.
2. Enter the Matrix (Respiratory), Extraction method (Respy_Vessel_Vessel). Verify the following volume:
 - a. Sample Volume (200µL)
 - b. Silica volume (50µL)
 - c. Elution volume (50µL)

- Enter or scan each sample name in the ID field and press enter (section 1). The requested extractions will appear in the request list (section 2).

The screenshot shows the EMAG V1 software interface. At the top, there is a navigation bar with icons for Instrument, Requests list, Prepare input, Load and run, Reports, Configuration, and Maintenance. Below this is the 'Extraction Requests list' form, which includes fields for ID, Matrix, Extraction method, Input volume (µl), Silica volume (µl), Elution volume (µl), and Output type (Vessel, Tube, PCR Strip). A '1' is placed to the left of this form. Below the form is a table titled 'Requests list' with 8 requests. A '2' is placed to the left of this table. The table has columns for ID, Input Type, Matrix, Extraction method, Modification date, Retest, Urgent, and Status. The status of the requests is: 8 (Created), 7 (Created), 6 (Prepared), 5 (Prepared), 4 (Prepared), 3 (Prepared). At the bottom of the interface, there is a 'User: fse' and 'EMAG V1 | 08:29'.



ID	Input Type	Matrix	Extraction method	Modification date	Retest	Urgent	Status
8	Sample	Plasma	B10t_On_Tub_SB_IC2	12/12/19, 8:16 AM			Created
7	Sample	Plasma	B10t_On_Tub_SB_IC2	12/12/19, 8:16 AM			Created
6	Sample	Plasma	B10t_On_Tub_SB_IC2	12/12/19, 8:16 AM			Prepared
5	Sample	Plasma	B10t_On_Tub_SB_IC2	12/12/19, 8:16 AM			Prepared
4	Sample	Plasma	B10t_On_Tub_SB_IC2	12/12/19, 8:16 AM			Prepared
3	Sample	Plasma	B10t_On_Tub_SB_IC2	12/12/19, 8:16 AM			Prepared



- Choose the Prepare Input icon.
- Begin preparing the vessel(s). Into each necessary well, pipette 20µL of Process Control (M5005) provided in the Lyra Influenza A+B Assay kit. (i.e., Five samples need five wells containing Process Control).
- Add 180µL of sample in the wells of the vessel(s) for a total of 200µL of liquid in each well.
- Scan the barcode on the first vessel. Each vessel will have samples individually assigned. (Depending on your barcode scanner, you may have to hit "Enter" after selecting the vessel tab).


- Select or check the boxes by the first set of samples (up to 8) listed for extraction that will go into the vessel indicated at the bottom of the screen.



- Click the small Up/Down  icon at the bottom of the list to add samples to the vessel.
- Click the  icon on the bottom right of the screen to add up to 8 more samples to a new vessel.
- Repeat steps 7 through 10 until all samples in the request list have been assigned to a vessel.

Section 4: Loading Sample Vessels, Aspirator Disposables, and Silica Beads



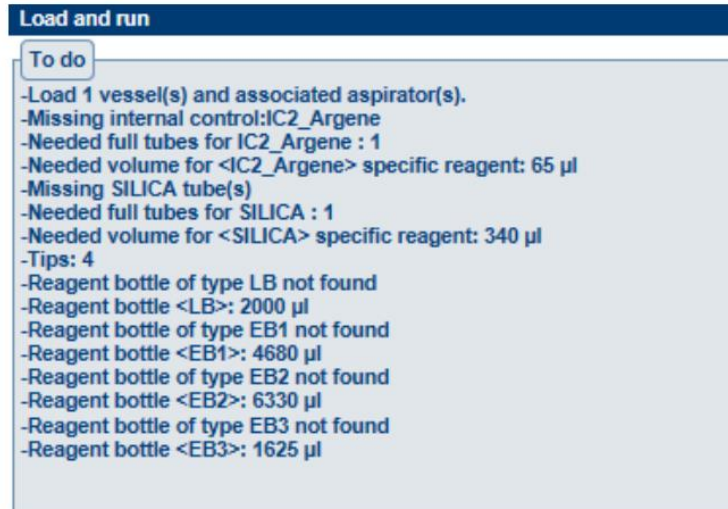
- Click or tap the “Load and Run” icon  at the top of the screen.

Note: In the “To Do” display, there is a guide that shows everything required to start a run.

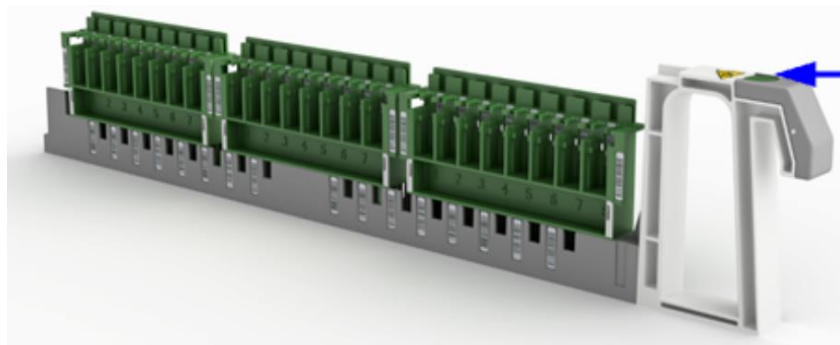
- Open the door to the working area.
- Load the sample vessels into the proper location according to the preparation tray locations. To load a sample vessel:
 - Hold it by the handle.
 - Slide it into position in the working area.
 - Push the vessel into position until the detection tab clicks into the slot at the back.
- Load the aspirator disposables.
- Close the working area door after all sample vessels and aspirator disposables are loaded. The EMAG instrument will now scan the vessel barcodes.
- Load the tubes of Silica.

Note: Before loading, check the number of Silica tubes needed to complete the run. This information is found in the “To Do” box.

Important: Only full (1.2mL) tubes of Silica should be loaded onto the instrument.



- a. To load Silica beads, pull out the reagent rack labeled with a green "R".



7. Vortex tubes immediately before inserting for 5 seconds. Insert the required Silica tubes (uncapped) into the slots of the green rack with the barcode facing the corresponding section (Left or Right).
8. Steadily slide the rack back into the instrument. The EMAG software will display the rack and the tubes.

Note: If a barcode is not read, the tube details can be entered manually by clicking on the segment and choosing the specific reagent. The reagent lot number and expiration date may be entered as well but is not mandatory to start the run.

- a. After the Reagent rack is back in place, Silica tubes will appear loaded on the EMAG software.

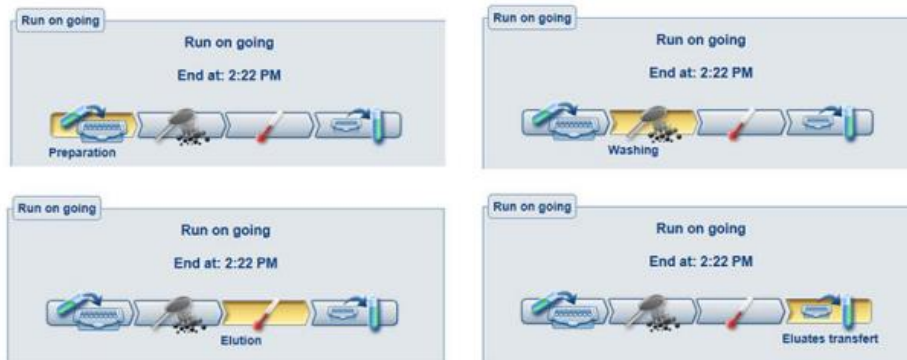
Important: The run must be started as soon as everything is loaded, and the system is ready.



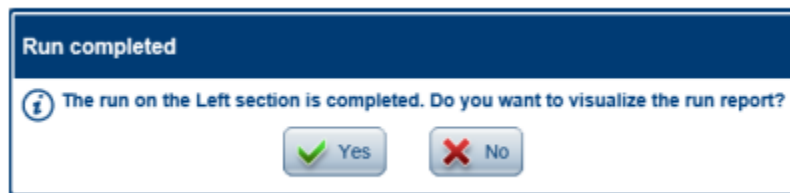
9. Click the Start button for the appropriate section (Left or Right). Each section of the instrument must be started separately.

Note: If any reagents or Silica tubes are missing, the Start button will not be available until all system requirements are met.

10. The EMAG software will show the progress of the run.



11. When the run is finished, the following pop-up box will be displayed. The run report can be viewed on the screen if desired.

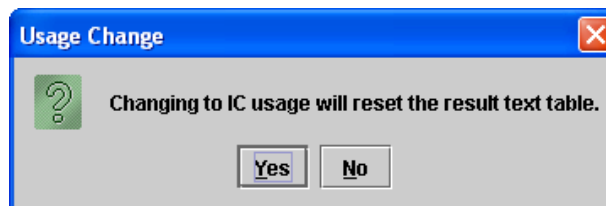


12. Upon completion of the run, remove the Reagent rack.
13. Transfer the purified nucleic acid from the vessel into nuclease-free tubes. Take care not to disturb the Silica that has been pulled to the front of the vessel.

