For use under the 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 nasopharyngeal and oropharyngeal swab specimens.

18 Contents

19 Intended Use 4
20 Summary and Explanation 4
21 Principle of the Procedure 4
22 Materials Provided 5
23 Materials Required But Not Provided 6
24 Warnings and Precautions 6
25 Storage and Handling of Kit Reagents 7
26 Specimen Collection, Storage and Handling 7
27 Nucleic Acid Extracts Storage 8
28 bioMérieux NucliSENS easyMAG Nucleic Acid Extraction Programming Instructions 8
29 Applied Biosystems 7500 Fast Dx Programming Instructions 10
30 Applied Biosystems 7500 Standard Programming Instructions 12
31 Roche's LightCycler® 480 Instrument II Programming Instructions 15
32 Creating a LC 480 II Assay Run Template 15
33 Qiagen Rotor-Gene Q Programming Instructions 16
34 Assay Procedure 18
35 Applied Biosystems® 7500 Fast Dx Thermocycler Test Procedure 19
36 Applied Biosystems® 7500 Standard Thermocycler Test Procedure 19
37 Creating a LC 480 II Assay Test Procedure 20
38 Qiagen Rotor-Gene Q Test Run 21
39 Quality Control 22
40 CLINICAL PERFORMANCE 23
41 ANALYTICAL PERFORMANCE 25
42 Level of Detection 25
43 Analytical Reactivity (Inclusivity) 28

44 Analytical Specificity (Cross-Reactivity) 28
45 Limitations 31
46 Customer and Technical Assistance 32
47 References 32
48 GLOSSARY 34
49
50

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 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 nasopharyngeal and oropharyngeal swab 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 positive 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. This virus, as with the novel coronavirus SARS-1 and MERS, is thought to have originated in bats, however the SARS-CoV-2 may have had an intermediary host such as pangolins, pigs or civets.[1] 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 bioMerieux 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 four thermocyclers: Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, Roche LightCycler 480, or Qiagen Rotor-Gene Q. 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.

96
97

Table 1. Lyra® SARS-CoV-2 Assay Probe Labels	
Target	**Dye**
Non-structural polyprotein (pp1ab)	FAM
Process Control (PRC)	Quasar® 670

98
99
100 The following is a summary of the procedure:
101
102 1. **Sample Collection:** Obtain nasopharyngeal or oropharyngeal swabs using standard techniques from
103 symptomatic patients. These specimens are transported, stored, and processed according to established
104 laboratory procedures.
105
106 2. **Nucleic Acid Extraction:** Extract nucleic acids from the specimens with the NucliSENS® easyMAG® or EMAG®
107 Systems following the manufacturer's instructions and using the appropriate reagents (See **Materials**
108 **Required but Not Provided**).
109
110 Prior to the extraction procedure add 20 µL of the Process Control (PRC) to each 180 µL aliquot of specimen
111 or controls. The PRC serves to monitor inhibitors in the extracted specimen, assures that adequate
112 amplification has taken place and confirms that the nucleic acid extraction was sufficient.
113
114 3. **Rehydration of Master Mix:** Rehydrate the lyophilized Master Mix using 135µL of Rehydration Solution.
115 The Master Mix contains oligonucleotide primers, fluorophore and quencher-labeled probes targeting
116 conserved regions of the SARS-CoV-2 as well as the process control sequence. The probes are dual labeled
117 with a reporter dye attached to the 5' end and a quencher attached to the 3' end. The rehydrated Master
118 Mix is sufficient for eight reactions.
119
120 4. **Nucleic Acid Amplification and Detection:** Add 15 µL of the rehydrated Master Mix to each plate well
121 (Applied Biosystems® 7500 Fast Dx, Applied Biosystems 7500 Standard, the Roche LightCycler 480) or tube
122 (Qiagen Rotor-Gene Q). 5 µL of extracted nucleic acids (specimen with PRC) is then added to the plate well
123 or tube. Place the plate or tube into the appropriate instrument.
124
125 Once the reaction plate or tubes are added to the instrument, the assay protocol is initiated. This protocol
126 initiates reverse transcription of the RNA targets generating complementary DNA, and the subsequent
127 amplification of the target sequences occurs. The Lyra SARS-CoV-2 Assay is based on TaqMan® chemistry,
128 and uses an enzyme with reverse transcriptase, DNA polymerase, and 5'-3' exonuclease activities. During
129 DNA amplification, this enzyme cleaves the probe bound to the complementary DNA sequence, separating
130 the quencher dye from the reporter dye. This step generates an increase in fluorescent signal upon
131 excitation by a light source of the appropriate wavelength. With each cycle, additional dye molecules are
132 separated from their quenchers resulting in additional signal. If sufficient fluorescence is achieved, the
133 sample is reported as positive for the detected target sequence.
134

135 ## Materials Provided

136 SKU # CE-M120
137

Table 2. Detection Kit (96 Reactions) – Store at 2°C to 8°C		
#	**Component**	**Quantity**
➊	**Rehydration Solution** Part M5003	1 vial/kit 1.9 mL

Table 2. Detection Kit (96 Reactions) – Store at 2°C to 8°C		
#	**Component**	**Quantity**
❷	**Lyra SARS-CoV-2 Master Mix** Part M5150 Lyophilized Contents: DNA polymerase enzyme with reverse transcriptase activity Oligonucleotide primer pairs; Oligonucleotide probes dNTPs (dATP, dCTP, dGTP, dUTP, dTTP) Stabilizers	12 vials/kit, 8 reactions/vial
CONTROL	**Process Control** Part M5005	1 vial/kit 2.0 mL
CONTROL +	**Positive Control** containing SARS-CoV-2 Synthetic RNA, Part M5153	1 vial/kit 1.0 mL
CONTROL –	**Negative Control** Part M5031	1 vial/kit 2.0 mL

138
139 • Lyra™ SARS-CoV-2 Assay Instructions for Use
140

141 Materials Required But Not Provided

142 • Micropipettors (range between 1 to 10 µL and 100 to 1000 µL)
143 • Non-aerosol pipette tips
144 • Applied Biosystems®7500Fast Dx, software version 1.4
145 • Applied Biosystems®Standard, software version 2.0.6
146 • Roche LightCycler® 480 Instrument II, software version 1.5.0.39
147 • Qiagen Rotor-Gene Q, software version 2.0.2.4
148 • 96 well PCR plate
149 • Optical plate films
150 • Qiagen Rotor-Disc
151 • Qiagen Rotor-Disc Heat Sealing Film
152 • Plate centrifuge for 96 well plate
153 • bioMérieux NucliSENS easyMAG software version 2.0
154 • bioMérieux EMAG software version 2.0
155 • bioMérieux NucliSENS easyMAG Buffers 1, 2, 3
156 • bioMérieux NucliSENS easyMAG Lysis Buffer
157 • bioMérieux NucliSENS easyMAG Silica Magnetic Beads
158 • bioMérieux NucliSENS easyMAG disposables
159 • Biohit pipettor
160

161 Warnings and Precautions

162 • For *In Vitro* Diagnostic Use under Emergency Use Authorization only.
163 • Positive results are indicative of the presence of SARS-CoV-2 RNA.
164 • Laboratories within the United States and its territories are required to report all positive results to the
165 appropriate public health authorities.
166 • The assay has been validated using bioMérieux NucliSENS easyMAG software version 2.0. Please contact
167 Quidel Technical Support prior to modifying or upgrading beyond this version of software.
168 • The assay has been validated using Applied Biosystems 7500Fast Dx software version 1.4. Please contact
169 Quidel Technical Support prior to modifying or upgrading beyond this version of software.

