SARS-CoV-2 RNA, Qualitative Real-Time RT-PCR (Test Code 39433)

Package Insert

For Emergency Use Only

For *In-vitro* Diagnostic Use - Rx Only

Intended Use

The Quest Diagnostics SARS-CoV-2 RNA, Qualitative Real-Time RT-PCR ("Quest SARS-CoV-2 rRT-PCR") is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, tracheal aspirates, and bronchoalveolar lavage) collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Quest Diagnostic Laboratories or other laboratories designated by Quest Diagnostics that are also 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 RNA is generally detectable in respiratory specimens during the acute phase of infection. Positive results 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. The agent detected may not be the definite cause of disease. 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 treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

Testing with the SARS-CoV-2 rRT-PCR test is intended for use by qualified and trained laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR assays. The SARS-CoV-2 rRT-PCR test is only for use under a Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation

An outbreak of pneumonia of unknown etiology in Wuhan City, Hubei Province, China was initially reported to WHO on December 31, 2019. Chinese authorities identified a novel coronavirus (SARS-CoV-2), which has resulted in thousands of confirmed human infections in multiple provinces throughout China and exported cases in several Southeast Asian countries and more recently in Europe and the United States. Cases of severe illness and some deaths have been reported.

The Quest Diagnostics SARS-CoV-2 RNA, Qualitative Real-Time RT-PCR aids in the detection of SARS-CoV-2 RNA and diagnosis COVID-19 and is a real-time reverse transcription polymerase chain reaction test. The test's primer and probe sets were designed to detect RNA from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to Quest Diagnostics laboratories in San Juan Capistrano CA, Chantilly VA, and Marlboro MA, or other laboratories designated by Quest Laboratories that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

Principles of the Procedure

The test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (for example, nasopharyngeal swabs, oropharyngeal swabs, sputum, BAL, and tracheal aspirates). The assay is composed of two principal steps: (1) extraction of RNA from patient specimens, (2) one-step reverse transcription and PCR amplification with SARS-CoV-2 specific primers and real-time detection with 2019-nCoV specific probes. The assay targets regions of the virus nucleocapsid gene (N1 & N3) and is designed for the detection of SARS-CoV-2. Amplification and detection are accomplished using TaqMan chemistry on the ABI 7500. To ensure the absence of non-specific PCR inhibition of a sample, an internal positive amplification control (IPC) is included with each specimen. A sample can be interpreted as negative only if the analysis of the IPC indicates that amplification has occurred in the reaction tube but no signal from target reporter dye has been detected. Detection of viral RNA not only aids in the diagnosis of illness but also provides epidemiological and surveillance information.

Materials Required (Provided)

- MagNA Pure 96 DNA and Viral NA – Small Volume Kit Roche Diagnostics #06 543 588 001 (3 x 192 isolations)
- 4X 1-Step RT-qPCR Master Mix, CG
- Exogenous NA Primer Pair
- Exogenous NA[1]
- TE Buffer, pH 8.0
- Quest V-C-M transport medium or other comparable transport medium that will be validated
- Poly (A)
- DEPC-water
- PBS, 1X
- MagNA Pure 96 External Lysis Buffer or other comparable lysis buffer that will be validated
- DTT

Reagents: RT-PCR Mix Primers and Probes
2019-nCoV_N1 Forward Primer
2019-nCoV_N1 Reverse Primer
2019-nCoV_N1 Probe
2019-nCoV_N3 Forward Primer
2019-nCoV_N3 Reverse Primer
2019-nCoV_N3 Probe

Reagent Preparation and Storage

Primer and Probe 10μM stocks in TE Buffer
Dilute Probes 100 µM stocks 1:10 in TE Buffer ex: 100 µL + 900 µL TE Buffer). Prepare aliquots in screw cap tubes.
Dilute Primers 200 µM stocks 1:10 in TE Buffer ex: 50 µL + 950 µL TE Buffer). Prepare aliquots in screw cap tubes.
Storage Store @ -60ºC to -90ºC
Stability 1 year from date of preparation.
Formulation sheet EFORM.129. 01480

4x RT-PCR enzyme mix 1 mL aliquots
Thaw / equilibrate 10mL bottle(s) of 4x RT-PCR enzyme mix to room temperature (protect from light). Mix bottle contents thoroughly by inversion and gentle swirling. Transfer 1.0 mL aliquots of mix to pre-labeled sterile screw cap tubes.
Storage Store @ -60ºC to -30ºC
Stability as specified by manufacturer on bottle

5 mg/mL poly (A)
Dissolve 100 mg of poly (A) in 20 mL of DEPC-water in a 50 mL sterile centrifuge tube. Vortex until completely dissolved. Prepare 1 mL aliquots in screw cap tubes.
Storage Store @ -60ºC to -90ºC
Stability 2 years from date of preparation.