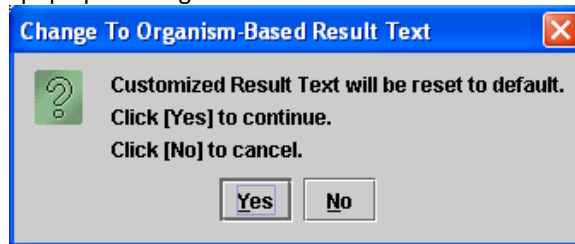
INITIAL THERMOCYCLER PROGRAMMING

SmartCycler II Programming Instructions

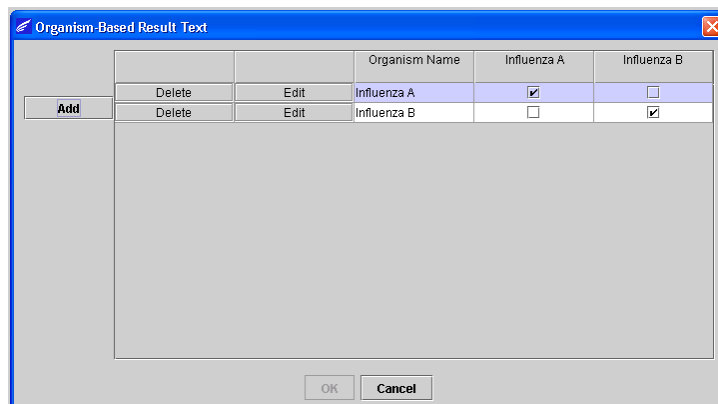
1. Launch the SmartCycler II software package (version 3.0b)
2. Create the Lyra Influenza A+B Assay
 - a. Select the **Define Assays** button from the top of the screen.
 - b. Name the assay.
 - i. Select the **New** button at the bottom left corner of the screen
 - ii. Type in 'Lyra Influenza A+B' and select the **OK**
 - iii. 'Lyra Influenza A+B' will be added to the top of the **Assay Name** list located on the upper left-hand of the screen
 - c. Set the analysis values: Under the **Assay Type: Research** section, select the **Analysis Settings** tab and make sure the following specifications are made:
 - i. Select **FATA25** from the **Dye Set** drop-down menu.
 - ii. The **Analysis Type** drop-down menu should be set to **Qualitative** (Default setting)
 - iii. In the **Channel Name** column, enter 'Influenza A' for FAM, 'Influenza B' for Alx532 and 'PRC' for Alx647
 - iv. In the **Usage** column, select **Target** from the drop down menus for Influenza A and Influenza B, and select **Internal Control** for PRC. When selecting **Internal Control** a window will pop up warning below. Select the **Yes** button.



- v. In the **Curve Analysis** column, enter **Primary Curve** for each channel (Influenza A, Influenza B, PRC) (Default setting).
- vi. In the **Thresh Setting** column, enter **Manual Threshold** for each channel (Influenza A, Influenza B, PRC) (Default setting).
- vii. In the **Manual Thresh Fluor Units** column, enter the following thresholds:
 - a. **Influenza A:** 20.0
 - b. **Influenza B:** 20.0
 - c. **PRC:** 20.0
- viii. In the **Valid Min Cyclers** column, enter **10** for each channel (Influenza A, Influenza B, PRC).
- ix. In the **Valid Max Cyclers** column, enter **45** for each channel (Influenza A, Influenza B, PRC).
- x. In the **Bkgn Sub** column, use "ON" for each channel (Influenza A, Influenza B, PRC) (Default setting).
- xi. In the **Bkgn Min Cycle** column, enter **5** for each channel (Influenza A, Influenza B, PRC) (Default setting).
- xii. In the **Bkgn Max Cycle** column, enter **45** for each channel (Influenza A, Influenza B, PRC).
- xiii. In the **Boxcar Avg cycles** column, keep **0** for each channel (Influenza A, Influenza B, PRC) (Default setting).
- xiv. In the **End Pt Threshold** column, enter **20** for each channel (Influenza A, Influenza B, PRC) (Default setting).
- xv. In the **NC IC%** column, keep "NA" for each channel (PRC) (Default setting).
- xvi. In the **IC Delta** column, keep "NA" for each channel (Influenza A, Influenza B) (Default setting).
- xvii. In the **Customize Result Text** section (below the table), select **Organism Based Result Text** from the drop-down menu. A window will pop up warning below. Select **Yes**.



- xviii. Select the **Customize** button to open the **Organism-Based Result Text** dialog window. Select the **Add** button, enter 'Influenza A' in the **Organism Name** column and check the **Influenza A** box. Select the **Add** button again, enter 'Influenza B' in the **Organism Name** column and check the **Influenza B** box.

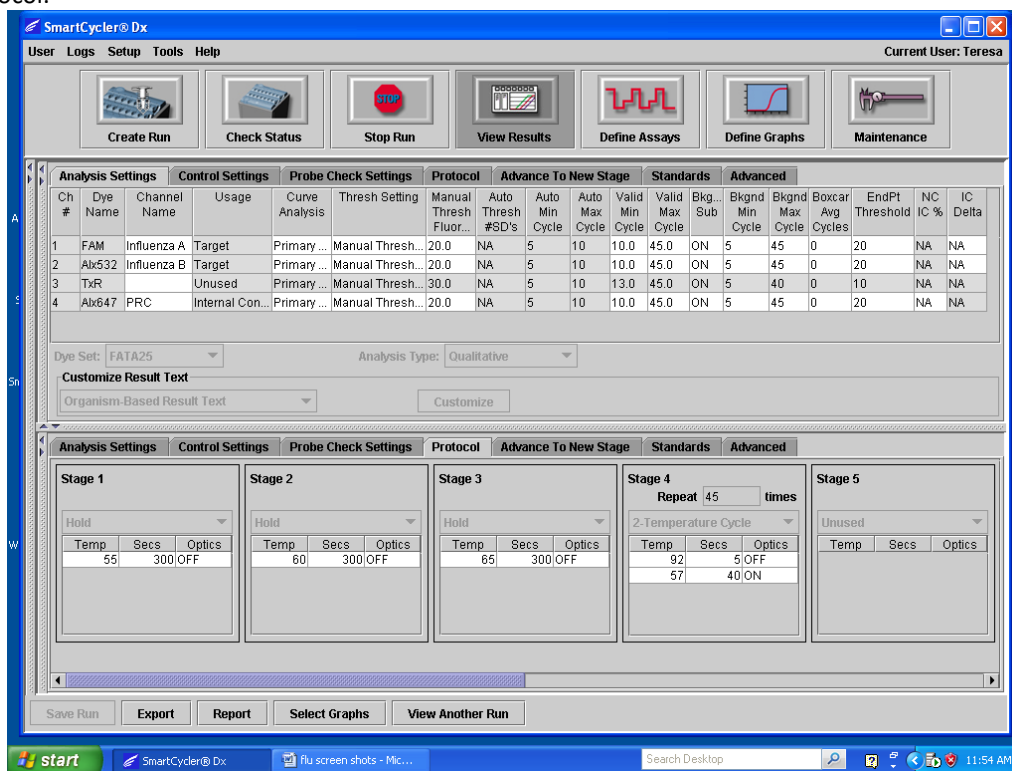


Click **OK** at the bottom of the pop-up window

- d. Set the RT-PCR cycling times and temperatures at the bottom of the screen as follows:
 - i. Stage 1
 - 1. Hold
 - 2. Temp: 55.0
 - 3. Secs: 300
 - 4. Optics: OFF
 - ii. Stage 2
 - 1. Hold
 - 2. Temp 60.0
 - 3. Secs: 300
 - 4. Optics: OFF

- iii. Stage 3
 - 1. Hold
 - 2. Temp: 65.0
 - 3. Secs: 300
 - 4. Optics: OFF
- iv. Stage 4
 - 1. 2-Temperature Cycle
 - 2. Times to Repeat: 45
 - 3. First Temperature Row:
 - a. Temp: 92.0
 - b. Secs: 5
 - c. Optics: OFF
 - 4. Second Temperature Row:
 - a. Temp: 57.0
 - b. Secs: 40
 - c. Optics: ON

3. Save the protocol by selecting the **Save** button at the bottom of the screen Figure of the completed Lyra Influenza A+B Protocol.



7500 Fast Dx Programming Instructions

1. Launch the 7500 Fast Dx software package.
2. The **Quick Startup document** dialog window will open. Select the **Create New Document** button to start the **New Document Wizard**. Follow each step to initiate the Lyra Influenza A+B protocol.
 - a. Define Document: Most of the following should be the default setting. If not, change accordingly. Confirm or enter the following information.

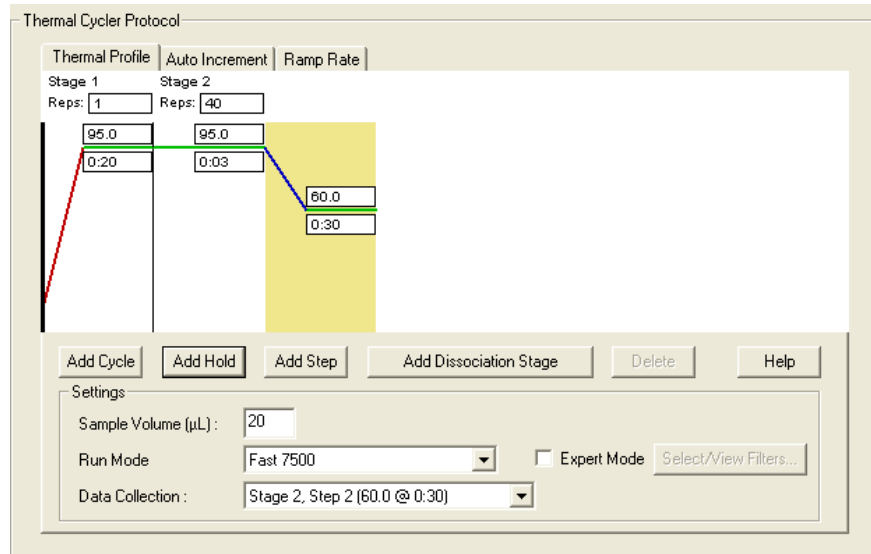
Assay:	Standard Curve (Absolute Quantitation)
Container:	96-Well Clear
Template:	Blank Document
Run Mode:	Fast 7500
Operator:	<i>your operator name</i>

Comments:	SDS v1.4.1
Plate Name:	'Lyra Influenza A+B'

- i. Select the **Next** button.
- b. **Select Detectors:** New detectors for Influenza A, Influenza B and the process control (PRC) must be added. For each target, select the **New Detector** button to open the **New Detector** pop-up window. Alternatively, use the **Create Another** button from within the **New Detector** pop-up window for the last two detectors.
 - i. Enter the following information for each detector.

Name	Reporter Dye	Quencher Dye	Color
Influenza A	FAM	(none)	(Select)
Influenza B	JOE	(none)	(Select)
PRC	Cy5	(none)	(Select)

- ii. Select a unique color to represent each detector.
- iii. Highlight the new detectors and add to the **Detectors in the Document** column using the **Add** button.
- iv. Select **(none)** from the **Passive Reference** drop-down menu.
- v. Select the **Next** button.
- vi. Select the **Finish** button without setting any wells.
- c. The wizard will close, and the software will open, starting with the **Setup** tab. This will show the sample plate that was setup during the quick start. For the initial setup, nothing needs to be changed here.
- d. Defining the Thermocycler Protocol: Select the **Instrument** tab to setup the Lyra Influenza A+B RT-PCR cycling times and temperatures. Under **Thermal Profile** there should be a default 2-stage protocol. Each stage will have 3 user-editable text boxes. The top box value represents the number of reps or cycles for that stage. The middle box value represents the temperature (°C) and the lowest box value represents the time (minutes: seconds).

