QUIDEL

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

Contents

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, or 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 persons 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:

1. **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]
2. **Nucleic Acid Extraction:** Extract nucleic acids from the specimens with the NucliSENS® easyMAG® 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.
3. **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.
4. **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 Cepheid SmartCycler II instrument, or place the plate into either the Applied Biosystems 7500 Fast Dx instrument or Life Technologies 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)	12 vials/kit, 8 reactions/vial
Stabilizers	
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
- Applied Biosystems 7500 Fast Dx software version 1.4
- Applied Biosystems 7500 Fast Dx 96 well PCR plate
- Applied Biosystems optical plate films
- Plate centrifuge for Applied Biosystems 96 well plate
- Life Technologies QuantStudio Dx software version 1.0 or higher
- bioMérieux NucliSENS easyMAG software version 2.0
- bioMérieux NucliSENS easyMAG Buffers 1, 2, 3
- bioMérieux NucliSENS easyMAG Lysis Buffer
- bioMérieux NucliSENS easyMAG Silica Magnetic Beads
- bioMérieux NucliSENS easyMAG disposables
- Biohit pipettor

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use
- The assay has been validated using bioMérieux NucliSENS easyMAG software version 2.0. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using Cepheid 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 Applied Biosystems 7500 Fast Dx software version 1.4. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using Life Technologies 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.
- 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.
- Wear suitable protective clothing, gloves, eye and face protection when using this kit.
- 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.
- Dispose of amplified material carefully and in accordance with local laws and regulations in order to minimize the risk of amplicon contamination.
- Do not use supplies dedicated for reagent or sample preparation for processing target nucleic acid.
- MSDS is available upon request or can be accessed on the product website.

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 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

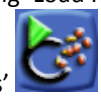
bioMérieux NucliSENS easMAG Nucleic Acid Extraction Programming Instructions

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. Vortex liquid stool specimen for 15 seconds to ensure complete mixing.

1. Turn on the instrument and wait for 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.
2. Barcode reagents after pressing the 'Instrument'  and 'Reagent Inventory'  buttons.
3. 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
4. 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.
5. 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.
6. 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  .
7. 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.
8. Add 180 µL of each sample to the appropriate well as designated.
9. Go to 'Load Run' by pressing the  button near the top of the screen. Insert tops and sample vessel(s) into the instrument.
10. Enter the barcode(s) of the sample vessel(s).
11. Enter the barcode(s) of silica beads to be used.
12. Close the instrument lid.
13. 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.

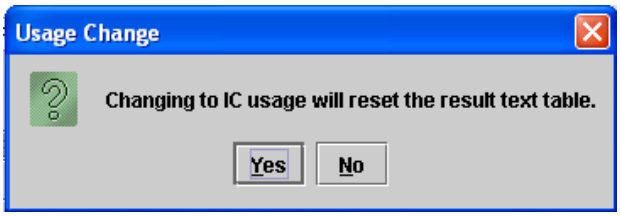
d. If the bead symbol to the right of number 5 in the picture below is selected, the silica bead lot number should be displayed for each sample.

14. Print work list by touching 'Load Run' icon followed by pressing the 'Print Work List' icon  .
15. Press the 'Dispense Lysis'  button. The on-board lysis will take approximately 12 minutes to complete.
16. 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.
17. Close the instrument and press the 'Start'  button to begin the run.
18. 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.