RNA Diluent P
Add 1 mL of 5 mg/mL poly (A) to 1 x 500 mL bottle of 1x PBS (new, unopened, without CA, Mg salts). Mix well. Prepare 40 mL aliquots in 50 mL sterile centrifuge tubes. The final concentration of poly (A) is 10 µg/mL.

[1] In the event that a RNA internal process control is temporarily unavailable, a DNA internal process control, exhibiting similar PCR performance, may be used temporarily.

Storage Store @ 2ºC to 8ºC
Stability 1 year from date of preparation.

DTT solution 500mM
Add 100 µL of nuclease free water to one microtube containing DTT and mix with pipette tip. Add the entire 100µL DTT solution into 5mL of cold sterile 0.01 M PBS (pH 7.2) and mix briefly. Discard any unused reconstituted DTT

SARS-CoV-2 PCR Mix
Combine the ingredients in the amounts listed below.
Dispense in **455** µ**L** aliquots label as: **NCOV PCR Mix**
Lot#/Prep: *(preparation date. initials)*
Exp. date: *(1 year from preparation date)*
Store at -60° to -90°C
Each aliquot is sufficient for up to 48 reactions.
Storage Store at -60° to -90°C.
Stability Expires 1 year after preparation.
See Formulation Sheet EFORM.129.001481

Item	**µL per reaction**	**Unit of Measure for 1,000 rxns.**	**Final Concentration per 25 µL reaction**
Sterile Nuclease Free Water	3.75	3.75 mL	---
2019-nCoV_N1 Forward Primer (10 µM in TE, pH 8.0)	1.00	1.00 mL	0.4 µM
2019-nCoV_N1 Reverse Primer (10 µM in TE, pH 8.0)	1.00	1.00 mL	0.4 µM
2019-nCoV_N1 Probe (10 µM in TE, pH 8.0)	0.25	0.25 mL	0.1 µM
2019-nCoV_N3 Forward Primer (10 µM in TE, pH 8.0)	1.00	1.0 mL	0.4 µM
2019-nCoV_N3 Reverse Primer (10 µM in TE, pH 8.0)	1.00	1.0 mL	0.4 µM
2019-nCoV_N3 Probe (10 µM in TE, pH 8.0)	0.25	0.25 mL	0.1 µM
50X Exogenous NA Primer/Probe Mix	0.50	0.50 mL	1X
Total	**8.750 µL**	**8.750 mL**	

Equipment and Supplies

- Applied Biosystems 7500 Real Time PCR System (or ABI 7500 fast system run as a standard ABI 7500)
- Roche MagNA Pure 96 System
- Bench-top centrifuge
- Serological Pipet (Pipette Aid)
- Sterile screw cap 15 mL conical tubes
- Sterile screw cap 50 mL conical tubes
- P10, P20, P200, P1000 pipettes
- P-10, P-20, P-200, P-1000 ART Plugged Tips
- 1.5 mL or 2 mL microcentrifuge tubes
- Metal tubes
- Standard absorbent wipes
- Latex gloves and other protective equipment (see Procedure)
- Biohazard Absorbent Wipes
- 96-Well Optical Reaction Plate
- Optical Adhesive Cover
- Vortexer
- Microcentrifuge

Warnings and Precautions

1. For *in vitro* diagnostic use (IVD).
2. For emergency use only.
3. Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
4. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
5. Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.
6. Specimen processing should be performed in accordance with national biological safety regulations.
7. If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
8. Performance characteristics have been determined with human upper respiratory specimens and lower respiratory tract specimens from human patients submitted for respiratory infection testing (and presumed to have signs and symptoms of disease).

Specimen Collection, Handling, and Storage

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13-A may be referenced as an appropriate resource.

- Collecting the Specimen
  - Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV) https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html
  - Follow specimen collection device manufacturer instructions for proper collection methods.
  - Swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media.
- Transporting Specimens
  - Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens. Store specimens at 2-8°C and ship overnight to CDC on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to CDC on dry ice
- Storing Specimens
  - Specimens can be stored at 2-8°C for up to 72 hours after collection.

- If a delay in extraction is expected, store specimens at -70°C or lower.
- Extracted nucleic acid should be stored at -70°C or lower.

Procedure

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor and in accordance with the Company's policies and Procedures.