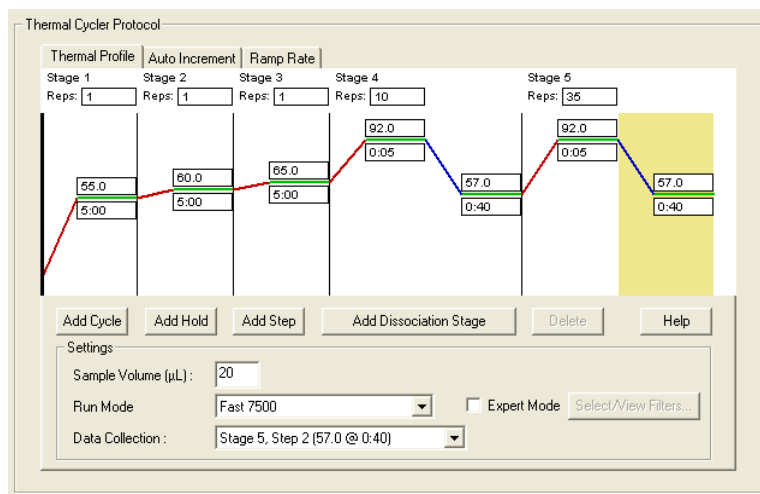


- i. Make the following changes to the default **Thermal Cycler Protocol**:
 1. Stage 1
 - a. Reps: 1
 - b. Temp: 55
 - c. Time: 5:00
 2. Select the bar between Stage 1 and Stage 2. Select the **Add Hold** button to add another stage.
 3. Stage 2
 - a. Reps: 1

- b. Temp: 60
- c. Time: 5:00
- 4. Select the bar between Stage 2 and Stage 3. Select the **Add Hold** button to add another stage.
- 5. Stage 3
 - a. Reps: 1
 - b. Temp: 65
 - c. Time: 5:00
- 6. Stage 4 (2-Step Dissociation Stage)
 - a. Reps: 10
 - b. Step 1
 - i. Temp: 92
 - ii. Time: 0:05
 - c. Step 2
 - i. Temp: 57
 - ii. Time: 0:40
- 7. Select the bar to the right of Stage 4. Select the **Add Cycle** button to add another stage.
- 8. Stage 5 (2-Step Dissociation Stage)
 - a. Reps: 35
 - b. Step 1
 - i. Temp: 92
 - ii. Time: 0:05
 - c. Step 2
 - i. Temp: 57
 - ii. Time: 0:40
- 9. If a wrong stage is added the stage can be removed by pressing the **Delete** button after highlighting the stage between the vertical lines.
- ii. Under **Settings** enter the following:

Sample Volume (µL):	20 (default)
Run Mode:	7500 Fast (default)
Data Collection:	Stage 5, Step 2 (57.0 @ 0:40)
NOTE: Do not check the check box next to 'Expert Mode.'	

iii. Final protocol



- e. Set threshold for each analyte.
 - i. Select the **Results** tab.
 - ii. Select the **Amplification Plot** tab.

- iii. Select Influenza A from the Detector tab in the top right corner.
- iv. In the **Analysis Settings** block, set the **Threshold** to **1.5e5**.
- v. Select the **Auto Baseline** radio button.
- vi. Repeat iii-v for Influenza B setting the **Threshold** to **1.2e5**.
- vii. Repeat iii-v for PRC setting the **Threshold** to **2.7e4**.

The image shows three panels of the software interface, each representing a different assay configuration. Each panel has a 'Data' dropdown set to 'Delta Rn vs Cycle', a 'Detector' dropdown, and a 'Line Color' dropdown set to 'Well Color'. Below these are 'Analysis Settings' with radio buttons for 'Auto Ct' and 'Manual Ct', a 'Threshold' text box, radio buttons for 'Auto Baseline' and 'Manual Baseline', and 'Start (cycle)' and 'End (cycle)' text boxes set to 'Auto'. At the bottom of each panel are 'Analyze' and 'Help' buttons.

- Panel 1 (Influenza A):** Detector: Influenza A, Threshold: 1.5e+005.
- Panel 2 (Influenza B):** Detector: Influenza B, Threshold: 1.2e+005.
- Panel 3 (PRC):** Detector: PRC, Threshold: 2.7e+004.

- f. Save the new protocol as a template for future use.
 - i. At the top of the screen select **File** and then **Save As**.
 - ii. **Save In:** D:\Applied Biosystems\7500 Fast System\Templates\
 - iii. **File name:** 'Lyra Influenza A+B'
 - iv. **Save as type:** 'SDS Templates (*.sdt)'
- g. Exit the software.

QUANTSTUDIO DX PROGRAMMING INSTRUCTIONS

Lyra provides a pre-defined template for the assay on a CD that must be uploaded to the QuantStudio Dx instrument. Please contact a Quidel Representative at 800.874.1517 (in the U.S.) or 858.552.1100, Monday through Friday, from 8:00 a.m. to 5:00 p.m., Eastern Time to obtain this CD. These templates contain the run parameters such that no instrument programming is needed to get started. To install a test definition document:

1. From the QuantStudio Dx Software Home tab, click Manage Test in the Tools panel.
2. From the Test Menu, click Install.
3. Navigate to your test definition document (.tdd) file, select the file, and click Open. The QuantStudio Dx Software automatically adds the selected test to the Test Menu.
4. Click Close to close the Test Menu and save your changes.

ASSAY PROCEDURE

Run the following procedures at controlled room temperature of 20°C to 25°C.

Master Mix Rehydration Procedure

1. Determine the number of extracted specimens to be tested, and obtain the correct number of eight-test lyophilized Master Mix vials for testing.
2. Return unused reagents to the appropriate storage conditions.
3. Open Master Mix carefully to avoid disruption of the pellet.
4. Add 135 µL of Rehydration Solution to the Master Mix.

- Place vial at room temperature for 1 to 2 minutes to allow rehydration of pellet.
- Gently pipette up and down 2 to 3 times (avoiding the formation of bubbles) prior to dispensing into the first PCR tube well.

Note: The rehydrated Master Mix is sufficient for eight reactions.

Note: The rehydrated Master Mix may be stored at room temperature (20°C to 25°C) for up to 24 hours. For longer storage, the rehydrated Master Mix should be recapped, sealed with parafilm and stored in an upright position at ≤-20°C for up to 14 days. Protect the Master Mix from light during storage.

RT-PCR Set-up Procedure:

- Add 15 µL of the rehydrated Master Mix to each reaction tube or plate well.
- Add 5 µL of extracted nucleic acid (specimen with the process control) into the reaction tube or plate well. Mixing of reagents is not required.
Note: Use a new micropipettor non-aerosol tip with each extracted specimen.
- Close the reaction tube or seal the plate.
Note: Quidel recommends that each RT-PCR run should include a tube or a plate well with an extracted influenza A/influenza B positive processing/extraction control (i.e. Quidel Molecular A+B Control Set, Cat. #M106, or previously characterized positive influenza A or influenza B specimen) and a tube or a plate well with a negative control. (i.e. viral transport media or previously characterized influenza A and influenza B negative specimen). Additional controls may be used in accordance with local, state, federal accrediting organizations, as applicable.
- Centrifuge the reaction tube or plate for a minimum of 15 seconds. Ensure that all liquid is at the bottom of the tube or plate wells.
- Insert tube or plate into the appropriate thermocycler.

AMPLIFICATION PROTOCOL ON THE SMARTCYCLER II INSTRUMENT

- Create a new run by clicking on the Create Run icon at the top of the screen. This will open the Create Run screen.
- Under Run Name in the left panel of the Create Run screen, enter a unique run identifier.
- Click on the Assay arrow in the left panel of the Create Run screen and select the Lyra Influenza A+B Protocol from the drop-down menu.
- Under Assay Information in the left panel of the Create Run screen, enter the Lot Number and Expiration Date of the Lyra Influenza A+B Assay.
- In the left panel of the Create Run screen, enter the number of specimens (including the Positive and Negative Control) and click on Apply. This will display the Site Table and the SmartCycler II Dx Software will automatically select the I-Core sites.
- In the Site Table under the Sample ID column, enter the Sample Identifier or Control Identifier for the appropriate I-Core sites.
- Insert each reaction tube into an I-Core site of the SmartCycler by pressing down firmly on all tubes and close each lid.
- Select the Start Run button located at the bottom left corner of the screen. Verify that the LED is on for the appropriate I-Core sites.
- Print Report
 - Click on Report at bottom of screen to open the Report Preview screen.
 - Click on the Print Icon at the top of the screen.

AMPLIFICATION PROTOCOL ON THE 7500 FAST DX THERMOCYCLER

- Switch on 7500 Fast Dx.
- Launch the 7500 Fast Dx software v1.4.1 package.
- The **Quick Startup document** dialog window will open.
- Click on **Create a new document**.
- Most of the following should be the default setting. If not, change accordingly.

Assay:	Standard Curve (Absolute Quantitation)
Container:	96-Well Clear
Template:	Lyra Influenza A+B
Run Mode:	Fast 7500
Operator:	<i>your operator name</i>

Comments:	SDS v1.4.1
Plate Name:	YYMMDD-Lyra Influenza A+B

6. Set Up Sample Plate
 - a. Under the **Setup** and **Plate** tabs the plate setup will appear.
 - b. Select all wells that will contain sample, right-click and select the **Well Inspector** from the drop-down menu. When the **Well Inspector** pop-up window opens, select the detectors for influenza A, influenza B and PRC.
 - c. Use the **Well Inspector** to enter the sample names. Patient IDs may be entered in the Well Inspector window. However, it is recommended that this is done prior to re-suspending the lyophilized master mix, post run, or using the import function to minimize the time the PCR reactions will sit at room temperature prior to starting the run.
 - d. Save the run as **YYMMDD-Lyra Influenza A+B.sds**.
 - e. A window will open asking for the "Reason for change of entry." Enter "**Setup**" and any other comments relevant to the run.
7. Starting the PCR
 - a. Select the **Instrument** tab.
 - b. Insert the 96 well PCR plate into the machine.
 - c. Under **Instrument Control**, select the **Start** button to initiate the run.
8. Post PCR
 - a. **IMPORTANT:** When the run is finished, press OK.
 - b. Analyze the data by pressing the "**Analyze**" button in the top menu and save the file. Save the file by pressing **Save Document** in the task bar. A window will open asking for the "Reason for change of entry."
 - c. Enter "**Data analysis post run**" and any other comments relevant to the run.

