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.

This test is also for use with anterior nasal swab specimens that are collected using the Quest Diagnostics Collection Kit for COVID-19 when used consistent with its authorization.

This test is for the qualitative detection of nucleic acid from the SARS-CoV-2 in pooled samples containing up to four of the individual upper respiratory swab specimens (nasopharyngeal, mid-turbinate nasal, anterior nasal or oropharyngeal swabs) that were collected in individual vials containing transport media from individuals suspected of COVID-19 by their healthcare provider. Negative results from pooled testing should not be treated as definitive. If patient's clinical signs and symptoms are inconsistent with a negative result or results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive, inconclusive, or invalid result must be tested individually prior to reporting a result. Specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.

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 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. The agent detected may not be the definite cause of disease. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information. Specimens that are collected will not be tested with an internal control to confirm that the specimen was properly collected. Collected specimens from SARS-CoV-2 positive individuals may yield negative results if the specimen was not collected properly.

Testing is limited to laboratories designated by Quest Diagnostics that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, and meet the requirements to perform high complexity tests.

Testing with the Quest 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 Quest 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.

In sample pooling, specimens are identified from populations based on positivity rate (for example, by county, zip code or by client), and up to four patient specimens are combined into a pool, and the pool is tested as described in this package insert. If the pool is positive or inconclusive or invalid, then each of the constituent samples is re-tested as a separate individual specimen. If the pool is negative, then each constituent sample is reported as negative.

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)

- MagnaPure Extraction
  - MagNA Pure 96 DNA and Viral NA – Small Volume Kit Roche Diagnostics #06 543 588 001 (3 x 192 isolations)
  - MagNA Pure 96 External Lysis Buffer or other comparable lysis buffer that will be validated
- Omega Extraction
  - Mag-Bind Viral RNA Xpress Kit (Omega Bio-Tek, Cat. M6219-2304)
- 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, Quest PBS Specimen Transport Tubes or other comparable transport medium that will be validated
- Poly (A)
- DEPC-water
- PBS, 1X
- DTT

Reagents:

nCoV 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.

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 µL** 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 reactions	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 (Magna Pure extraction)
- Hamilton MagEx STAR (Omega extraction)
- 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

Home Collection Kits

Quest Diagnostics Collection kit for COVID-19

Warnings and Precautions
1. For *in vitro* diagnostic use (IVD).
2. This product has not been FDA cleared or approved, but has been authorized by FDA under an Emergency Use

Authorization (EUA) for use by authorized laboratories; laboratories designated by Quest Diagnostics that are certified under CLIA, and meet the requirements to perform high complexity tests.

3. This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens
4. The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
5. Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
6. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
7. 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.
8. Specimen processing should be performed in accordance with national biological safety regulations.
9. 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.
10. 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 1-3 ml of viral transport media (or PBS or saline if better alternatives are not available)
- 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 on ice pack. If a specimen is frozen at -70°C or lower, ship overnight on dry ice
- Storing Specimens
  - Specimen stability after collection: 14 days at 18°C to 25°C, 14 days at 2°C to 8°C, 14 days at -10°C to -30°C, or at -70°C or lower.
  - 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.

Inspection of Returned Anterior Nasal Swab Specimens Collected Using the Quest Diagnostics Collection Kit for COVID-19

Specimens that are received through collection programs using the Quest Diagnostics Collection Kit for COVID-19 are checked for integrity of packaging, adequacy of sample, verification of patient information, and acceptable time window between specimen collection and receipt at the laboratory before entering the work flow (according to the Unobserved Collected Sample Processing for COVID-19 Molecular Testing Non-Technical Standard Operating Procedure (SOP)), please refer to the SOP for full sample processing procedures.

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. When performing pooling, laboratories will monitor sample pooling in accordance with Attachment 1 – Protocol for Monitoring of Sample Pooling Testing Strategies.

<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><td>A</td><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><td>B</td><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><td>C</td><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><td>D</td><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><td>E</td><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><td>F</td><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><td>G</td><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><td>H</td><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
a. Add ~ 250 µL of sample to a metal bead tube.
b. Add 350 µL PBS to each tube containing viscous specimen.
c. Vortex tubes for about 20 seconds, repeat if needed.
d. Quick spin to deposit debris in the bottom of the tube.
<u>Alternative method:</u>
a. Rehydrate Thermo Scientific Pierce DTT (Dithiothreitol by adding 100 µ or nuclease- free water to one microtube containing DTT and gently mix with pipette tip to completely dissolve (500mM final concentration).
b. Add the entire 100 µL of freshly prepared DTT to 5 mL of cold Sterile 0.01M PBS (pH 7.2) and mix briefly.
c. 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.
d. Incubate at room temperature with intermittent mixing until the sample is liquified (up to 30 minutes).
e. Liquefied specimen can be used for downstream nucleic acid extraction.
f.</td></tr>
<tr><td>6</td><td>For sample pooling, first pipette 250 µL MagNA Pure External Lysis Buffer into the appropriate well of a MagNA Pure 96 Processing Cartridge. For sample pooling, add equal amounts of each specimen (for 4 specimens, add 50 µL each) pipette up and down at least once after each addition (performed in BSC 2). The decision to pool specimens should be based on the positivity rate of the location. Pooling is permitted for NP, OP, AN and MT swabs. For laboratories considering pooling, please see Attachment 1 for monitoring requirements</td></tr>
<tr><td>7</td><td>For controls and all other patient specimens that are not run in a pool, first pipette 250 µL 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 and pooled specimens, mixing by pipette at least once after each addition (performed in BSC 2).</td></tr>
<tr><td>8</td><td>Visually check the level of samples and controls in the MagNA Pure cartridge to ensure sample was added to the appropriate wells.</td></tr>
<tr><td>9</td><td>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.</td></tr>
</table>

