

This Procedural Bulletin is intended to provide a ready outline reference for performance of the assay. These abbreviated directions for use are not intended to replace the complete Package Insert. It is the obligation of every manufacturer of medical devices labeled FOR *IN VITRO* DIAGNOSTIC USE to provide a complete Package Insert in accordance with FDA labeling regulation (21 CFR 809.10).

Quidel Corporation provides CLSI procedures for your use. The procedures are required to include the same information as listed in the Package Insert. Any modifications to this document are the sole responsibility of the Laboratory.

Lyra® Parainfluenza Virus Assay

CLIA Complexity: High

INTENDED USE

The Lyra Parainfluenza Virus Assay is a Real-Time PCR assay for the qualitative detection and identification of human parainfluenza virus types 1, 2 and 3 viral RNA from nasal and nasopharyngeal swab specimens from symptomatic patients. It is intended for use as an aid in the differential diagnosis of parainfluenza virus types 1, 2 and 3. This test is not intended to detect Parainfluenza 4a or Parainfluenza 4b Viruses.

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

SUMMARY AND EXPLANATION

Parainfluenza viruses (family *Paramyxoviridae*) are enveloped viruses with a single, negative strand RNA genome. The 4 different types cause croup and viral pneumonia in children under the age of 5 years and upper respiratory illness in adults. Parainfluenza is the second leading cause of lower respiratory illness in children after RSV. Outbreaks caused by parainfluenza viruses usually occur in the fall during alternate years (P1 and P2) or throughout the year, with increased activity in the spring (P3).¹

The Lyra Parainfluenza Virus Assay only detects and identifies human parainfluenza type 1, 2 and 3 viral RNA from nasal and nasopharyngeal swab specimens from symptomatic patients. This test is not intended to detect Parainfluenza 4a or Parainfluenza 4b Viruses.

PRINCIPLE OF THE PROCEDURE

The assay detects viral nucleic acids that have been extracted from a patient sample. A multiplex Real-time RT-PCR reaction is carried out under optimized conditions in a single tube generating amplicons for PIV-1, PIV-2, PIV-3 and the Process Control (PRC). Identification of PIV-1, PIV-2, PIV-3 and the PRC occurs by the use of target-specific primers and fluorescent-labeled probes that hybridize to conserved regions in the genomes of PIV-1, PIV-2, PIV-3 and the PRC.

Lyra Probe Labels	
Target	Dye
PIV-1	FAM
PIV-2	JOE
PIV-3	Tex Red
PRC	CY5

The following is a summary of the procedure:

1. **Sample Collection:** Obtain nasal or nasopharyngeal swabs specimens using standard techniques from symptomatic patients. Transport, store, and process these specimens according to established laboratory procedures.²
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**). Use of other extraction systems with the Lyra Parainfluenza assay has not been validated. Validation of these other systems is the responsibility of the end user.

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 conserved regions of PIV-1, PIV-2, PIV-3, as well as the Process Control sequence.
4. **Nucleic Acid Amplification and Detection:** Add 15 µL of the rehydrated Master Mix to each plate well. Then add 5 µL of extracted nucleic acids (specimen with PRC) to the plate well. Place plate into the Applied Biosystems® (ABI) 7500 Fast Dx instrument.

Once the reaction plate is added to the instrument, initiate the assay protocol. This protocol initiates reverse transcription of the RNA targets generating complementary DNA, and the subsequent amplification of the target amplicons occurs. The Lyra Parainfluenza 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 nucleic acid.

MATERIALS PROVIDED

Cat. #M114

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

Component	Quantity
Rehydration Solution Part M5003	1 vial/kit 1.9 mL
Lyra Parainfluenza Master Mix Part M5035 Lyophilized Contents: DNA polymerase enzyme with reverse transcriptase activity Primers and probes dNTPs Stabilizers	12 vials/kit, 8 reactions/vial
Process Control Part M5005	1 vial/kit 2.0 mL

OPTIONAL MATERIALS

External controls for PIV-1, PIV-2, PIV-3 (i.e. Lyra Parainfluenza Control Set #M115, 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
- Applied Biosystems 7500 Fast Dx or 7500 Fast instrument with software version 1.4 or later
- 96 well PCR plate
- Optical plate films
- Plate centrifuge for ABI 96 well plate
- bioMerieux NucliSENS easyMAG software version 2.0
- bioMerieux NucliSENS easyMAG Buffers 1, 2, 3
- bioMerieux NucliSENS easyMAG Lysis Buffer
- bioMerieux NucliSENS easyMAG Silica Magnetic Beads
- bioMerieux NucliSENS easyMAG disposables

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use
- 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 extraction systems other than the NucliSENS easyMAG System has not been validated. Validation of these systems is the responsibility of the end-user.
- Using cycling conditions other than those indicated in the **Thermocycler Programming Instructions** section may give erroneous results.
- 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 kept at room temperature for up to 2 hours, at 2°C to 8°C for up to 4 hours or ≤-20°C for up to 2 days. (See “Storage and Handling of Kit Reagents” section for additional storage options) The rehydrated Master Mix should be recapped, sealed with parafilm, and stored in an upright position. Protect the Master Mix from light during storage.