170 • The assay has been validated using Applied Biosystems Standard software version 2.0.6. Please contact
171 Quidel Technical Support prior to modifying or upgrading beyond this version of software.
172 • The assay has been validated using Roche LightCycler® 480 Instrument II, software version 1.5.0.39
173 Please contact Quidel Technical Support prior to modifying or upgrading beyond this version of software.
174 • The assay has been validated using Qiagen Rotor-Gene Q, software version 2.0.2.4. Please contact Quidel
175 Technical Support prior to modifying or upgrading beyond this version of software.
176 • Performance characteristics of this test have been established with the specimen types listed in the
177 **Intended Use Section** only. The performance of this assay with other specimen types or samples has not
178 been evaluated.
179 • Use of this product should be limited to personnel with sufficient training in PCR and RT-PCR techniques.
180 • Treat all specimen/samples as potentially infectious. Follow universal precautions when handling
181 samples, this kit and its contents.
182 • Proper sample collection, storage and transport are essential for correct results.
183 • Store assay reagents as indicated on their individual labels.
184 • Wear suitable protective clothing, gloves, eye and face protection when using this kit.
185 • For accurate results, pipette carefully using only calibrated equipment.
186 • Thoroughly clean and disinfect all surfaces with a 10% bleach solution followed by molecular grade water.
187 • Use micropipettes with an aerosol barrier or positive displacement tips for all procedures.
188 • Avoid microbial and cross contamination of the kit reagents. Follow Good Laboratory Procedures.
189 • Do not mix reagents from kits with different lot numbers.
190 • Do not use reagents from other manufacturers with this kit.
191 • Do not use product after its expiration date.
192 • Proper workflow planning is essential to minimize contamination risk. Always plan laboratory workflow in
193 a uni-directional manner, beginning with pre-amplification and moving through amplification and
194 detection.
195 • Use dedicated supplies and equipment in pre-amplification and amplification areas.
196 • Do not allow cross movement of personnel or equipment between areas.
197 • Keep amplification supplies separate from pre-amplification supplies at all times.
198 • Do not open sample tubes or unseal plates post amplification.
199 • Dispose of amplified material carefully and in accordance with local laws and regulations in order to
200 minimize the risk of amplicon contamination.
201 • Do not use supplies dedicated for reagent or sample preparation for processing target nucleic acid.
202 • MSDS is available upon request or can be accessed on the product website.
203

204 ## Storage and Handling of Kit Reagents

205 • Store the unopened kit at 2°C to 8°C until the expiration date listed on the outer kit box.
206 • The rehydrated Master Mix may be stored at room temperature (20°C to 25°C) for up to 24 hours. For
207 longer storage the rehydrated Master Mix should be recapped, sealed with parafilm and stored in an
208 upright position at ≤–20°C for up to 14 days. Protect the Master Mix from light during storage.
209

210 **Indications of Instability or Deterioration of Reagents:** Cloudiness of the Rehydration Solution, when within
211 expiration, may indicate deterioration of this reagent. Contact Quidel Technical Assistance for a replacement.
212

213 **Specimen Collection, Storage and Handling**
214 Nasopharyngeal or oropharyngeal specimens should be collected, transported, stored, and processed
215 according to CLSI M41-A[2]. Specimens should be stored at 2°C to 8°C until tested. If specimens cannot be
216 tested within 72 hours of collection, they should be frozen at -70°C or colder until tested.
217

218 The following viral transport media (M4, M4-RT, M5, M6, and UTM) (1 mL and 3 mL) are compatible with the
219 Lyra respiratory assays.

220

221 **Nucleic Acid Extracts Storage**
222 Eluates from the NucliSENS easyMAG can be stored at room temperature (20°C to 25°C) for 2 hours, at 2°C to
223 8°C for 24 hours and 1 month at –20°C to –70°C.
224

225 **bioMérieux NucliSENS easyMAG Nucleic Acid Extraction Programming Instructions**
226

227 Note: A Positive Control (i.e. Lyra SARS-CoV-2, Positive Control #M5153), and a negative process control (i.e.,
228 Lyra SARS-CoV-2, Negative Control #M5031) should be included in each extraction run.

229 1. Turn on the instrument and wait for instrument light to appear orange. Then switch on the
230 computer/launch easyMAG software. Do not log into software until the light on the instrument has
231 turned green.

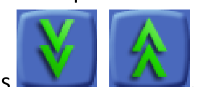
232 2. Barcode reagents after pressing the 'Instrument' and 'Reagent Inventory' buttons.

233 3. To enter samples, press the 'Daily Use' button, which will default to the 'Define Request'
234 screen. Select the following settings:
235 a. Sample ID: Enter the **sample name** using the keyboard.
236 b. Matrix: Select **Other** from the drop-down menu
237 c. Request: Select **Generic** from the drop-down menu
238 d. Volume (mL): Select **0.200** from the drop-down menu
239 e. Eluate (µL): Select **50** from the drop-down menu
240 f. Type: Primary
241 g. Priority: Normal

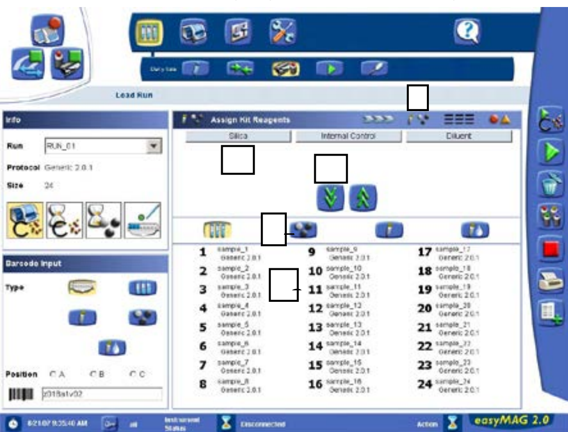
242 4. Upon pressing the 'Save' button, the sample will appear in the 'Unassigned Sample' window on
243 the left side of the screen. Press the 'Enter New Extraction Request' button, and repeat the
244 process for additional samples. Alternatively multiple samples can be entered by pressing the 'Auto
245 Create New Extraction Requests' button.

246 5. Once all samples are created, go to 'Organize Runs' by clicking on the icon near the top of the
247 page. Create a run by pressing the 'Create Run' button. Enter a run name or use the default.

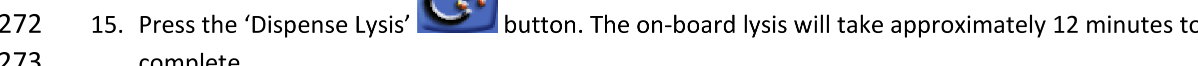
248 6. Add samples to the run by using the 'Auto Fill Run' button (auto fills up to 24 samples from the
249 'Unassigned Sample list' on the left hand side of the screen). Alternatively, individual samples can be
250 moved into and out of the run by using the left and right 'Positioning icons' after selecting

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.

278 b. Vortex the magnetic silica. Using 1 tip and Program 1, aspirate 550 µL of magnetic silica, dispense
279 into the water and mix by vortexing.
280 c. Using 1 tip and Program 2, aspirate 1050 µL of the magnetic silica mixture and dispense 25 µL
281 back into the same tube.
282 d. Dispense 125 µL magnetic silica mixture each into 8 wells of an ELISA strip plate. Discard tip.
283 e. After Lysis is complete (NB: the 'Instrument Status' at the bottom of the screen must be '**IDLE**'!),
284 using 8 tips and Program 3, aspirate 100 µL of magnetic silica mixture in strip wells, dispense 100
285 µL of magnetic silica mixture in strip wells, and aspirate 100 µL of magnetic silica mixture in strip
286 wells.
287 f. Insert tips into liquid within the sample vessels. Aspirate 800 µL then dispense 900 µL of
288 magnetic silica mixture back into vessel. Aspirate 1000 µL of magnetic silica mixture from vessel
289 and dispense 1000 µL of magnetic silica back into vessel. Repeat aspiration / dispensing of 1000
290 µL two more times.

291 17. Close the instrument and press the 'Start' button to begin the run.
292 18. Upon completion of run, transfer purified nucleic acid to nuclease-free tubes. Eluates from the easyMAG
293 can be stored at room temperature (20°C to 25°C) for 2 hours, at 2°C to 8°C for 8 hours and 1 month at -
294 20°C to –70°C.