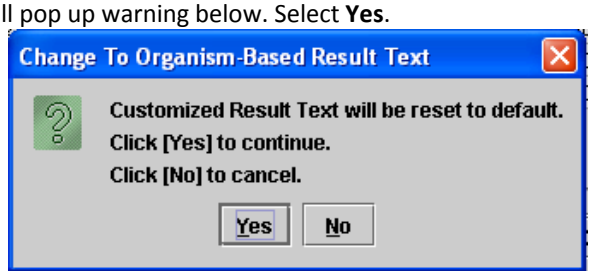
INITIAL THERMOCYCLER PROGRAMMING

Cepheid SmartCycler II Programming Instructions

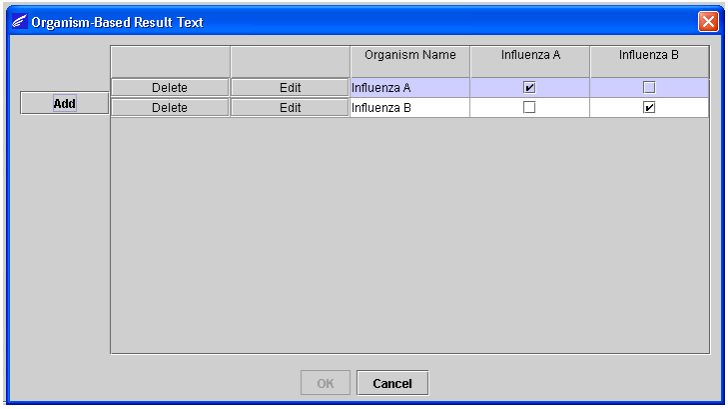
1. Launch the SmartCycler II software package (version 3.0b)
2. Create the Lyra Influenza A+B Assay
   a. Select the **Define Assays** button from the top of the screen
   b. Name the assay
      i. Select the **New** button at the bottom left corner of the screen
      ii. Type in 'Lyra Influenza A+B' 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 Cycler** column, enter **10** for each channel (Influenza A, Influenza B, PRC).
      ix. In the **Valid Max Cycler** column, enter **45** for each channel (Influenza A, Influenza B, PRC).
      x. In the **Bkgnd Sub** column, use "ON" for each channel (Influenza A, Influenza B, PRC) (Default setting).
      xi. In the **Bkgnd Min Cycle** column, enter **5** for each channel (Influenza A, Influenza B, PRC) (Default setting).
      xii. In the **Bkgnd 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**.



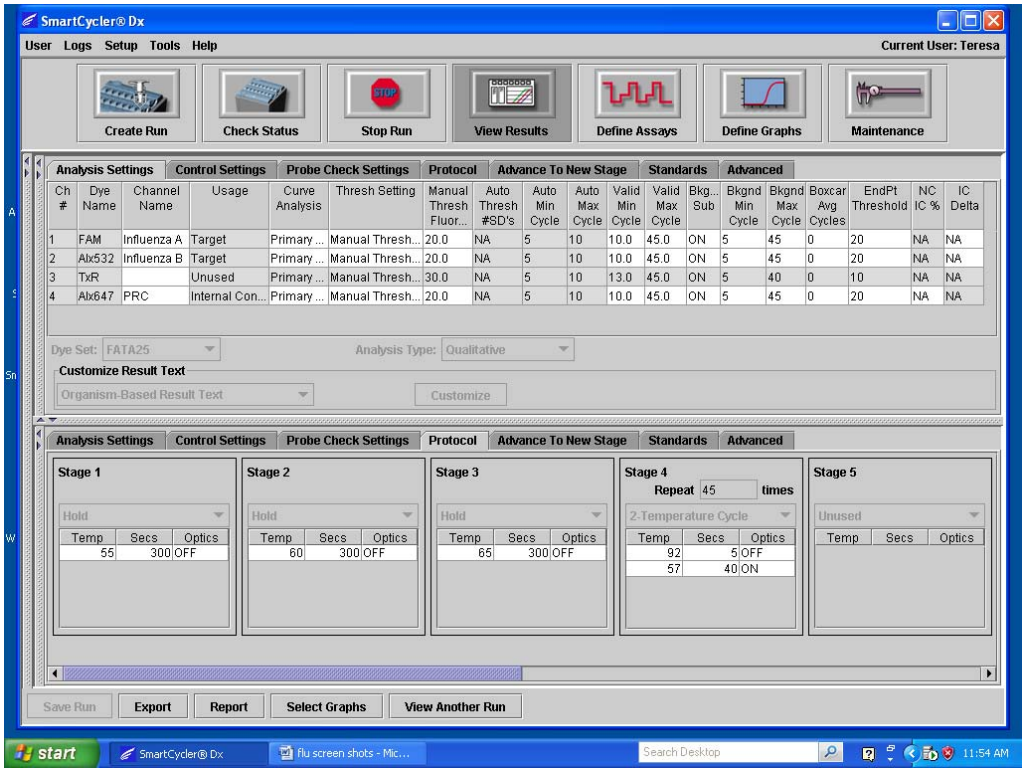
      xviii. Select the **Customize** button to open the **Organism-Based Result Text** dialog window. Select the **Add** button, enter 'Influenza A' in the **Organism Name** column and check the **Influenza A** box. Select the **Add** button again, enter 'Influenza B' in the **Organism Name** column and check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