INDICATIONS OF INSTABILITY OR DETERIORATION OF REAGENTS

Cloudiness of the Rehydration Solution may indicate deterioration of this reagent. Contact Quidel Technical Support for a replacement.

SPECIMEN COLLECTION, STORAGE, AND HANDLING

Specimens used for the validation of the Lyra Parainfluenza Virus Assay were obtained using standard techniques from patients with upper respiratory tract infection symptoms. These specimens were collected, transported, stored, and processed according to CLSI M41-A. Briefly, samples should be transported refrigerated at 2°C to 8°C and stored refrigerated (2°C to 8°C) for 72 hours before processing. Any additional leftover specimen should be stored ≤-70°C.

A series of studies were performed evaluating a number of routinely used viral transport medium at a volume of 2 mL: M4, M4-RT, M5, M6, and UTM. No significant difference in assay performance was seen between the five different types of viral transport media.

NUCLEIC ACID EXTRACTS STORAGE



Eluates can be stored up to 30 days at room temperature, 4°C, -20°C and -80°C and be used with up to 3 freeze-thaw cycles.

NUCLEIC ACID EXTRACTION PROGRAMMING INSTRUCTIONS

Note: A PIV-1, PIV-2, PIV-3 positive processing/extraction control (i.e. Lyra Parainfluenza Control Set #M115 or previously characterized positive PIV-1, PIV-2, or PIV-3 specimen) and a negative process control (i.e. viral transport media or previously characterized PIV-1, PIV-2, or PIV-3 negative specimen) should be included in each extraction run.













1. Turn on the instrument and wait for instrument light to appear orange. Then switch on the computer/launch easyMAG software.

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 on 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 to 24 samples from the 'Unassigned Sample list' on the left hand side of the screen). Alternatively, individual samples may be moved into and out of the run by using the left and right 'Positioning icons'   after selecting the appropriate sample. The sample order within the run may 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 tips 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. 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 pipettor 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 8 times into 8 wells of an ELISA strip plate. Discard tip.
- e. After Lysis is complete (NB: 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.

17. Upon completion of the run, transfer purified nucleic acid to nuclease-free tubes. Eluates can be stored at room temperature (20°C to 25°C) for up to 4 hours, at 2°C to 8°C for 6 hours and at -20°C for 1 month. The extracted RNA is stable for up to three freeze/thaws cycles when stored at -20°C.

INITIAL THERMOCYCLER PROGRAMMING

1. Launch the ABI 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 Parainfluenza protocol.
 - a. Define Document: Most of the following should be the default setting. If not, change accordingly.
 - i. Confirm or enter the following information:

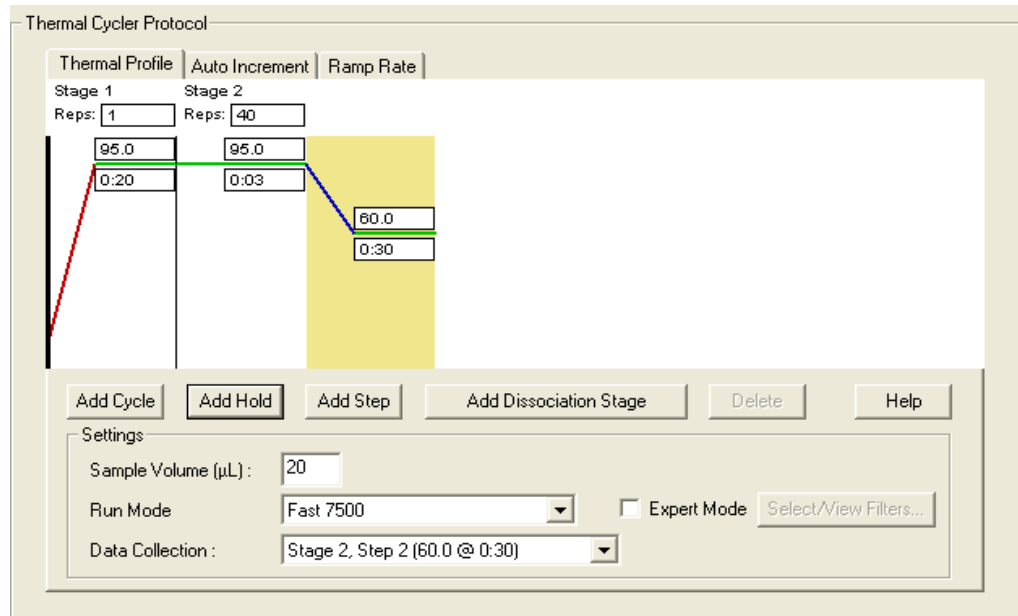
Assay:	Standard Curve (Absolute Quantitation)
Container:	96-Well Clear
Template:	Blank Document
Run Mode:	Fast 7500
Operator:	<i>your operator name</i>
Comments:	SDS v1.4
Plate Name:	'Lyra PIV'