AMPLIFICATION PROTOCOL ON THE QUANTSTUDIO DX

1. Switch on QuantStudio Dx.
2. Choose IVD mode on the instrument.
3. Launch the QuantStudio Dx IVD software package.
4. Enter the system **Username** and **Password** when prompted.
5. The **Home screen** window will open.
6. In the **Setup** box, highlight the previously loaded test name "**Lyra Influenza A + B Assay.**"
7. Click the **Setup** button to begin a run.
8. The **Setup, Test Properties** screen will be displayed. Enter run information accordingly.
 - a. Enter the **Experiment Name** (default setting launches the run with a date and time stamp).
 - b. Enter the **Plate Barcode** information.
 - c. Record material lot numbers under **Reagent Information**.
 - d. Save the run with a unique identifier as an ".sds" file (e.g., **YYMMDD-runID#-Lyra Influenza A+B.sds**).
 - e. A window will open asking for the "Reason for change of entry." Enter "**Setup**" and any other comments relevant to the run.
9. In the left menu bar, select **Define**.
10. Edit sample information.
 - a. Enter specific sample information for each well by deleting the default identifier (Patient 1, Patient 2, etc.) and entering new information, OR
 - b. Select **Import from File** across the top of the display to upload a predefined plate map from a Text (tab delimited) file.
11. In the left menu bar, select **Assign** to verify proper plate setup.
12. Loading the sample plate.
 - a. Eject the instrument tray.
 - b. Insert the 96 well PCR plate into the machine with the A1 well positioned in the top, left corner.
 - c. Retract the instrument tray.
13. Starting the run.
 - a. In the left menu bar, select **Run**.
 - b. Click the green **Start Run** button at the top of the screen.
 - i. If prompted, select the serial number specific to the instrument being used.
14. When the run is complete, select **Analysis** in the left menu bar.

- a. Save the file by pressing **Save** in the task bar. A window will open asking for the “Reason for change of entry.” Enter “**Data analysis post run**” and any other comments relevant to the run.
 - b. The **Amplification Plot** will show by default. To view other plot types, select them from the left menu bar.
 - c. To view run information with Ct values, select the **Well Table** tab on the right side of the screen.
15. Printing a report.
- a. In the top menu bar, select **Print Report**. Customize the report contents by selecting or deselecting boxes from the report window.
 - b. Select the “**Print Report**” button at the bottom of the dialogue box.
16. Exporting data files.
- a. In the left menu bar, select **Export**.
 - b. Enter the **Export File Location** OR click **Browse** to locate the desired path.
 - c. The **Export File Name** will default to that of the saved run.
 - d. Select Excel as the file type.
 - e. Customize the exported data report by toggling across the provided tabs and selecting or deselecting options.
 - f. Select **Start Export** along the bottom of the screen.

INTERPRETATION OF RESULTS

Interpretation of Results using the SmartCycler II

Interpretation of the Lyra Influenza A+B Assay Results using the SmartCycler II			
Detector: Influenza A	Detector: Influenza B	Detector: Process Control	Interpretation of Results
Negative	Negative	PASS	Negative - No Influenza A or Influenza B viral RNA detected; PRC detected
Positive	Negative	NA*	Influenza A Positive - Influenza A viral RNA detected
Negative	Positive	NA*	Influenza B Positive - Influenza B viral RNA detected
Negative	Positive	NA*	Influenza A and B Positive - Influenza A and Influenza B viral RNA detected**
Negative	Negative	FAIL	Invalid - No Influenza A or Influenza B and no PRC viral RNA detected, invalid test. Retest the same purified sample. If the test is also invalid, re-extract and retest another aliquot of the same sample or obtain a new sample and retest.

*No Ct value is required for the Process Control to make a positive call.

**Dual infections are rare. Repeat testing using the same purified sample. If the retest confirms this result, collect and test a new specimen. Contact Quidel if multiple samples provide this result.

Error Code 3079: Warning/Error Code 3079 may be observed with influenza A and influenza B positive samples. Warning/Error Code 3079 occurs when the fluorescence (RFU) signal is too high. In this case, all results for that sample are reported by the Dx software as ND (Not Determined). Repeat testing using the same purified sample. If the retest confirms this result, collect and test a new specimen. Contact Quidel if multiple samples provide this result.

Interpretation of Results using the 7500 Fast Dx Thermocycler

Interpretation of the Lyra Influenza A+B Assay Results using the 7500 Fast Dx			
Detector: Influenza A	Detector: Influenza B	Detector: Process Control	Interpretation of Results
Ct <5.0 or Ct >35.0	Ct <5.0 or Ct >35.0	5.0 ≤ Ct ≤ 35.0	Negative - No Influenza A or Influenza B viral RNA detected; PRC detected
5.0 ≤ Ct ≤ 35.0	Ct <5.0 or Ct >35.0	NA*	Influenza A Positive - Influenza A viral RNA detected
Ct <5.0 or Ct >35.0	5.0 ≤ Ct ≤ 35.0	NA*	Influenza B Positive - Influenza B viral RNA detected
5.0 ≤ Ct ≤ 35.0	5.0 ≤ Ct ≤ 35.0	NA*	Influenza A and B Positive - Influenza A and Influenza B viral RNA detected**

Interpretation of the Lyra Influenza A+B Assay Results using the 7500 Fast Dx			
Detector: Influenza A	Detector: Influenza B	Detector: Process Control	Interpretation of Results
Ct <5.0 or C t>35.0	Ct <5.0 or Ct >35.0	Ct <5.0 or Ct >35.0	Invalid - No Influenza A or Influenza B and no PRC viral RNA detected; invalid test. Retest the same purified sample. If the test is also invalid, re-extract and retest another aliquot of the same sample or obtain a new sample and retest.
Undetermined	Undetermined	Undetermined	Invalid - Not Determined. Retest the same purified sample. If the test is also invalid, re-extract and retest another aliquot of the same sample or obtain a new sample and retest.

*No Ct value is required for the Process Control to make a positive call.

**Dual infections are rare. Repeat testing using the same purified sample. If the retest confirms this result, collect and test a new specimen. Contact Quidel if multiple samples provide this result.

Interpretation of Results using the QuantStudio Dx Real-Time PCR Instrument

Interpretation of the Lyra Influenza A+B Assay Results using the QuantStudio Dx			
Detector: Influenza A	Detector: Influenza B	Detector: Process Control	Interpretation of Results
No Ct-value Reported	No Ct-value Reported	Ct-value Reported	Negative - No Influenza A or Influenza B viral RNA detected; PRC detected
Ct-value Reported	No Ct-value Reported	NA*	Influenza A Positive - Influenza A viral RNA detected
No Ct-value Reported	Ct-value Reported	NA*	Influenza B Positive - Influenza B viral RNA detected
Ct-value Reported	Ct-value Reported	NA*	Influenza A and B Positive - Influenza A and Influenza B viral RNA detected**
No Ct-value Reported	No Ct-value Reported	No Ct-value Reported	Invalid - No Influenza A or Influenza B and no PRC viral RNA detected; invalid test. Retest the same purified sample. If the test is also invalid, re-extract and retest another aliquot of the same sample or obtain a new sample and retest.

*No Ct value is required for the Process Control to make a positive call.

**Dual infections are rare. Repeat testing using the same purified sample. If the retest confirms this result, collect and test a new specimen. Contact Quidel if multiple samples provide this result.

QUALITY CONTROL

The Lyra Influenza A+B Assay incorporates several controls to monitor assay performance. Prior to evaluating the patient results, the Positive and Negative Control should be interpreted using the interpretation table below.

1. Failure of either the Positive Control or the Negative Control invalidates the RT-PCR run and results should not be reported. The RT-PCR run should be repeated with the extracted controls and specimens first. Re-extract and retest another aliquot of the controls and the samples or obtain new samples and retest as necessary if the controls fail again.
2. The Process Control should be used during extraction and amplification in the assay. This control should be added to each sample aliquot prior to extraction.
3. Commercially available external positive influenza A/B controls, (i.e., Quidel Molecular A+B Control Set, Cat. #M106 which serves as an external processing and extraction control) should be treated as a patient specimen and be included in every extraction and PCR run. Previously characterized positive influenza A or influenza B specimens may be used in lieu of the commercial external positive influenza A/B control.
4. Viral transport media or previously characterized negative specimen may be used as an external negative control. This must be treated as a patient specimen and be included in every extraction and PCR run.
5. Additional controls may be used in accordance with local, state, federal accrediting organizations, as applicable.

LIMITATIONS

- This test is not intended to differentiate influenza A subtypes. Additional testing is required if subtype differentiation is required.
- Negative results do not preclude infection with influenza virus and should not be the sole basis of a patient treatment decision.
- Improper collection, storage or transport of specimens may lead to false negative results.
- Inhibitors present in the sample and/or errors in following the assay procedure may lead to false negative results.
- Recent patient exposure to LAIV (FluMist) may cause inaccurate dual positive results.
- A trained health care professional should interpret assay results in conjunction with the patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
- Analyte targets (viral sequences) may persist *in vivo*, independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(s) are infectious, nor are the causative agents for clinical symptoms.
- There is a risk of false positive values resulting from cross-contamination by target organisms, their nucleic acids or amplified product, or from non-specific signals in the assay.
- There is a risk of false negative values due to the presence of sequence variants in the viral targets of the assay.
- The performance of the assay has not been established in individuals who received nasally administered Influenza A vaccine.
- The assay performance was not established in immunocompromised patients.
- Positive and negative predictive values are highly dependent on prevalence. The assay performance was established during the 2011 and 2013 influenza seasons. The performance may vary depending on the prevalence and population tested.

EXPECTED VALUES

The Lyra Influenza A+B Assay was evaluated in a clinical study in the winter of 2011 (January 2011 to March 2011) using the SmartCycler II platform and the 7500 Fast Dx. Testing was performed with prospective specimens received from throughout the United States. The table below provides the expected value for each virus on the two instruments.

