For use under Emergency Use Authorization (EUA) only

For in vitro diagnostic use

Rx Only

Lyra® SARS-CoV-2 Assay
Instructions for Use

For the qualitative detection of human coronavirus SARS-CoV-2 viral RNA extracted from nasal, nasopharyngeal and oropharyngeal swab specimens.
# Contents

Intended Use ........................................................................................................................................... 4

Summary and Explanation ...................................................................................................................... 4

Principle of the Procedure ...................................................................................................................... 4

Materials Provided ................................................................................................................................. 5

Materials Required But Not Provided .................................................................................................... 6

Warnings and Precautions ...................................................................................................................... 7

Storage and Handling of Kit Reagents .................................................................................................... 8

Specimen Collection, Storage and Handling .......................................................................................... 8

Nucleic Acid Extracts Storage ................................................................................................................. 8

bioMérieux NucliSENS easyMAG Nucleic Acid Extraction Programming Instructions ...................... 9

bioMérieux NucliSENS EMAG Nucleic Acid Extraction Programming Instructions ........................... 11

Assay Procedure ................................................................................................................................... 20

Quality Control ..................................................................................................................................... 20

Interpretation of Results from Patient Specimens ............................................................................... 21

Performance Characteristics ................................................................................................................ 22

  Clinical Performance with Anterior Nasal Swabs ............................................................................. 22

  Clinical Performance with Nasopharyngeal Swabs ........................................................................... 23

  Level of Detection ............................................................................................................................. 24

  Analytical Reactivity (Inclusivity) ...................................................................................................... 29

  Analytical Specificity (Cross-Reactivity) ........................................................................................... 30

Limitations ............................................................................................................................................ 32

Conditions of Authorization for the Labs ............................................................................................ 33

Customer and Technical Assistance ..................................................................................................... 34

Intellectual Property ............................................................................................................................. 34

References ............................................................................................................................................ 34
INTENDED USE

The Lyra SARS-CoV-2 Assay is a real-time RT-PCR assay intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasal, nasopharyngeal (NP), or oropharyngeal (OP) swab specimens from patients suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 is generally detectable in upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Lyra SARS-CoV-2 Assay is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Lyra SARS-CoV-2 Assay is only for use under the Food and Drug Administration’s Emergency Use Authorization.

SUMMARY AND EXPLANATION

SARS-CoV-2, also known as the COVID-19 virus, was first identified in Wuhan, Hubei Province, China December 2019. By the start of March 2020, human infection has spread to over 74 countries, infected over 92,000 people and has killed over 3100 people.1 On March 11, the WHO had declared the SARS-CoV-2 as a global pandemic.

The median incubation time is estimated to be 5.1 days with symptoms expected to be present within 12 days of infection.2 The symptoms of COVID-19 are similar to other viral respiratory diseases and include fever, cough and shortness of breath.3

The Lyra SARS-CoV-2 Assay has been designed to specifically detect SARS-CoV-2 RNA.

PRINCIPLE OF THE PROCEDURE

The Lyra SARS-CoV-2 Assay detects SARS-CoV-2 viral RNA that has been extracted from a patient sample using either the bioMérieux NucliSENS® easyMAG® system or EMAG® system. A multiplex real-time RT-PCR reaction is carried out under optimized conditions in a single tube generating amplicons for the targeted virus (if present) and the Process Control (PRC) present in the sample. This reaction is performed utilizing one of six thermocyclers: Applied Biosystems® (also referred to as ABI) 7500 Fast Dx, Applied Biosystems 7500 Standard, Roche LightCycler® 480, Qiagen Rotor-Gene® Q, Bio-Rad CFX96 Touch™, Thermo Fisher QuantStudio® 7 Pro. Identification of the SARS-CoV-2 virus occurs by the use of target specific primers and fluorescent-labeled probes that hybridize to a conserved region of the non-structural polyprotein of the SARS-CoV-2 virus.

<table>
<thead>
<tr>
<th>Target</th>
<th>Dye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-structural polyprotein (pp1ab)</td>
<td>FAM</td>
</tr>
<tr>
<td>Process Control (PRC)</td>
<td>Quasar® 670</td>
</tr>
</tbody>
</table>
The following is a summary of the procedure:

1. **Sample Collection:** Obtain nasopharyngeal, oropharyngeal, or nasal swabs using standard techniques from symptomatic patients. These specimens are transported, stored, and processed according to established laboratory procedures.

2. **Nucleic Acid Extraction:** Extract nucleic acids from the specimens with the NucliSENS easyMAG or EMAG Systems 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 or controls. 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 135µL of Rehydration Solution. The Master Mix contains oligonucleotide primers, fluorophore and quencher-labeled probes targeting conserved regions of the SARS-CoV-2 as well as the process control sequence. The probes are dual labeled with a reporter dye attached to the 5' end and a quencher attached to the 3' end. The rehydrated Master Mix is sufficient for eight reactions.

4. **Nucleic Acid Amplification and Detection:** Add 15 µL of the rehydrated Master Mix to each plate well (Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, the Roche LightCycler 480) or tube (Qiagen Rotor-Gene Q). 5 µL of extracted nucleic acids (specimen with PRC) is then added to the plate well or tube. Place the plate or tube into the appropriate instrument.

   Once the reaction plate or tubes are 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 SARS-CoV-2 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**
SKU # CE-M120

| Table 2. Detection Kit (96 Reactions) – Store at 2°C to 8°C |
| --- | --- | --- |
| # | Component | Quantity |
| 1 | Rehydration Solution Part M5003 | 1 vial/kit 1.9 mL |
| 2 | Lyra SARS-CoV-2 Master Mix Part M5150 | 12 vials/kit, 8 reactions/vial |
|  | Lyophilized Contents: |  |
|  | DNA polymerase enzyme with reverse transcriptase activity |  |
|  | Oligonucleotide primer pairs; Oligonucleotide probes |  |
|  | dNTPs (dATP, dCTP, dGTP, dUTP, dTTP) |  |
Table 2. Detection Kit (96 Reactions) – Store at 2°C to 8°C

<table>
<thead>
<tr>
<th>#</th>
<th>Component</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stabilizers</td>
<td></td>
</tr>
<tr>
<td>CONTROL</td>
<td>Process Control Part M5005</td>
<td>1 vial/kit 2.0 mL</td>
</tr>
<tr>
<td>CONTROL+</td>
<td>Positive Control containing SARS-CoV-2 Synthetic RNA, Part M5153</td>
<td>1 vial/kit 1.0 mL</td>
</tr>
<tr>
<td>CONTROL−</td>
<td>Negative Control Part M5031</td>
<td>1 vial/kit 2.0 mL</td>
</tr>
</tbody>
</table>

- Lyra SARS-CoV-2 Assay Instructions for Use

MATERIALS REQUIRED BUT NOT PROVIDED

- Micropipettors (range between 1 to 10 μL and 100 to 1000 μL)
- Non-aerosol pipette tips
- Applied Biosystems 7500Fast Dx, software version 1.4, or later
- Applied Biosystems Standard, software version 2.0.6, or later. This instrument is currently for “Research Use Only,” but is authorized for use with the Lyra SARS-CoV-2 Assay under the COVID-19 Emergency Use Authorization.
- Roche LightCycler 480 Instrument II, software version 1.5.0.39 or later. This instrument is currently for “Research Use Only,” but is authorized for use with the Lyra SARS-CoV-2 Assay under the COVID-19 Emergency Use Authorization.
- Qiagen Rotor-Gene Q, software version 2.0.2.4, or later
- Bio-Rad CFX96 Touch, software version 3.1, or later. This instrument is currently for “Research Use Only,” but is authorized for use with the Lyra SARS-CoV-2 Assay under the COVID-19 Emergency Use Authorization.
- Thermo Fisher QuantStudio 7 Pro, software version 2.0, or later. This instrument is currently for “Research Use Only”, but is authorized for use with the Lyra SARS-CoV-2 Assay under the COVID-19 Emergency Use Authorization.
- 96 well PCR plate #:
  - Applied Biosystems 7500 Fast Dx: 4346906
  - Applied Biosystems Standard: N8010560
  - Roche LightCycler 480: 04729692001, foil included
  - Bio-Rad CFX96 Touch: HSP9631, seals MSB1001
  - Thermo Fisher QuantStudio 7 Pro: 4483354
- Optical plate films
- Qiagen Rotor-Disc®
- Qiagen Rotor-Disc Heat Sealing Film
- Plate centrifuge for 96 well plate
- bioMérieux NucliSENS easyMAG software version 2.0
- bioMérieux EMAG software version 1.3.2-9
- bioMérieux NucliSENS easyMAG or EMAG Buffers 1, 2, 3
- bioMérieux NucliSENS easyMAG or EMAG Lysis Buffer
- bioMérieux NucliSENS easyMAG or EMAG Silica Magnetic Beads
- bioMérieux NucliSENS easyMAG or EMAG disposables
- Biohit pipettor
- Biohit Tips for use with EasyMAG
- EMAG Tips 1000 μL for use with EMAG
WARNINGS AND PRECAUTIONS

- For In Vitro Diagnostic Use.
- For use under Emergency Use Authorization only.
- For prescription use only.
- This test has not been FDA cleared or approved; This test has been authorized by FDA under an EUA for use by authorized laboratories.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- This test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- The assay has been validated using bioMérieux NucliSENS easyMAG software version 2.0 and EMAG software 1.3.2.9. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated with a custom extraction program defined in the EMAG procedures below. The performance of this assay with other extraction programs used on this instrument have not been evaluated.
- The assay has been validated using Applied Biosystems 7500Fast 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 Applied Biosystems Standard software version 2.0.6. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using Roche LightCycler 480 Instrument II, software version 1.5.0.39 Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using Qiagen Rotor-Gene Q, software version 2.0.2.4. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using Bio-Rad CFX96 Touch, software version 3.1. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- The assay has been validated using Thermo Fisher QuantStudio 7 Pro, software version 2.0. Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
- 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 Assistance for a replacement.

SPECIMEN COLLECTION, STORAGE AND HANDLING
Nasopharyngeal, oropharyngeal, or nasal specimens should be collected, transported, stored, and processed according to CLSI M41-A2. The following viral transport media (M4, M4-RT, M5, M6, MTM and UTM) (1 mL and 3 mL) are compatible with the Lyra respiratory assays. CDC Viral Transport Medium (https://www.cdc.gov/coronavirus/2019-ncov/downloads/Viral-Transport-Medium.pdf) and Quidel Transport Medium (QTM) are also compatible with the Lyra SARS-CoV-2 Assay. Specimens should be stored according to the following recommendations:

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Transport Medium</th>
<th>Storage Temperature</th>
<th>Storage Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasopharyngeal, Oropharyngeal, or Nasal Swab</td>
<td>M4, M4-RT, M5, M6, MTM, UTM (1 mL and 3 mL) and CDC Viral Transport Medium</td>
<td>2°C to 8°C</td>
<td>Up to 72 hours after collection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-70°C</td>
<td>Up to 6 days after collection</td>
</tr>
<tr>
<td></td>
<td>Quidel Transport Medium</td>
<td>2°C to 25°C</td>
<td>Up to 72 hours after collection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-70°C</td>
<td>Up to 6 days after collection</td>
</tr>
</tbody>
</table>

NUCLEIC ACID EXTRACTS STORAGE
Eluates from the NucliSENS easyMAG and EMAG can be stored at room temperature (20°C to 25°C) for 2 hours, at 2°C to 8°C for 24 hours and 1 month at –20°C to –70°C.
BIOMÉRIEUX NUCLISENS EASYMAG NUCLEIC ACID EXTRACTION PROGRAMMING

INSTRUCTIONS

Note: A Positive Control (i.e. Lyra SARS-CoV-2, Positive Control #M5153), and a negative process control (i.e., Lyra SARS-CoV-2, Negative Control #M5031) 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. 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 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 can 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 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 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. 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 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 each 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.