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

297 1. Launch the 7500 Fast Dx software package.
298 2. The **Quick Startup document** dialog window will open. Select the **Create New Document** button to start
299 the **New Document Wizard**. Follow each step to initiate the Lyra™ SARS-CoV-2 Assay protocol.
300 a. Define Document: Most of the following should be the default setting. If not, change accordingly.
301 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'

302 ii. Select the **Next** button.
303
304 b. Select Detectors: New detectors for SARS-CoV-2 and the process control (PRC) must be added. For
305 each target, select the **New Detector** button to open the **New Detector** pop-up window.
306 Alternatively, use the **Create Another** button from within the **New Detector** pop-up window for
307 the last two detectors.
308
309 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)

310
311 ii. Select a unique color to represent each detector.
312 iii. Highlight the new detectors and add to the **Detectors in Document** column using the **Add**
313 button.
314 iv. Select **(none)** from the **Passive Reference** drop-down menu.
315 v. Select the **Next** button.
316 vi. Select the **Finish** button without setting any wells.
317 c. The wizard will close and the software will open, starting with the **Setup** tab. This will show the
318 sample plate that was set up during the quick start. For the initial set up, nothing needs to be
319 changed here.
320 d. <u>Defining the Thermocycler Protocol:</u> Select the **Instrument** tab to set up the Lyra™ SARS-CoV-2
321 Assay RT-PCR cycling times and temperatures. Under **Thermal Profile** there should be a default 2-
322 stage protocol. Each stage will have 3 user-editable text boxes. The top box value represents the
323 number of reps or cycles for that stage. The middle box value represents the temperature (˚C) and
324 the lowest box value represents the time (minutes: seconds).
325
326 i. Make the following changes to the default **Thermal Cycler Protocol**:
327 1. Stage 1
328 a. Reps: 1
329 b. Temp: 55
330 c. Time: 5:00
331 2. Select the bar between Stage 1 and Stage 2. Select the **Add Hold** button to add
332 another stage.
333 3. Stage 2
334 a. Reps: 1
335 b. Temp: 60
336 c. Time: 5:00
337 4. Select the bar between Stage 2 and Stage 3. Select the **Add Hold** button to add
338 another stage.
339 5. Stage 3
340 a. Reps: 1
341 b. Temp: 65
342 c. Time: 5:00
343 6. Stage 4 (2-Step Dissociation Stage)
344 a. Reps: 10
345 b. Step 1
346 i. Temp: 92
347 ii. Time: 0:05
348 c. Step 2
349 i. Temp: 57
350 ii. Time: 0:40
351 7. Select the bar to the right of Stage 4. Select the **Add Cycle** button to add another
352 stage.
353 8. Stage 5 (2-Step Dissociation Stage)
354 a. Reps: 30

355 b. Step 1
356 i. Temp: 92
357 ii. Time: 0:05
358 c. Step 2
359 i. Temp: 57
360 ii. Time: 0:40
361 9. If a wrong stage is added the stage can be removed by pressing the **Delete** button
362 after highlighting the stage between the vertical lines
363 ii. Under **Settings** enter the following:

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

364
365 e. Set threshold for each analyte.
366 i. Select the **Results** tab.
367 ii. Select the **Amplification Plot** tab.
368 iii. Select SARS-CoV-2 from the Detector tab in the top right corner.
369 iv. In the **Analysis Settings** block, set the **Threshold** to **7.5e+004**.
370 v. Select the **Auto Baseline** radio button.
371 vi. Select PRC from the Detector tab in the top right corner.
372 vii. In the **Analysis Settings** block, set the **Threshold** to **1.0e+004**.
373 viii. Select the **Auto Baseline** radio button.
374
375 f. Save the new protocol as a template for future use.
376 i. At the top of the screen select **File** and then **Save As.**
377 ii. **Save In:** D:\Applied Biosystems\7500 Fast System\Templates\
378 iii. **File name:** 'Lyra SARS-CoV-2'
379 iv. **Save as type:** 'SDS Templates (*.sdt)'
380 g. Exit the software.

381 **Applied Biosystems 7500 Standard Programming Instructions**
382 Refer to User Manual Part Number 4387783 rev C for additional information.

383 1. Launch the ABI 7500 software package.
384 2. Select the **Advanced Setup** button to open Setup and Experiment Properties. Follow each step to
385 initiate the Lyra SARS-CoV-2 protocol.
386 a. Experiment Name: Enter Experiment Name as SARS-CoV-2. Leave the Barcode, User
387 Name, and Comments fields blank
388 b. Define Experiment Setup: Select 7500 (96 Wells), Quantitation- Standard Curve, TaqMan®
389 Reagents, and Standard (~2 hours to complete a run)
390 3. In the upper left menu select **Plate Setup**
391 a. Define Targets: New detectors for SARS-CoV-2, and the process control (PRC) must be
392 added.

393 i. Enter the following information for each detector.
394

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

395
396 ii. Select **Add New Target** button for each target.
397 iii. From each drop down menu select reporter, quencher, and color
398 iv. Select a unique color to represent each detector
399 b. Assign Targets and Samples: Under this tab in the bottom left corner, select **none** as the
400 Passive Reference.
401 4. Select **Run Method** from the upper left menu
402 a. Set the **Reaction Volume** per Well to 20 µL under the **Graphical** or **Tabular View**
403 b. Define the Thermocycler Protocol: Under the **Graphical** or **Tabular View** the default
404 profile should be 2 holding stages and a 2-step cycling protocol. Each stage will have 3 user-
405 editable text boxes. The first box value represents the Ramp Rate (%) for that stage, the
406 second box value represents the temperature (°C) and the third box value represents the
407 time (minutes:seconds).
408
409 i. Make the following changes to the default Thermocycler protocol:
410 1. Stage 1 First **Holding Stage**
411 a. Ramp Rate: 100%
412 b. Temp: 55
413 c. Time: 5:00
414 2. Step 1 Second **Holding Stage**.
415 a. Ramp Rate: 100%
416 b. Temp: 60
417 c. Time: 5:00
418 3. Highlight the second **Holding Stage** and select the **Add Stage** button. In the drop
419 down menu select **Holding**
420 4. Step 1 **Third Holding Stage**
421 a. Ramp Rate: 100%
422 b. Temp: 65
423 c. Time: 5:00
424 5. First **2-Step Cycling Stage**
425 a. Number of cycles: 10
426 b. Do NOT check Enable Auto Delta
427 c. Step 1
428 i. Ramp Rate: 100%
429 ii. Temp: 92
430 iii. Time: 0:05
431 d. Step 2
432 i. Ramp Rate: 100%
433 ii. Temp: 57

434 iii. Time: 0:40
435 iv. Turn data collection “Off” by selecting the **Data Selection** button
436 at the bottom of the step.
437 6. Highlight step 2 and select the **Add Stage** button. In the drop down menu select
438 **Cycling**
439 7. Second 2-Step **Cycling Stage**
440 a. Number of cycles: 30
441 b. Do NOT check Enable Auto Delta
442 c. Step 1
443 i. Ramp Rate: 100%
444 ii. Temp: 92
445 iii. Time: 0:05
446 d. Step 2
447 i. Ramp Rate: 100%
448 ii. Temp: 57
449 iii. Time: 0:40
450 iv. Ensure the data collection has been turned “On” for this step
451 (default setting)
452 8. If a wrong stage is added the stage can be removed by pressing the **Undo “Add**
453 **Stage”** button immediately after adding the stage or highlight the stage between
454 the vertical lines and select the **Delete Selected** button
455
456 5. Set threshold for each analyte
457 a. Select the **Analysis** tab in the upper left menu.
458 b. Select **Analysis Settings** button in the top right corner.
459 c. Highlight SARS-CoV-2 and deselect the **Use Default Settings** box. De-select **Automatic**
460 **Threshold** and change threshold to 75,000. Leave **Automatic Baseline** selected.
461 d. Highlight PRC and de-select the **Use Default Settings** box. De-select **Automatic Threshold**
462 and change threshold to 10,000. Leave **Automatic Baseline** selected.
463 e. At the bottom of the box select **Apply Analysis Settings** button
464

Target	Threshold	Baseline Start	Baseline End
SARS-CoV-2	75,000	Auto	Auto
PRC	10,000	Auto	Auto

465
466 i. Save the new protocol as a template for future use.
467 i. At the top of the screen select the drop down menu next to **Save**
468 ii. Choose **Save as Template**
469 iii. Save in an appropriate folder
470 iv. **File name:** ‘Lyra SARS-CoV-2’
471 v. **Save as type: ‘**Experiment Document Template files (*.edt)’
472 vi. Exit the software.