<table>
<tr><th colspan="2">SPECIMEN TRANSFER PROCEDURE (to be performed in Extraction Room)</th></tr>
<tr><td>1.</td><td>Total nucleic acids (DNA and RNA) are extracted from patient specimens and assay controls using the Roche MagNA Pure 96 DNA and Viral NA Small Volume kit and the Roche MagNA Pure 96 System. Refer to PROC.129.01298- Nucleic Acid Isolation on the MagNA Pure 96 Instrument for general instructions on using the MagNA Pure 96 Instrument.</td></tr>
<tr><td>2.</td><td>Decontaminate work area by wiping down work surface and pipettes with 10% bleach. Let soak for 1 minute. Wipe down work surface and pipettes with 70% ethanol and dry with paper towels.</td></tr>
<tr><td>3.</td><td>Remove one aliquot of NCOV Positive Control and NCOV Negative Control from the freezer and let thaw at room temperature. Vortex and spin down.</td></tr>
<tr><td>4.</td><td>Create MagNA Pure and AB7500 setup maps for the samples to be tested. 94 patient specimens can be run in a single set-up, along with 2 controls (1 Positive Control and 1 Negative control per batch). Example of a MagNA Pure set-up map is provided below.
<table>
<tr><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th></tr>
<tr><th>A</th><td>Pos</td><td>8</td><td>16</td><td>24</td><td>32</td><td>40</td><td>48</td><td>56</td><td>64</td><td>72</td><td>80</td><td>88</td></tr>
<tr><th>B</th><td>1</td><td>9</td><td>17</td><td>25</td><td>33</td><td>41</td><td>49</td><td>57</td><td>65</td><td>73</td><td>81</td><td>89</td></tr>
<tr><th>C</th><td>2</td><td>10</td><td>18</td><td>26</td><td>34</td><td>42</td><td>50</td><td>58</td><td>66</td><td>74</td><td>82</td><td>90</td></tr>
<tr><th>D</th><td>3</td><td>11</td><td>19</td><td>27</td><td>35</td><td>43</td><td>51</td><td>59</td><td>67</td><td>75</td><td>83</td><td>91</td></tr>
<tr><th>E</th><td>4</td><td>12</td><td>20</td><td>28</td><td>36</td><td>44</td><td>52</td><td>60</td><td>68</td><td>76</td><td>84</td><td>92</td></tr>
<tr><th>F</th><td>5</td><td>13</td><td>21</td><td>29</td><td>37</td><td>45</td><td>53</td><td>61</td><td>69</td><td>77</td><td>85</td><td>93</td></tr>
<tr><th>G</th><td>6</td><td>14</td><td>22</td><td>30</td><td>38</td><td>46</td><td>54</td><td>62</td><td>70</td><td>78</td><td>86</td><td>94</td></tr>
<tr><th>H</th><td>7</td><td>15</td><td>23</td><td>31</td><td>39</td><td>47</td><td>55</td><td>63</td><td>71</td><td>79</td><td>87</td><td>Neg</td></tr>
</table></td></tr>
<tr><td>5.</td><td>For viscous samples (sputum and bronchial wash): Performed inside BSC 2

▪ Add ~ 250 µl of sample to a metal bead tube.
▪ Add 350 µL PBS to each tube containing viscous specimen.
• Vortex tubes for about 20 seconds, repeat if needed.
• Quick spin to deposit debris in the bottom of the tube.

Alternative method:
• Rehydrate Thermo Scientific™ Pierce™ DTT (Dithiothreitol by adding 100 µl or nuclease- free water to one microtube containing DTT and gently mix with pipette tip to completely dissolve (500mM final concentration).
• Add the entire 100 µl of freshly prepared DTT to 5 mL of cold Sterile 0.01M PBS (pH 7.2) and mix briefly. In a microcentrifuge tube, add the diluted DTT solution to an equal volume of specimen (e.g. 250 µL of fresh 500mM DTT solution to 250 µL of sample). Note: Use DTT immediately. Discard any unused reconstituted DTT.
• Incubate at room temperature with intermittent mixing until the sample is liquified (up to 30 minutes).
• Liquefied specimen can be used for downstream nucleic acid extraction.</td></tr>
<tr><td>6.</td><td>For controls and all other patient specimens, first pipette 250 ul MagNA Pure External Lysis Buffer into the appropriate well of a MagNA Pure 96 Processing Cartridge. Next, add 200 µL of controls and patient specimens, mixing by pipet 3-5 times after each addition.</td></tr>
</table>

SPECIMEN TRANSFER PROCEDURE (to be performed in Extraction Room)	
7.	Visually check the level of samples and controls in the MagNA Pure cartridge to ensure sample was added to the appropriate wells.
8.	Cover the MagNA Pure cartridge with an absorbent wipe and put into a clean biohazard bag then seal before transporting to the MagNA Pure 96 instrument.