Applied BioSystem 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:	*your operator name*
Comments:	SDS v1.4
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.

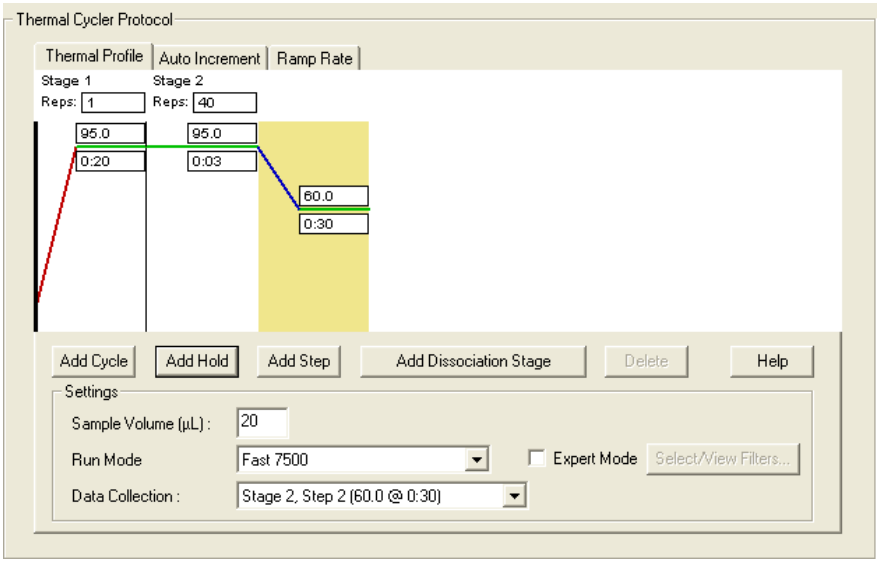
Name	Reporter Dye	Quencher Dye	Color
Influenza A	FAM	(none)	(Select)
Influenza B	JOE	(none)	(Select)
PRC	Cy5	(none)	(Select)

      ii. Select a unique color to represent each detector.
      iii. Highlight the new detectors and add to the **Detectors in 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 Theromocycler 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).



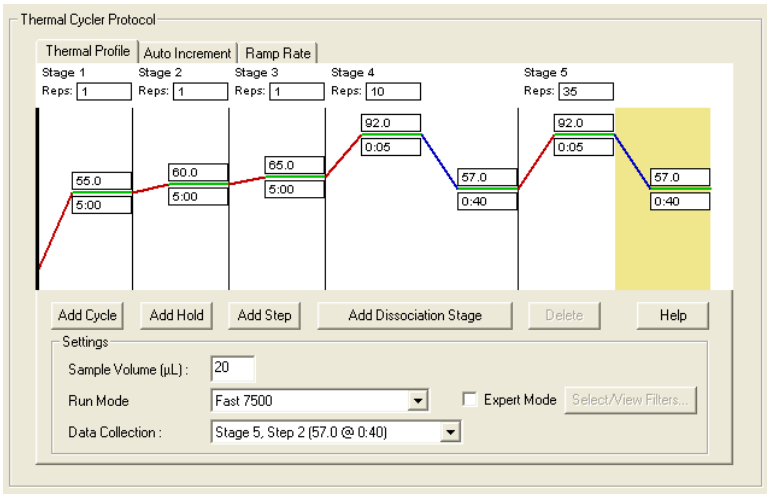
i. Make the following changes to the default **Thermal Cycler Protocol**:
   1. Stage 1
      a. Reps: 1
      b. Temp: 55
      c. Time: 5:00
   2. Select the bar between Stage 1 and Stage 2. Select the **Add Hold** button to add another stage.
   3. Stage 2
      a. Reps: 1
      b. Temp: 60
      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.