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 24 hours and 1 month at -20°C to –70°C.

BIOMÉRIEUX NUCLISENS EMAG NUCLEIC ACID EXTRACTION PROGRAMMING INSTRUCTIONS

Note: A SARS-CoV-2 positive processing/extraction control (i.e., Lyra SARS-CoV-2 kit provided control Catalog #M5153 or previously characterized positive SARS-CoV-2 specimen) and a negative process control (i.e., viral transport media or previously characterized SARS-CoV-2 negative specimen) should be included in each extraction run.

The assay has been validated with a custom extraction program defined in the EMAG procedures below. The performance of this assay with other extraction programs used on this instrument have not been evaluated.

Section 1: EMAG SYSTEM STARTUP

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

2. Click the “Load and Run” icon.
3. Click the “Load” button on the screen under Reagents Display. Scan in the reagent bottles for either the left, right, or both sections depending on your use.

4. Verify that the tips are loaded and there are enough tips for the extraction run. If tips need to be loaded, press Load button on the screen under the Tips display. If tips need loaded, ensure that the barcode is facing the corresponding barcode readers for the instrument side.

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

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

1. Navigate to the Configuration screen.


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

3. Click the “Duplicate” button to create a copy of this extraction method and a General tab window will pop up.
4. Under the **General** tab Name this new method “Respy_Vessel_Vessel”.

5. Select “Next” to move to the **Input** tab.

6. Ensure only “Other” and “Respiratory” are selected in the Allowed in Laboratory Column.

7. Ensure the valid input volumes are set to from 200 to 200 and the default input volume is set to 200 as well.

8. Select “Next” to move to the **Preparation** tab.

9. Under the Preparation Protocol Steps Column, select “Transfer internal control to well” and click the back arrow to remove this step. This step will be completed off-board during sample loading.

10. Ensure the following items remain under the Preparation Protocol Steps Column:
    a. Distribute reagent bottle to well.
    b. Incubate at room temperature.
    c. Transfer silica to well
    d. Incubate at room temperature
11. Select “Next” to move to the Extraction tab.
12. Ensure the Extraction Protocol is set to Generic_3.0.4 and elution volumes are set to 50.

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

14. Select “Keep eluates in vessel”.

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

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

Section 3: Loading an EMAG Extraction Run

1. To create a Request List, Navigate to the Request List screen.

2. Enter the Matrix (Respiratory), Extraction method (Respy_Vessel_Vessel). Verify the following volume:
   a. Sample Volume (200µL)
   b. Silica volume (50µL)
   c. Elution volume (50µL)
3. Enter or scan each sample name in the ID field and press enter (section 1). The requested extractions will appear in the request list (section 2).

4. Choose the Prepare Input icon.

5. Begin preparing the vessel(s). Into each necessary well, pipette 20µL of Process Control (M5005) provided in the Lyra SARS-CoV2 Assay kit (i.e., five samples need five wells containing Process Control).

6. Add 180µL of sample in the wells of the vessel(s) for a total of 200µL of liquid in each well.

7. Scan the barcode on the first vessel. Each vessel will have samples individually assigned. (Depending on your barcode scanner, you may have to hit “Enter” after selecting the vessel tab).
8. Select or check the boxes by the first set of samples (up to 8) listed for extraction that will go into the vessel indicated at the bottom of the screen.

9. Click the small Up/Down icon at the bottom of the list to add samples to the vessel.

10. Click the icon on the bottom right of the screen to add up to 8 more samples to a new vessel.

11. Repeat steps 7 through 10 until all samples in the request list have been assigned to a vessel.

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

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

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

2. Open the door to the working area.

3. Load the sample vessels into the proper location according to the preparation tray locations. To load a sample vessel:
   a. Hold it by the handle.
   b. Slide it into position in the working area.
   c. Push the vessel into position until the detection tab clicks into the slot at the back.

4. Load the aspirator disposables.
5. Close the working area door after all sample vessels and aspirator disposables are loaded. The EMAG instrument will now scan the vessel barcodes.

6. Load the tubes of Silica.

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

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

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

7. Vortex tubes immediately before inserting for 5 seconds. Insert the required Silica tubes (uncapped) into the slots of the green rack with the barcode facing the corresponding section (Left or Right).

8. Steadily slide the rack back into the instrument. The EMAG software will display the rack and the tubes.

   Note: If a barcode is not read, the tube details can be entered manually by clicking on the segment and choosing the specific reagent. The reagent lot number and expiration date may be entered as well but is not mandatory to start the run.
a. After the Reagent rack is back in place, Silica tubes will appear loaded on the EMAG software.

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

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

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

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

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

12. Upon completion of the run, remove the Reagent rack.

13. Transfer the purified nucleic acid from the vessel into nuclease-free tubes. Take care not to disturb the Silica that has been pulled to the front of the vessel.
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 specimens extracted 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 plate well or tube.
   
   Note: The rehydrated Master Mix is sufficient for 8 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 plate well or tube.
2. Add 5 µL of extracted nucleic acid (specimen with the process control) into the plate well or tube. Mixing of reagents is not required.
   
   Note: Use a new barrier micropipettor tip with each extracted specimen.
3. Seal the plate or tubes.
4. Centrifuge the plate or tubes for a minimum of 15 seconds. Ensure that all liquid is at the bottom of the plate wells or tubes.
5. Turn on the appropriate thermocycler.
6. Insert plate or tubes into the appropriate thermocycler.

   NOTE: Refer to Appendix for specific programming and testing protocols of each thermocycler.

QUALITY CONTROL
The Lyra SARS-CoV-2 Assay incorporates several controls to monitor assay performance.

1. The Process Control (PRC) consists of an inactivated and stabilized MS2 Bacteriophage that contains an RNA genome. It must be used during extraction and amplification in the assay. This control should be added to each sample aliquot prior to extraction. 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.

2. The Positive Control (containing SARS-CoV-2 Synthetic RNA, Part M5153) must be treated as a patient specimen and be included in every extraction and RT-PCR run.

3. The Negative Control (Part M5031) must be treated as a patient specimen and be included in every extraction and PCR run.

4. 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 specimens or obtain new samples and retest if the controls fail again.
Table 3. Expected Results from Controls (Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, Bio-Rad CFX96 Touch, Qiagen Rotor-Gene Q, or Thermo Fisher QuantStudio-7 Pro)

<table>
<thead>
<tr>
<th>Control Type/ Name</th>
<th>Used to Monitor</th>
<th>SARS-CoV-2 Expected Ct Values</th>
<th>PRC</th>
<th>Expected Ct Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>Substantial reagent failure including primer and probe integrity</td>
<td>+</td>
<td>5.0 ≤ Ct ≤ 30.0</td>
<td>+/-</td>
</tr>
<tr>
<td>Negative Control</td>
<td>Reagent and/or environmental contamination</td>
<td>-</td>
<td>None detected</td>
<td>+</td>
</tr>
</tbody>
</table>

Expected Results from Controls (Roche LightCycler 480)

<table>
<thead>
<tr>
<th>Control Type/ Name</th>
<th>Used to Monitor</th>
<th>SARS-CoV-2 Expected Ct Values</th>
<th>PRC</th>
<th>Expected Ct Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>Substantial reagent failure including primer and probe integrity</td>
<td>+</td>
<td>5.0 ≤ Ct ≤ 40.0</td>
<td>+/-</td>
</tr>
<tr>
<td>Negative Control</td>
<td>Reagent and/or environmental contamination</td>
<td>-</td>
<td>None detected</td>
<td>+</td>
</tr>
</tbody>
</table>

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

INTERPRETATION OF RESULTS FROM PATIENT SPECIMENS

Table 4. Interpretation of the Lyra SARS-CoV-2 Assay Results on the Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, Bio-Rad CFX96 Touch, Qiagen Rotor-Gene Q, or Thermo Fisher QuantStudio-7 Pro

<table>
<thead>
<tr>
<th>Assay Result</th>
<th>Detector: SARS-CoV-2</th>
<th>Detector: Process Control</th>
<th>Interpretation of Results</th>
<th>Notes and Special Guidance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No Ct detected</td>
<td>5.0 ≤ Ct ≤ 30.0</td>
<td>No SARS-CoV-2 viral RNA detected; PRC Detected.</td>
<td></td>
</tr>
<tr>
<td>SARS-CoV-2 Positive</td>
<td>5.0 ≤ Ct ≤ 30.01</td>
<td>NA2</td>
<td>SARS-CoV-2 Virus viral RNA detected.</td>
<td></td>
</tr>
<tr>
<td>Invalid</td>
<td>No Ct detected</td>
<td>No Ct detected</td>
<td>No SARS-CoV-2 viral RNA and no PRC RNA detected.</td>
<td>Invalid test. Retest the same purified sample. If the test is also invalid, re-extract and retest another aliquot of the same specimen or obtain a new specimen and retest.</td>
</tr>
</tbody>
</table>

Interpretation of the Lyra SARS-CoV-2 Assay Results on the Roche LightCycler 480
Table 4. Interpretation of the Lyra SARS-CoV-2 Assay Results on the Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, Bio-Rad CFX96 Touch, Qiagen Rotor-Gene Q, or Thermo Fisher QuantStudio-7 Pro

<table>
<thead>
<tr>
<th>Assay Result</th>
<th>Detector: SARS-CoV-2</th>
<th>Detector: Process Control</th>
<th>Interpretation of Results</th>
<th>Notes and Special Guidance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No Ct detected</td>
<td>5.0≤ Ct ≤40.0</td>
<td>No SARS-CoV-2 viral RNA detected; PRC Detected.</td>
<td></td>
</tr>
<tr>
<td>SARS-CoV-2 Positive</td>
<td>5.0≤ Ct ≤40.0¹</td>
<td>NA²</td>
<td>SARS-CoV-2 Virus viral RNA detected.</td>
<td></td>
</tr>
<tr>
<td>Invalid</td>
<td>No Ct detected</td>
<td>No Ct detected</td>
<td>No SARS-CoV-2 viral RNA and no PRC RNA detected.</td>
<td>Invalid test. Retest the same purified sample. If the test is also invalid, re-extract and retest another aliquot of the same specimen or obtain a new specimen and retest.</td>
</tr>
</tbody>
</table>

¹ For samples generating a Ct value between 0 (Undetermined) and 5, perform 1:10 and 1:100 dilutions using uninoculated transport media. Extract and test the diluted samples according to the instructions above. Interpret the results according to Table 4 above. Note that if review of the original amplification curve from the neat sample is not suggestive of a positive result, processing of the diluted specimens may be deferred. If the diluted samples generate Ct values between 0 (Undetermined) and 5 again, please contact a Quidel Representative at (800) 874-1517 (toll-free in the U.S.) or (858) 552-1100 (outside of U.S.), Monday through Friday, between 8:00 a.m. and 5:00 p.m., Eastern for additional instructions.