Expected Values for the Winter of 2011		
Instrument	Influenza A virus	Influenza B virus
SmartCycler II	21.5% (165/767)	19.7% (151/767)
7500 Fast Dx	21.6% (147/682)	17.2% (117/682)

The Lyra Influenza A+B Assay was also evaluated in a clinical study in the winter of 2013 (January 2013 to March 2013) using the QuantStudio Dx. Testing was performed with prospective specimens received from throughout the United States. The number and percentage of the influenza A and influenza B RNA positive cases, calculated by age group, are presented in the following table:

Expected Values for Winter 2013						
Age Group	Influenza A			Influenza B		
	Number of Patients	Number of Positives	Prevalence	Number of Patients	Number of Positives	Prevalence
≤5 year	219	81	37.0%	219	35	16.0%
6-21 years	193	101	52.3%	193	60	31.1%
22-59 years	113	32	28.3%	113	17	15.0%
≥60 years	94	23	24.5%	94	4	4.3%
Total	619	237	38.3%	619	116	18.7%

CLINICAL PERFORMANCE

Prospective Clinical Study – SmartCycler II

Performance characteristics of the Lyra Influenza A+B Assay using the SmartCycler II instrument were established in a prospective study during the 2011 influenza virus season (January to March 2011). Samples used for this study were fresh nasal (427) and nasopharyngeal (352) swab specimens that were collected for routine influenza testing at thirteen sites across the United States. A single specimen was collected per patient and tested within 72 hours of collection. A

comparator method (a high-performance FDA-cleared Influenza A and B molecular test) was used in the evaluation of the Lyra Influenza A+B Assay. Testing of the comparator method was performed at one central location.

The gender and age demographics of the patients enrolled in the study are shown below.

Age and Gender Distribution		
Age Group	Female	Male
≤5 years	188 (50.9%)	199 (48.5%)
6 to 21 years	97 (26.2%)	132 (32.2%)
22 to 59 years	66 (17.9%)	68 (16.6%)
≥60 years	18 (4.9%)	11 (2.7%)
Total	369	410

Seven hundred and seventy-nine (779) fresh specimens (427 nasal swabs and 352 nasopharyngeal swabs) were tested by both Lyra Influenza A+B and the comparator device for influenza A and influenza B viral RNA. Twelve (12) of these specimens were invalid on initial testing with the Quidel assay (1.5%, 95% CI: 0.9% to 2.7%). Re-testing of the specimens according to the Interpretation algorithm described above also yielded invalid results. Twenty-three (23) specimens were invalid on initial and repeat testing (as per the device's PI) on the comparator device (3.0%, 95% CI: 2.0% to 4.4%). Nine (9) specimens were invalid in both devices; therefore, a total of 26 invalid specimens have been removed from additional analysis. The table below details the performance of the Lyra Influenza A+B Assay on the SmartCycler II instrument with the remaining 753 specimens when compared to a commercially available FDA-cleared RT-PCR influenza detection device.

Influenza A			
Fresh nasal & nasopharyngeal swabs (N=753)	Comparator: FDA-cleared RT-PCR device		
Lyra Influenza A+B Assay	Positive	Negative	Total
Positive	157	8*	165
Negative	0	588	588
Total	157	596	753
95% CI			
Positive Percent Agreement	157/157	100%	97.7% to 100%
Negative Percent Agreement	588/596	98.7%	97.4% to 99.4%

*Eight (8) specimens were negative by FDA-cleared RT-PCR device but positive for influenza A by sequence analysis.

Influenza B			
Fresh nasal & nasopharyngeal swabs (N=753)	Comparator: FDA-cleared RT-PCR device		
Lyra Influenza A+B Assay	Positive	Negative	Total
Positive	123	28*	151
Negative	2	600	602
Total	125	628	753
95% CI			
Positive Percent Agreement	123/125	98.4%	94.3% to 99.8%
Negative Percent Agreement	600/628	95.5%	93.6% to 97.0%

*Twenty-six (26) specimens were negative by FDA-cleared RT-PCR device but positive for influenza B by sequence analysis. Two (2) specimens were negative by FDA-cleared RT-PCR device but negative by sequence analysis for influenza B.

The prospective clinical study had a dual infection rate for Influenza A and Influenza B of 2.4% (18/753, 95% CI: 1.5% to 3.7%) using the Lyra Influenza A+B Assay. Three (3) of these dual infections were concordant with the FDA-cleared RT-PCR

device comparator assay. Three (3) of these dual infections were discordant with the Influenza A results from the FDA-cleared RT-PCR device comparator assay. Twelve (12) of these dual infections were discordant with the Influenza B results from the FDA-cleared RT-PCR device comparator assay.

Retrospective Study – SmartCycler II

Performance characteristics of the Lyra Influenza A+B Assay using the SmartCycler II instrument were also evaluated during a retrospective study of frozen specimens collected during the 2011 influenza virus season (January to March of 2011). Samples tested in this study were frozen nasopharyngeal (356) swab specimens that were collected for routine influenza testing. These specimens represent a subset (356/376) of specimens collected in the Retrospective Study – 7500 Fast Dx study. For this study the comparator method was a high performance FDA-cleared influenza A and B molecular device.

Three hundred fifty-six (356) frozen nasopharyngeal swabs were tested by both the subject and the comparator device for influenza A and influenza B viral RNA. Two (2) of these specimens were invalid on initial testing with the subject device (0.6%, 95% CI: 0.2% to 2.0%). Re-testing of the specimens according to the Interpretation algorithm described above also yielded invalid results. Two (2) specimens were invalid on initial and repeat testing on the comparator device (0.6%, 95% CI: 0.2% to 2.0%) (per the device's Instructions for Use). The invalid specimens were removed from performance analyses. The table below details the performance of the Lyra Influenza A+B Assay on the SmartCycler II instrument with the remaining 352 specimens when compared to a commercially available FDA-cleared RT-PCR influenza detection device.

Influenza A			
Frozen nasopharyngeal swabs (N=352)	Comparator: FDA-cleared RT-PCR device		
Lyra Influenza A+B Assay	Positive	Negative	Total
Positive	37	0	37
Negative	0	315	315
Total	37	315	352
95% CI			
Positive Percent Agreement	37/37	100%	90.5% to 100%
Negative Percent Agreement	315/315	100%	98.8% to 100%

Influenza B			
Frozen nasopharyngeal swabs (N=352)	Comparator: FDA-cleared RT-PCR device		
Lyra Influenza A+B Assay	Positive	Negative	Total
Positive	37	5*	42
Negative	1	309	310
Total	38	314	352
95% CI			
Positive Percent Agreement	37/38	97.4%	86.2% to 99.9%
Negative Percent Agreement	309/314	98.4%	96.3% to 99.5%

*Five specimens were negative by FDA-cleared RT-PCR device but positive for influenza B by sequence analysis.

Prospective Clinical Study – 7500 Fast Dx

Performance characteristics of the Lyra Influenza A+B Assay using the 7500 Fast Dx platform were established during a prospective study during the 2011 influenza season (January to March 2011). Samples used for this study were fresh nasal (373) and nasopharyngeal (313) swab specimens that were collected for routine influenza testing at thirteen sites across the United States. A single specimen was collected per patient and tested within 72 hours of collection. These specimens represent a subset (686/779) of specimens collected for the Prospective Clinical Study – SmartCycler II. A comparator method (a high-performance FDA-cleared Influenza A and B molecular test) was used in the evaluation of the Lyra Influenza A+B Assay.

The gender and age demographics of the patients enrolled in this study are shown below.

Age and Gender Distribution		
Age Group	Female	Male
≤5 years	164 (50.6%)	176 (48.6%)
6 to 21 years	85 (26.2%)	113 (31.2%)
22 to 59 years	58 (17.9%)	62 (17.1%)
≥60 years	17 (5.2%)	11 (3.0%)
Total	324	362

These specimens represent a subset (686/779) of specimens collected for the Prospective Clinical Study – SmartCycler II.

Six hundred and eighty-six (686) fresh specimens (373 nasal swabs and 313 nasopharyngeal swabs) were tested by both the subject and the comparator device for influenza A and influenza B viral RNA. Four (4) of these specimens were invalid on initial testing with the subject device (0.6%, 95% CI: 0.2% to 1.5%). Re-testing of the specimens according to the Interpretation algorithm described above also yielded invalid results. Seventeen (17) specimens were invalid on initial and repeat testing (as per the device's PI) on the comparator device (2.5%, 95% CI: 1.6% to 3.9%). Three specimens were invalid in both devices; therefore, a total of 18 specimens were removed from additional analysis. The table below details the performance of the Lyra Influenza A+B Assay on the 7500 Fast Dx instrument with the remaining 668 specimens when compared to a commercially available FDA-cleared RT-PCR influenza detection device.

Influenza A			
Fresh nasal & nasopharyngeal swabs (N=668)	Comparator: FDA-cleared RT-PCR device		
	Lyra Influenza A+B Assay	Positive	Negative
Positive	139	8*	147
Negative	0	521	521
Total	139	529	668
95% CI			
Positive Percent Agreement	139/139	100%	97.4% to 100%
Negative Percent Agreement	521/529	98.5%	97.0% to 99.3%

*Seven (7) specimens were negative by FDA-cleared RT-PCR device but positive for influenza A by sequence analysis. One (1) specimen was negative by FDA-cleared RT-PCR device and negative by sequence analysis for influenza A.

Influenza B			
Fresh nasal & nasopharyngeal swabs (N=668)	Comparator: FDA-cleared RT-PCR device		
	Lyra Influenza A+B Assay	Positive	Negative
Positive	105	12*	117
Negative	5	546	551
Total	110	558	668
95% CI			
Positive Percent Agreement	105/110	95.5%	89.7% to 98.5%
Negative Percent Agreement	546/558	97.8%	96.3% to 98.9%

*Twelve (12) specimens were negative by FDA-cleared RT-PCR device but positive for influenza B by sequence analysis.

The prospective clinical study had a dual infection rate for influenza A and influenza B of 1.8% (12/668, 95% CI: 1.0% to 3.1%) using the Lyra Influenza A+B Assay. Three (3) of these dual infections were concordant with the FDA-cleared RT-PCR device comparator assay. Five (5) of these dual infections were discordant with the influenza A results from the FDA-cleared RT-PCR device comparator assay. Four (4) of these dual infections were discordant with the influenza B results from the FDA-cleared RT-PCR device comparator assay.