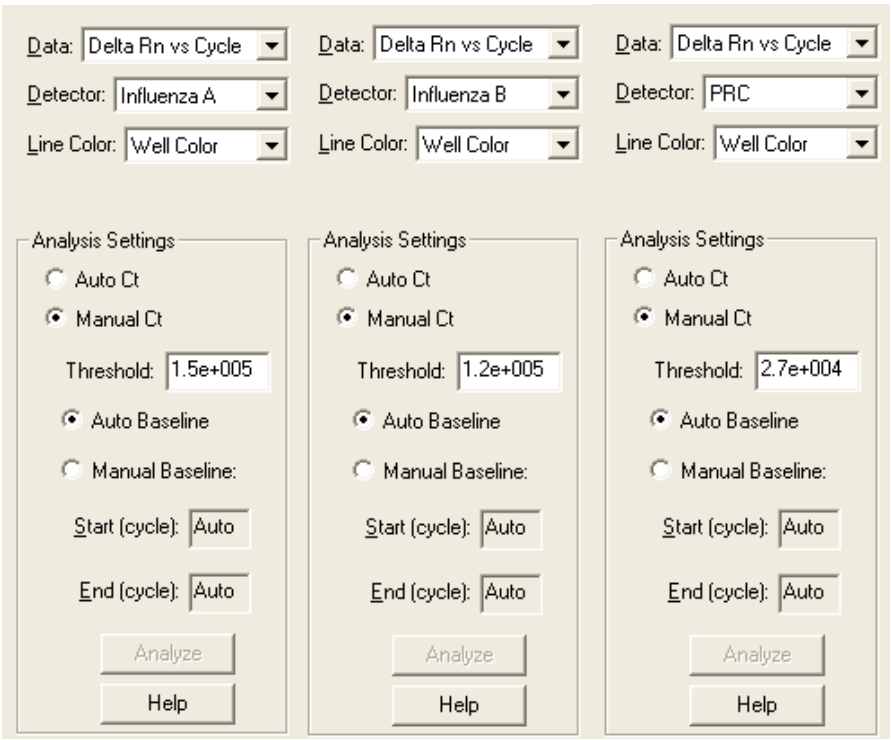
ii. Under **Settings** enter the following:

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**.



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.

LIFE TECHNOLOGIES QUANTSTUDIO Dx PROGRAMMING INSTRUCTIONS

Lyra provides a pre-defined template for the assay on a CD that must be uploaded to the Life Technologies 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 Life Technologies 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 Life Technologies 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.
5. Place vial at room temperature for 1 to 2 minutes to allow rehydration of pellet.
6. 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:

1. Add 15 µL of the rehydrated Master Mix to each reaction tube or plate well.
2. 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.
3. 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.
4. 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.
5. Insert tube or plate into the appropriate thermocycler.

AMPLIFICATION PROTOCOL ON THE SMARTCYCLER II INSTRUMENT

1. Create a new run by clicking on the Create Run icon at the top of the screen. This will open the Create Run screen.
2. Under Run Name in the left panel of the Create Run screen, enter a unique run identifier.
3. 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.
4. 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.
5. 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.
6. In the Site Table under the Sample ID column, enter the Sample Identifier or Control Identifier for the appropriate I-Core sites.
7. Insert each reaction tube into an I-Core site of the SmartCycler by pressing down firmly on all tubes and close each lid.
8. 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.
9. Print Report
   a. Click on Report at bottom of screen to open the Report Preview screen.
   b. Click on the Print Icon at the top of the screen.