² No Ct value is required for the Process Control to make a positive call.

PERFORMANCE CHARACTERISTICS

Clinical Performance with Anterior Nasal Swabs
Anterior nares (nasal) swab samples from individuals suspected of COVID-19 were collected in transport medium. Each sample was tested for SARS-CoV-2 using an FDA EUA authorized, highly sensitive molecular comparator test and the Lyra SARS-CoV-2 Assay (ABI 7500 Fast Dx or QuantStudio 7 workflow). Clinical performance is summarized in Table 5 below. The clinical study included a sufficient number of weak positive specimens, as determined by the FDA EUA authorized, highly sensitive molecular comparator test.
**Table 5. Lyra SARS-CoV-2 Assay Performance Compared to Highly Sensitive FDA Authorized RT-PCR Assay**

<table>
<thead>
<tr>
<th>Comparator Results</th>
<th>95% CI (Wilson Score Method)</th>
</tr>
</thead>
<tbody>
<tr>
<td>POS</td>
<td>NEG</td>
</tr>
<tr>
<td>POS</td>
<td>54</td>
</tr>
<tr>
<td>NEG</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
</tr>
</tbody>
</table>

PPA 98.2% (54/55) 90.4%-99.7%
NPA 99.6% (272/273) 98.0%-99.9%

**Clinical Performance with Nasopharyngeal Swabs**

The clinical performance of the Lyra SARS-CoV-2 Assay was evaluated using two different studies:

- A study using two hundred sixty-five fresh or frozen nasopharyngeal swab specimens collected in UTM (36 and 229, respectively) from patients located in the USA.
- A fully contrived positive specimen study using nasopharyngeal swab specimens.

All two hundred sixty-five specimens were negative for SARS-CoV-2 when extracted with the easyMAG system and tested by the Lyra SARS-CoV-2 Assay.

One hundred twenty-four specimens included in this study were positive for other circulating respiratory viruses as identified by FDA-cleared assays:

<table>
<thead>
<tr>
<th>Circulating Virus</th>
<th># of positive specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A</td>
<td>30</td>
</tr>
<tr>
<td>RSV</td>
<td>4</td>
</tr>
<tr>
<td>Coronavirus Seasonal (not identified)</td>
<td>10</td>
</tr>
<tr>
<td>Coronavirus 229e</td>
<td>20</td>
</tr>
<tr>
<td>Coronavirus OC43</td>
<td>20</td>
</tr>
<tr>
<td>Coronavirus NL63</td>
<td>20</td>
</tr>
<tr>
<td>Coronavirus HKU1</td>
<td>20</td>
</tr>
</tbody>
</table>

Viral RNA was obtained from BEI Resources for use in the contrived clinical study. The genomic RNA was extracted from a preparation of cell lysate and supernatant from Cercopithecus aethiops kidney epithelial (Vero E6, ATCC® CRL-1586) cells infected with SARS-related coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020, using QIAamp® Viral RNA Mini Kit (Qiagen 52904). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA. The Genome Copy Number was established using Bio-Rad QX200 Droplet Digital PCR (ddPCR®) System.

Ninety-two positive contrived samples were created by spiking ninety-two individual clinical samples determined to be negative for SARS-CoV-2 by the Lyra SARS-CoV-2 Assay prior to spiking with one of three concentrations of genomic SARS-CoV-2 RNA. Forty-four specimens were spiked with 1x LoD
(8.00E-01 cp/µL) of RNA. Twenty-four additional specimens were spiked with 3x LoD (2.40E00 cp/µL) of RNA. Twenty-four additional specimens were spiked with 5x LoD (4.00E00 cp/µL) of RNA. All samples were extracted and tested according to the Lyra SARS-CoV-2 Assay package insert.

All ninety-two contrived samples were positive in the Lyra SARS-CoV-2 Assay. The results for the contrived positive specimens are shown in the table below:

<table>
<thead>
<tr>
<th>Sample RNA Concentration</th>
<th># Positives/# Tested</th>
<th>Mean SARS-CoV-2 Ct</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>unspiked</td>
<td>0/92</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>1.0x LoD</td>
<td>44/44</td>
<td>26.9</td>
<td>5.7</td>
</tr>
<tr>
<td>3x LoD</td>
<td>24/24</td>
<td>22.8</td>
<td>3.4</td>
</tr>
<tr>
<td>5x LoD</td>
<td>24/24</td>
<td>22.4</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Performance against the expected results are:
Positive Percent Agreement  92/92 = 100% (95% CI: 95.99%-100%)
Negative Percent Agreement  92/92 = 100% (95% CI: 95.99%-100%)

**Level of Detection**
The Limit of Detection of the Lyra SARS-CoV-2 Assay utilized limiting dilutions of genomic SARS-CoV-2 RNA in negative nasopharyngeal matrix. Each dilution was extracted using the NucliSENS easyMAG System and tested on Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, Roche LightCycler 480, Qiagen Rotor-Gene Q, Bio-Rad CFX96 Touch, or Thermo Fisher QuantStudio 7 Pro. Analytical sensitivity (LoD) is defined as the lowest concentration at which at least 95% of all replicates tested positive.

The genomic RNA was extracted from a preparation of cell lysate and supernatant from Cercopithecus aethiops kidney epithelial (Vero E6, ATCC CRL-1586) cells infected with SARS-related coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020, using QIAamp Viral RNA Mini Kit (Qiagen 52904). The viral genomic RNA is in a background of cellular nucleic acid and carrier RNA. The Genome Copy Number was established using Bio-Rad QX200 Droplet Digital PCR (ddPCR) System.

This study established the LoD for the Lyra SARS-CoV-2 Assay as 8.00E-01 genomic RNA copies/µL, subsequently confirmed by testing 20 replicates.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Replicate</th>
<th>SARS-CoV-2 Ct</th>
<th>PRC Ct</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.00E-01 genomic RNA copies/µL</td>
<td>1</td>
<td>23.95</td>
<td>18.54</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>26.59</td>
<td>18.28</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>26.19</td>
<td>18.32</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>25.13</td>
<td>18.41</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>24.88</td>
<td>18.74</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>24.84</td>
<td>19.18</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>25.51</td>
<td>18.82</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>25.20</td>
<td>18.58</td>
<td>Positive</td>
</tr>
</tbody>
</table>
### Table 7. LoD in Nasopharyngeal specimens with Applied Biosystems 7500 Fast Dx

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Replicate</th>
<th>SARS-CoV-2 Ct</th>
<th>PRC Ct</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>24.69</td>
<td>18.71</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>24.57</td>
<td>18.67</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>23.86</td>
<td>18.75</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>24.58</td>
<td>18.91</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>25.19</td>
<td>19.03</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>25.84</td>
<td>19.05</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>26.58</td>
<td>19.10</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>26.72</td>
<td>19.15</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>24.16</td>
<td>19.06</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>25.15</td>
<td>18.91</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>25.51</td>
<td>19.05</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>24.41</td>
<td>19.07</td>
<td>Positive</td>
<td></td>
</tr>
</tbody>
</table>

### Table 8. LoD in Oropharyngeal specimens with Applied Biosystems 7500 Fast Dx

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Replicate</th>
<th>SARS-CoV-2 Ct</th>
<th>PRC Ct</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.00E-01 genomic RNA copies/µL</td>
<td>1</td>
<td>27.26</td>
<td>19.38</td>
<td>Positive</td>
</tr>
<tr>
<td>2</td>
<td>28.99</td>
<td>19.22</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>27.3</td>
<td>19.51</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>26.09</td>
<td>19.27</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>26.88</td>
<td>19.61</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>26.02</td>
<td>19.19</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>26.37</td>
<td>19.21</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>25.01</td>
<td>19.30</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>25.14</td>
<td>19.06</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>26.21</td>
<td>19.03</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>27.79</td>
<td>19.27</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>28.83</td>
<td>19.12</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>28.83</td>
<td>19.19</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>26.81</td>
<td>19.50</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>25.11</td>
<td>19.30</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>26.21</td>
<td>19.11</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>26.74</td>
<td>19.00</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>25.28</td>
<td>19.13</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>26.27</td>
<td>19.31</td>
<td>Positive</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>26.37</td>
<td>19.24</td>
<td>Positive</td>
<td></td>
</tr>
</tbody>
</table>
### Table 9. LoD in Nasopharyngeal specimens with Applied Biosystems 7500 Standard

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Replicate</th>
<th>SARS-CoV-2 Ct</th>
<th>PRC Ct</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.00E-01 genomic RNA copies/µL</td>
<td>1</td>
<td>26.63</td>
<td>19.26</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>29.15</td>
<td>19.28</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>25.67</td>
<td>19.69</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>25.53</td>
<td>20.07</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>26.15</td>
<td>20.50</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>7</td>
<td>26.11</td>
<td>19.14</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>26.94</td>
<td>19.18</td>
<td>Positive</td>
</tr>
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<td></td>
<td>9</td>
<td>25.62</td>
<td>18.64</td>
<td>Positive</td>
</tr>
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<td>10</td>
<td>25.80</td>
<td>18.80</td>
<td>Positive</td>
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<td>11</td>
<td>26.76</td>
<td>19.15</td>
<td>Positive</td>
</tr>
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<td>12</td>
<td>26.15</td>
<td>19.63</td>
<td>Positive</td>
</tr>
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<td></td>
<td>13</td>
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</tr>
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</tr>
<tr>
<td></td>
<td>18</td>
<td>27.71</td>
<td>19.27</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>26.51</td>
<td>18.86</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Undetermined</td>
<td>19.11</td>
<td>Negative</td>
</tr>
</tbody>
</table>

### Table 10. LoD in Nasopharyngeal specimens with Roche LightCycler 480*

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Replicate</th>
<th>SARS-CoV-2 Ct*</th>
<th>PRC Ct*</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.00E-01 genomic RNA copies/µL</td>
<td>1</td>
<td>32.91</td>
<td>31.73</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>34.54</td>
<td>32.9</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>34.83</td>
<td>32.25</td>
<td>Positive</td>
</tr>
<tr>
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<td>4</td>
<td>34.94</td>
<td>31.7</td>
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</tr>
<tr>
<td></td>
<td>7</td>
<td>33.90</td>
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<td>Positive</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>33.83</td>
<td>32.80</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>9</td>
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<td>34.28</td>
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<td>Positive</td>
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<td></td>
<td>11</td>
<td>33.63</td>
<td>32.81</td>
<td>Positive</td>
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<td>12</td>
<td>33.72</td>
<td>32.45</td>
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</tr>
<tr>
<td></td>
<td>13</td>
<td>34.86</td>
<td>33.17</td>
<td>Positive</td>
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</table>
Table 10. LoD in Nasopharyngeal specimens with Roche LightCycler 480*

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Replicate</th>
<th>SARS-CoV-2 Ct*</th>
<th>PRC Ct*</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14</td>
<td>34.57</td>
<td>32.64</td>
</tr>
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<td></td>
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<td>32.92</td>
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<td>16</td>
<td>33.61</td>
<td>32.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>33.87</td>
<td>33.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18</td>
<td>34.44</td>
<td>33.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>19</td>
<td>34.22</td>
<td>32.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>33.77</td>
<td>32.97</td>
</tr>
</tbody>
</table>
* Results include 10 cycles not captured by the other instruments