473 **Roche's LightCycler® 480 Instrument II Programming Instructions**
474 Refer to User Manual Part Number 05152062001 0208 for additional information.
475
476 **Creating a LC 480 II Assay Run Template**
477 1. Launch the LightCycler (LC) 480 software package
478 2. The **Detection Format** must be established to specify the channels in which fluorescence will be read
479 **a.** Select **Tools** in the startup screen in the lower right of the screen
480 b. Select **Detection Formats** then choose **New**
481 c. Name the format Lyra ® SARS-CoV-2
482 d. In the **Filter Combination Selection** window select 465-510 and 618-660
483 e. In the **Selected Filter Combination List** window under name type in SARS-CoV-2 for 465-510 and
484 PRC for 618-660
485 f. Leave all default setting values to 1 under Melt Factor, Quant Factor, and Max Integration Time
486 g. Select **Close** to save the new detection format and return to startup screen
487 h. To access this newly created **Detection Format**, the LC 480 software must be closed, then reloaded
488 3. After closing and reloading the software select **White Plates** and **New Experiment** under Experiment
489 Creation window
490 4. On the next screen select "Lyra ® SARS-CoV-2" from the pull-down menu under **Detection Formats**
491 5. Enter **20ul** as the **Reaction Volume** in the upper right of the screen
492 6. Enter the names for each of the RT-PCR programs
493 a. Under **Program Name** enter **Stage 1**, under **Cycles** enter **1**, and in **Analysis Mode** select **none**
494 b. Select the **"+"** icon to add a program
495 c. Name the next program **Stage 2**, under **Cycles** enter **1**, and in the **Analysis Mode** select **none**
496 d. Select the **"+"** icon to add a program
497 e. Name the next program **Stage 3**, under **Cycles** enter **1**, and in the **Analysis Mode** select **none**
498 f. Select the **"+"** icon to add a program
499 g. Name the next program **Stage 4**, under **Cycles** enter **40**, and in the **Analysis Mode** select
500 **quantification**
501 7. Set the RT-PCR cycling times and temperatures
502 a. Highlight **Stage 1** under **Program Name** and change **Stage 1 Temperature Targets** as follows:
503 i. **Target (°C)** set to **55**
504 ii. **Acquisition Mode** select **none**
505 iii. **Hold** (hh:mm:ss) set to **5:00**
506 iv. **Ramp Rate (°C/s)** to 4.4
507 v. **Sec Target (°C)**, **Step Size (°C),** and **Step Delay (cycles)** will be left at 0 for stages 1-4.
508 b. Highlight **Stage 2** under **Program Name** and change **Stage 2 Temperature Targets** as follows:
509 i. **Target (°C)** set to **60**
510 ii. **Acquisition Mode** select **none**
511 iii. **Hold** (hh:mm:ss) set to **5:00**
512 iv. **Ramp Rate (°C/s)** to 4.4
513 c. Highlight **Stage 3** under **Program Name** and change **Stage 3 Temperature Targets** as follows:
514 i. **Target (°C)** set to **65**
515 ii. **Acquisition Mode** select **none**
516 iii. **Hold** (hh:mm:ss) set to **5:00**

517 iv. **Ramp Rate (°C/s)** to 4.4
518 d. Highlight **Stage 4** under **Program Name** and change **Stage 4 Temperature Targets** as follows:
519 i. The first step:
520 1. **Target (°C)** set to **92**
521 2. **Acquisition Mode** select **none**
522 3. **Hold** (hh:mm:ss) set to **0:05**
523 4. **Ramp Rate (°C/s)** to 4.4
524 ii. Select the "+" icon to add a step and set the second step:
525 1. **Target (°C)** set to **57**
526 2. **Acquisition Mode** select **single**
527 3. **Hold** (hh:mm:ss) set to **0:40**
528 4. **Ramp Rate (°C/s)** to 2.2
529 8. Save the new protocol as a run template for future use.
530 **a.** In the lower left corner of the screen select the pull-down menu next to the **Apply Template** button
531 **b.** Choose **Save As Template**
532 **c.** Select the **Templates Folder**
533 **d.** Highlight **Run Templates Folder**
534 **e.** Name the template Lyra ® SARS-CoV-2 run template and click the "check" button
535 9. Exit the software.

536 **Qiagen Rotor-Gene Q Programming Instructions**

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

538 **Programming Instructions:**

539 1. Launch the Rotor-Gene Q software package
540 2. In the **New Run** pop-up window select the **Advanced** tab on the top of the screen
541 3. Select **Empty Run** and then **New** on the lower right of the pop-up window to start the **Advanced Run Wizard**
542 **a.** Select the appropriate rotor size in the **Advanced Run Wizard** on the upper left of the screen
543 **b.** Check the box that states the **Locking Ring** is **Attached** and select **Next**
544 c. Leave the **Operator** and **Notes** sections empty
545 d. Enter **20ul** as the **Reaction Volume** in the lower left of the screen
546 e. For the **Sample Layout** choose **1, 2, 3…** and then select **Next**
547 f. Under **Channel Setup** select **Create New** to enter information for each detector
548 i. Under **Name** enter **SARS-CoV-2**
549 ii. **Source** select 470nm
550 iii. **Detector** select 510nm
551 iv. Do not adjust the default **Gain** setting of 7 as this will be set in a later step
552 v. Select **OK**
553 g. Repeat the step above by selecting **Create New**
554 i. Under **Name** enter **PRC**
555 ii. **Source** select 625nm
556 iii. **Detector** select 660nm
557 iv. Do not adjust the default **Gain** setting of 7 as this will be set in a later step
558 v. Select **OK**

559 h. Select the **Edit Profile** button in the middle of the window to setup a cycling profile
560 i. In the **Edit Profile** window go to the upper left of the screen to **New** and in the drop-down
561 menu select **Cycling**. A hold and three step cycling stage should appear.
562 ii. Modify the hold stage to have a temperature at **55°C** and a time of **5:00 minutes**
563 iii. Select the **Insert After** button in the middle of the pop-up window and then select **New**
564 **Hold at Temperature**
565 iv. Modify the second hold stage to have a temperature at **60°C** and a time of **5:00 minutes**
566 v. Select the **Insert After** button in the middle of the pop-up window and then select **New**
567 **Hold at Temperature** to insert a third hold stage
568 vi. Modify the third hold stage to have a temperature at **65°C** and a time of **5:00 minutes**
569 vii. Highlight the first **cycling stage** and modify it as follows:
570 1. This cycle repeats **10** time(s)
571 2. Select **Timed Step** from the drop-down menu in the middle left of the screen
572 3. Do <u>not</u> select **Long Range** or **Touchdown** on the left of the screen
573 4. The first step:
574 a. **92°C**
575 b. **5 seconds**
576 c. **Not Acquiring**
577 5. Select step two and set as follows:
578 a. **57°C**
579 b. **40 seconds**
580 c. **Not Acquiring**
581 6. Highlight step three and delete it by selecting the **"-"** button in the middle of the
582 window
583 7. Select the **Insert After** button in the middle of the pop-up window and then
584 select **New Cycling**
585 viii. Highlight the second **cycling stage** and modify it as follows:
586 1. This cycle repeats **30** time(s)
587 2. Select **Timed Step** from the drop-down menu in the middle left of the screen
588 3. Do <u>not</u> select **Long Range** or **Touchdown** on the left of the screen
589 4. The first step:
590 a. **92°C**
591 b. **5 seconds**
592 c. **Not Acquiring**
593 5. Select step two and set as follows:
594 a. **57°C**
595 b. **40 seconds**
596 c. Select **Acquiring to Cycling A**
597 i. **Under Acquiring Channels** highlight the default channel name
598 (Green) and select the **<** button to move it over to the
599 **Available Channels** list
600 ii. In the **Available Channels** list select **SARS-CoV-2** and select the
601 **>** button to move it over to the **Acquiring Channels** list
602 iii. Repeat the step above for the **PRC** and then select **OK**

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

628 ## Assay Procedure

629 Run the following procedures at controlled room temperature of 20°C to 25°C.
630

631 **Master Mix Rehydration Procedure**

632 1. Determine the number of specimens extracted to be tested and obtain the correct number of eight-
633 test lyophilized Master Mix vials for testing.
634 2. Return unused reagents to the appropriate storage conditions.
635 3. Open Master Mix carefully to avoid disruption of the pellet.
636 4. Add 135 µL of Rehydration Solution to the Master Mix.
637 5. Place vial at room temperature for 1 to 2 minutes to allow rehydration of pellet.
638 6. Gently pipette up and down 2 to 3 times avoiding the formation of bubbles prior to dispensing into
639 the first plate well or tube.
640 **Note**: The rehydrated Master Mix is sufficient for 8 reactions.
641 **Note**: The rehydrated Master Mix may be stored at room temperature (20°C to 25°C) for up to 24
642 hours. For longer storage the rehydrated Master Mix should be recapped, sealed with parafilm and
643 stored in an upright position at ≤–20°C for up to 14 days. Protect the Master Mix from light during
644 storage.
645

646 **RT-PCR Set-up Procedure:**

647 1. Add 15 µL of the rehydrated Master Mix to each plate well or tube.
648 2. Add 5 µL of extracted nucleic acid (specimen with the process control) into the plate well or tube.
649 Mixing of reagents is not required.