- ii. Select the **Next** button.
- iii. Select Detectors: New detectors for PIV-1, PIV-2, PIV-3 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.

Enter the following information for each detector:

Name	Reporter Dye	Quencher Dye	Color
PIV-1	FAM	(none)	(Select)
PIV-2	JOE	(none)	(Select)
PIV-3	Texas Red	(none)	(Select)
PRC	Cy5	(none)	(Select)

- iv. Select a unique color to represent each detector.
 - v. Highlight the new detectors and add to the **Detectors in Document** column using the **Add** button.
 - vi. Select **(none)** from the **Passive Reference** drop-down menu.
 - vii. Select the **Next** button.
 - viii. Select the Finish button without setting any wells.
- b. The wizard will close, and the software will open, starting with the **Setup** tab. This will show the sample plate that was set up during the quick start. For the initial set up, nothing needs to be changed here.
 - c. Define the Thermocycler Protocol:
 - i. Select the **Instrument** tab to set up the Lyra Parainfluenza RT-PCR cycling times and temperatures.
 - ii. 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).



- iii. 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 Amplification 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 Amplification Stage)
 - a. Reps: 35
 - b. Step 1
 - i. Temp: 92
 - ii. Time: 0:05

c. Step 2

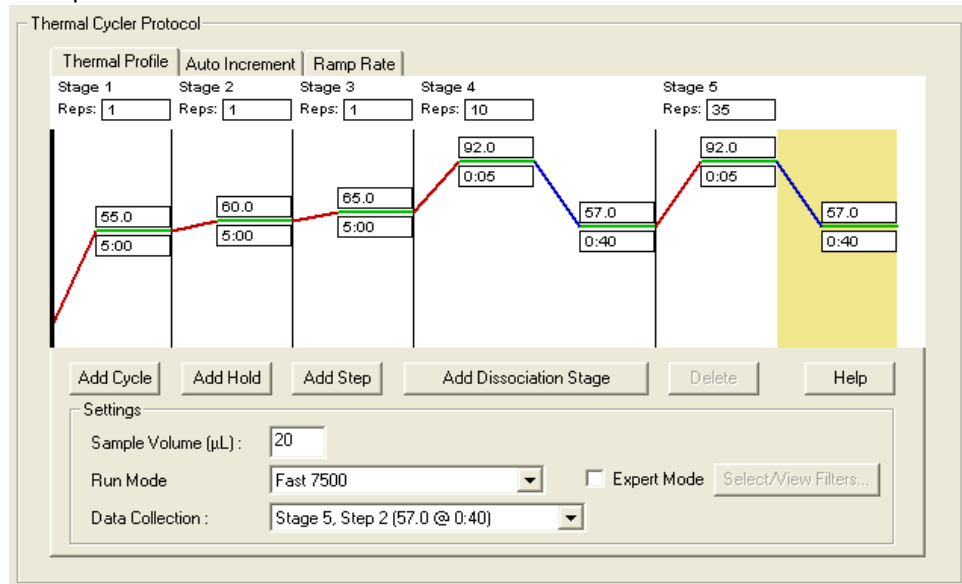
- i. Temp: 57
- ii. Time: 0:40

9. If a wrong stage is added, the stage may be removed by pressing the **Delete** button after highlighting the stage between the vertical lines.

iv. Under **Settings**, enter the following:

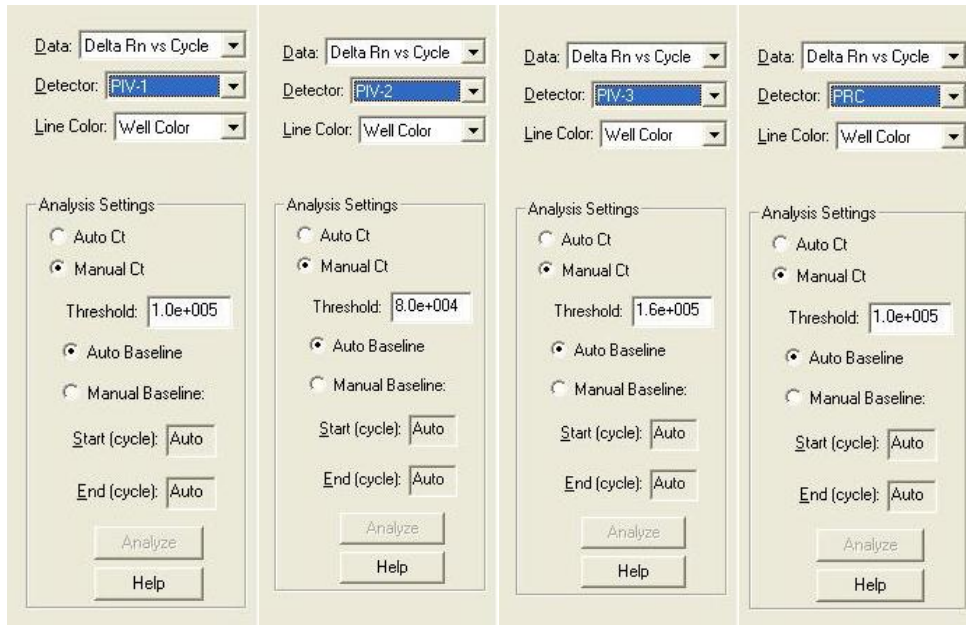
Sample Volume (µL):	20 (default)
Run Mode:	7500 Fast (default)
Data Collection:	Stage 5, Step 2(57.0 @ 0:40)
NOTE: Do not check the check box next to 'Expert Mode.'	

v. Final protocol



d. Set threshold for each analyte as follows:

- i. Select the **Results** tab.
- ii. Select the **Amplification Plot** tab.
- iii. Select PIV-1 from the Detector tab in the top right corner.
- iv. In the **Analysis Settings** block, set the **Threshold** to **1.0e005**.
- v. Select the **Auto Baseline** radio button.
- vi. Repeat iii-v for PIV-2 setting the **Threshold** to **8.0e004**.
- vii. Repeat iii-v for PIV-3 setting the **Threshold** to **1.6e005**.
- viii. Repeat iii-v for PRC setting the **Threshold** to **1.0e005**.



- e. Save the new protocol as a template for future uses.
 - 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 PIV.'
 - iv. **Save as type:** 'SDS Templates (*.sdt).'
- f. Exit the software.

ASSAY PROCEDURE

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

Nucleic Acid Extraction Procedure

Refer to the bioMerieux NucliSENS easyMAG System Programming Instructions above.

1. Add 20 µL of the Process Control to the sample extraction well.
2. Add 180 µL of patient sample or external control to a sample extraction well.
3. Follow extraction procedure as per manufacturer's instructions.
4. Eluates can be stored up to 30 days at room temperature, 4°C, -20°C and -80°C and be used with up to 3 freeze-thaw cycles.

Master Mix Rehydration Procedure

1. Determine the number of 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 1-2 minutes to allow rehydration of pellet.
6. Gently pipette up and down 2-3 times (avoiding the formation of bubbles) prior to dispensing into the first PCR tube or plate well.

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

Note: The rehydrated Master Mix may be kept at room temperature for up to 2 hours, at 2°C to 8°C for up to 4 hours or ≤-20°C for up to 2 days. (See "Storage and Handling of Kit Reagents" section for additional storage options) The rehydrated Master Mix should be recapped, sealed with parafilm, and stored in an upright position. Protect the Master Mix from light during storage.

RT-PCR Set-up Procedure:

1. Add 15 μ L of the rehydrated Master Mix to each reaction tube or plate well.
2. Add 5 μ L of extracted nucleic acid (specimen with the process control) into the plate wells. Mixing of reagents is not required.
Note: Use a micropipettor with a new non-aerosol tip with each extracted specimen.
3. Seal the plate.
Note: Quidel suggests each thermocycler run should include a reaction tube or well with PIV External Positive Control and Negative Control. Run controls in keeping with your lab practices and policies.
4. Centrifuge the plate for a minimum of 15 seconds. Ensure that all liquid is at the bottom of the tube.
5. Insert plate into the thermocycler.