Table 11. LoD in Nasopharyngeal specimens with Qiagen Rotor-Gene Q

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Replicate</th>
<th>SARS-CoV-2 Ct</th>
<th>PRC Ct</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.00E-01 genomic RNA copies/µL</td>
<td>1</td>
<td>24.01</td>
<td>19.08</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>24.04</td>
<td>19.36</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>24.85</td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>23.23</td>
<td>19.13</td>
<td>Positive</td>
</tr>
<tr>
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<td>5</td>
<td>24.39</td>
<td>19.07</td>
<td>Positive</td>
</tr>
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<td>6</td>
<td>23.89</td>
<td>18.94</td>
<td>Positive</td>
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<tr>
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<td>7</td>
<td>23.78</td>
<td>18.80</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>9</td>
<td>23.87</td>
<td>18.83</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>24.05</td>
<td>18.90</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>11</td>
<td>23.28</td>
<td>18.84</td>
<td>Positive</td>
</tr>
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<td></td>
<td>12</td>
<td>24.36</td>
<td>18.71</td>
<td>Positive</td>
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<td>18.88</td>
<td>Positive</td>
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<td>15</td>
<td>24.84</td>
<td>19.20</td>
<td>Positive</td>
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<td>18</td>
<td>23.47</td>
<td>19.01</td>
<td>Positive</td>
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<td>19</td>
<td>23.58</td>
<td>18.94</td>
<td>Positive</td>
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<tr>
<td></td>
<td>20</td>
<td>23.89</td>
<td>19.02</td>
<td>Positive</td>
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</table>
### Table 12. LoD in Nasopharyngeal specimens with Bio-Rad CFX96 Touch

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Replicate</th>
<th>SARS-CoV-2 Ct</th>
<th>PRC Ct</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.00E-01 genomic RNA copies/µL</td>
<td>1</td>
<td>27.19</td>
<td>21.25</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>25.57</td>
<td>21.35</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>25.80</td>
<td>22.68</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>27.93</td>
<td>21.3</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>29.03</td>
<td>21.09</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>25.79</td>
<td>21.45</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>25.65</td>
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</tr>
<tr>
<td></td>
<td>8</td>
<td>26.26</td>
<td>21.16</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>29.46</td>
<td>21.41</td>
<td>Positive</td>
</tr>
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<td>10</td>
<td>25.09</td>
<td>21.45</td>
<td>Positive</td>
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<tr>
<td></td>
<td>11</td>
<td>25.68</td>
<td>21.36</td>
<td>Positive</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>13</td>
<td>25.5</td>
<td>21.97</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>26.81</td>
<td>21.36</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>18</td>
<td>25.54</td>
<td>21.26</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>25.77</td>
<td>22.29</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>25.59</td>
<td>22.16</td>
<td>Positive</td>
</tr>
</tbody>
</table>

### Table 13. LoD in Nasopharyngeal specimens with Thermo Fisher QuantStudio-7 Pro

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Replicate</th>
<th>SARS-CoV-2 Ct</th>
<th>PRC Ct</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.00E-01 genomic RNA copies/µL</td>
<td>1</td>
<td>24.25</td>
<td>20.21</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>26.7</td>
<td>20.9</td>
<td>Positive</td>
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<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>4</td>
<td>27.28</td>
<td>20.81</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>29.60</td>
<td>20.78</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
<td></td>
<td>7</td>
<td>28.75</td>
<td>20.82</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>27.63</td>
<td>20.76</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
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<td>29.80</td>
<td>20.65</td>
<td>Positive</td>
</tr>
<tr>
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<td>10</td>
<td>26.60</td>
<td>20.55</td>
<td>Positive</td>
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<tr>
<td></td>
<td>11</td>
<td>27.23</td>
<td>20.54</td>
<td>Positive</td>
</tr>
<tr>
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<td>12</td>
<td>29.81</td>
<td>20.73</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>13</td>
<td>26.59</td>
<td>20.88</td>
<td>Positive</td>
</tr>
</tbody>
</table>
The Lyra SARS-CoV-2 Assay was evaluated using the FDA SARS-CoV-2 Reference Panel. The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. The results are summarized in the Table 14. The samples were extracted using the bioMérieux NucliSENS easyMAG and amplified and detected with the Thermo Fisher QuantStudio 7 Pro.

### Table 14. Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

<table>
<thead>
<tr>
<th>Reference Materials Provided by FDA</th>
<th>Specimen Type</th>
<th>Product LoD</th>
<th>Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS-CoV-2</td>
<td>NPS</td>
<td>6.0x10^3 NDU/mL</td>
<td>N/A</td>
</tr>
<tr>
<td>MERS-CoV</td>
<td>N/A</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

NDU/mL: RNA NAAT detectable units/mL
N/A: Not Applicable
ND: Not Detected

### Analytical Reactivity (Inclusivity)

The inclusivity of the Lyra SARS-CoV-2 Assay was established by testing Genomic RNA from the SARS-related coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020, via in-silico analysis. The in-silico analysis demonstrated the Lyra SARS-CoV-2 primers are >95% conserved to 41,270 and 301,386 SARS-CoV-2 sequences available from NCBI (December 17, 2020) and GISAID (December 30, 2020), respectively.

As of December 30, 2020, GISAID had 4,529 UK variant VU1 202012/01 and South Africa variant 501Y.V2 sequences. The in-silico analysis demonstrated the Lyra SARS-CoV-2 primers are >95% conserved to these variants. 5 UK variants have a SNP aligned to the 3' end. An RNA sequence representing a UK variant sequence with the SNP and one representing a control sequence (MN908947.3) were ordered and tested on the Lyra SARS-CoV-2 assay. Both RNA templates yielded identical results indicating that the SNP aligned to the 3’ end will not interfere with detection. This result correlates with previous studies on SNPs aligned to the 3’ end of a Lyra primer.

The Lyra SARS-COV-2 primers are 95-100% conserved to 342,656 SARS-COV-2 sequences available from NCBI and GISAID as of December 30, 2020.
Analytical Specificity (Cross-Reactivity)

The Analytical Specificity of the assay was established by both direct testing of organisms in the assay (“wet” testing) and in silico analysis. The wet testing used 25 micro-organisms, in high concentrations, identified by the FDA as high priority for evaluation due to the reasonable likelihood they may be present in upper respiratory samples. All micro-organisms were undetectable with the Lyra SARS-CoV-2 Assay when wet tested as shown below.

### Table 15. Cross-reactivity test results

<table>
<thead>
<tr>
<th>Virus/Bacteria/Parasite</th>
<th>Strain</th>
<th>Source/Sample type</th>
<th>Concentration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>Type 1</td>
<td>Isolate</td>
<td>1 x 10^{7.53} U/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>229e</td>
<td>Isolate</td>
<td>1 x 10^{6.10} U/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>OC43</td>
<td>Isolate</td>
<td>9.55 x 10^6 TCID_{50}/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Coronavirus</td>
<td>NL63</td>
<td>Isolate</td>
<td>1 x 10^{4.67} U/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>MERS-CoV (heat-inactivated)</td>
<td>Florida/USA-2, Saudia Arabia_2014</td>
<td>Isolate</td>
<td>4.17 x 10^5 TCID_{50}/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>SARS -1</td>
<td>2003-00592</td>
<td>Inactivated virus</td>
<td>Not available</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>M129</td>
<td>Isolate</td>
<td>3 x 10^7 CCU/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>Z018</td>
<td>Isolate</td>
<td>3.8 x 10^9 cfu/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Influenza A H3N2</td>
<td>Brisbane/10/07</td>
<td>Isolate</td>
<td>1 x 10^{5.07} U/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Influenza A H1N1</td>
<td>New Caledonia/20/99</td>
<td>Isolate</td>
<td>1 x 10^{6.66} U/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Influenza B</td>
<td>Brisbane/33/08</td>
<td>Isolate</td>
<td>1 x 10^{5.15} U/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>Type 1</td>
<td>Isolate</td>
<td>1 x 10^{5.01} U/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>Type 2</td>
<td>Isolate</td>
<td>1 x 10^{5.34} U/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>Type 3</td>
<td>Isolate</td>
<td>8.51 x 10^7 TCID_{50}/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Parainfluenza</td>
<td>Type 4b</td>
<td>Isolate</td>
<td>1 x 10^{7.53} U/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Enterovirus</td>
<td>Type 68</td>
<td>Isolate</td>
<td>1 x 10^{5.5} U/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Human Metapneumovirus</td>
<td>A1 (IA10-s003)</td>
<td>Isolate</td>
<td>1 x 10^{5.55} U/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Respiratory Syncytial Virus</td>
<td>Type A (3/2015 Isolate #3)</td>
<td>Isolate</td>
<td>1 x 10^{5.62} U/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Human Rhinovirus</td>
<td>N/A</td>
<td>Inactivated virus</td>
<td>Not available</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Chlamyphila pneumoniae</td>
<td>AR-39</td>
<td>Isolate</td>
<td>2.9 x 10^7 IFU/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>Type b; Eagan</td>
<td>Isolate</td>
<td>7.87 x 10^8 cfu/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Legionella pneumophila</td>
<td>Philadelphia</td>
<td>Isolate</td>
<td>6.82 x 10^9 cfu/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>Z022; 19f</td>
<td>Isolate</td>
<td>2.26 x 10^9 cfu/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>Isolate</td>
<td></td>
<td></td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Pneumocystis jirovecii-S. cerevisiae Recombinant</td>
<td>W303-Pji</td>
<td>Isolate</td>
<td>1.56 x 10^8 cfu/mL</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Negative Nasopharyngeal Matrix</td>
<td>MTM</td>
<td>N/A</td>
<td>N/A</td>
<td>Neg, Neg, Neg</td>
</tr>
</tbody>
</table>
Table 15. Cross-reactivity test results

<table>
<thead>
<tr>
<th>Virus/Bacteria/Parasite</th>
<th>Strain</th>
<th>Source/ Sample type</th>
<th>Concentration</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Nasopharyngeal Matrix</td>
<td>MTM</td>
<td>N/A</td>
<td>N/A</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Negative Nasal Matrix</td>
<td>CDC Viral Transport</td>
<td>N/A</td>
<td>N/A</td>
<td>Neg, Neg, Neg</td>
</tr>
<tr>
<td>Negative Oropharyngeal Matrix</td>
<td>CDC Viral Transport</td>
<td>N/A</td>
<td>N/A</td>
<td>Neg, Neg, Neg</td>
</tr>
</tbody>
</table>

The *in silico* analysis focused on 32 micro-organisms identified by the FDA as high priority for assessment due to their potential presence in upper respiratory samples.