Retrospective Study – 7500 Fast Dx

Performance characteristics of the Lyra Influenza A+B Assay using the 7500 Fast Dx platform were also evaluated during a retrospective study of frozen specimens collected during the 2011 influenza virus season (January to March of 2011). Samples tested in this study were frozen nasopharyngeal (376) swab specimens that were collected for routine influenza testing. These specimens include the specimens tested during the Retrospective Study – SmartCycler II. For this study, the comparator method was a high-performance FDA-cleared influenza A and B molecular device.

Three hundred and seventy-six (376) frozen nasopharyngeal swabs were tested by both the subject and comparator devices for influenza A and influenza B virus viral RNA. Two (2) of these specimens were invalid on initial testing with the subject device (0.5%, 95% CI: 0.1% to 1.9%). Re-testing of the specimens according to the Interpretation algorithm described above also yielded invalid results. Two (2) specimens were invalid on initial and repeat testing (as per the device's PI) on the comparator device (0.5%, 95% CI: 0.1% to 1.9%). The invalid specimens were removed from performance analyses. The table below details the results for the performance of the Lyra Influenza A+B Assay on the 7500 Fast Dx instrument with the remaining 372 specimens when compared to a commercially available FDA-cleared RT-PCR influenza detection device.

Influenza A			
Frozen nasopharyngeal swabs (N=372)	Comparator: FDA-cleared RT-PCR Device		
Lyra Influenza A+B Assay	Positive	Negative	Total
Positive	37	0	37
Negative	0	335	335
Total	37	335	372
95% CI			
Positive Percent Agreement	37/37	100%	90.5% to 100%
Negative Percent Agreement	335/335	100%	98.9% to 100%

Influenza B			
Frozen nasopharyngeal swabs (N=372)	Comparator: FDA-cleared RT-PCR Device		
Lyra Influenza A+B Assay	Positive	Negative	Total
Positive	37	2*	39
Negative	1	332	333
Total	38	334	372
95% CI			
Positive Percent Agreement	37/38	97.4%	86.2% to 99.9%
Negative Percent Agreement	332/334	99.4%	97.9% to 99.9%

*Two (2) specimens were negative by FDA-cleared RT-PCR device but positive for influenza B by sequence analysis.

Prospective Clinical Study – QuantStudio Dx

Performance characteristics of the Lyra Influenza A+B Assay using the QuantStudio Dx were established in a prospective study during the 2013 influenza season (January to March 2013). Six hundred and thirty-one (631) fresh swab specimens were collected for routine influenza virus testing at three distinct geographical locations in the United States. These specimens were tested at three clinical sites in the United States. A single specimen was collected per patient. The specimens were extracted with the NucliSENS easyMAG and tested with the Lyra Influenza A+B Assay using the QuantStudio Dx. The specimens were also tested with a high-performance FDA-cleared Influenza A and B molecular test.

The gender and age demographics of the patients enrolled in this study are shown below.

Age and Gender Distribution		
Age Group	Female	Male

≤ 5 years	124 (36.6%)	103 (35.3%)
6 to 21 years	90 (26.5%)	103 (35.3%)
22 to 59 years	67 (19.8%)	47 (16.1%)
≥ 60 years	58 (17.1%)	39 (13.4%)
Total	339	292

Six hundred and thirty-one (631) fresh swab specimens were tested by both the subject and comparator device for influenza A and influenza B viral RNA. Four (4) of these specimens were invalid on initial testing with the subject device (0.6%, 95% CI: 0.2% to 1.6%). Re-testing of the specimens according to the Interpretation algorithm described above also yielded invalid results. Eight (8) specimens were invalid on initial and repeat testing (as per the device's Package Insert) on the comparator device (1.3%, 95% CI: 0.6% to 2.5%). A total of twelve (12) invalid specimens have been removed from additional analysis. The table below details the performance of the Lyra Influenza A+B Assay on the QuantStudio Dx with the remaining 619 specimens when compared to a commercially available FDA-cleared RT-PCR influenza detection device.

Influenza A			
Fresh nasal & nasopharyngeal swabs (N=619)	Comparator: FDA-cleared RT-PCR device		
Lyra Influenza A+B Assay	Positive	Negative	Total
Positive	204	33	237
Negative	0	382	382
Total	204	415	619
95% CI			
Positive Percent Agreement	204/204	100%	98.2% to 100%
Negative Percent Agreement	382/415	92.0%	89.0% to 94.3%

Influenza B			
Fresh nasal & nasopharyngeal swabs (N=619)	Comparator: FDA-cleared RT-PCR device		
Lyra Influenza A+B Assay	Positive	Negative	Total
Positive	106	10	116
Negative	1	502	503
Total	107	512	619
95% CI			
Positive Percent Agreement	106/107	99.1%	94.9% to 99.8%
Negative Percent Agreement	502/512	98.0%	96.4% to 98.9%

The prospective clinical study had a dual infection rate for Influenza A and Influenza B of 1.8% (11/631, 95% CI: 1.0% to 3.1%) using the Lyra Influenza A+B Assay. Three (3) of these dual infections were concordant with the FDA-cleared RT-PCR device comparator assay. Six (6) of these dual infections were discordant with the Influenza A results from the FDA-cleared RT-PCR device comparator assay. Two (2) of these dual infections were discordant with the Influenza B results from the FDA-cleared RT-PCR device comparator assay.

ANALYTICAL PERFORMANCE

Level of Detection

The analytical sensitivity (limit of detection or LOD) of the Lyra Influenza A+B Assay was determined using quantified (TCID₅₀/mL) cultures of five (5) influenza A strains and three (3) influenza B strains, serially diluted in negative nasopharyngeal matrix. Each dilution was extracted using the NucliSENS easyMAG System in replicates of 20 per concentration of virus and tested on both the SmartCycler II, the 7500 Fast Dx, and the QuantStudio Dx platforms. Analytical sensitivity (LOD) is defined as the lowest concentration at which at least 95% of all replicates tested positive. The demonstrated LOD for each of the three (3) instruments is shown below.

Level of Detection			
Strain	Final TCID ₅₀ /mL LOD		
	SmartCycler II	7500 Fast Dx	QuantStudio Dx
A/Mexico/4108/2009 (H1N1)	2.40E+01	4.80E+01	2.0E+01
A1/Mal/302/54 (H1N1)	7.00E+00	1.60E+01	N/A
A/Victoria/3/75 (H3N2)	3.10E+01	9.20E+01	N/A
A/Brisbane/59/2007 (H1N1)	N/A	3.33E+01	1.00E+02
A/Brisbane (H3N2)	N/A	1.00E+01	5.00E+01
B/RCHIN 8/05	1.80E+00	1.20E+01	N/A
B/Brisbane '09-'10 Vaccine Strain	N/A	1.50E+02	1.00E+02
B/Florida/04/2006	6.00E+00	4.30E+01	1.00E+02
B/Malaysia/25/06/04	1.30E+00	5.70E+00	1.00E+00

Analytical Reactivity (Inclusivity)

The reactivity of the Lyra Influenza A+B Assay was evaluated against multiple strains of influenza A and influenza B viruses. The clinical influenza panel consisted of ten (10) Influenza A subtype H1N1, two (2) Influenza A subtype 2009H1N1, eight (8) Influenza A subtype H3N2, two (2) Influenza A subtype H5N1, and 13 Influenza B strains. An additional panel of non-clinical restricted isolates was also tested. Each panel member was extracted using the NucliSENS easyMAG instrument and tested in triplicate on both the SmartCycler II and 7500 Fast Dx platforms.

In 2013 additional studies were performed using two new and unique strains of influenza A virus (H3N2v and H7N9). Six (6) isolates of the H3N2v strain were extracted using the NucliSENS easyMAG instrument and tested in triplicate on both the QuantStudio Dx and the 7500 Fast Dx platforms. An inactivated isolate of H7N9 was extracted using the NucliSENS easyMAG instrument and tested in triplicate on both the QuantStudio Dx and 7500 Fast Dx platforms.

The Lyra Influenza A+B Assay detected 100% of the influenza A (45/45) and influenza B strains (15/15) at 2x to 3x LOD levels including pandemic and avian influenza A strains and recent circulating influenza A variant strains.

Influenza A Viruses				
Subtype	Strain	TCID ₅₀ /mL	A	B
2009 H1N1	H1N1 A/California/07/2009	1.45E+02	Positive	Negative
H1N1	A/New Caledonia/20/1999	1.12E+02	Positive	Negative
H1N1	A/New Jersey/8/76	3.80E+02	Positive	Negative
H1N1	A/PR/8/34	5.89E+02	Positive	Negative
H1N1	A/NWS/33	NA	Positive	Negative
H1N1	A/Denver/1/57	1.26E+02	Positive	Negative
H1N1	A/FM/1/47	3.80E+02	Positive	Negative
2009 H1N1	A/Mexico/4108/2009	1.40E+02	Positive	Negative
H1N1	A1/Mal/302/54	4.19E+02	Positive	Negative
H1N1	A/Taiwan/42/06	3.39E+02	Positive	Negative
H1N1	A/Brisbane/59/07	7.24E+01	Positive	Negative
H1N1	A/Solomon Islands/3/06	1.41E+01	Positive	Negative
H3N2	A/WI/629-2/2008 (H3N2)	2.00E+02	Positive	Negative
H1N1	A/WI/629-S7(D02473)/2009 (H1N1pdm)	2.00E+02	Positive	Negative
H1N1	A/WI/629-S5 (D02312)/2009 (H1N1pdm)	2.00E+02	Positive	Negative
H3N2	A/Hong Kong/8/68	1.15E+02	Positive	Negative
H3N2	A/Wisconsin/67/2005	7.24E+02	Positive	Negative
H3N2	A/Aichi/2/68	4.17E+02	Positive	Negative
H3N2	A/Port Chalmers/1/73	4.57E+02	Positive	Negative
H3N2	A/Perth/16/2009	9.83E+02	Positive	Negative
H3N2	A/Uruguay/7/16/2007	1.03E+02	Positive	Negative
H3N2	A/Victoria/3/75	2.19E+02	Positive	Negative
H3N2	A/Brisbane/10/07	4.17E+02	Positive	Negative

Influenza A Viruses				
Subtype	Strain	TCID ₅₀ /mL	A	B
H3N2v	A/Indiana/10/11	5.90E+01	Positive	Negative
H3N2v	A/Kansas/13/9	4.90E+01	Positive	Negative
H3N2v	A/Pennsylvania/14/10	4.80E+01	Positive	Negative
H3N2v	A/Victoria/361/11	5.20E+01	Positive	Negative
H3N2v	A/Minnesota/11/10	4.60E+01	Positive	Negative
H3N2v	A/West Virginia/6/11	5.00E+01	Positive	Negative
H7N9*	A/ANHUI/1/2013	3.95E+03*	Positive	Negative

*Inactivated virus – relative EID₅₀ Titer/mL

Influenza B Viruses			
Strain	TCID ₅₀ /mL	A	B
B/HongKong/5/72	6.67E+02	Negative	Positive
B/Panama/45/90	1.02E+02	Negative	Positive
B/Florida/02/2006	3.16E+02	Negative	Positive
B/Florida/04/2006	3.80E+02	Negative	Positive
B/Florida/07/2004	1.26E+02	Negative	Positive
B/Malaysia/25/06/04	3.41E+02	Negative	Positive
B/Maryland/1/59	1.15E+02	Negative	Positive
B/Allen/45	4.17E+02	Negative	Positive
B/Taiwan/2/62	1.51E+02	Negative	Positive
B/Russia/69	2.19E+02	Negative	Positive
B/Mass/3/66	1.38E+02	Negative	Positive
B/Lee/40	1.95E+02	Negative	Positive
B/GL/1739/54	6.30E+02	Negative	Positive

The following avian strains were tested as cultured isolates in a BS-3 facility.