AMPLIFICATION PROTOCOL ON THE ABI 7500 FAST Dx THERMOCYCLER

1. Switch on Applied Biosystems 7500 Fast Dx.
2. Launch the Applied Biosystems 7500 Fast Dx software v1.4 package.
3. The **Quick Startup document** dialog window will open.
4. Click on **Create a new document**.
5. 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:	*your operator name*
Comments:	SDS v1.4
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 LIFE TECHNOLOGY 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 in 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 Cepheid 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 Applied Biosystems 7500 Fast Dx Thermocycler

Interpretation of the Lyra Influenza A+B Assay Results using the ABI 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**
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.

Interpretation of the Lyra Influenza A+B Assay Results using the ABI 7500 Fast Dx			
Detector: Influenza A	**Detector: Influenza B**	**Detector: Process Control**	**Interpretation of Results**
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 Cepheid SmartCycler II platform and the Applied Biosystems 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**
Cepheid SmartCycler II	21.5% (165/767)	19.7% (151/767)
Applied Biosystems 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 Life Technologies 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 – Cepheid SmartCycler II

Performance characteristics of the Lyra Influenza A+B Assay using the Cepheid 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 Cepheid 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-PCRdevice 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 – Cepheid SmartCycler II

Performance characteristics of the Lyra Influenza A+B Assay using the Cepheid 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 – Applied Biosystems 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 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 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 Cepheid 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 - Applied Biosystems 7500 Fast Dx

Performance characteristics of the Lyra Influenza A+B Assay using the Applied Biosystems 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 – Cepheid 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 – Cepheid 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 18 specimens were removed from additional analysis. The table below details the performance of the Lyra Influenza A+B Assay on the Applied Biosystems 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	Total
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	Total
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 of these dual infections were concordant with the FDA-cleared RT-PCR device comparator assay. Five 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 – Applied Biosystems 7500 Fast Dx

Performance characteristics of the Lyra Influenza A+B Assay using the Applied Biosystems 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 – Cepheid 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 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 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 Applied Biosystems 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 – Life Technologies QuantStudio Dx

Performance characteristics of the Lyra Influenza A+B Assay using the Life Technologies 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 bioMérieux easyMAG and tested with the Lyra Influenza A+B Assay using the Life Technologies 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 PI) 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 Life Technologies 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 ($TCID_{50}$/mL) cultures of five influenza A strains and three 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 Cepheid SmartCycler II, the Applied Biosystems 7500 Fast Dx, and the Life Technologies 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 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 Influenza A subtype H1N1, two Influenza A subtype 2009H1N1, eight Influenza A subtype H3N2, two 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 Cepheid SmartCycler II and Applied Biosystems 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 Life Technologies QuantStudio Dx and the Applied Biosystems 7500 Fast Dx platforms. An inactivated isolate of H7N9 was extracted using the NucliSENS easyMAG instrument and tested in triplicate on both the Life Technologies QuantStudio Dx and Applied Biosystems 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

Influenza A Viruses				
Subtype	**Strain**	**$TCID_{50}$/mL**	**A**	**B**
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
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 EID_{50} 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 Cepheid SmartCycler II, one panel for the Applied Biosystems 7500 Fast Dx, and one panel for the Life Technologies QuantStudio Dx) of eight simulated samples. The panels consisted of 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 operators per instrument for five 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 bioMérieux easyMAG system and tested on either the Cepheid SmartCycler II, the Applied Biosystems 7500 Fast Dx, or the Life Technologies QuantStudio Dx platform.