MagNA Pure 96 Nucleic Acid Isolation	
1.	Refer to PROC.129.01298, Nucleic Acid Isolation on the MagNA Pure 96 Instrument for general instructions on using the MagNA Pure 96 Instrument
2.	All of the following steps are performed in the Specimen Preparation Area.
3.	Perform beginning of run maintenance on the MagNA Pure 96 instrument (as described in PROC.129.01298).
4.	In the Overview tab, select Enter Order, and select “External Lysis 450 µL ”.
5	The following parameters should be loaded: MagNA Pure Kit Name: “DNA/Viral NA SV 2.0” Protocol: “DNA BLOOD EXT LYS SV 3.1” Sample volume: 450 µL Elution volume: 50 µL Internal Control should be “IPC”
6.	Click on the “…” button next to the IPC dropdown. Scan the barcode on the vial of internal control. Note the fill volume (3.1mL) and number of samples in the batch. Set the expiration date.
7.	Set the total number of specimens and controls to match the current batch.
8.	Save the order to move to next screen.
9.	Refer to the software for the correct volumes and placement of the liquid reagents and disposable plastic supplies. Label the sample elution cartridge with the batch ID.
10.	Carefully place the loaded sample cartridge on the MagNA Pure 96 instrument.
11.	Confirm proper placement on the screen.
12.	Start the run by clicking **Start**.
13.	At the completion of the run cover the sample elution cartridge with an adhesive plate sealer and transfer the cartridge to the PCR set up area if processing immediately (within 30 minutes) or to frozen storage (< -70^{o}C) for up to one week.
14.	Tips and unused reagents may stay on the system for the next run. Cover reagents with a foil sealing cover if not using immediately.
15.	Perform end of run maintenance on MagNAPure 96 instrument as in PROC.129.01298.

Setting Up Real-Time RT-PCR Reactions	
1.	In the Reagent Room, thaw two vials of 2019-NCOV PCR MIX for every 96 samples (94 specimens + 2 controls) in the assay run**. DO NOT thaw on a heat block or on the blowers of a biosafety hood.**
2.	**Add 325 µl of 4X TaqPath Enzyme for every 455 µl tube of NCOV PCR MIX.** **Vortex to mix and spin down.**
3.	**KEEP 96 WELL PLATE CHILLED ON A COLD BLOCK.**

Setting Up Real-Time RT-PCR Reactions	
4.	To each well of the 96-well reaction plate, add 15 µL of 2019-NCOV PCR MIX. Transfer the 96-well Optical Reaction Plate to the PCR Setup Room. Example of an AB7500 set-up map is provided below.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Pos	8	16	24	32	40	48	56	64	72	80	88
B	1	9	17	25	33	41	49	57	65	73	81	89
C	2	10	18	26	34	42	50	58	66	74	82	90
D	3	11	19	27	35	43	51	59	67	75	83	91
E	4	12	20	28	36	44	52	60	68	76	84	92
F	5	13	21	29	37	45	53	61	69	77	85	93
G	6	14	22	30	38	46	54	62	70	78	86	94
H	7	15	23	31	39	47	55	63	71	79	87	Neg

5.	Add 10 µL of extracted nucleic acid (from patient specimens and controls) to the appropriate well of the 96-well Optical Reaction Plate. Note: Make sure to follow the Reaction Plate tray map.
6.	Cover the plate with Optical Adhesive Cover. Note: Make sure to handle the Optical Adhesive Cover on the edge only. Do not touch the middle part of the cover.
7.	Briefly centrifuge the plate to collect the reactions at the bottom of the wells and to eliminate any air bubbles.
8.	Take the covered reaction plate to the AB7500.
9.	Open up the "**7500 System Software**" program on computer.
10.	A new window will appear. Select "**Absolute quantification**" from the "**Assay Type**" drop-down menu. Select "**96-Well Clear**" from the "**Container**" drop-down menu. Select "COVID RNA TEMPLATE" from the "**Template**" menu. Click "**Finish**" at the bottom of the window. **The 2019-NCOV PCR MIX parameters are:** **NCOV1= FAM** **NCOV3=TAMRA** **IPC = Q670**
10.	Real-time RT-PCR parameters using the AB 7500 Stage 1: 50ºC for 15 min Stage 2: 95ºC for 2 min Stage 3: 95ºC for 15 sec; 55ºC for 35 sec; 50 cycles. Sample volume is set at 25 µL Choose **Standard** for correct thermal profile parameters.
11.	Review the run parameters to make sure they are correct. **The passive reference should be set to "ROX."**
12.	Go to the Well Inspector Menu (Double-click on 1 of the wells). Highlight the wells not being used and click on **"Omit"**. Wells not in use will then contain an X.
13.	Refer to PROC.129.028 for details on how to run the plate on the AB 7500 Real Time PCR systems.

Analyzing the Run Data, Exporting Results and Printing	
1.	When the run finishes, click OK from the window.