650 **Note**: Use a new barrier micropipettor tip with each extracted specimen.
651 3. Seal the plate or tubes.
652 4. Centrifuge the plate or tubes for a minimum of 15 seconds. Ensure that all liquid is at the bottom of
653 the plate wells or tubes.
654 5. Turn on the appropriate thermocycler.
655 6. Insert plate or tubes into the appropriate thermocycler.
656

657 Applied Biosystems® 7500 Fast Dx Thermocycler Test Procedure

658 1. Launch the Applied Biosystems® 7500 Fast Dx software v1.4 package.
659 2. The **Quick Startup document** dialog window will open.
660 3. Click on **Create a new document.**
661 4. Most of the following should be the default setting. If not, change accordingly.

Assay:	Standard Curve (Absolute Quantitation)
Container:	96-Well Clear
Template:	Lyra SARS-CoV-2
Run Mode:	Fast 7500
Operator:	*your operator name*
Comments:	SDS v1.4
Plate Name:	**YYMMDD-** Lyra SARS-CoV-2

662 5. Set Up Sample Plate
663 a. Under the **Setup** and **Plate** tabs the plate setup will appear.
664 b. Select all wells that will contain sample, right-click and select the **Well Inspector** from the drop-
665 down menu. When the **Well Inspector** pop-up window opens, select the detectors for SARS-CoV-
666 2 and PRC.
667 c. Use the **Well Inspector** to enter the sample names. Patient IDs can be entered in the Well Inspector
668 window. However, it is recommended that this is done prior to re-suspending the lyophilized
669 master mix, post run or using the import function to minimize the time the PCR reactions will sit
670 at room temperature prior to starting the run.
671 d. Save the run as **YYMMDD- Lyra SARS-CoV-2.sds**.
672 e. A window will open asking for the “Reason for change of entry”. Enter “**Setup**” and any other
673 comments relevant to the run.
674 6. Starting the PCR
675 a. Select the **Instrument** tab.
676 b. Insert the 96 well PCR plate into the machine.
677 c. Under **Instrument Control**, select the **Start** button to initiate the run.
678 7. Post PCR
679 **IMPORTANT:** When the run is finished press OK.
680 a. Analyze the data by pressing the **“Analyze”** button in the top menu and save the file.
681 b. Save the file by pressing **Save Document** in the task bar. A window will open asking for the
682 “Reason for change of entry”.
683 c. Enter “**Data analysis post run**” and any other comments relevant to the run.

684 Applied Biosystems® 7500 Standard Thermocycler Test Procedure

685 1. Launch the Applied Biosystems® 7500 Standard software v2.06 package.
686 2. The **Quick Startup document** dialog window will open.

687 3. Click on **Create a new document.**
688 4. Most of the following should be the default setting. If not, change accordingly.

Assay:	Standard Curve (Absolute Quantitation)
Container:	96-Well Clear
Template:	Lyra SARS-CoV-2
Run Mode:	Fast 7500
Operator:	*your operator name*
Comments:	SDS v1.4
Plate Name:	**YYMMDD-** Lyra SARS-CoV-2

689 5. Set Up Sample Plate
690 a. Under the **Setup** and **Plate** tabs the plate setup will appear.
691 b. Select all wells that will contain sample, right-click and select the **Well Inspector** from the drop-
692 down menu. When the **Well Inspector** pop-up window opens, select the detectors for SARS-CoV-
693 2 and PRC.
694 c. Use the **Well Inspector** to enter the sample names. Patient IDs can be entered in the Well Inspector
695 window. However, it is recommended that this is done prior to re-suspending the lyophilized
696 master mix, post run or using the import function to minimize the time the PCR reactions will sit
697 at room temperature prior to starting the run.
698 d. Save the run as **YYMMDD- Lyra SARS-CoV-2.sds**.
699 e. A window will open asking for the "Reason for change of entry". Enter "**Setup**" and any other
700 comments relevant to the run.
701 6. Starting the PCR
702 a. Select the **Instrument** tab.
703 b. Insert the 96 well PCR plate into the machine.
704 c. Under **Instrument Control**, select the **Start** button to initiate the run.
705 7. Post PCR
706 **IMPORTANT:** When the run is finished press OK.
707 a. Analyze the data by pressing the **"Analyze"** button in the top menu and save the file.
708 b. Save the file by pressing **Save Document** in the task bar. A window will open asking for the
709 "Reason for change of entry".
710 c. Enter "**Data analysis post run**" and any other comments relevant to the run.

711 **Creating a LC 480 II Assay Test Procedure**

712 1. Load the Lyra SARS-CoV-2 run template.
713 2. Press Start.
714 3. The analysis template can only be established after the initial experiment has completed
715 4. On the Lyra ® SARS-CoV-2 run select the **Analysis** button in the module bar
716 a. Choose **Abs Quant/Fit Points**
717 b. In the **Create New Analysis** pop-up window select your pre-defined subset from the **subset** drop
718 down menu and then select the "check" button
719 c. Set the **Background** to 2-10 for all analytes
720 i. Set **Min Offset** to 1
721 ii. Set **Max Offset** to 9
722 d. In the center bottom of the screen ensure that **Color Compensation** is off for all analytes

723 e. Leave the default settings as **First Cycle** 1 and **Last Cycle** 40
724 5. At the top middle of the screen select **Noise Band**
725 6. Choose the pull-down menu next to the **Noise Band** button and select **Noise Band Fluorescence**
726 7. For each analyte under the **Filter Comb** button, set the noise band as follows:
727 a. SARS-CoV-2set to 1.95
728 b. PRC set to 1.4619
729 8. Choose **Calculate** in the bottom left of the screen
730 9. Save the new analysis protocol as a template for future use
731 a. In the lower left corner of the screen select the pull-down menu next to the **Apply Template** button
732 b. Choose **Save As Template**
733 c. Select the **Templates Folder**
734 d. Highlight **Analysis Templates Folder**
735 e. Name the template Lyra ® SARS-CoV-2 analysis template and click the "check" button
736 10. Create a report
737 a. Select the **Save** icon on the global action bar on the right side of the screen
738 b. Choose the **Report** button on the module bar on the left of the screen
739 c. Select the appropriate settings and press the **Generate** button
740 11. To apply an Analysis Template to subsequent runs
741 a. Once the run has finished select the **Analysis** button in the module bar
742 b. Choose **Abs Quant/Fit Points**
743 c. In the **Create New Analysis** pop-up window select your pre-defined subset from the **subset** drop
744 down menu and then select the "check" button
745 d. Select the **Apply Template** button on the far left of the screen and choose the Lyra ® SARS-CoV-2
746 analysis template from the **Analysis Templates Folder**
747 e. Select yes in the pop-up window
748 12. Interpretation of results (See Table 4)

749 ## Qiagen Rotor-Gene Q Test Run
750 **Analysis Instructions:**

751 1. In the New Run Wizard load the SARS-CoV-2 Template.
752 2. Press Start.
753 3. Open the run file that needs to be analyzed
754 4. In the upper menu toolbar select the **Analysis** button
755 a. Select **Quantitation**, then **Cycling A. SARS-CoV-2**, and **Show**
756 b. The threshold needs to be set for SARS-CoV-2
757 i. In the far right bottom of the screen under **CT Calculation** enter **0.03** for the **SARS-CoV-2**
758 **Threshold**
759 ii. In the **Eliminate Cycles before** box ensure the default of **1** is entered
760 iii. Ensure the amplification graph is set to **Log Scale** (toggle button on the bottom left of the
761 graph states Linear Scale or Log Scale)
762 c. Select **Quantitation**, then **Cycling A. PRC**, and **Show**
763 d. The threshold needs to be set for PRC
764 i. In the far right bottom of the screen under **CT Calculation** enter **0.05** for the **PRC**
765 **Threshold**
766 ii. In the **Eliminate Cycles before** box ensure the default of **1** is entered