Table 16. Cross-Reactivity Organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total # Sequences</th>
<th># Complete Genomes</th>
<th># WGS Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus</td>
<td>532</td>
<td>532</td>
<td>0</td>
</tr>
<tr>
<td>Coronavirus (Seasonal)</td>
<td>288</td>
<td>288</td>
<td>0</td>
</tr>
<tr>
<td>Enterovirus(^B)</td>
<td>2708</td>
<td>2674</td>
<td>34</td>
</tr>
<tr>
<td>Influenza A Virus(^A,B)</td>
<td>172455</td>
<td>21444 (+39 A/Mexico/4108/2009)</td>
<td>108</td>
</tr>
<tr>
<td>Influenza B Virus(^A,B)</td>
<td>53952</td>
<td>6755 (+16 B/Florida/4/2006)</td>
<td>0</td>
</tr>
<tr>
<td>Influenza C Virus(^B)</td>
<td>2205</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Human Metapneumovirus</td>
<td>145</td>
<td>145</td>
<td>0</td>
</tr>
<tr>
<td>Human Parainfluenza Virus 1-4</td>
<td>439</td>
<td>439</td>
<td>0</td>
</tr>
<tr>
<td>Human Parechovirus</td>
<td>124</td>
<td>124</td>
<td>0</td>
</tr>
<tr>
<td>Human Respiratory Syncytial Virus(^B)</td>
<td>1275</td>
<td>1275</td>
<td>0</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>214</td>
<td>214</td>
<td>0</td>
</tr>
<tr>
<td>SARS-1</td>
<td>236(^C)</td>
<td>232 (+4 pp1ab sequences)</td>
<td>0</td>
</tr>
<tr>
<td><em>Bacillus anthracis</em></td>
<td>4152</td>
<td>69</td>
<td>86</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>1541</td>
<td>59</td>
<td>34</td>
</tr>
<tr>
<td><em>Chlamydia pneumoniae</em></td>
<td>466</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td><em>Chlamydia psittaci</em></td>
<td>11179</td>
<td>23</td>
<td>45</td>
</tr>
<tr>
<td><em>Corynebacterium diphtheriae</em></td>
<td>20797</td>
<td>17</td>
<td>194</td>
</tr>
<tr>
<td><em>Coxiella burnetii</em></td>
<td>419</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>45267</td>
<td>61</td>
<td>692</td>
</tr>
<tr>
<td><em>Legionella</em>(^B)</td>
<td>4843</td>
<td>98</td>
<td>65</td>
</tr>
<tr>
<td><em>Leptospira</em>(^B)</td>
<td>64456</td>
<td>133</td>
<td>266</td>
</tr>
<tr>
<td><em>Moraxella catarrhalis</em>(^B)</td>
<td>8333</td>
<td>11</td>
<td>184</td>
</tr>
</tbody>
</table>
TABLE 16. Cross-Reactivity Organisms

<table>
<thead>
<tr>
<th>Organism</th>
<th>Total # Sequences</th>
<th># Complete Genomes</th>
<th># WGS Strains</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>194</td>
<td>194</td>
<td>0</td>
</tr>
<tr>
<td><em>Mycoplasma pneumoniae</em></td>
<td>808</td>
<td>51</td>
<td>45</td>
</tr>
<tr>
<td><em>Neisseria elongata &amp; N. meningitidis</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>312050</td>
<td>116</td>
<td>1318</td>
</tr>
<tr>
<td><em>Pneumocystis jirovecii</em></td>
<td>487</td>
<td>15</td>
<td>3</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>195</td>
<td>195</td>
<td>0</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>634</td>
<td>634</td>
<td>0</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>61880</td>
<td>23</td>
<td>508</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1633369</td>
<td>107</td>
<td>8526</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46153</td>
<td>201</td>
<td>1733</td>
</tr>
<tr>
<td><em>Streptococcus salivarius</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9417</td>
<td>18</td>
<td>48</td>
</tr>
</tbody>
</table>

<sup>A</sup> Genome counts for Influenza A and Influenza B were attained for strains that included all 8 segments, except for A/Mexico/4108/2009(H1N1) and B/Florida/4/2006; all available gene sequences were included.

<sup>B</sup> For BLAST, ‘Max Target Seqs’ was set to 5000. See Table 2.

<sup>C</sup> 4 polyprotein cds sequences were also included.

The in-silico analysis demonstrated < 80% homology with all organisms except for the following: three Enterovirus sequences are 80.9% conserved to the reverse primer, however, the forward primer is only 76% conserved and the probe alignment had an overall homology of 56%. The SARS-1 sequences are ≥80% conserved to both primers, however, the last base on the 3’ ends of both primers are not conserved. The wet testing of the only available SARS-1 strain was non-detectable.

LIMITATIONS

- Negative results do not preclude infection with SARS-CoV-2 and should not be the sole basis of a patient treatment decision.
- This test is intended to be used for the detection of SARS-CoV-2 RNA in nasopharyngeal, oropharyngeal, and nasal swab samples. Testing of other sample types may result in inaccurate results.
- Mid-turbinate nasal swabs are considered an acceptable specimen type for use with the Lyra SARS-CoV-2 Assay but performance with this specimen type has not been established. Testing of mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA’s FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.
- 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.
- 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(es) 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.

• The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

• The assay performance was not established in immunocompromised patients.

• Specimens may be freeze/thawed no more than once prior to testing.

CONDITIONS OF AUTHORIZATION FOR THE LABS


However, to assist clinical laboratories using the Lyra SARS-CoV-2 Assay, the relevant Conditions of Authorization are listed below.

• Authorized laboratories using the Lyra SARS-CoV-2 Assay must include with result reports of the Lyra SARS-CoV-2 Assay test, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

• Authorized laboratories using the Lyra SARS-CoV-2 Assay must perform the Lyra SARS-CoV-2 Assay as outlined in the Lyra SARS-CoV-2 Assay Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to perform the Lyra SARS-CoV-2 Assay are not permitted.

• Authorized laboratories that receive the Lyra SARS-CoV-2 Assay must notify the relevant public health authorities of their intent to run the test prior to initiating testing.

• Authorized laboratories using the Lyra SARS-CoV-2 Assay must have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

• Authorized laboratories must collect information on the performance of the test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Quidel (QDL.COV2.test.event.report@quidel.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.

• All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use the test in accordance with the authorized labeling.
• Quidel, its authorized distributor(s) and authorized laboratories using the Lyra SARS-CoV-2 Assay must ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records must be made available to FDA for inspection upon request.

1 For ease of reference, the letter of authorization refers to, “United States (U. S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests” as “authorized laboratories.”

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REFERENCES
APPENDIX I

Applied Biosystems 7500 Fast Dx Programming Instructions
Refer to User Manual Part Number 4406991 for additional information.

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 SARS-CoV-2 Assay 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:      | your operator name                     |
         | Comments:      | SDS v1.4                               |
         | Plate Name:    | ‘Lyra SARS-CoV-2 Assay’                |
      ii. Select the Next button.
   b. Select Detectors: New detectors for SARS-CoV-2 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 |
         | SARS-CoV-2 | FAM     | (none)   | (Select) |
         | PRC     | Quasar 670 | (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 set up during the quick start. For the initial set up, nothing needs to be changed here.
   d. Defining the Thermocycler Protocol: Select the Instrument tab to set up the LyraSARS-CoV-2 Assay 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: 30
   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:

<table>
<thead>
<tr>
<th>Sample Volume (μL):</th>
<th>20 (default)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Run Mode:</td>
<td>7500 Fast (default)</td>
</tr>
<tr>
<td>Data Collection:</td>
<td>Stage 5, Step 2(57.0 @ 0:40)</td>
</tr>
</tbody>
</table>

**NOTE:** Do not check the check box next to ‘Expert Mode’.
e. **Set threshold for each analyte.**
   i. Select the **Results** tab.
   ii. Select the **Amplification Plot** tab.
   iii. Select SARS-CoV-2 from the Detector tab in the top right corner.
   iv. In the **Analysis Settings** block, set the **Threshold** to **7.5e+004**.
   v. Select the **Auto Baseline** radio button.
   vi. Select PRC from the Detector tab in the top right corner.
   vii. In the **Analysis Settings** block, set the **Threshold** to **1.0e+004**.
   viii. Select the **Auto Baseline** radio button.

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 SARS-CoV-2’
   iv. **Save as type:** ‘SDS Templates (*.sdt)’

g. Exit the software.

**Applied Biosystems 7500 Fast Dx Thermocycler Test Procedure**

1. Launch the Applied Biosystems 7500 Fast Dx software v1.4 package.
2. The **Quick Startup document** dialog window will open.
3. Click on **Create a new document**.
4. Most of the following should be the default setting. If not, change accordingly.
   
<table>
<thead>
<tr>
<th>Assay:</th>
<th>Standard Curve (Absolute Quantitation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Container:</td>
<td>96-Well Clear</td>
</tr>
<tr>
<td>Template:</td>
<td>Lyra SARS-CoV-2</td>
</tr>
<tr>
<td>Run Mode:</td>
<td>Fast 7500</td>
</tr>
<tr>
<td>Operator:</td>
<td>your operator name</td>
</tr>
<tr>
<td>Comments:</td>
<td>SDS v1.4</td>
</tr>
<tr>
<td>Plate Name:</td>
<td>YYMMDD- Lyra SARS-CoV-2</td>
</tr>
</tbody>
</table>

5. **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 SARS-CoV-2 and PRC.
   c. Use the **Well Inspector** to enter the sample names. Patient IDs can 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 SARS-CoV-2.sds**.
   e. A window will open asking for the “Reason for change of entry”. Enter “**Setup**” and any other comments relevant to the run.

6. **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.
7. Post PCR

**IMPORTANT:** When the run is finished press OK.

a. 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”.
c. Enter “**Data analysis post run**” and any other comments relevant to the run.

---

**Applied Biosystems 7500 Standard Programming Instructions**

Refer to User Manual Part Number 4387783 rev C for additional information.

1. Launch the ABI 7500 software package.

2. Select the **Advanced Setup** button to open Setup and Experiment Properties. Follow each step to initiate the Lyra SARS-CoV-2 protocol.

   a. Experiment Name: Enter Experiment Name as SARS-CoV-2. Leave the Barcode, User Name, and Comments fields blank
   
   b. Define Experiment Setup: Select 7500 (96 Wells), Quantitation- Standard Curve, TaqMan Reagents, and Standard (~2 hours to complete a run)

3. In the upper left menu select **Plate Setup**

   a. Define Targets: New detectors for SARS-CoV-2, and the process control (PRC) must be added.

      i. Enter the following information for each detector.

      | Name     | Reporter Dye | Quencher Dye | Color   |
      |----------|--------------|--------------|---------|
      | SARS-CoV-2| FAM          | (none)       | (Select) |
      | PRC      | Quasar 670   | (none)       | (Select) |

      ii. Select **Add New Target** button for each target.

      iii. From each drop down menu select reporter, quencher, and color

      iv. Select a unique color to represent each detector

   b. Assign Targets and Samples: Under this tab in the bottom left corner, select **none** as the Passive Reference.