Analyzing the Run Data, Exporting Results and Printing	
2.	Click on “Analysis”, and then click on “Analysis Settings”. **For 2019-NCOV PCR MIX** **Manual Ct should be selected** **Set the threshold at 0.1 for NCOV1, automatic baseline** **Set the threshold at 0.01 for NCOV3, automatic baseline** **Set the threshold at 0.05 for IPC, automatic baseline**
3.	Click the Analyze icon (□) from the toolbar. *Note: Wait approximately one minute for the analysis process to be completed.*
4.	Click the Results Tab.
5.	Click the Amplification Plot tab.
6.	Choose NCOV1, NCOV2, and IPC from the Detector window.
7.	Click one well containing a specimen at a time and look at the Amplification plot and Component plot to check for the accuracy of the result.
8.	Choose Save option from the File menu after analyzing all the wells.
9.	To export the results to the LIS, start by highlighting the wells containing NCOV1, NCOV3, and IPC reactions from the plate grid.
10.	From the File menu, choose Export and then Results.
11.	Select “Taqman on ‘samba **(**\\lis.focusdx.priv**)**’ M in the Look in window.
12.	Type in the name of the plate in the File Name window (e.g.: QINF 031918 EXPORT). *Note: Make sure to export the files as Text file (Tab-delimited file) and include the word “EXPORT” in the file name to help distinguish exported file from imported file.*
13.	Click the Save button. *Note: It will take approximately 15 minutes for LIS to download the results. Do not open the exported file while it is being transferred.*
14.	To print the results, choose Print from the File menu.
15.	Click Print and then click O.K.

NOTE: In the event that the test system becomes inoperable, notify supervision or designee for further direction. Patient specimens must be stored in a manner that maintains the integrity of the specimen.

Interpretation of Results and Reporting

Review patient results for unusual patterns, trends or distributions in patient results, such as an unusually high percentage of abnormal results, or unusually high percentage of non-reactive, or indeterminate, or reactive results. Computer aided tools should be used when available. Refer to SOP Quality Control Program and Molecular Infectious Diseases Department. Real-Time Group Results Review and Release Process.

Report atypical or unexpected results or trends for this test to appropriate supervisory personnel, prior to releasing results.

- When all controls exhibit the expected performance (Acceptance Criteria for Controls), a specimen is considered negative if all SARS-CoV-2 markers (N1, N3) cycle threshold amplification curves do not cross the threshold and the IPC amplification curve does cross the threshold line within the acceptance range.

- When all controls exhibit the expected performance, a specimen is considered Detected for SARS-CoV-2 if all markers (N1, N3) cycle threshold amplification curves cross the threshold line (<40.00 Ct). The IPC may or may not be positive as described above, but the SARS-CoV-2 result is still valid.

- When all controls exhibit the expected performance and the amplification curves for the SARS-CoV-2 markers (N1, N3) and the IPC amplification curve does not cross the threshold line within the acceptance range, possible PCR inhibition has occurred for the specimen. Specimen should be re-tested. If upon repeat testing the same situation occurs the patient result is reported as “Indeterminate due to inhibition” (TNP1146).

- When all controls exhibit the expected performance and the cycle threshold amplification curve for any one or two markers, (N1, N3) but not all two crosses the threshold line (< 40.00 Ct), the result is inconclusive for SARS-CoV-2. The sample should be rerun. If upon repeat testing the same situation occurs the patient result is reported as “Inconclusive”.

Specimen Result Interpretation*				
nCoV-N1	**nCoV-N3**	**IPC**	**Interpretation**	**Actions**
ND	ND	Within +/- 3 Ct of Negative Control	**NOT DETECTED**	None
DET	DET	Not Applicable (+/-)	**DETECTED**	Store Samples at -70 to refer to the appropriate Public Health laboratory if requested.
Only one of two SARS-nCoV-2 targets are Detected		Not Applicable (+/-)	**INCONCLUSIVE**	Repeat extraction and RT-PCR. If the repeated result remains Inconclusive, Store Samples at -70 to refer to the appropriate Public Health laboratory if requested.
ND	ND	Undetermined or IPC out of range (>3Ct)	**Indeterminate due to inhibition**	Repeat extraction and RT-PCR. If upon repeat testing the same situation occurs the patient result is reported as “Unable to report” due to inhibition (TNP1146).

Quality Control

Run/assay acceptability criteria:

One replicate of the positive control and one replicate of the negative control are tested in each batch. Each control is processed as a sample, through nucleic acid isolation and amplification/detection. Controls results (detection cycle or Ct) are generated for the two SARS-CoV-2 targets, and the Internal Control target. Acceptable control results for the SARS-CoV-2 and internal control are required for the run to be acceptable. An example of acceptable control results is shown in the table below. If the Positive Control criteria are not met, the batch is invalid and all specimens must be repeated. If the Negative Control has a Ct value $\leq$ 40.00 (and has a valid amplification curve) for one or more of the SARS-CoV-2 targets, then this control is invalid. This indicates possible contamination of prepared samples. Positive patient results cannot be reported. Positive specimens on this run must be repeated. Negative specimens may be reported given that all other assay run criteria are met.