Amplification Protocol on the Applied Biosystem 7500 Fast Dx Thermocycler

1. Switch on 7500 Fast Dx.
2. Launch the 7500 Fast Dx software 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 PIV
Run Mode:	Fast 7500
Operator:	<i>your operator name</i>
Comments:	SDS v1.4 (<i>add more if needed</i>)
Plate Name:	YYMMDD-Lyra PIV

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 PIV-1, PIV-2, PIV-3 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 resuspending 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 PIV.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. Analyze the data by pressing the "**Analyze**" button in the top menu, and save the file.

- b. Save the file by pressing **Save Document** 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.

INTERPRETATION OF RESULTS

Interpretation of the Lyra Parainfluenza Assay Results on the 7500 Fast Dx Thermocycler					
Assay Result	Detector: PIV-1	Detector: PIV-2	Detector: PIV-3	Detector: Process Control	Interpretation of Results
Negative	Ct <5.0 or Ct >35.0	Ct <5.0 or Ct >35.0	Ct <5.0 or Ct >35.0	5.0 ≤ Ct ≤ 35.0	No PIV-1, PIV-2, or PIV-3 viral RNA detected; PRC Detected
PIV-1 Positive	5.0 ≤ Ct ≤ 35.0	Ct <5.0 or Ct >35.0	Ct <5.0 or Ct >35.0	NA*	PIV-1 viral RNA detected
PIV-2 Positive	Ct <5.0 or Ct >35.0	5.0 ≤ Ct ≤ 35.0	Ct <5.0 or Ct >35.0	NA*	PIV-2 viral RNA detected
PIV-3 Positive	Ct <5.0 or Ct >35.0	Ct <5.0 or Ct >35.0	5.0 ≤ Ct ≤ 35.0	NA*	PIV-3 viral RNA detected
PIV-1 and PIV-2 Positive	5.0 ≤ Ct ≤ 35.0	5.0 ≤ Ct ≤ 35.0	Ct <5.0 or Ct >35.0	NA*	PIV-1 and PIV-2 viral RNA detected
PIV-1 and PIV-3 Positive	5.0 ≤ Ct ≤ 35.0	Ct <5.0 or Ct >35.0	5.0 ≤ Ct ≤ 35.0	NA*	PIV-1 and PIV-3 viral RNA detected
PIV-2 and PIV-3 Positive	Ct <5.0 or Ct >35.0	5.0 ≤ Ct ≤ 35.0	5.0 ≤ Ct ≤ 35.0	NA*	PIV-2 and PIV-3 viral RNA detected
PIV-1, PIV-2 and PIV-3 Positive	5.0 ≤ Ct ≤ 35.0	5.0 ≤ Ct ≤ 35.0	5.0 ≤ Ct ≤ 35.0	NA*	PIV-1, PIV-2 and PIV-3 viral RNA detected
Invalid	Ct <5.0 or Ct >35.0	Ct <5.0 or Ct >35.0	Ct <5.0 or Ct >35.0	Ct <5.0 or Ct >35.0	No PIV-1, PIV-2, or PIV-3 viral RNA detected and no PRC detected; invalid test. Retest the same purified sample. If this 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.

QUALITY CONTROL

The Lyra Parainfluenza Virus Assay incorporates several controls to monitor assay performance.

1. 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.
2. Commercially available external positive parainfluenza controls may be treated as a patient specimen and should be used in accordance with your lab standards. Previously characterized positive parainfluenza specimens may be used in lieu of a commercial parainfluenza control.

3. 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 should be performed in accordance with currently lab standards.

LIMITATIONS

- This test is not intended to detect Parainfluenza 4a or Parainfluenza 4b Viruses.
- Negative results do not preclude infection with parainfluenza and should not be the sole basis of a treatment decision.
- As with other assays of this type, there is a risk of false negative results due to the presence of sequence variants in the viral target.
- For all virus combinations, detectable interference near the LOD of the assay for each virus (PIV-1, PIV-2, and PIV-3) was seen in analytical studies when the competing virus concentration was equal to or greater than 4.0×10^6 TCID₅₀.
- Improper collection, storage, or transport 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.
- 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.
- The performance of the assay has not been established in individuals who received nasally administered corticosteroids.
- The performance of the assay has not been established in individuals who received nasally administered Influenza vaccine.

EXPECTED VALUES

Clinical studies were performed with the Lyra Parainfluenza assay using the Applied Biosystems 7500 Fast Dx. Testing was performed with prospective specimens received from throughout the United States in the winter of 2013 (January 2013 to March 2013, and October 2013 to December 2013). The table below provides the expected value for each virus.