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

769 ## Quality Control

770 The Lyra SARS-CoV-2 Assay incorporates several controls to monitor assay performance.
771
772 1. The **Process Control (PRC)** consists of an inactivated and stabilized MS2 Bacteriophage that contains
773 an RNA genome. It must be used during extraction and amplification in the assay. This control should
774 be added to each sample aliquot prior to extraction. The PRC serves to monitor inhibitors in the
775 extracted specimen, assures that adequate amplification has taken place and confirms that the
776 nucleic acid extraction was sufficient.
777
778 2. The **Positive Control** (containing SARS-CoV-2 Synthetic RNA, Part M5153) must be treated as a
779 patient specimen and be included in every extraction and RT-PCR run.
780
781 3. The **Negative Control** (Part M5031) must be treated as a patient specimen and be included in every
782 extraction and PCR run.
783
784 4. Failure of either the **Positive Control** or the **Negative Control** invalidates the RT-PCR run and results
785 should not be reported. The RT-PCR run should be repeated with the extracted controls and
786 specimens first. Re-extract and retest another aliquot of the controls and the specimens or obtain
787 new samples and retest if the controls fail again.
788

Table 3. Expected Results from Controls (Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, or Qiagen Rotor-Gene Q)

Control Type/ Name	Used to Monitor	SARS-CoV-2	Expected Ct Values	PRC	Expected Ct Values
Positive Control	Substantial reagent failure including primer and probe integrity	+	5.0≤ Ct ≤30.0	+/-	NA[1]
Negative Control	Reagent and/or environmental contamination	-	None detected	+	5.0≤ Ct ≤30.0

Expected Results from Controls (Roche LightCycler 480)

Control Type/ Name	Used to Monitor	SARS-CoV-2	Expected Ct Values	PRC	Expected Ct Values
Positive Control	Substantial reagent failure including primer and probe integrity	+	5.0≤ Ct ≤40.0	+/-	NA[1]
Negative Control	Reagent and/or environmental contamination	-	None detected	+	5.0≤ Ct ≤40.0

789 [1]No Ct value is required for the Process Control to make a positive call.
790

791 ## Interpretation of Results from Patient Specimens

792

Table 4. Interpretation of the Lyra SARS-CoV-2 Assay Results on the Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500 Standard, or Qiagen Rotor-Gene Q

Assay Result	Detector: SARS-CoV-2	Detector: Process Control	Interpretation of Results	Notes and Special Guidance
Negative	No Ct detected	5.0≤ Ct ≤30.0	No SARS-CoV-2 viral RNA detected; PRC Detected.	
SARS-CoV-2 Positive	5.0≤ Ct ≤30.0	NA[1]	SARS-CoV-2 Virus viral RNA detected.	
Invalid	No Ct detected	No Ct detected	No SARS-CoV-2 viral RNA and no PRC RNA detected.	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.

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

Assay Result	Detector: SARS-CoV-2	Detector: Process Control	Interpretation of Results	Notes and Special Guidance
Negative	No Ct detected	5.0≤ Ct ≤40.0	No SARS-CoV-2 viral RNA detected; PRC Detected.	
SARS-CoV-2 Positive	5.0≤ Ct ≤40.0	NA[1]	SARS-CoV-2 Virus viral RNA detected.	
Invalid	No Ct detected	No Ct detected	No SARS-CoV-2 viral RNA and no PRC RNA detected.	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.

793 [1] No Ct value is required for the Process Control to make a positive call.

794 **CLINICAL PERFORMANCE**
795
796 The clinical performance of the Lyra SARS-CoV-2 Assay was evaluated using two different studies:
797 • A study using two hundred sixty-five fresh or frozen nasopharyngeal swab specimens
798 collected in UTM (36 and 229, respectively) from patients located in the USA.
799 • A fully contrived positive specimen study using nasopharyngeal swab specimens.
800 All two hundred sixty-five specimens were negative for SARS-CoV-2 when extracted with the
801 easyMAG system and tested by the Lyra SARS-CoV-2 Assay.
802
803 One hundred twenty-four specimens included in this study were positive for other circulating
804 respiratory viruses as identified by FDA-cleared assays:

805

Circulating Virus	# of positive specimens
Influenza A	30
RSV	4
Coronavirus Seasonal (not identified)	10
Coronavirus 229e	20
Coronavirus OC43	20
Coronavirus NL63	20
Coronavirus HKU1	20

806
807 Viral RNA was obtained from BEI Resources for use in the contrived clinical study. The genomic RNA
808 was extracted from a preparation of cell lysate and supernatant from Cercopithecus aethiops kidney
809 epithelial (Vero E6, ATCC® CRL-1586™) cells infected with SARS-related coronavirus 2 (SARS-CoV-2),
810 isolate USA-WA1/2020, using QIAamp® Viral RNA Mini Kit (Qiagen 52904). The viral genomic RNA is
811 in a background of cellular nucleic acid and carrier RNA. The Genome Copy Number was established
812 using BioRad QX200 Droplet Digital PCR (ddPCR™) System.
813
814 Ninety-two positive contrived samples were created by spiking ninety-two individual clinical samples
815 determined to be negative for SARS-CoV-2 by the Lyra SARS-CoV-2 Assay prior to spiking with one of
816 three concentrations of genomic SARS-CoV-2 RNA. Forty-four specimens were spiked with 1x LoD
817 (8.00E-01 cp/µL) of RNA. Twenty-four additional specimens were spiked with 3x LoD (2.40E00
818 cp/µL) of RNA. Twenty-four additional specimens were spiked with 5x LoD (4.00E00 cp/µL) of RNA.
819 All samples were extracted and tested according to the Lyra SARS-CoV-2 Assay package insert.
820
821 All ninety-two contrived samples were positive in the Lyra SARS-CoV-2 Assay. The results for the
822 contrived positive specimens are shown in the table below:
823

Table 5. Clinical evaluation in spiked nasopharyngeal swab specimens			
Sample RNA Concentration	# Positives/# Tested	Mean SARS-CoV-2 Ct	%CV
unspiked	0/92	NA	NA
1 .0x LoD	44/44	26.9	5.7
3x LoD	24/24	22.8	3.4
5x LoD	24/24	22.4	3.0

824 Performance against the expected results are:
825 Positive Percent Agreement 92/92 = 100% (95% CI: 95.99%-100%)
826 Negative Percent Agreement 92/92 = 100% (95% CI: 95.99%-100%)
827
828

829 **ANALYTICAL PERFORMANCE**
830 **Level of Detection**
831
832 The Limit of Detection of the Lyra SARS-CoV-2 Assay utilized limiting dilutions of genomic SARS-CoV-
833 2 RNA in negative nasopharyngeal matrix. Each dilution was extracted using the NucliSENS
834 easyMAG System and tested on Applied Biosystems 7500 Fast Dx, Applied Biosystems 7500
835 Standard, Roche LightCycler 480, or Qiagen Rotor-Gene Q. Analytical sensitivity (LoD) is defined as
836 the lowest concentration at which at least 95% of all replicates tested positive.

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

843 This study established the LoD for the Lyra SARS-CoV-2 Assay as 8.00E-01 genomic RNA copies/µL,
844 subsequently confirmed by testing 20 replicates.
845

Table 6. LoD in Nasopharyngeal specimens with Applied Biosystems 7500 Fast Dx

Concentration	Replicate	SARS-CoV-2 Ct	PRC Ct	Interpretation
8.00E-01 genomic RNA copies/µL	1	23.95	18.54	Positive
	2	26.59	18.28	Positive
	3	26.19	18.32	Positive
	4	25.13	18.41	Positive
	5	24.88	18.74	Positive
	6	24.84	19.18	Positive
	7	25.51	18.82	Positive
	8	25.20	18.58	Positive
	9	24.69	18.71	Positive
	10	24.57	18.67	Positive
	11	23.86	18.75	Positive
	12	24.58	18.91	Positive
	13	25.19	19.03	Positive
	14	25.84	19.05	Positive
	15	26.58	19.10	Positive
	16	26.72	19.15	Positive
	17	24.16	19.06	Positive
	18	25.15	18.91	Positive
	19	25.51	19.05	Positive
	20	24.41	19.07	Positive

Table 7. LoD in Oropharyngeal specimens with Applied Biosystems 7500 Fast Dx				
Concentration	Replicate	SARS-CoV-2 Ct	PRC Ct	Interpretation
8.00E-01 genomic RNA copies/µL	1	27.26	19.38	Positive
	2	28.99	19.22	Positive
	3	27.3	19.51	Positive
	4	26.09	19.27	Positive
	5	26.88	19.61	Positive
	6	26.02	19.19	Positive
	7	26.37	19.21	Positive
	8	25.01	19.30	Positive
	9	25.14	19.06	Positive
	10	26.21	19.03	Positive
	11	27.79	19.27	Positive
	12	28.83	19.12	Positive
	13	28.83	19.19	Positive
	14	26.81	19.50	Positive
	15	25.1	19.30	Positive
	16	26.2	19.11	Positive
	17	26.74	19.00	Positive
	18	25.28	19.13	Positive
	19	26.27	19.31	Positive
	20	26.37	19.24	Positive