Examples of Acceptance Criteria for Controls (Detection cycle target ranges for controls)			
Control	nCoV-N1	nCov-N3	IPC
nCoV Positive	26.85-32.85	26.20-32.20	26-32*
nCoV Negative	Not Detected	Not Detected	26-32*

*Acceptance range for IPC is determined by negative control value in each run ± 3 Ct

Limitations

1. All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently. Quest will limit the distribution of this device to only those users who have successfully completed training provided by Quest.
2. Performance of the test has only been established in upper and lower respiratory specimens (such as nasopharyngeal or oropharyngeal swabs, sputum, tracheal aspirates, and bronchoalveolar lavage/wash).
3. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
4. A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is moderate to low.
5. Do not use any reagent past the expiration date.
6. If the virus mutates in the rRT-PCR target region, SARS-CoV-2 may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result. An interference study evaluating the effect of common cold medications was not performed.
7. Test performance can be affected because the epidemiology and clinical spectrum of infection caused by 2019-nCoV is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and when during the course of infection these specimens are most likely to contain levels of viral RNA that can be readily detected.
8. Quest Diagnostics did not independently evaluate Specimen Stability and Fresh-frozen Testing. Quest Diagnostics adopted standard practices recommended by the CDC EUA.
9. Quest Diagnostics did not perform an interfering substances study. The assay uses conventional well-established nucleic acid extraction methods and based on our experience with other similar assays, e.g. Influenza A and B Real-Time PCR. We do not anticipate interference from common endogenous substances. Interference studies have not been performed for this assay.
10. Quest Diagnostics did not independently evaluate *in silico* sensitivity or specificity. Quest Diagnostics adopted the evaluation performed by the Centers for Disease Control and Prevention.

Conditions of Authorization for the Laboratory

The Quest SARS-CoV-2 rRT-PCR test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#coronavirus2019
To assist clinical laboratories running the test, the relevant Conditions of Authorization are listed verbatim below, and are required to be met by laboratories performing the EUA test.

- Authorized laboratories[1] using Quest SARS-CoV-2 rRT-PCR test will include with result reports of the COVID-19 RT-PCR 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 Quest SARS-CoV-2 rRT-PCR test will perform the COVID-19 RT-PCR Test as outlined in the COVID-19 RT-PCR test 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 COVID-19 RT-PCR Test are not permitted.

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

- Authorized laboratories using Quest SARS-CoV-2 rRT-PCR test will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

- Authorized laboratories will 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 Quest Diagnostics (XXXXXXX) 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.

- Quest Diagnostics and authorized laboratories using Quest SARS-CoV-2 rRT-PCRT test will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1]Authorized Laboratories: For ease of reference, the Letter of Authorization refers to "Quest Diagnostic Laboratories or other laboratories designated by Quest Diagnostics that are also certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests" as "authorized laboratories."

Performance Characteristics

1) Limit of Detection

The Limit of Detection (LOD) is defined as the lowest SARS-CoV-2 RNA concentration that is successfully detected with a probability of 95% or greater. Sensitivity standards were prepared by serially diluting the SARS-CoV-2 viral RNA transcript containing an 1100 nucleotide region from the N gene in stabilizing buffer RNA Diluent P to the following concentrations: 2,580, 968, 363, 136, and 51 copies/mL. RNA was quantified by an RNA fluorometric method (Qubit HS Assay). The LOD was evaluated by testing the sensitivity standards over three separate runs using the SARS-CoV-2 RNA Qualitative RT-PCR assay. In each run, 7 replicates at each concentration level were purified using the MagNA Pure 96 and each replicate was then tested in the ABI 7500 to yield a total of 21 replicate results at each concentration level.

The Limit of Detection study results are shown in the table below. The concentration of SARS-CoV-2 RNA that was successfully detected with at least a 95% detection rate was calculated as 136 copies/mL for nCoV-N1 and nCoV-N3 primer/probe sets. The LOD of the test is established at 136 copies/mL.

Table 1. Sensitivity Results for nCoV RNA Qualitative RT-PCR

			nCoV N1		nCoV N3	
sample ID	nCoV copies/mL	nCoV log copies/mL	mean Ct	detection rate*	mean Ct	detection rate*
LOD 1	2,580	3.41	30.43	100%	29.77	100%
LOD 2	968	2.99	31.95	100%	31.02	100%
LOD 3	363	2.56	33.31	100%	32.44	100%
LOD 4	**136**	2.13	34.88	**95%**	34.27	**100%**
LOD 5	51	1.71	35.85	81%	34.93	86%

* samples with nCoV Ct < 40.00 cycles are considered detected (positive), nCoV Ct ≥ 40.00 are considered not detected (negative)

2) *In silico* inclusivity testing.