Expected Values for the Winter of 2013 and 2014 (Combined)			
Instrument	PIV-1	PIV-2	PIV-3
Applied Biosystems 7500 Fast Dx	1.0% (13/1241)	0.4% (5/1241)	1.8% (22/1241)

CLINICAL PERFORMANCE

Performance characteristics of the Lyra Parainfluenza Virus assay using the Applied Biosystems 7500 Fast Dx platform were established during a prospective study during the 2013 respiratory virus season (January 2013 to March 2013, and October 2013 to December 2013). One thousand two hundred and forty-one (1241) fresh specimens used for this study were swab specimens that were collected for routine respiratory virus testing at three (3) sites across the United States. A single specimen was collected per patient and tested (direct specimen DFA and culture with DFA) immediately after collection. The specimens were extracted with the bioMérieux easyMAG and tested with Lyra Parainfluenza Virus Assay.

The age demographics

Combined Study – Age and Gender Distribution		
Gender	Female	Male
Total	651	590
Age		
<2 years	111	158
2 to 5 years	130	126
6 to 21 years	150	129
22 to 59 years	160	108
≥60 years	100	69

Prospective Clinical Study

The evaluation of the Lyra Parainfluenza Virus Assay occurred in two separate studies: a prospective multi-center study using one thousand two hundred and forty-one (1241) fresh specimens from the upper respiratory tract; and a retrospective study using one hundred five (105) frozen specimens from the upper respiratory tract. In both studies the specimens were processed the bioMérieux NucliSENS® easyMag® at all sites for the extraction of nucleic acids from the clinical specimens. The Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument was used with the Quidel assay for the amplification and detection of the target nucleic acids with the Lyra Parainfluenza Virus Assay. The prospective specimens were also processed and tested with direct specimen fluorescent antibody (DSFA) and cell culture with DFA (CCFA).

One thousand two hundred and forty-one (1241) fresh specimens were collected and transported to each laboratory for testing with the Lyra Parainfluenza Virus Assay. The specimens shipped daily with cold packs for DSFA and CCFA to the central location and were tested within 72-hours of collection. The table below details the PIV-1 results for the specimens.

PIV-1			
Lyra Parainfluenza Virus Assay	Comparator: DSFA and Culture with DFA		
	Positive	Negative	Total
Positive	10	3*	13
Negative	0	1228	1228
Total	10	1231	1241
95% CI			
Sensitivity	10/10	100%	72.2% to 100%
Specificity	1228/1231	99.8%	99.3 % to 99.9%

*Two (2) of the three (3) positives were positive by an additional RT-PCR assay.

The table below details the PIV-2 results for the specimens.

PIV-2			
Lyra Parainfluenza Virus Assay	Comparator: DSFA and Culture with DFA		
	Positive	Negative	Total
Positive	5	0	5
Negative	0	1236	1236
Total	5	1236	1241
95% CI			
Sensitivity	5/5	100%	56.6% to 100%
Specificity	1236/1236	100%	99.7% to 100%

The table below details the PIV-3 results for the specimens.

PIV-3			
Lyra Parainfluenza Virus Assay	Comparator: DSFA and Culture with DFA		
	Positive	Negative	Total
Positive	17	5*	22
Negative	0	1219	1219
Total	17	1224	1241
95% CI			
Sensitivity	17/17	100%	81.6% to 100%
Specificity	1219/1224	99.6%	99.0% to 99.8%

*Five (5) of the five (5) positives were positive by an additional RT-PCR assay.

Retrospective Study

Due to the low prevalence of parainfluenza virus at the clinical sites during the study period, a retrospective study was conducted with specimens obtained from a pediatric hospital in the Southwest United States. One hundred five (105) frozen specimens from the upper respiratory tract were tested concurrently with the Lyra Parainfluenza Virus Assay and an additional FDA-cleared molecular device.

PIV-1			
Lyra Parainfluenza Virus Assay	Comparator: Prodesse ProParaFlu+ assay		
	Positive	Negative	Total
Positive	24	1*	25
Negative	0	80	80
Total	24	81	105
95% CI			
Positive Percent Agreement	24/24	100%	86.2% to 100%
Negative Percent Agreement	80/81	98.8%	93.3% to 99.8%

*One (1) of one (1) positive was positive by an additional RT-PCR assay.

PIV-2			
Lyra Parainfluenza Virus Assay	Comparator: Prodesse ProParaFlu+ assay		
	Positive	Negative	Total
Positive	22	5*	27
Negative	0	78	78
Total	22	83	105
95% CI			
Positive Percent Agreement	22/22	100%	85.1% to 100%
Negative Percent Agreement	78/83	94.0%	86.7% to 97.4%

*Five (5) of five (5) positives were positive by an additional RT-PCR assay.