Non-clinical Restricted Influenza A Viruses				
Subtype	Strain	TCID ₅₀ /mL	A	B
H2N2	A/Mallard/NY/6750/78 (H2N2)	2.00E+02	Positive	Negative
H7N3	A/Chicken/NJ/15086-3/94 (H7N3)	2.00E+02	Positive	Negative
H9N2	A/Chicken/NJ/12220/97 (H9N2)	2.00E+02	Positive	Negative
H4N8	A/Mallard/OH/338/86 (H4N8)	2.00E+02	Positive	Negative
H6N2	A/Chicken/CA/431/00 (H6N2)	2.00E+02	Positive	Negative
H8N4	A/Blue Winged Teal/LA/B174/86 (H8N4)	2.00E+02	Positive	Negative
H5N1	A/Anhui/01/2005(H5N1)-PR8-IBCDC-RG5	2.00E+02	Positive	Negative
H10N7	A/GWT/LA/169GW/88 (H10N7)	2.00E+02	Positive	Negative
H11N9	A/Chicken/NJ/15906-9/96 (H11N9)	2.00E+02	Positive	Negative
H12N5	A/Duck/LA/188D/87 (H12N5)	2.00E+02	Positive	Negative
H13N6	A/Gull/MD/704/77 (H13N6)	2.00E+02	Positive	Negative
H14N5	A/Mallard/GurjevRussia/262/82 (H14N5)	2.00E+02	Positive	Negative
H15N9	A/Shearwater/Australia/2576/79 (H15N9)	2.00E+02	Positive	Negative
H16N3	A/Shorebird/DE/172/2006(H16N3)	2.00E+02	Positive	Negative

REPRODUCIBILITY STUDY

The reproducibility of the Lyra Influenza A+B Assay was evaluated at three laboratory sites. Reproducibility was assessed using three panels (one panel for the SmartCycler II, one panel for the 7500 Fast Dx, and one panel for the QuantStudio Dx) of eight simulated samples. The panels consisted of two (2) positive samples (above LOD), a high negative (0.3x LOD) sample and a negative sample for each virus, influenza A (A/Mexico/4108/2009) and influenza B (B/Florida/04/2006).

Panels and controls were tested at each site by two (2) operators per instrument for five (5) days, each sample tested in three (3) replicates, for a total of 90 results per level for each virus for each instrument (2 operators x 5 days x 3 sites x 3

replicates). Each panel and controls were extracted using the NucliSENS easyMAG system and tested on either the SmartCycler II, the 7500 Fast Dx, or the QuantStudio Dx platform.

Reproducibility Results –SmartCyclerII												
Panel Member ID (TCID ₅₀ / mL)	Site 1			Site 2			Site 3			Combined Site Data		
	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV
Influenza A High Negative 0.3x LOD (7.2E+00)	3/30 (3 positive results)	44.3*	1.2	4/30 (4 positive results)	43.5*	4.5	2/30 (2 positive results)	42.7*	0.50	9/90	43.5*	3.2
Influenza A Low Positive 2x LOD (4.8E+01)	30/30	37.7	2.7	30/30	38.1	3.8	30/30	37.4	4.5	90/90	37.7	3.7
Influenza A Med Positive 5x LOD (1.2E+02)	30/30	35.4	1.9	30/30	36.1	3.3	30/30	35.0	1.6	90/90	35.5	2.7
Influenza A Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
Influenza B High Negative 0.3x LOD (1.8E+00)	0/30	N/A	N/A	1/30 (1 positive result)	39.9*	N/A	4/30 (4 positive results)	42.4*	3.2	5/90	41.9*	3.9
Influenza B Low Positive 2x LOD (1.2E+01)	30/30	35.4	1.14	30/30	36.2	3.1	30/30	34.9	3.6	90/90	35.4	3.3
Influenza B Med Positive 5x LOD (3.0E+01)	30/30	33.5	1.2	30/30	33.9	1.6	30/30	33.0	1.3	90/90	33.4	1.7
Influenza B Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
Influenza A Positive Control	30/30	29.2	1.2	30/30	29.5	1.9	30/30	29.3	1.5	90/90	29.3	1.6
Influenza B Positive Control	30/30	27.8	1.1	30/30	28.1	2.7	30/30	27.6	1.5	90/90	27.8	2.0
Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A

*Average Ct of only positive results

Reproducibility Results – 7500 Fast Dx												
Panel Member ID (TCID ₅₀ /mL)	Site 1			Site 2			Site 3			Total Results		
	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV
Influenza A High Negative (1.44E+01)	4/30 (4 positive results)	34.0*	2.0	8/30 (8 positive results)	34.1*	1.9	0/30	N/A	N/A	12/90	34.1	1.8

Reproducibility Results – 7500 Fast Dx												
Panel Member ID (TCID ₅₀ /mL)	Site 1			Site 2			Site 3			Total Results		
	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV
Influenza A Low Positive (9.6E+01)	30/30	27.3	3.5	30/30	27.3	6.3	30/30	29.2	7.0	90/90	27.9	6.7
Influenza A Med Positive (2.4E+02)	30/30	25.3	2.9	30/30	25.2	5.1	30/30	26.8	5.5	90/90	25.7	5.4
Influenza A Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
Influenza B High Negative (1.3E+01)	0/30	N/A	N/A	3/30 (3 positive results)	34.2*	1.2	0/30	N/A	N/A	3/90	34.2	1.2
Influenza B Low Positive (8.6E+01)	30/30	24.7	2.6	30/30	24.6	4.8	30/30	25.7	5.1	90/90	25.0	4.7
Influenza B Med Positive (2.2E+02)	30/30	22.9	2.0	30/30	22.7	2.6	30/30	23.5	2.9	90/90	23.0	3.0
Influenza B Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
Influenza A Positive Control	30/30	12.4	1.6	30/30	11.8	2.2	30/30	12.1	1.1	90/90	12.1	2.4
Influenza B Positive Control	30/30	15.2	2.6	30/30	14.9	3.1	30/30	15.1	1.4	90/90	15.1	2.5
Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A

*Average Ct of only positive results

Reproducibility Results – QuantStudio Dx												
Panel Member ID	Site 1			Site 2			Site 3			Combined Site Data		
	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV
Influenza A High Negative (0.3 x LOD)	25/30	37.4*	3.8	13/30	38.2*	2.6	4/28**	37.5*	4.2	42/88**	37.2	3.6
Influenza A Positive 1	30/30	27.5	2.5	30/30	27.7	2.1	29/29**	27.9	4.3	89/89**	27.7	3.1
Influenza A Positive 2	30/30	26.4	1.7	30/30	26.4	1.8	30/30	27.0	6.1	90/90	26.6	3.9
Influenza A Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
Influenza B High Negative (0.3 x LOD)	28/30	37.7*	3.1	23/30	37.8*	3.6	14/28**	37.7*	2.7	65/88**	37.7	3.2
Influenza B Positive 1	30/30	25.8	1.8	30/30	25.4	1.5	29/29	25.9	8.7	89/89**	25.7	5.2
Influenza B Positive 2	30/30	24.4	1.8	30/30	24.0	1.7	30/30	24.9	8.8	90/90	24.4	5.5
Influenza B Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A

Reproducibility Results – QuantStudio Dx												
Panel Member ID	Site 1			Site 2			Site 3			Combined Site Data		
	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV
Influenza A Positive Control	30/30	28.7	2.7	30/30	30.7	2.1	30/30	31.7	9.2	90/90	30.4	7.1
Influenza B Positive Control	30/30	27.9	1.2	30/30	27.5	1.8	30/30	29.9	6.9	90/90	28.7	6.6
Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A

*Average Ct of positive results only

**One or more of the replicates was invalid due to non-detection of the PRC

Upon review of the QuantStudio Dx reproducibility data it was determined that the concentration of the positive samples was higher than expected. A supplemental study was conducted internally using a near-LOD specimen. In this study, a sample with a low positive concentration (2x LOD) for influenza A and for influenza B and a negative sample were each extracted on three NucliSENS easyMAG systems and then tested on three (3) QuantStudio Dx platforms. The two (2) samples and controls were tested by two (2) operators per instrument for five (5) days, each sample tested in three (3) replicates, for a total of 90 results per sample for each virus for each instrument (2 operators x 5 days x 3 instruments x 3 replicates). This data is presented separately as a supplemental study.