Quest Diagnostics is using the same sequences as CDC therefore, additional *in silico* studies were not performed. CDC performed an alignment with the oligonucleotide primer and probe sequences of the CDC 2019 nCoV Real-Time RT-PCR Diagnostic Panel with all publicly available nucleic acid sequences for 2019-nCoV in GenBank as of February 1, 2020 to demonstrate the predicted inclusivity of the CDC 2019 nCoV Real-Time RT-PCR Diagnostic panel. All the alignments showed 100% identity of the CDC panel to the available 2019-nCoV sequences with the exception of one nucleotide mismatch with the N1 forward primer in one deposited sequence. Similarly, a single mismatch is observed in the alignment of the N3 probe. The risk assessment of these single mismatches resulting in a significant loss in reactivity, and false negative result, is low due to the design of the primers and probes with melting temperatures > 60°C and run conditions of the assay with annealing temperature at 55°C to tolerate one to two mismatches.

3) Cross-reactivity

Organisms in the a commercially available Respiratory Verification Panel were extracted and tested with the Quest SARS-CoV-2 Real-Time RT-PCR assay to demonstrate analytical specificity and exclusivity. The commercially available panel comprised of 22 individual inactivated respiratory related pathogens (purified, intact virus particles and bacterial cells) manufactured specifically for use as positive controls in nucleic acid tests. There was no cross-reactivity observed for any of the tested pathogens.

Pathogen	Strain
Human coronavirus 229E	229E
Human coronavirus OC43	OC43

Human coronavirus HKU1	HKU1
Human coronavirus NL63	NL63
Adenovirus (e.g. C1 Ad. 71)	Type 3
Human Metapneumovirus	8, Peru6-2003
Parainfluenza virus 1-4	Parainfluenza 1-4
Influenza A	A/Brisbane/10/07
Influenza B	B/Florida/02/06
Respiratory syncytial virus	A
Rhinovirus	1A
Chlamydia pneumoniae	M129
Bordetella pertussis	A639
Mycoplasma pneumoniae	M129

4) Interfering substances study

The assay uses conventional well-established nucleic acid extraction methods and based on our experience with other similar assays, e.g. Influenza A and B Real-Time PCR. We do not anticipate interference from common endogenous substances. Interference studies have not been performed for this assay.

5) *In silico* cross-reactivity testing

Cross-reactivity is defined as the amplification and detection of related viruses or other pathogens by the SARS-CoV-2 RNA Qualitative RT-PCR assay. CDC determined that the 2019-nCoV rRT-PCR assay N1 and N3, designed for the detection of SARS-CoV-2, showed no significant combined homologies with human genome, other coronaviruses (with the exception of N3 with SARS homology), or human microflora that would predict potential false positive rRT-PCR results. The N3 RT-PCR is expected to cross-react with human SARS coronavirus and bat SARS-like coronaviruses. Quest Diagnostics is using the same sequences as CDC, therefore, additional *in silico* studies were determined to be unnecessary.

6) Specimen Stability and Fresh-frozen Testing

Quest Diagnostics intends to follow the CDC's specimen collection and transport guidance contained in CDC EUA IFU under Specimen Collection, Handling, and Storage and the CDC website for guidance on specimen collection handling and storage (https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html).

7) Inter-assay Precision

Inter-assay precision is defined as the reproducibility of a sample between assay runs and was evaluated by testing 3 replicates of 3 separate precision standards in three separate runs using the SARS-CoV-2 RNA Qualitative RT-PCR assay. The precision standards were aliquots of the same standards prepared as described in the Intra-assay Precision section. Precision standard replicate CV's ranged from 0.1% to 0.7%, with mean overall inter-assay precision equal to 0.3%. The individual precision standard replicate results were within 0.20 detection cycles of their respective mean values.

8) Intra-assay Precision

Intra-assay precision is defined as the reproducibility of a sample within an assay run and was evaluated by testing 3 replicates of 3 separate precision standards in a single run using the SARS-CoV-2 RNA Qualitative RT-PCR assay. The precision standards were prepared by diluting the SARS-CoV-2 viral RNA transcript containing an 1100 nucleotide region from the N gene in stabilizing buffer RNA Diluent P to final concentrations of 44,000 copies/mL (high), 13,200 copies/mL (mid), and 5,657 copies/mL (low). Aliquots of each standard were prepared and stored frozen until the time of testing. Precision standard replicate CV's ranged from 0.0% to 0.4%, with mean overall intra-assay precision equal to 0.3%. The individual precision standard replicate results were within 0.13 detection cycles of their respective mean values. The intra-assay precision data is included in the table below.