PIV-3			
Lyra Parainfluenza Virus Assay	Comparator: Prodesse ProParaFlu+ assay		
	Positive	Negative	Total
Positive	24	0	24
Negative	0	81	81
Total	24	81	105
95% CI			
Positive Percent Agreement	24/24	100%	86.2% to 100%
Negative Percent Agreement	81/81	100%	95.5% to 100%

ANALYTICAL PERFORMANCE

Level of Detection

The analytical sensitivity (limit of detection or LOD) of the Lyra Parainfluenza Virus assay was determined using quantified (TCID₅₀/mL) cultures of parainfluenza virus types 1, 2, and 3 serially diluted in negative nasopharyngeal matrix. Each dilution was extracted in replicates of 20 per concentration of virus using the NucliSENS easyMAG System and tested on the three platforms. Analytical sensitivity (LOD) is defined as the lowest concentration at which 95% of all replicates tested positive.

	TCID ₅₀ /mL
Parainfluenza virus type 1 (C-35 strain)	2.50 x 10 ⁰
Parainfluenza virus type 2 (Greer strain)	2.50 x 10 ²
Parainfluenza virus type 3 (C-243 strain)	8.00 x 10 ¹

Analytical Reactivity (Inclusivity)

To verify the Lyra Parainfluenza Virus Assay detects multiple strains of Parainfluenza Type 1 (HPIV-1), Type 2 (HPIV-2) and Type 3 (HPIV-3). The number of characterized strains of Parainfluenza is very limited. *In silico* analysis was performed to demonstrate that primers are representative of the genetic diversity in the chosen target region for each Parainfluenza Virus type identified by the assay.

All full-genome Genbank annotation files for Human Parainfluenza virus Types 1, 2, and 3 were downloaded from NCBI. A summary of the number of sequences evaluated for each virus type is in the table below.

HPIV Type	Subtyping HPIV Type	Total Number of Sequences
HPIV1	HPIV2	308
	HPIV3	
	HPIV4	
HPIV2	HPIV1	458
	HPIV3	
	HPIV4	
HPIV3	HPIV1	509
	HPIV2	
	HPIV4	

Reproducibility Study

The reproducibility of the Lyra Influenza Parainfluenza assay was evaluated at three (3) laboratory sites. Reproducibility was assessed using a panel of four (4) simulated samples that include medium positive and low positive, high negative PIV-1, PIV-2, PIV-3 and negative samples. Panels and controls were tested at each site by two (2) operators for 5-days (triplicate testing x 2 operators x 5 days x 3 sites = 90 results per level for each virus). The LOD values were based on the values obtained in the LOD study. The panels and controls were extracted using the bioMérieux easyMAG system and tested on the Applied Biosystems 7500 Fast Dx.

	Percent Agreement (CI95)	Percent Agreement (CI95)	Percent Agreement (CI95)	Percent Agreement (CI95)
	Site 1	Site 2	Site 3	Combined
PIV-1 High Negative	40% (CI95 24.6% to 57.7%)	76.6% (CI95 59.1% to 88.2%)	63.3% (CI95 45.5% to 78.1%)	60% (CI95 49.7% to 69.5%)
PIV-1 Low Positive	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 95.6% to 100%)
PIV-1 Moderate Positive	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 95.6% to 100%)
PIV-1 Negative	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100%* (CI95 88.3% to 100%)	100% (CI95 95.6% to 100%)
PIV-2 High Negative	20% (CI95 9.5% to 37.3%)	93.3% (CI95 78.7% to 98.2%)	80% (CI95 62.7% to 90.5%)	64.4% (CI95 54.1% to 73.6%)
PIV-2 Low Positive	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 95.6% to 100%)
PIV-2 Moderate Positive	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 95.6% to 100%)
PIV-2 Negative	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100%* (CI95 88.3% to 100%)	100% (CI95 95.6% to 100%)
PIV-3 High Negative	0% (CI95 0% to 11.4%)	3.3% (CI95 0.6% to 16.7%)	53.3% (CI95 36.1% to 69.8%)	18.9% (CI95 12.1% to 28.2%)
PIV-3 Low Positive	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 95.6% to 100%)
PIV-3 Moderate Positive	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 95.6% to 100%)
PIV-3 Negative	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100%* (CI95 88.3% to 100%)	100% (CI95 95.6% to 100%)
PIV-1 Positive Control	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 95.6% to 100%)
PIV-2 Positive Control	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 95.6% to 100%)
PIV-3 Positive Control	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 95.6% to 100%)
Negative Control	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 88.6% to 100%)	100% (CI95 95.6% to 100%)

*One (1) replicate had an invalid PRC value and was removed for analysis.