Reproducibility Results – QuantStudio Dx – Supplemental Study												
Panel Member ID (TCID ₅₀ /mL)	QuantStudio #1			QuantStudio #2			QuantStudio #3			Combined Instrument Data		
	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV	Rate of Detection	AVE Ct	%CV
Influenza A Low Positive (3.90E+01)	30/30	35.1	3.5	30/30	33.7	4.6	30/30	35.7	3.1	90/90	34.8	4.4
Influenza A Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
Influenza B Low Positive (2.01E+02)	30/30	35.2	3.1	30/30	34.5	3.2	30/30	35.6	2.7	90/90	35.1	3.2
Influenza B Negative	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A
Influenza A Positive Control	30/30	31.0	4.6	30/30	30.9	6.1	30/30	31.3	4.0	90/90	31.1	5.0
Influenza B Positive Control	30/30	27.7	1.5	30/30	27.7	3.2	30/30	28.1	1.3	90/90	27.8	2.3
Negative Control	0/30	N/A	N/A	0/30	N/A	N/A	0/30	N/A	N/A	0/90	N/A	N/A

ANALYTICAL SPECIFICITY – CROSS-REACTIVITY

The analytical specificity of the Lyra Influenza A+B Assay was evaluated by testing a panel consisting of 26 viruses, 24 bacteria, and one (1) yeast strain representing common respiratory pathogens or flora commonly present in the nasopharynx. Bacteria and yeast were tested at concentrations of 10⁵ to 10¹⁰ CFU/mL. Viruses were tested at concentrations of 10³ to 10⁶ TCID₅₀/mL. Samples were extracted using the NucliSENS easyMAG instrument and tested in triplicate on the 7500 Fast Dx platform. Analytical specificity of the Lyra Influenza A+B Assay was 100%.

Cross-reactivity			
Organism ID	Final Conc.	Influenza A Result	Influenza B Result
hMPV A1	3.70E+04	Negative	Negative
hMPV B1	2.37E+04	Negative	Negative
RSV Long	4.40E+04	Negative	Negative
RSV Washington	1.75E+03	Negative	Negative
Adenovirus 1/Adenoid 71	5.67E+04	Negative	Negative
Coronavirus 229E	1.70E+06	Negative	Negative
Coronavirus OC43	1.67E+06	Negative	Negative
Coxsackievirus B4	2.43E+06	Negative	Negative
Coxsackievirus B5/10/2006	2.28E+06	Negative	Negative
Cytomegalovirus	8.76E+05	Negative	Negative
Echovirus 7	5.38E+08	Negative	Negative
Echovirus 9	1.50E+06	Negative	Negative
Echovirus 6	1.05E+08	Negative	Negative
Echovirus 11	1.50E+05	Negative	Negative
Enterovirus 71	2.68E+03	Negative	Negative
Enterovirus 70	1.66E+05	Negative	Negative
Epstein Barr Virus	5,000cp/mL	Negative	Negative
HSV Type 1 MacIntyre strain	1.95E+06	Negative	Negative
HSV Type 2 G strain	3.67E+06	Negative	Negative
Rubeola	3.78E+05	Negative	Negative
Mumps virus	8.43E+04	Negative	Negative
Parainfluenza Type 1	2.50E+05	Negative	Negative
Parainfluenza Type 2	2.20E+04	Negative	Negative
Parainfluenza Type 3	9.10E+05	Negative	Negative
Parainfluenza Type 4	9.57E+06	Negative	Negative
Varicella Zoster Virus	7.50E+02	Negative	Negative
<i>Bordetella pertussis</i>	1.04E+07	Negative	Negative
<i>Bordetella bronchiseptica</i>	2.55E+07	Negative	Negative
<i>Chlamydia trachomatis</i>	2.10E+05	Negative	Negative
<i>Legionella pneumophila</i>	2.05E+08	Negative	Negative
<i>Mycobacterium intracellulare</i>	6.90E+08	Negative	Negative
<i>Mycobacterium tuberculosis</i>	6.60E+07	Negative	Negative
<i>Mycobacterium avium</i>	1.36E+10	Negative	Negative
<i>Haemophilus influenzae</i>	5.90E+07	Negative	Negative
<i>Pseudomonas aeruginosa</i>	5.15E+07	Negative	Negative
<i>Proteus vulgaris</i>	2.65E+08	Negative	Negative
<i>Proteus mirabilis</i>	2.75E+07	Negative	Negative
<i>Neisseria gonorrhoeae</i>	2.15E+07	Negative	Negative
<i>Neisseria meningitidis</i>	1.85E+08	Negative	Negative
<i>Neisseria mucosa</i>	1.85E+08	Negative	Negative
<i>Klebsiella pneumoniae</i>	3.30E+07	Negative	Negative
<i>Escherichia coli</i>	6.80E+07	Negative	Negative
<i>Moraxella catarrhalis</i>	5.85E+07	Negative	Negative

Cross-reactivity			
Organism ID	Final Conc.	Influenza A Result	Influenza B Result
<i>Corynebacterium diptheriae</i>	6.0E+05	Negative	Negative
<i>Lactobacillus plantarum</i>	1.03E+08	Negative	Negative
<i>Streptococcus pneumoniae</i>	4.5E+07	Negative	Negative
<i>Streptococcus pyogenes</i>	2.05E+08	Negative	Negative
<i>Streptococcus salivarius</i>	2.50E+06	Negative	Negative
<i>Staphylococcus epidermidis</i>	2.6E+07	Negative	Negative
<i>Staphylococcus aureus</i>	5.15E+08	Negative	Negative
<i>Candida albicans</i>	1.07E+06	Negative	Negative

ANALYTICAL SPECIFICITY – INTERFERING SUBSTANCES

The performance of Lyra Influenza A+B Assay was evaluated with potentially interfering substances that may be present in nasopharyngeal specimens. The potentially interfering substances were evaluated with influenza A (A/Mexico/4108/2009), and influenza B (B/Florida/04/2006) at a concentration of 3x LOD on the 7500 Fast Dx platform. There was no evidence of interference caused by the substances tested at the concentrations shown below.

Substance Name	Concentration Tested	Influenza A Result (3x LOD)	Influenza B Result (3x LOD)
Mucin (Bovine Submaxillary Gland, type I-S)	60 µg/mL	Positive	Positive
Blood (human), EDTA anticoagulated	2% (vol/vol)	Positive	Positive
Neo-Syneprine	15% (vol/vol)	Positive	Positive
Afrin Nasal Spray	15% (vol/vol)	Positive	Positive
Zicam Homeopathic Non-Drowsy Allergy Relief No Drip Liquid Nasal Gel	5% (vol/vol)	Positive	Positive
Saline Nasal Spray	15% (vol/vol) of dose	Positive	Positive
Throat Lozenges	0.68 g/mL; 1/18 drop, crushed; active ingredients: 1.7 mg/mL menthol	Positive	Positive
Zanamivir	3.3 to 5 mg/mL	Positive	Positive
Tobramycin	4.0 µg/mL	Positive	Positive
Mupirocin	6.6 to 10 mg/mL	Positive	Positive
Oseltamivir phosphate	7.5 to 25 mg/mL	Positive	Positive

CARRYOVER AND CROSS-CONTAMINATION STUDIES

An internal study was completed with the SmartCycler II, the 7500 Fast Dx, and QuantStudio Dx platform where a number of PCR reactions were performed in five separate extractions and PCR runs. Each extraction run had alternating high positive and high negative samples within the same disposable. Each PCR run had alternating high positive and negative samples. All the high positive samples were positive for influenza A and influenza B (100%). All of the high negative samples were negative for influenza A and influenza B. The data demonstrates that no carry-over or cross contamination was observed with the NucliSENS easyMAG automated nucleic acid extraction instrument and the SmartCycler II instrument, the 7500 Fast Dx, or QuantStudio Dx platform.

ASSISTANCE

If you have any questions regarding the use of this product, please contact Quidel Technical Support at 1.800.874.1517 (in the U.S.) or technicalsupport@quidel.com. If outside the U.S., further information can be obtained from your distributor, or directly from Quidel at one of the numbers listed below. Reference quidel.com to see more options for Support.

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REFERENCES

- 1 Atmar, R.L. and Lindstrom, S.E. 2011. Influenza Viruses in *Manual of Clinical Microbiology*. 10th Edition. 1333–1334.
- 2 <http://1918.pandemicflu.gov> accessed on 6/9/11
- 3 <http://www.who.int/mediacentre/factsheets/fs211/en/>
- 4 http://www.cdc.gov/flu/about/disease/us_flu-related_deaths.htm accessed on 6/9/11
- 5 <http://www.cdc.gov/flu/about/disease/symptoms.htm>
- 6 Clinical and Laboratory Standards Institute. Viral Culture; Approved Guidelines. CLSI document M41-A [ISBN 1562386239] Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA 2006.

ADDITIONAL SOURCE MATERIAL

1. Guidance on Class II Special Controls Guidance Document: Reagents for Detection of Specific Novel Influenza A Viruses (March 2006) – <http://www.fda.gov/cdrh/oivd/guidance/1596.pdf>.
2. Guidance on In Vitro Diagnostic Devices to Detect Influenza A Viruses: Labeling and Regulatory Path (April 2006) – <http://www.fda.gov/cdrh/oivd/guidance/1594.pdf>.
3. Guidance on Informed Consent for In Vitro Diagnostic Device Studies Leftover Human Specimens that are Not Individually Identifiable (April 2006) – <http://www.fda.gov/cdrh/oivd/guidance/1588.pdf>.
4. Draft Guidance on Nucleic Acid Based In Vitro Diagnostic Devices for Detection of Microbial Pathogens (Dec 2005) – <http://www.fda.gov/cdrh/oivd/guidance/1560.html>.
5. CLSI EP17-A: Guidance for Protocols for Determination of Limits of Detection and Limits of Quantitations (Vol. 2, No. 34) (Oct 2004).
6. CLSI MM13-A: Guidance for the Collection, Transport, Preparation and Storage of Specimens for Molecular Methods (Vol. 25, No. 31) (Dec 2005).
7. CLSI EP7-A2: Guidance for Interference Testing in Clinical Chemistry (Vol. 25, No. 27 Second Ed) (Nov 2005).
8. CLSI EP12-A: Guidance for User Protocol for Evaluation of Qualitative Test Performance (Vol. 22, No. 14) (Sept 2002).
9. CLSI MM6-A: Guidance for the Quantitative Molecular Methods for Infectious Diseases (Vol. 23, No. 28) (Oct 2003).
10. CLSI EP5-A2: Guidance for Evaluation of Precision Performance of Quantitative Measurement Methods (Vol. 24, No. 25 Second Ed.) (Aug 2004).



M100 – Lyra Influenza A+B Assay



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EFM1560800EN00 (01/23)

SYMBOLS GLOSSARY

REF

Catalogue number

LOT

Batch code



Use by



Manufacturer



Temperature limitation



Intended use

R_x ONLY

Prescription use only



Consult e-labeling
instructions for use



Biological risks

IVD

For *In Vitro* diagnostic use



Contains sufficient for 96 determinations

CONT

Contents/Contains

CONTROL

Control