9) Clinical Evaluation:

The clinical evaluation consisted of 30 SARS-COV-2 RNA-positives and 30 SARS-COV-2 RNA-negatives (negatives were RNA Diluent P buffer). SARS-COV-2 RNA-positives consisted of 24 virus-positive RNA preparations derived from clinical specimens, with 6 randomly selected and run in duplicate for a total of 30 positives. RNA preparations were obtained from a well-established clinical laboratory located in the Republic of Korea (originating lab) and consisted of 12 paired extracted patient samples from both an upper respiratory (NP/OP swabs) and lower respiratory source (sputum). Extraction from patient specimens was performed using the MagNA Pure 96 system and MagNA Pure 96 DNA and Viral NA Small Volume Kit. Amplification was performed using a RT-PCR kit commercially available in the Republic of Korea to identify the paired rRT-PCR positives.

The samples were randomized, blind-labeled and tested using the SARS-CoV-2 RNA Qualitative RT-PCR assay. Considering the SARS-CoV-2 RNA positive RNA extracts would degrade during the nucleic acid extraction step of the assay only the RT-PCR amplification and detection step was performed in this study. RT-PCR Mix 1 and RT-PCR Mix 2 were formulated to include the RNA internal positive amplification control (RIPC) at a final concentration that is comparable to the expected concentration in MP96-extracted preparations. There was 100% agreement (30/30, 95% CI 88.7-100%) for the positive samples and 100% agreement for the negative samples (30/30, 95% CI 88.7-100%).

		Comparator RT-PCR Test	
		Positive	Negative
Quest SARS-CoV-2 rRT-PCR Test	Positive	30	0
	Negative	0	30

Positive Percent Agreement: 100% (95% CI 88.7-100%)
Negative Percent Agreement: 100% (95% CI 88.7-100%)
Overall Agreement: 100% (95% CI 93.98-100%)

10) Specificity in a Presumed Negative Population (Upper Respiratory)

Quest Diagnostics randomly selected 72 presumed-negative nasopharyngeal/throat swabs submitted for respiratory pathogen testing in October 2019 and stored at < -10°C. One specimen was initially indeterminant, and upon re-peat testing, RNA was not detected. SARS-CoV-2 RNA was not detected in any of the samples tested for a specificity of 100% (72/72, 95% CI 95-100%).

11) Specificity in a Presumed Negative Population (Lower Respiratory)

Quest Diagnostics randomly selected 30 presumed-negative lower respiratory specimens (and one upper respiratory specimen) submitted for respiratory pathogen testing during January and early February 2020, including 22 BAL specimens, 8 sputum specimen remnants, and one M4 swab specimen. The sputum and M4 swab remnants were tested in duplicate and the first result was used for the analysis. SARS-CoV-2 RNA was not detected

in any of the replicates tested. One of the sputum specimens had invalid results for the internal control in all RT-PCR reactions (replicates were out of range by about 1 cycle). The invalid sputum sample was noted to be highly mucopurulent in both the raw and pre-processed states, possibly causing the inhibitory result. Excluding the one invalid result, the specificity with presumed negative lower respiratory specimens was 100% (29/29, 95%CI 88.1-100%).

12) Post-CLIA Validation Confirmation with a Public Health RT-PCR

After the assay's CLIA validation was completed and the clinical laboratory testing service was made commercially available, Quest Diagnostics sent the first five positive specimens and the first five negative specimens that had been submitted for clinical testing to a county public health laboratory located in Southern California for confirmation testing with a CDC-based RT-PCR. The public health laboratory results agreed with the Quest assay results: 100% (5/5, 95% CI 47.8-100%) agreement with the positives and 100% (5/5, 95% CI 47.8-100%) agreement with negatives.

13) Comparison with the Prior Version of the Quest SARS-CoV-2 rRT-PCR test (n = 460)

Quest Diagnostics selected a total of 460 de-identified specimens from its clinical laboratory testing runs and compared the new version of the assay containing N1 and N3 targets ("New RT-PCR") versus the initial version of the test containing the N1 and N2 targets ("Comparator RT-PCR"). using the same extracted specimen. For each specimen, the N1 and N3 targets were performed together in a well, and the N2 target was performed in a separate well. Of the 460 specimens, the results for the Comparator RT-PCR was 35 detected, 421 undetected, and four inconclusive. Of the 35 specimens that were detected in the Comparator RT-PCR, the New RT-PCR agreed 100% (35/35, 95%CI 90.0-100%). Of the 421 specimens that were undetected in the Comparator RT-PCR, the New RT-PCR agreed 100% (421/421, 95%CI 99.1-100%). Of the four specimens that were inconclusive in the Comparator RT-PCR, three of the four were detected and one was inconclusive in the New RT-PCR.

Comparison with the Prior Version of the Quest SARS-CoV-2 rRT-PCR test (n = 460)

New RT-PCR (N1+N3)	Comparator RT-PCR (N1+N2)		
	Detected	Inconclusive	Not Detected
Detected	35	3	0
Inconclusive	0	1	0
Not Detected	0	0	421

References

Contact Information, Laboratory Service Ordering, and Support

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