4. Select **Run Method** from the upper left menu

   a. Set the **Reaction Volume** per Well to 20 μL under the **Graphical** or **Tabular View**

   b. Define the Thermocycler Protocol: Under the **Graphical** or **Tabular View** the default profile should be 2 holding stages and a 2-step cycling protocol. Each stage will have 3 user-editable text boxes. The first box value represents the Ramp Rate (%) for that stage, the second box value represents the temperature (°C) and the third box value represents the time (minutes:seconds).

      i. Make the following changes to the default Thermocycler protocol:

         1. Stage 1 First **Holding Stage**
            a. Ramp Rate: 100%
            b. Temp: 55
            c. Time: 5:00

         2. Step 1 Second **Holding Stage**
            a. Ramp Rate: 100%
b. Temp: 60
  c. Time: 5:00
3. Highlight the second **Holding Stage** and select the **Add Stage** button. In the drop down menu select **Holding**

4. Step 1 **Third Holding Stage**
   a. Ramp Rate: 100%
   b. Temp: 65
   c. Time: 5:00

5. First **2-Step Cycling Stage**
   a. Number of cycles: 10
   b. Do NOT check Enable Auto Delta
   c. Step 1
      i. Ramp Rate: 100%
      ii. Temp: 92
      iii. Time: 0:05
   d. Step 2
      i. Ramp Rate: 100%
      ii. Temp: 57
      iii. Time: 0:40
      iv. Turn data collection “Off” by selecting the **Data Selection** button at the bottom of the step.

6. Highlight step 2 and select the **Add Stage** button. In the drop down menu select **Cycling**

7. Second 2-Step **Cycling Stage**
   a. Number of cycles: 30
   b. Do NOT check Enable Auto Delta
   c. Step 1
      i. Ramp Rate: 100%
      ii. Temp: 92
      iii. Time: 0:05
   d. Step 2
      i. Ramp Rate: 100%
      ii. Temp: 57
      iii. Time: 0:40
      iv. Ensure the data collection has been turned “On” for this step (default setting)

8. If a wrong stage is added the stage can be removed by pressing the **Undo “Add Stage”** button immediately after adding the stage or highlight the stage between the vertical lines and select the **Delete Selected** button

5. Set threshold for each analyte
   a. Select the **Analysis** tab in the upper left menu.
   b. Select **Analysis Settings** button in the top right corner.
c. Highlight SARS-CoV-2 and deselect the Use Default Settings box. De-select Automatic Threshold and change threshold to 75,000. Leave Automatic Baseline selected.

d. Highlight PRC and de-select the Use Default Settings box. De-select Automatic Threshold and change threshold to 10,000. Leave Automatic Baseline selected.

e. At the bottom of the box select Apply Analysis Settings button

<table>
<thead>
<tr>
<th>Target</th>
<th>Threshold</th>
<th>Baseline Start</th>
<th>Baseline End</th>
</tr>
</thead>
<tbody>
<tr>
<td>SARS-CoV-2</td>
<td>75,000</td>
<td>Auto</td>
<td>Auto</td>
</tr>
<tr>
<td>PRC</td>
<td>10,000</td>
<td>Auto</td>
<td>Auto</td>
</tr>
</tbody>
</table>

i. Save the new protocol as a template for future use.
   i. At the top of the screen select the drop down menu next to Save
   ii. Choose Save as Template
   iii. Save in an appropriate folder
   iv. File name: ‘Lyra SARS-CoV-2’
   v. Save as type: ‘Experiment Document Template files (*.edt)’
   vi. Exit the software.

Applied Biosystems 7500 Standard Thermocycler Test Procedure

1. Launch the Applied Biosystems 7500 Standard software v2.06 package.
2. The Quick Startup document dialog window will open.
3. Click on Create a new document.
4. Most of the following should be the default setting. If not, change accordingly.

<table>
<thead>
<tr>
<th>Assay:</th>
<th>Standard Curve (Absolute Quantitation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Container:</td>
<td>96-Well Clear</td>
</tr>
<tr>
<td>Template:</td>
<td>Lyra SARS-CoV-2</td>
</tr>
<tr>
<td>Run Mode:</td>
<td>Fast 7500</td>
</tr>
<tr>
<td>Operator:</td>
<td>your operator name</td>
</tr>
<tr>
<td>Comments:</td>
<td>SDS v1.4</td>
</tr>
<tr>
<td>Plate Name:</td>
<td>YYMMDD- Lyra SARS-CoV-2</td>
</tr>
</tbody>
</table>

5. 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 SARS-CoV-2 and PRC.
   c. Use the Well Inspector to enter the sample names. Patient IDs can 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 SARS-CoV-2.sds.
   e. A window will open asking for the “Reason for change of entry”. Enter “Setup” and any other comments relevant to the run.

6. 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.

7. Post PCR
   **IMPORTANT:** When the run is finished press OK.
   a. 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”.
   c. Enter “Data analysis post run” and any other comments relevant to the run.

Bio-Rad CFX96 Touch Thermocycler Programming Procedure
Refer to User Manual Part Number 10010424 Rev D for additional information.

**Programming Instructions:**

1. Launch the CFX96 Touch software package
2. In the Startup Wizard pop-up window **Select instrument** to be CFX96 from the drop down menu
3. Under **Select Run Type** press the **User-defined** button
4. Create a new thermocycler protocol by selecting **Create New** from the Run Setup window
5. Make the following changes to the cycling conditions in the Protocol Editor:
   a. Change the **Sample Volume** to **20ul**
   b. Under **Tools** in the top left toolbar select **Run Time Calculator** and check **96 Wells-All Channels**
   c. **Step 1** (Hold)
      i. Reps: 1
      ii. Temp: 55C
      iii. Time: 5:00
   d. **Step 2** (Hold)
      i. Reps: 1
      ii. Temp: 60C
      iii. Time: 5:00
   e. **Step 3** (Hold)
      i. Reps: 1
      ii. Temp: 65C
      iii. Time: 5:00
      iv. Remove the plate read from this stage by selecting the **Remove Plate Read** button on the lower left
   f. **Step 4** (2-Step Amplification Stage)
      i. Highlight **step 3** and go to the lower left of the window and select **Insert Step** for a total of 2 times until step 5 is reached (ensure in the upper left of the window the drop-down menu for **Insert Step** has **After** selected).
      ii. Highlight **step 4** and set as follows:
         1. Temp: 92C
         2. Time: 0:05
      iii. Highlight **step 5** and set as follows:
         1. Temp: 57C
         2. Time: 0:40
         3. Go to the left of the screen and select **Remove Plate Read** button
      iv. Select **step 6**, the **GOTO step**, and change to state **GOTO step 4** and change the times to repeat to 9
g. **Step 7 (2-Step Amplification Stage)**
   i. With step 6 highlighted select **Insert Step** button, on the lower left of the window, for a total of 2 times (until step 8 is reached)
   ii. Highlight **step 7** and set as follows:
       1. Temp: 92°C
       2. Time: 0:05
   iii. Highlight **step 8** and set as follows:
       1. Temp: 57°C
       2. Time: 0:40
       3. In the left of the window select **Add Plate Read to Step** button
       4. Highlight **step 8** and select **Insert GOTO** button on the lower left of the window
   iv. Select **step 9**, the **GOTO step**, and change to **GOTO step 7** and times to repeat to 29

h. Save the new cycling conditions as protocol for future use
   i. At the upper left of the screen select the **Save** button
   ii. Save in the **ExpressLoad** folder
   iii. Name the file ‘Lyra SARS-CoV-2’
   iv. **Save as type** ‘Protocol File (*.prcl)’
   v. Select **Save**
   vi. Click **Ok** in the protocol editor window

6. Define the plate setup
   a. In the **Run Setup** window select the **Plate** tab
   b. Under **Express Load** in the drop-down menu select **Quick Plate 96 wells All Channels.pltd**
   c. Select the **Edit Selected** button to customize the plate setup
   d. In the upper toolbar select **Settings**. The default settings need to be set.
      i. **Plate Size** select 96 Wells
      ii. **Plate Type** select BR Clear
      iii. **Number Convention** select Scientific Notation
      iv. **Units** select Copy Number
   e. Leave the **Scan Mode** set to **All Channels** at the top of the window
   f. Select the **Select Fluorophores** button on the upper right of the Plate Editor window
      i. De-select all default fluorophores
      ii. Select FAM, and Cy5 and click Ok
   g. In the **Plate Editor** window highlight the whole plate and click the check box in front of all fluorophores: FAM and Cy5
   h. Select the **Experiment Settings** button in order to define the Targets
      i. In the lower left of the **Experiment Settings** window in the **New** box type in SARS-CoV-2 and select **Add**
      ii. Repeat this for the PRC
      iii. Select **Ok**
   i. In the **Plate Editor** window next to FAM in the drop-down menu under **Target Name** select SARS-CoV-2 and for Cy5 select PRC
   j. Save the new plate setup for future use
      i. At the upper left of the screen select the **Save** button
      ii. Save in the **ExpressLoad** folder
      iii. Name the file ‘Lyra SARS-CoV-2 plate’
iv. Save as type ‘Plate File (*.pltd)’
v. Select Save
vi. Click Ok in the Plate Editor window
k. Exit the software

Bio-Rad CFX96 Touch Thermocycler Test Procedure

Analysis Instructions:

1. Open the run file that needs to be analyzed
2. In the upper left select the Quantification Tab
3. On the Amplification curve check the box in front of Log Scale
4. Select Settings in the toolbar in the upper left of the screen
   a. For the Cq Determination Mode select Single Threshold
   b. Under the Baseline Setting choose Baseline Subtracted Curve Fit
   c. For Analysis Mode select Target
   d. Under Cycles to Analyze choose 1-30 and then click Ok
   e. The baseline cycles and the threshold for each target need to be set
      i. Ensure that only the SARS-CoV-2 box is checked in the amplification plot
      ii. Go up to Settings in the toolbar and select Baseline Threshold
         1. At the top of the box select Auto Calculated for the Baseline Cycles
         2. For the Single Threshold at the bottom of the box select User Defined
            a. Set this to 164
            b. Select Ok
      iii. Uncheck the SARS-CoV-2 box and check the PRC box in the amplification plot
      iv. Go up to Settings in the toolbar and select Baseline Threshold
         1. At the top of the box select Auto Calculated for the Baseline Cycles
         2. For the Single Threshold at the bottom of the box select User Defined
            a. Set this to 100
            b. Select Ok

5. Exit the software

Qiagen Rotor-Gene Q Programming Instructions

Refer to User Manual Part Number 1065453EN for additional information.