Reproducibility Results – Cepheid SmartCycler II												
Panel	**Site 1**			**Site 2**			**Site 3**			**Combined Site Data**		
Member ID ($TCID_{50}$/ mL)	**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 – Applied Biosystems 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
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 – Life Technologies 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
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 Life Technologies 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 bioMérieux easyMAG systems and then tested on three Life Technologies QuantStudio Dx platforms. The two samples and controls were tested by two operators per instrument for five 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 - Life Technologies 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	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 - Life Technologies 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
Negative												
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 yeast strain representing common respiratory pathogens or flora commonly present in the nasopharynx. Bacteria and yeast were tested at concentrations of 10^5 to 10^{10} CFU/mL. Viruses were tested at concentrations of 10^3 to 10^6 $TCID_{50}$/mL. Samples were extracted using the NucliSENS easyMAG instrument and tested in triplicate on the Applied Biosystems 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 Maclnytre 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

Cross-reactivity			
Organism ID	**Final Conc.**	**Influenza A Result**	**Influenza B Result**
Parainfluenza Type 3	9.10E+05	Negative	Negative
Parainfluenza Type 4	9.57E+06	Negative	Negative
Varicella Zoster Virus	7.50E+02	Negative	Negative
Bordetella pertussis	1.04E+07	Negative	Negative
Bordetella bronchiseptica	2.55E+07	Negative	Negative
Chlamydia trachomatis	2.10E+05	Negative	Negative
Legionella pneumophila	2.05E+08	Negative	Negative
Mycobacterium intracellulare	6.90E+08	Negative	Negative
Mycobacterium tuberculosis	6.60E+07	Negative	Negative
Mycobacterium avium	1.36E+10	Negative	Negative
Haemophilus influenzae	5.90E+07	Negative	Negative
Pseudomonas aeruginosa	5.15E+07	Negative	Negative
Proteus vulgaris	2.65E+08	Negative	Negative
Proteus mirabilis	2.75E+07	Negative	Negative
Neisseria gonorrhoeae	2.15E+07	Negative	Negative
Neisseria menigitidis	1.85E+08	Negative	Negative
Neisseria mucosa	1.85E+08	Negative	Negative
Klebsiella pneumoniae	3.30E+07	Negative	Negative
Escherichia coli	6.80E+07	Negative	Negative
Moraxella catarrhalis	5.85E+07	Negative	Negative
Corynebacterium diptheriae	6.0E+05	Negative	Negative
Lactobacillus plantarum	1.03E+08	Negative	Negative
Streptococcus pneumoniae	4.5E+07	Negative	Negative
Streptococcus pyogenes	2.05E+08	Negative	Negative
Streptococcus salivarius	2.50E+06	Negative	Negative
Staphylococcus epidermidis	2.6E+07	Negative	Negative
Staphylococcus aureus	5.15E+08	Negative	Negative
Candida albicans	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 concentrations of 3x LOD on the Applied Biosystems 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-Synephrine	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

Substance Name	Concentration Tested	Influenza A Result (3x LOD)	Influenza B Result (3x LOD)
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 Cepheid SmartCycler II, the Applied Biosystems 7500 Fast Dx, and Life Technologies 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 bioMérieux NucliSENS easyMAG automated nucleic acid extraction instrument and the Cepheid SmartCycler II instrument, the Applied Biosystems 7500 Fast Dx, or Life Technologies QuantStudio Dx platform.

CUSTOMER AND TECHNICAL ASSISTANCE

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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 kit

MDSS GmbH
Schiffgraben 41
30175 Hannover,
Germany

Quidel Corporation
2005 East State Street, Suite 100
Athens, OH 45701 USA
quidel.com **M100en v2014SEPT14**

REF Catalogue number	CE CE mark of conformity
EC REP Authorized Representative in the European Community	LOT Batch code
 Use by	 Manufacturer
 Temperature limitation	 Intended use
 Consult e-labeling instructions for use	IVD For *In Vitro* diagnostic use
 Contains sufficient for 96 determinations	CONT Contents/Contains