846

Table 8. LoD in Nasopharyngeal specimens with Applied Biosystems 7500 Standard				
Concentration	Replicate	SARS-CoV-2 Ct	PRC Ct	Interpretation
8.00E-01 genomic RNA copies/µL	1	26.63	19.26	Positive
	2	29.15	19.28	Positive
	3	25.67	19.69	Positive
	4	25.53	20.07	Positive
	5	26.15	20.50	Positive
	6	26.71	20.50	Positive
	7	26.11	19.14	Positive
	8	26.94	19.18	Positive
	9	25.62	18.64	Positive
	10	25.80	18.80	Positive
	11	26.76	19.15	Positive
	12	26.15	19.63	Positive

Table 8. LoD in Nasopharyngeal specimens with Applied Biosystems 7500 Standard				
Concentration	Replicate	SARS-CoV-2 Ct	PRC Ct	Interpretation
	13	27.42	19.44	Positive
	14	27.51	19.99	Positive
	15	26.07	19.9	Positive
	16	25.92	18.81	Positive
	17	27.95	20.02	Positive
	18	27.71	19.27	Positive
	19	26.51	18.86	Positive
	20	Undetermined	19.11	Negative

847

Table 9. LoD in Nasopharyngeal specimens with Roche LightCycler 480*				
Concentration	Replicate	SARS-CoV-2 Ct*	PRC Ct*	Interpretation
8.00E-01 genomic RNA copies/µL	1	32.91	31.73	Positive
	2	34.54	32.9	Positive
	3	34.83	32.25	Positive
	4	34.94	31.7	Positive
	5	33.81	32.14	Positive
	6	34.36	32.37	Positive
	7	33.90	32.10	Positive
	8	33.83	32.80	Positive
	9	33.8	31.86	Positive
	10	34.28	32.27	Positive
	11	33.63	32.81	Positive
	12	33.72	32.45	Positive
	13	34.86	33.17	Positive
	14	34.57	32.64	Positive
	15	34.48	32.92	Positive
	16	33.61	32.82	Positive
	17	33.87	33.34	Positive
	18	34.44	33.36	Positive
	19	34.22	32.55	Positive
	20	33.77	32.97	Positive

848 * Results include 10 cycles not captured by the other instruments

Table 10. LoD in Nasopharyngeal specimens with Qiagen Rotor-Gene Q				
Concentration	Replicate	SARS-CoV-2 Ct	PRC Ct	Interpretation
8.00E-01 genomic RNA copies/µL	1	24.01	19.08	Positive
	2	24.04	19.36	Positive
	3	24.85	19.44	Positive
	4	23.23	19.13	Positive
	5	24.39	19.07	Positive
	6	23.89	18.94	Positive
	7	23.78	18.80	Positive
	8	24.82	18.86	Positive
	9	23.87	18.83	Positive
	10	24.05	18.90	Positive
	11	23.28	18.84	Positive
	12	24.36	18.71	Positive
	13	23.85	18.87	Positive
	14	23.54	18.88	Positive
	15	24.84	19.20	Positive
	16	23.63	19.01	Positive
	17	24.18	18.97	Positive
	18	23.47	19.01	Positive
	19	23.58	18.94	Positive
	20	23.89	19.02	Positive

849

850 **Analytical Reactivity (Inclusivity)**

851

852 The inclusivity of the Lyra SARS-CoV-2 Assay was established by testing Genomic RNA from the
853 SARS-related coronavirus 2 (SARS-CoV-2), isolate USA-WA1/2020, via *in-silico* analysis. The *in-silico*
854 analysis demonstrated the Lyra SARS-CoV-2 primers are 100% conserved to 257 SARS-CoV-2
855 sequences available from NCBI and GISAID as of March 5, 2020.

856 **Analytical Specificity (Cross-Reactivity)**

857

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

863

Table 11. Cross-reactivity test results				
Virus/Bacteria/Parasite	Strain	Source/ Sample type	Concentration	Results
Adenovirus	Type 1	Isolate	$1 \times 10^{7.53}$ U/mL	Neg, Neg, Neg
Coronavirus	229e	Isolate	$1 \times 10^{6.10}$ U/mL	Neg, Neg, Neg
Coronavirus	OC43	Isolate	9.55×10^{6} $TCID_{50}$/mL	Neg, Neg, Neg
Coronavirus	NL63	Isolate	$1 \times 10^{4.67}$ U/mL	Neg, Neg, Neg
MERS-CoV (heat-inactivated)	Florida/USA-2_Saudia Arabia_2014	Isolate	4.17×10^{5} $TCID_{50}$/mL	Neg, Neg, Neg
SARS -1	2003-00592	Inactivated virus	Not available	Neg, Neg, Neg
Mycoplasma pneumoniae	M129	Isolate	3×10^{7} CCU/mL	Neg, Neg, Neg
Streptococcus pyogenes	Z018	Isolate	3.8×10^{9} cfu/mL	Neg, Neg, Neg
Influenza A H3N2	Brisbane/10/07	Isolate	$1 \times 10^{5.07}$ U/mL	Neg, Neg, Neg
Influenza A H1N1	New Caledonia/20/99	Isolate	$1 \times 10^{6.66}$ U/mL	Neg, Neg, Neg
Influenza B	Brisbane/33/08	Isolate	$1 \times 10^{5.15}$ U/mL	Neg, Neg, Neg
Parainfluenza	Type 1	Isolate	$1 \times 10^{8.01}$ U/mL	Neg, Neg, Neg
Parainfluenza	Type 2	Isolate	$1 \times 10^{6.34}$ U/mL	Neg, Neg, Neg
Parainfluenza	Type 3	Isolate	8.51 x 107 TCID50/mL	Neg, Neg, Neg
Parainfluenza	Type 4b	Isolate	$1 \times 10^{7.53}$ U/mL	Neg, Neg, Neg
Enterovirus	Type 68	Isolate	$1 \times 10^{6.5}$ U/mL	Neg, Neg, Neg
Human Metapneumovirus	A1 (IA10-s003)	Isolate	$1 \times 10^{5.55}$ U/mL	Neg, Neg, Neg
Respiratory Syncytial Virus	Type A (3/2015 Isolate #3)	Isolate	$1 \times 10^{5.62}$ U/mL	Neg, Neg, Neg
Human Rhinovirus	N/A	Inactivated virus	Not available	Neg, Neg, Neg
Chlamydophila pneumoniae	AR-39	Isolate	2.9 x 107 IFU/mL	Neg, Neg, Neg
Haemophilus influenzae	Type b; Eagan	Isolate	7.87×10^{8} cfu/mL	Neg, Neg, Neg
Legionella pneumophila	Philadelphia	Isolate	6.82×10^{9} cfu/mL	Neg, Neg, Neg
Streptococcus pneumoniae	Z022; 19f	Isolate	2.26×10^{9} cfu/mL	Neg, Neg, Neg
Bordetella pertussis		Isolate		Neg, Neg, Neg
Pneumocystis jirovecii-S. cerevisiae Recombinant	W303-Pji	Isolate	1.56×10^{8} cfu/mL	Neg, Neg, Neg

864

865 The *in silico* analysis focused on 32 micro-organisms identified by the FDA as high priority for
866 assessment due to their potential presence in upper respiratory samples.
867

TABLE 12. Cross-Reactivity Organisms			
Organism	Total # Sequences	# Complete Genomes	# WGS Strains
Adenovirus	532	532	0
Coronavirus (Seasonal)	288	288	0

TABLE 12. Cross-Reactivity Organisms			
Organism	**Total # Sequences**	**# Complete Genomes**	**# WGS Strains**
Enterovirus [B]	2708	2674	34
Influenza A Virus[A B]	172455	21444 (+39 A/Mexico/4108/2009)	108
Influenza B Virus[A B]	53952	6755 (+16 B/Florida/4/2006)	0
Influenza C Virus [B]	2205	N/A	N/A
Human Metapneumovirus	145	145	0
Human Parainfluenza Virus 1-4	439	439	0
Human Parechovirus	124	124	0
Human Respiratory Syncytial Virus [B]	1275	1275	0
Rhinovirus	214	214	0
SARS-1	236[C]	232 (+4 pp1ab sequences)	0
Bacillus anthracis	4152	69	86
Candida albicans	1541	59	34
Chlamydia pneumoniae	466	5	20
Chlamydia psittaci	11179	23	45
Corynebacterium diptheriae	20797	17	194
Coxiella burnetii	419	28	3
Haemophilus influenzae	45267	61	692
Legionella [B]	4843	98	65
Leptospira [B]	64456	133	266
Moraxella catarrhalis [B]	8333	11	184
Mycobacterium tuberculosis	194	194	0
Mycoplasma pneumoniae	808	51	45
Neisseria elongata & N. meningitidis [B]	312050	116	1318
Pneumocystis jirovecii	487	15	3
Pseudomonas aeruginosa	195	195	0
Staphylococcus aureus	634	634	0
Staphylococcus epidermidis [B]	61880	23	508
Streptococcus pneumoniae [B]	1633369	107	8526
Streptococcus pyogenes [B]	46153	201	1733
Streptococcus salivarius [B]	9417	18	48
[A] 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.			
[B] For BLAST, 'Max Target Seqs' was set to 5000. See Table 2.			
[C] 4 polyprotein cds sequences were also included.			