MagNA Pure 96 Nucleic Acid Extraction	
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°C or colder) 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.

	SPECIMEN TRANSFER PROCEDURE for the Omega Method using the HAMILTON STAR (to be performed in Extraction Room)
1.	Total nucleic acids (DNA and RNA) are extracted from patient specimens and assay controls using the Mag-Bind Viral RNA Xpress Kit (Omega Bio-Tek) on the Hamilton MagEx STAR Autoload automated platform. Refer to PROC.129.01744- *Hamilton MagEx STAR Autoload System Use and Maintenance* for general instructions on using the Hamilton STAR.
2.	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.
3.	Remove one aliquot of NCOV Positive Control and NCOV Negative Control from the freezer and let thaw at room temperature. Vortex and spin down.
4.	Create Hamilton and AB7500 setup maps for the samples to be tested. For example: 94 patient specimens can be run in a single set-up, along with 2 controls (1 Positive Control, 1 Negative, and 94 patient specimens per 96 wells).

	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

	Rotating positional control based on the load number will be used for specific identification of the extraction plate and prevention of plate switch. Refer to additional preventive measures on PROC.129.01038 and PROC.129.01611
5.	For controls and **upper respiratory** patient specimens, pipette **240 uL Hamilton Lysis Buffer** followed by **200 uL of specimen** into the appropriate well of a Hamilton deep well plate. ***Note: The Hamilton is not for use with Sputum or BAL specimens.***
6.	Visually check the level of samples and controls in the deep well plate to ensure sample was added to the appropriate wells.
7.	Cover the deep well plate with an absorbent wipe and put into a clean biohazard bag then seal before transporting to the Hamilton STAR.
8	Label the load name on the extraction and output to each corresponding plate that will be loaded on the HamiltonCheck the position of the corresponding plates on the Hamilton deck.

	Omega Method using the Hamilton STAR Nucleic Acid Isolation
1.	Refer to PROC.129.01744, *Hamilton MagEx STAR Autoload System Use and Maintenance* for general instructions on using the Hamilton STAR.
2.	All of the following steps are performed in the Specimen Preparation Area.
3.	Perform daily maintenance on the Hamilton instrument.
4.	In the Method Launcher desktop application located on the desktop, click on the "Omega extraction" tab, and select the number of plates to be run (up to 4 plates). If a partial plate is run, the instrument will process as full.
5.	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.
6.	Confirm proper placement on the screen.
7.	Start the run by clicking the green arrow located in the upper left-hand corner of the application .
8	Double check the accuracy of the matching load number across the extraction and output plates.
9.	At the completion of the run cover the sample elution plate with an adhesive plate sealer and store at 2-8°C until needed for PCR set-up.
10.	Tips and unused reagents may stay on the system for the next run. Cover reagents with a foil sealing cover if not using immediately.
11.	Perform end of run maintenance on the Hamilton STAR. Start the UV Decontamination by clicking on the "StarUVLight Software" prompt located on the desktop. Set time for 30 minutes between each run. The UV Decontamination must be performed once each 8-hour shift for a full 60 minutes on instruments used for the SARS-CoV-2 assay.

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.**

4. To each well of the 96-well reaction plate, add 15 µL of 2019-NCOV PCR MIX as needed. 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. 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=TAM**
   - **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	
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, NCOV3, 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* (for specimens that are not pooled)				
nCoV-N1	**nCoV-N3**	**IPC**	**Interpretation**	**Actions**
ND	ND	Within +/- 3 Ct of Negative Control	**NOT DETECTED**	Report to public health authorities.
DET	DET	Not Applicable (+/-)	**DETECTED**	Report to public health authorities. Store Samples at -70°C or colder 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°C or colder to refer to the appropriate Public Health laboratory if requested.
ND	ND	Undetermined or IPC out of range (>3Ct)	**INVALID**	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).