Programming Instructions:

1. Launch the Rotor-Gene Q software package
2. In the New Run pop-up window select the Advanced tab on the top of the screen
3. Select Empty Run and then New on the lower right of the pop-up window to start the Advanced Run Wizard
   a. Select the appropriate rotor size in the Advanced Run Wizard on the upper left of the screen
   b. Check the box that states the Locking Ring is Attached and select Next
   c. Leave the Operator and Notes sections empty
   d. Enter 20ul as the Reaction Volume in the lower left of the screen
   e. For the Sample Layout choose 1, 2, 3... and then select Next
   f. Under Channel Setup select Create New to enter information for each detector
      i. Under Name enter SARS-CoV-2
ii. **Source** select 470nm  

iii. **Detector** select 510nm  

iv. Do not adjust the default **Gain** setting of 7 as this will be set in a later step  

v. Select **OK**  

g. Repeat the step above by selecting **Create New**  

i. Under **Name** enter **PRC**  

ii. **Source** select 625nm  

iii. **Detector** select 660nm  

iv. Do not adjust the default **Gain** setting of 7 as this will be set in a later step  

v. Select **OK**  

h. Select the **Edit Profile** button in the middle of the window to setup a cycling profile  

i. In the **Edit Profile** window go to the upper left of the screen to **New** and in the drop-down menu select **Cycling**. A hold and three step cycling stage should appear.  

ii. Modify the hold stage to have a temperature at 55°C and a time of **5:00 minutes**  

iii. Select the **Insert After** button in the middle of the pop-up window and then select **New Hold at Temperature**  

iv. Modify the second hold stage to have a temperature at 60°C and a time of **5:00 minutes**  

v. Select the **Insert After** button in the middle of the pop-up window and then select **New Hold at Temperature** to insert a third hold stage  

vi. Modify the third hold stage to have a temperature at 65°C and a time of **5:00 minutes**  

vii. Highlight the first **cycling stage** and modify it as follows:  

1. This cycle repeats **10** time(s)  

2. Select **Timed Step** from the drop-down menu in the middle left of the screen  

3. Do not select **Long Range** or **Touchdown** on the left of the screen  

4. The first step:  
   a. **92°C**  
   b. **5 seconds**  
   c. **Not Acquiring**  

5. Select step two and set as follows:  
   a. **57°C**  
   b. **40 seconds**  
   c. **Not Acquiring**  

6. Highlight step three and delete it by selecting the “-” button in the middle of the window  

7. Select the **Insert After** button in the middle of the pop-up window and then select **New Cycling**  

viii. Highlight the second **cycling stage** and modify it as follows:  

1. This cycle repeats **30** time(s)  

2. Select **Timed Step** from the drop-down menu in the middle left of the screen  

3. Do not select **Long Range** or **Touchdown** on the left of the screen  

4. The first step:  
   a. **92°C**
b. 5 seconds  
c. Not Acquiring  

5. Select step two and set as follows:  
   a. 57°C  
   b. 40 seconds  
   c. Select Acquiring to Cycling A  
      i. Under Acquiring Channels highlight the default channel name (Green) and select the < button to move it over to the Available Channels list  
      ii. In the Available Channels list select SARS-CoV-2 and select the > button to move it over to the Acquiring Channels list  
      iii. Repeat the step above for the PRC and then select OK  

6. Highlight step three and delete it by selecting the “-” button in the middle of the window  
   ix. In the Edit Profile window select OK  
   i. In the New Run Wizard window select Gain Optimisation  
      i. In the middle of the Auto-Gain Optimisation Setup window select the drop-down menu under Channel Settings and select SARS-CoV-2.  
      ii. Select the Add button on the right  
         1. In the Auto-Gain Optimisation Channel Settings window ensure that the SARS-CoV-2 Tube Position is set to 1. This requires that a positive control, containing SARS-CoV-2 and PRC, be tested with each PCR run and placed in the first tube. Failure to do so may cause the gain to be incorrectly set.  
         2. Leave the Target Sample Range and the Acceptable Gain Range set to the defaults, 5-10Fl and -10 to 10 respectively.  
         3. Select OK  
         4. Repeat steps 3. j. ii. 1-3. for the PRC  
      iii. In the Auto-Gain Optimisation Setup window check the box next to Perform Optimisation Before 1st Acquisition  
      iv. Select Close  
   j. In the New Run Wizard window select the Next button  
   k. Save the new protocol as a template for future use  
      i. On the bottom right of the window select the Save Template button  
      ii. Save In: C:\Program Files\Rotor-Gene Q Software\Templates  
      iii. File name: ‘Lyra SARS-CoV-2’  
      iv. Save as type: ‘Template (*.ret)’  
   l. Exit the software  

**Qiagen Rotor-Gene Q Test Run**  
**Analysis Instructions:**  

1. In the New Run Wizard load the SARS-CoV-2 Template.  
2. Press Start.  
3. Open the run file that needs to be analyzed  
4. In the upper menu toolbar select the Analysis button  
   a. Select Quantitation, then Cycling A. SARS-CoV-2, and Show  
   b. The threshold needs to be set for SARS-CoV-2
i. In the far right bottom of the screen under **CT Calculation** enter 0.03 for the **SARS-CoV-2 Threshold**

ii. In the **Eliminate Cycles before** box ensure the default of 1 is entered

iii. Ensure the amplification graph is set to **Log Scale** (toggle button on the bottom left of the graph states Linear Scale or Log Scale)

c. Select **Quantitation**, then **Cycling A. PRC**, and **Show**

d. The threshold needs to be set for PRC

   i. In the far right bottom of the screen under **CT Calculation** enter 0.05 for the **PRC Threshold**

   ii. In the **Eliminate Cycles before** box ensure the default of 1 is entered

   iii. Ensure the amplification graph is set to **Log Scale** (toggle button on the bottom left of the graph states Linear Scale or Log Scale)

---

**Roche’s LightCycler (LC) 480 Instrument II Programming Instructions**

Refer to User Manual Part Number 05152062001 0208 for additional information.

**Creating a LC 480 II Assay Run Template**

1. Launch the LC 480 software package

2. The **Detection Format** must be established to specify the channels in which fluorescence will be read

   a. Select **Tools** in the startup screen in the lower right of the screen

   b. Select **Detection Formats** then choose **New**

   c. Name the format **Lyra SARS-CoV-2**

   d. In the **Filter Combination Selection** window select 465-510 and 618-660

   e. In the **Selected Filter Combination List** window under name type in **SARS-CoV-2** for 465-510 and **PRC** for 618-660

   f. Leave all default setting values to 1 under Melt Factor, Quant Factor, and Max Integration Time

   g. Select **Close** to save the new detection format and return to startup screen

   h. To access this newly created **Detection Format**, the LC 480 software must be closed, then reloaded

3. After closing and reloading the software select **White Plates** and **New Experiment** under Experiment Creation window

4. On the next screen select “Lyra SARS-CoV-2” from the pull-down menu under **Detection Formats**

5. Enter **20ul** as the **Reaction Volume** in the upper right of the screen

6. Enter the names for each of the RT-PCR programs

   a. Under **Program Name** enter **Stage 1**, under **Cycles** enter 1, and in **Analysis Mode** select none

   b. Select the “+” icon to add a program

   c. Name the next program **Stage 2**, under **Cycles** enter 1, and in the **Analysis Mode** select none

   d. Select the “+” icon to add a program

   e. Name the next program **Stage 3**, under **Cycles** enter 1, and in the **Analysis Mode** select none

   f. Select the “+” icon to add a program

   g. Name the next program **Stage 4**, under **Cycles** enter 40, and in the **Analysis Mode** select **quantification**

7. Set the RT-PCR cycling times and temperatures
a. Highlight Stage 1 under Program Name and change Stage 1 Temperature Targets as follows:
   i. Target (°C) set to 55
   ii. Acquisition Mode select none
   iii. Hold (hh:mm:ss) set to 5:00
   iv. Ramp Rate (°C/s) to 4.4
   v. Sec Target (°C), Step Size (°C), and Step Delay (cycles) will be left at 0 for stages 1-4.

b. Highlight Stage 2 under Program Name and change Stage 2 Temperature Targets as follows:
   i. Target (°C) set to 60
   ii. Acquisition Mode select none
   iii. Hold (hh:mm:ss) set to 5:00
   iv. Ramp Rate (°C/s) to 4.4

c. Highlight Stage 3 under Program Name and change Stage 3 Temperature Targets as follows:
   i. Target (°C) set to 65
   ii. Acquisition Mode select none
   iii. Hold (hh:mm:ss) set to 5:00
   iv. Ramp Rate (°C/s) to 4.4

d. Highlight Stage 4 under Program Name and change Stage 4 Temperature Targets as follows:
   i. The first step:
      1. Target (°C) set to 92
      2. Acquisition Mode select none
      3. Hold (hh:mm:ss) set to 0:05
      4. Ramp Rate (°C/s) to 4.4
   ii. Select the “+” icon to add a step and set the second step:
      1. Target (°C) set to 57
      2. Acquisition Mode select single
      3. Hold (hh:mm:ss) set to 0:40
      4. Ramp Rate (°C/s) to 2.2

8. Save the new protocol as a run template for future use.
   a. In the lower left corner of the screen select the pull-down menu next to the Apply Template button
   b. Choose Save As Template
   c. Select the Templates Folder
   d. Highlight Run Templates Folder
   e. Name the template Lyra SARS-CoV-2 run template and click the “check” button

9. Exit the software.

Creating a LightCycler 480 II Assay Test Procedure
1. Load the Lyra SARS-CoV-2 run template.
2. Press Start.
3. The analysis template can only be established after the initial experiment has completed
4. On the Lyra SARS-CoV-2 run select the Analysis button in the module bar
   a. Choose Abs Quant/Fit Points
b. In the Create New Analysis pop-up window select your pre-defined subset from the subset drop down menu and then select the “check” button

c. Set the Background to 2-10 for all analytes
   i. Set Min Offset to 1
   ii. Set Max Offset to 9

d. In the center bottom of the screen ensure that Color Compensation is off for all analytes

   e. Leave the default settings as First Cycle 1 and Last Cycle 40

5. At the top middle of the screen select Noise Band

6. Choose the pull-down menu next to the Noise Band button and select Noise Band Fluorescence

7. For each analyte under the Filter Comb button, set the noise band as follows:
   a. SARS-CoV-2 set to 1.95
   b. PRC set to 1.4619

8. Choose Calculate in the bottom left of the screen

9. Save the new analysis protocol as a template for future use
   a. In the lower left corner of the screen select the pull-down menu next to the Apply Template button
   b. Choose Save As Template
   c. Select the Templates Folder
   d. Highlight Analysis Templates Folder
   e. Name the template Lyra SARS-CoV-2 analysis template and click the “check” button

10. Create a report
    a. Select the Save icon on the global action bar on the right side of the screen
    b. Choose the Report button on the module bar on the left of the screen
    c. Select the appropriate settings and press the Generate button

11. To apply an Analysis Template to subsequent runs
    a. Once the run has finished select the Analysis button in the module bar
    b. Choose Abs Quant/Fit Points
    c. In the Create New Analysis pop-up window select your pre-defined subset from the subset drop down menu and then select the “check” button
    d. Select the Apply Template button on the far left of the screen and choose the Lyra SARS-CoV-2 analysis template from the Analysis Templates Folder
    e. Select yes in the pop-up window

12. Interpretation of results (See Table 4)

Thermo Fisher QuantStudio 7 Pro Programming Instructions
Refer to User Manual Part Number 4489822 Revision A for additional information.