868

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

874

875 **Limitations**

876 • Negative results do not preclude infection with SARS-CoV-2 and should not be the sole basis of a
877 patient treatment decision.
878 • This test is intended to be used for the detection of SARS-CoV-2 RNA in nasopharyngeal and
879 oropharyngeal swab samples. Testing of other sample types may result in inaccurate results.
880 • Improper collection, storage or transport of specimens may lead to false negative results.
881 • Inhibitors present in the sample and/or errors in following the assay procedure may lead to false
882 negative results.
883 • A trained health care professional should interpret assay results in conjunction with the
884 patient's medical history, clinical signs and symptoms, and the results of other diagnostic tests.
885 • Analyte targets (viral sequences) may persist *in vivo*, independent of virus viability. Detection of
886 analyte target(s) does not imply that the corresponding virus(es) are infectious, nor are the
887 causative agents for clinical symptoms.
888 • There is a risk of false positive values resulting from cross-contamination by target organisms,
889 their nucleic acids or amplified product, or from non-specific signals in the assay.
890 • There is a risk of false negative values due to the presence of sequence variants in the viral
891 targets of the assay.
892 • The assay performance was not established in immunocompromised patients.
893

894 **Conditions of Authorization for the Labs**

895 The Lyra® SARS-CoV-2 Assay Letter of Authorization, along with the authorized Fact Sheet for Healthcare
896 Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website:
897 https://www.fda.gov/MedicalDevices/Safety/ EmergencySituations/ucm161496.htm.

898 However, to assist clinical laboratories using the Lyra® SARS-CoV-2 Assay, the relevant Conditions of
899 Authorization are listed below.
900

901 • Authorized laboratories[1] using the Lyra SARS-CoV-2 Assay will include with result reports of the Lyra
902 SARS-CoV-2 Assay test, all authorized Fact Sheets. Under exigent circumstances, other appropriate
903 methods for disseminating these Fact Sheets may be used, which may include mass media.

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

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

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

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

918 • All laboratory personnel using the test must be appropriately trained in RT-PCR techniques and use
919 appropriate laboratory and personal protective equipment when handling this kit, and use the test in
920 accordance with the authorized labeling.

921 • Quidel, its authorized distributor(s) and authorized laboratories using the Lyra SARS-CoV-2 Assay will
922 ensure that any records associated with this EUA are maintained until otherwise notified by FDA.
923 Such records will be made available to FDA for inspection upon request.

924 [1] For ease of reference, the letter of authorization refers to, "United States (U. S.) laboratories certified under the
925 Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as
926 "authorized laboratories."

927 **Customer and Technical Assistance**

928 To place an order or for technical support, please contact a Quidel Representative at (800) 874-1517 (toll-free
929 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.,
930 Eastern Time. Orders may also be placed by fax at (740) 592-9820. For e-mail support contact: customer
931 service@dhiusa.com or technical_services@dhiusa.com. For services outside the U.S., please contact your local
932 distributor. Additional information about Quidel, our products, and our distributors can be found on our website
933 quidel.com.
934
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937 trademark of Roche. Rotor-Gene is a registered trademark of Qiagen. Q Dye compounds in this product are
938 sold under license from BioSearch Technologies, Inc. and protected by U.S. and world-wide patents either
939 issued or under application. The license covers R&D use and human in vitro diagnostic (IVD) applications.
940

941 **References**

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951

M120 – Lyra SARS-CoV-2 Assay kit

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Athens, OH 45701 USA
quidel.com

952
953

GLOSSARY

iu Intended use	REF Catalog number
CONT Contents / Contains	Σ 96 Contains sufficient for 96 determinations
	CONTROL Control
LOT Batch code	EXP Use by
	Consult e-labeling instructions for use
Manufacturer	Temperature limitation

*** DO NOT DISCARD: Important product-specific information ***

Lyra® SARS-CoV-2 Assay – Verification Requirements

Please consult the following guidance from CMS regarding Emergency Use Authorized diagnostic tests: https://www.cms.gov/Medicare/Provider-Enrollment-and- Certification/SurveyCertificationGenInfo/Policy-and-Memos-to-States-and-Regions- Items/QSO18-19-CLIA

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 nasopharyngeal or oropharyngeal 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 nasopharyngeal and oropharyngeal swab 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 positive 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.

REQUIRED MATERIALS

The SARS-CoV-2 Positive Control is provided with the Lyra SARS-CoV-2 Assay and ready to process according to the package insert. The positive control consists synthetic RNA to the Lyra SARS-CoV-2 target region.

Approximately 2-mL of an upper respiratory specimen (e.g. nasopharyngeal swabs (NPS) in viral transport media) will be needed for assay verification testing. Specimens may be pooled if less than 2-mL of one specimen is available.

PRECAUTIONS

Lyra SARS-CoV-2 Assay should be handled in an approved BSL-2 handling area to avoid contamination of laboratory equipment and reagents that could cause false positive results. The positive control is an RNA transcript and is non-infectious. However, all testing should be handled in accordance with Good Laboratory Practices.

Store the Lyra SARS-CoV-2 Assay at 2° to 8°C. Bring all reagents to room temperature (20° C to 25°C) before beginning the assay. Please use standard precautions when handling respiratory specimens.

INSTRUCTIONS FOR PREPARING TEST VERIFICATION SAMPLES BEFORE EXTRACTION bioMerieux NucliSENS® easyMAG® system or EMAG® system

Moderate Concentration Positive:

1. Add 170-µL of upper respiratory specimen (e.g. NPS in viral transport media) into each of the three labeled tubes.
2. Add 10-µL of the SARS-CoV-2 Positive Control to each labeled tube.
3. Add 20-µL of the SARS-CoV-2 Process Control to each labeled tube.
4. Proceed to step 12.

Low Concentration Positive:

5. Add 175-µL of upper respiratory specimen (e.g. NPS in viral transport media) into each of the three labeled tubes.
6. Add 5-µL of the SARS-CoV-2 Positive Control to each labeled tube.
7. Add 20-µL of the SARS-CoV-2 Process Control to each labeled tube.
8. Proceed to step 12.

Negative Sample:

9. Add 170-µL of upper respiratory specimen (e.g. NPS in viral transport media) into each of the three labeled tubes.
10. Add 10-µL of the SARS-CoV-2 Negative Control to each labeled tube.
11. Add 20-µL of the SARS-CoV-2 Process Control to each labeled tube.
12. Perform extractions of all nine samples according to the Lyra SARS-CoV-2 Assay instructions for use

PROCEDURE

Follow the Lyra SARS-CoV-2 Assay instructions for use for testing the 9 extracted samples at least once.

EXPECTED RESULTS

1. Moderate SARS-CoV-2 samples should be positive with Lyra SARS-CoV-2 Assay ($5.0 \leq$ Ct ≤ 30.0).
2. Low SARS-CoV-2 samples should be positive with Lyra SARS-CoV-2 Assay ($5.0 \leq$ Ct ≤ 30.0).
3. Negative upper respiratory samples should be negative for with Lyra SARS-CoV-2 Assay (No Ct Detected).

100% of Moderate positive samples should agree with expected results. ≥ 90% of Low positive samples should agree with expected results. If testing results do not meet this criterion, contact Quidel Corporation at technicalsupport@quidel.com.