The data from the combined sites indicates that the Lyra Parainfluenza Virus Assay, on the Applied Biosystems 7500 Fast Dx, generates reproducible results for the detection of parainfluenza virus types 1, 2, 3, and the internal control.

Analytical Specificity – Cross-reactivity

The analytical specificity of the Lyra Parainfluenza assay was evaluated by testing a panel consisting of 28 viruses, 26 bacteria and 1 yeast strain representing common respiratory pathogens or flora commonly present in nasopharynx. Samples were extracted using the NucliSENS easyMAG instrument and tested in triplicate. Analytical specificity of the Lyra Parainfluenza assay was 100%.

Organisms used for the study were the following:

Organisms Evaluated for Cross-Reactivity		
<i>Bordetella pertussis</i>	<i>Candida albicans</i>	RSV A (Long)
<i>Bordetella bronchiseptica</i>	Adenovirus 1	RSV B (Wash/18537/62)
<i>Chlamydophila pneumonia</i>	Coronavirus 229E	Varicella Zoster Virus
<i>Chlamydia trachomatis</i>	Coronavirus NL63	
<i>Legionella pneumophila</i>	Coronavirus OC43	
<i>Mycobacterium intracellulare</i>	Coxsackievirus B4	
<i>Mycobacterium tuberculosis</i>	Coxsackievirus B5/10/2006	
<i>Mycobacterium avium</i>	Cytomegalovirus	
<i>Mycoplasma pneumoniae</i>	Echovirus 6	
<i>Haemophilus influenzae</i>	Echovirus 7	
<i>Pseudomonas aeruginosa</i>	Echovirus 9	
<i>Proteus vulgaris</i>	Echovirus 11	
<i>Proteus mirabilis</i>	Enterovirus 70	
<i>Neisseria gonorrhoeae</i>	Enterovirus 71	
<i>Neisseria meningitidis</i>	Epstein Barr Virus	
<i>Neisseria mucosa</i>	HSV Type 1 MacIntyre Strain	
<i>Klebsiella pneumoniae</i>	HSV Type 2 Strain G	
<i>Escherichia coli</i>	Human Metapneumovirus (A1)	
<i>Moraxella catarrhalis</i>	Human Rhinovirus 45	
<i>Corynebacterium diphtheriae</i>	Human Rhinovirus 52	
<i>Lactobacillus plantarum</i>	Influenza A/Mexico/4108/2009	
<i>Streptococcus pneumoniae</i>	Influenza A/Port Chalmers	
<i>Streptococcus pyogenes</i>	Influenza B/Florida/04/2006	
<i>Streptococcus salivarius</i>	Measles/7/2000	
<i>Staphylococcus epidermidis</i>	Mumps Virus	
<i>Staphylococcus aureus</i>	Parainfluenza Type 4A	

Analytical Specificity – Interfering Substances

The performance of Lyra Parainfluenza Virus assay was evaluated with potentially interfering substances that may be present in nasopharyngeal specimens. The potentially interfering substances were evaluated using parainfluenza virus types 1, 2, and 3 at a concentration of 2x LOD. There was no evidence of interference caused by the substances tested at 2x LOD.

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

Carryover and Cross-contamination Studies

In an internal study there was no evidence of carry-over/cross contamination with the Lyra Parainfluenza assay using the NucliSens easyMAG automated nucleic acid extraction instrument and the Applied Biosystems 7500 Fast Dx.

ADDITIONAL SOURCE MATERIAL

1. 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>.
2. 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>.
3. CLSI EP17-A: Guidance for Protocols for Determination of Limits of Detection and Limits of Quantitations (Vol. 2, No. 34) (Oct 2004).
4. CLSI MM13-A: Guidance for the Collection, Transport, Preparation and Storage of Specimens for Molecular Methods (Vol. 25, No. 31) (Dec 2005).
5. CLSI EP7-A2: Guidance for Interference Testing in Clinical Chemistry (Vol. 25, No.27 Second Ed) (Nov 2005).
6. CLSI EP12-A: Guidance for User Protocol for Evaluation of Qualitative Test Performance (Vol. 22, No. 14) (Sept 2002).
7. CLSI MM6-A: Guidance for the Quantitative Molecular Methods for Infectious Diseases (Vol. 23, No.28) (Oct 2003).
8. CLSI EP5-A2: Guidance for Evaluation of Precision Performance of Quantitative Measurement Methods (Vol. 24, No. 25 Second Ed.) (Aug 2004).

CUSTOMER AND TECHNICAL SUPPORT

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1. Easton, A.J., Eglin, R.P. Epidemiology of Parainfluenza virus type 3 in England and Wales over a 10 year period. *Epidemiol. Infect.*, 1989;102:531-535.
2. 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.