Thermo Fisher QuantStudio 7 Pro Test Run Programming Instructions:
1. Open the Design and Analysis Software
2. Select the “SET UP PLATE” option
3. From the side bar on the screen, select the following properties to filter:
   a. Instrument – QuantStudio 7 Pro
   b. Block – 96-Well 0.2 mL
   c. Run Mode – Fast
   d. Analysis options are left blank
4. From the plate selections present on the screen, select the System Template “PCR Only” and the system will automatically navigate to the “Run Method” tab
5. Run Method
   a. Change the Reaction Volume to 20.0 uL
   b. The temperature of the enabled heated cover will remain at 105.0 degrees C
   c. Scroll over the Hold stage present in the cycling parameters and
      addition/subtraction buttons will become visible at both the top and bottom of the
      first stage.
   d. Left click the right addition button at the top and a list of Stage choices will become
      visible. Scroll down and choose Hold.
   e. Repeat the previous steps so there are three Hold stages present in the cycling
      parameters.
   f. Scroll over to the PCR stage and addition/subtraction buttons will become visible at
      both the top and bottom. Left click the right addition button at the top and a list of
      Stage choices will become visible. Scroll down and choose PCR.
   
g. Going back to the first stage enter the following parameters:
   i. Stage 1 Hold
      1. 2.63 ramp rate
      2. 55°C
      3. 5 minutes
   ii. Stage 2 Hold
      1. 2.63 ramp rate
      2. 60°C
      3. 5 minutes
   iii. Stage 3 Hold
      1. 2.63 ramp rate
      2. 65°C
      3. 5 minutes
   iv. Stage 4 PCR
      1. Step 1:
         a. 2.63 ramp rate
         b. 92°C
         c. 5 seconds
      2. Step 2:
         a. 2.32 ramp rate
         b. 57°C
         c. 40 seconds
         d. Click on the camera icon under Step 2. A window will pop
            up asking for confirmation to turn off data collection during
            this step. Click “Ok”.
   v. Located at the bottom of Stage 4 PCR change the number of cycles to 10
   vi. Stage 5 PCR
      1. Step 1:
         a. 2.63 ramp rate
         b. 92°C
c. 5 seconds

2. Step 2:
   a. 2.32 ramp rate
   b. 57°C
   c. 40 seconds
   d. Ensure the camera icon image is bold/on for data collection during the 30 cycles of Stage 5, Step 2.

   vii. Located at the bottom of Stage 4 PCR change the number of cycles to 30

h. Scroll up and choose the “Plate Setup” tab near the top of the screen.

6. Plate Setup
   a. Change the Passive Reference to “NONE”
   b. On the lower right side of screen, ensure the Targets Tab is chosen then highlight and press the addition button to add “Target 1“. Press again to add “Target 2”
   c. Click on the “Target 1” box and change the name to CoV-2.
   d. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose FAM.
   e. Click on the “Target 2” box and change the name to PRC.
   f. Click the associated reporter box below the Reporter tab and, from the drop down menu, choose CY5.
   g. Highlight the “Actions” button located in the upper right side of the screen and press the drop down button. In the drop down menu choose “Analysis Setting”
   h. Under Analysis Setting, disable the following for all targets:
      i. Use Default Column
      ii. Auto Threshold Column
      iii. Auto Baseline Column
      iv. The Baseline Start and Baseline End should default to 3 and 15
   i. Under “Threshold” click on the box associate with the CoV target and enter 70000.
   j. Under “Threshold” click on the box associated with the PRC target and enter 20000
   k. Click “Save”
   l. Navigate back to the “Actions” button and press the drop down button, choosing “Save As”. This will save your template to a location of choice. Save the template as “Lyra SARS Cov-2 Assay”.

**Creating a Thermo Fisher QuantStudio 7 Pro Test Procedure**

Note: These instructions are based upon the user not having the QuantStudio 7 Pro Real-Time PCR instrument and the ABI Design and Analysis 2.2 software connected. The user must open the Lyra SARS CoV-2 template created previously with the software and save any newly created sample run template onto a USB and transfer the template to the instrument.

For connectivity related to the software and the instrument please contact your Thermo Fisher QuantStudio representative.

1) Open the Lyra SARS CoV-2 Assay Template previously generated.
2) Click on the Plate Setup Tab located near the top of the screen.
3) On the right side of the screen ensure the “Samples” tab is highlighted and press the addition button to add the number of samples being tested.
4) Click on the “Sample 1” box to rename the sample. Repeat this step for all subsequent samples being entered.
5) Click the well located in the plate map then check the box next to the sample name from the right side bar to associate the name to the well.
   a. User also has the option to highlight the well location in the plate map and click on the “Enter sample” box. Enter the sample ID and press tab to continue to the next well in the plate map. This will automatically load the sample name into the sidebar.
6) Once samples names have been entered, the wells may be highlighted by left clicking the mouse over starting well and dragging the mouse across all wells associated in run. The targets are then chosen by clicking the check boxes next to each target in the side bar.
7) Click on the Actions button located top right of the screen and choose “Save As” in the dropdown menu.
   a. A pop-up window will appear directing the user to title the file according to information pertaining to the sample run and the location of the file to be saved.
   b. Save the newly named (.edt) run file to a USB that is inserted into the computer.
8) Transfer the USB to the port on the front of the instrument.
9) From the options on the instrument’s screen press “Load plate file”. The QuantStudio 7 Pro is a touchscreen device.
10) From the “Run Queue” screen, press “USB drive” on the right side. This will bring up any plate files saved on the USB.
11) Press the plate file associated with the run to be performed.
12) A new window will appear requesting location of results once the run is complete.
   a. Press the “USB drive Connected” if the icon is not already highlighted and press “Done”.
13) Centrifuge the 96-well sample plate to ensure all liquid is toward the bottom of each well.
   a. Ensure the centrifuge is properly balanced.
   b. Gently pull the plate from the centrifuge to ensure all liquids remain at the bottom of the wells.
14) Press the double-arrow icon located at the top right sided corner of the screen on the instrument.
   a. The instrument drawer will open from the front.
15) Place the centrifuged plate into the plate holder ensuring proper orientation of the plate.
   a. A1 well should be in the position of the top left corner
   b. The plate will appear slightly suspended above the block due to two silicone strips above and below this plate. This is to be expected and the instrument lid will press the plate down once the drawer has closed.
16) Press “Start Run” on the screen of the instrument.
   a. A pop-up window will appear asking the user to confirm the plate has been loaded.
   b. If the plate has been loaded, press “Start Run” again or press “Open Drawer” to place the plate into the block and then press “Start Run”
APPENDIX II

Instruments Qualification Method for the Lyra SARS-CoV-2 Assay

Purpose: This appendix is intended to provide a qualification procedure describing how to test a panel of specimens for use in verifying performance of the Applied Biosystems 7500 Standard, Roche LightCycler 480, Bio-Rad CFX96 Touch, Thermo Fisher QuantStudio 7 Pro with the Lyra SARS-CoV-2 Assay by the end user. Qualification of these instruments with the Lyra SARS-CoV-2 Assay (meeting the acceptance criteria listed below) must be achieved prior to usage for diagnostic testing.

Acceptance Criteria:

1. Specimen processing according to this protocol, in combination with an authorized thermocycler, detects the following when tested with the Lyra SARS-CoV-2 Assay:
   a. 3 out of 3 replicates in Panel Members 1 and 3;
   b. At least 2 out of 3 replicates in Panel Members 2 and 4.

Protocol: Quidel has generated a panel of four samples. The panel is comprised of two positive samples diluted with a contrived negative nasopharyngeal matrix to approximately 3x and 1x LoD of the Lyra SARS-CoV-2 Assay (based on Ct value). The lab will perform three (3) replicates of each specimen using this protocol. Each replicate will be tested according to the Lyra SARS-CoV-2 Assay IFU using the thermocycler pending qualification.

1. Sample processing and extraction:
   NOTE: Samples will be shipped on dry ice to the testing laboratory. The aliquot should be stored at -70°C or colder until tested.
   a. Thaw each sample aliquot in a 35°C water bath or similar device.
   b. Mix well by inversion 10 times.
   c. Extract three (3) replicates of the aliquot according to established laboratory procedure.

2. PCR set-up:
   a. Two vials of Lyra SARS-CoV-2 Master Mix will be rehydrated with 135µL of Rehydration Solution per vial.
   b. Vials will be placed at room temperature for 1-2 minutes to allow rehydration of pellet then gently pipetted up and down 2-3 times (avoiding formation of bubbles).
   c. Fifteen microliters (15µL) of Master mix and 5µL of the processed sample (in triplicate), following the IFU, will be loaded into each of the wells on the PCR plate.
   d. The plate will be centrifuged to ensure liquid is at the bottom of the wells.

3. PCR Amplification:
   a. Plate will be inserted into the thermocycler and amplification initiated using the applicable program parameters according to the IFU:
   b. The instrument software is used to detect amplification in real time.

4. Data analysis:
   a. Analysis is performed according to the IFU, and is briefly described in the table below:
### Interpretation of the Lyra SARS-CoV-2 Assay Results on the Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, Bio-Rad CFX96 Touch, Qiagen Rotor-Gene Q, or Thermo Fisher QuantStudio-7 Pro

<table>
<thead>
<tr>
<th>Assay Result</th>
<th>Detector: SARS-CoV-2</th>
<th>Detector: Process Control</th>
<th>Interpretation of Results</th>
<th>Notes and Special Guidance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No Ct detected</td>
<td>5.0≤ Ct ≤30.0</td>
<td>No SARS-CoV-2 viral RNA detected; PRC Detected.</td>
<td></td>
</tr>
<tr>
<td>SARS-CoV-2 Positive</td>
<td>5.0≤ Ct ≤30.0</td>
<td>NA¹</td>
<td>SARS-CoV-2 Virus RNA detected.</td>
<td></td>
</tr>
<tr>
<td>Invalid</td>
<td>No Ct detected</td>
<td>No Ct detected</td>
<td>No SARS-CoV-2 viral RNA and no PRC RNA detected.</td>
<td>Invalid test. Retest the same processed sample. If the test is also invalid, re-process and retest another aliquot of the same specimen or obtain a new specimen and retest.</td>
</tr>
</tbody>
</table>

### Interpretation of the Lyra SARS-CoV-2 Assay Results on the Roche LightCycler 480

<table>
<thead>
<tr>
<th>Assay Result</th>
<th>Detector: SARS-CoV-2</th>
<th>Detector: Process Control</th>
<th>Interpretation of Results</th>
<th>Notes and Special Guidance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>No Ct detected</td>
<td>5.0≤ Ct ≤40.0</td>
<td>No SARS-CoV-2 viral RNA detected; PRC Detected.</td>
<td></td>
</tr>
<tr>
<td>SARS-CoV-2 Positive</td>
<td>5.0≤ Ct ≤40.0</td>
<td>NA¹</td>
<td>SARS-CoV-2 Virus RNA detected.</td>
<td></td>
</tr>
<tr>
<td>Invalid</td>
<td>No Ct detected</td>
<td>No Ct detected</td>
<td>No SARS-CoV-2 viral RNA and no PRC RNA detected.</td>
<td>Invalid test. Retest the same purified sample. If the test is also invalid, re-extract and retest another aliquot of the same specimen or obtain a new specimen and retest.</td>
</tr>
</tbody>
</table>

¹ No Ct value is required for the Process Control to make a positive call.
## Results:

<table>
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<tr>
<th>Panel Member</th>
<th>SARS-CoV-2 Ct</th>
<th>PRC Ct</th>
<th>SARS-CoV-2 Ct</th>
<th>PRC Ct</th>
<th>SARS-CoV-2 Ct</th>
<th>PRC Ct</th>
<th>Interpretation</th>
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</table>

## Conclusion:

Upon successful testing of the panel, meeting the acceptance criteria listed above, the instrument(s) may be used for diagnostic testing with the Lyra SARS-CoV-2 Assay. The instrument should be affixed with the below label. If the instruments include labeling indicating “For Research Use Only”, please cover with the below “Emergency Use Only” labeling.

---

**Emergency Use Only.**

This instrument is authorized for use with the Lyra SARS-CoV-2 Assay.
M120 – Lyra SARS-CoV-2 Assay kit
## GLOSSARY

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="iu" /></td>
<td>Intended use</td>
</tr>
<tr>
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