Specimen Result Interpretation for Pooled Specimens				
nCoV-N1	**nCoV-N3**	**IPC**	**Interpretation**	**Actions**
ND	ND	Within +/- 3 Ct of Negative Control	**NOT DETECTED**	Report to public health authorities.
DET	DET	Not Applicable (+/-)	**POOLED POSITIVE – DO NOT REPORT**	Repeat each constituent specimen in the pool as a separate unpooled specimen.
Only one of two SARS-nCoV-2 targets are Detected		Not Applicable (+/-)	**POOLED INCONCLUSIVE – DO NOT REPORT**	Repeat each constituent specimen in the pool as a separate unpooled specimen.
ND	ND	Undetermined or IPC out of range (>3Ct)	**INVALID**	Repeat each constituent specimen in the pool as a separate unpooled specimen.

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 ≤ 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 SARS-CoV-2 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.
11. The performance of this test was established based on the evaluation of a limited numberof clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
11. Samples should only be pooled when testing demand exceeds laboratory capacity and/or when testing reagents are in short supply
12. Sample pooling has only been validated using upper respiratory swab specimens.
13. Specimens that are collected using the Quest Diagnostics Collection Kit for COVID-19 will not be tested with an internal control to confirm that the specimen was properly collected. Specimens collected using the Quest Diagnostics Collection Kit for COVID-19 from SARS-CoV-2 positive individuals may yield negative results if the specimen was not collected properly.
14. The requirement to run a sample adequacy control for all samples that were collected using the Quest Diagnostics Collection Kit for COVID-19 when unobserved by a healthcare professional will be waived provided that the following disclosure has been acknowledged by the entity utilizing the Quest Diagnostics Collection Kit for COVID-19 (or notified of the disclosure via contractual notice):

> Acknowledgement
>
> (Insert Client name) acknowledges it has received the disclosure below:
>
> *Specimens that are self-collected using the Quest Diagnostics Collection Kit for COVID-19 will not be tested with an internal control to confirm that the specimen was properly collected. Specimens collected using the Quest Diagnostics Collection Kit for COVID-19 from SARS-CoV-2 positive individuals may yield negative results if the specimen was not collected properly.*

Note that acknowledgements consistent with prior authorized labeling and made prior to October 1, 2021 are also effective.

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 other authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas
To assist clinical laboratories running the test, the relevant Conditions of Authorization are listed below, and are required to be met by laboratories performing the EUA test.

- Authorized laboratories[1] using the Quest SARS-CoV-2 rRT-PCR test must include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

- Authorized laboratories using the Quest SARS-CoV-2 rRT-PCR test must use the Quest SARS-CoV-2 rRT-PCR test as outlined in the authorized labeling. 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 use the Quest SARS-CoV-2 rRT-PCR test are not permitted.

- Authorized laboratories testing authorized specimens collected using the Quest Diagnostics Collection Kit for COVID-19 must follow the Quest Diagnostics' "Unobserved Collected Sample Processing for COVID-19 Molecular Testing Non-Technical SOP," when accepting specimens for testing.

- Authorized laboratories testing authorized specimens collected using the Quest Diagnostics Collection Kit for COVID-19 must include in the test report for specific patients whose specimen(s) were collected without observation the following limitation: "*Specimens that are collected using the Quest Diagnostics Collection Kit for COVID-19 were not tested with an internal control to confirm that the specimen was properly collected. As such, unobserved collected specimens using the Quest Diagnostics Collection Kit for COVID-19 from SARS-CoV-2 positive individuals may yield negative results if the specimen was not collected properly*".

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

- Authorized laboratories must collect information on the performance of the Quest SARS-CoV-2 rRT-PCR test and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Quest Diagnostics (via email: michael.j.wagner@questdiagnostics.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the test of which they become aware.

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

- Quest Diagnostics and authorized laboratories using Quest SARS-CoV-2 rRT-PCR test must 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.

- Authorized laboratories using specimen pooling strategies when testing patient specimens with your product must include with negative test result reports for specific patients whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that "*In very rare cases, estimated at about 1 in 1,000 (0.1%) or less patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing*."

- Authorized laboratories implementing pooling strategies for testing patient specimens must use the "Protocol for Monitoring of Specimen Pooling Testing Strategies" recommendations available in the authorized labeling to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.

- Authorized laboratories must keep records of specimen pooling strategies implemented including type of strategy, date implemented, and quantities tested, and test result data generated as part of the "Protocol for Monitoring of Specimen Pooling Testing Strategies". For the first 12 months from the date of their creation, such records will be made available to FDA within 48 business hours for inspection upon request, and will be made available within a reasonable time after 12 months from the date of their creation.

[1]Authorized Laboratories: For ease of reference, the Letter of Authorization refers to "Laboratories designated by Quest Diagnostics that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, and meet the requirements 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 exps 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

16) Pooling Validation/ Sensitivity for Pools with One Positive Sample and Three Negative Samples (n = 101)

Quest Diagnostics evaluated the sensitivity of sample pooling using positive samples collected from three different populations with the following positivity rates: 0-3% (n=30), 3-6% (n=36) and 6-10% (n=35). The samples were sequentially selected from de-identified specimen remnants that had been previously tested individually using the Quest Diagnostics SARS-2-CoV RT-PCR molecular assay. Sample pools were made by combining one positive sample and three negative samples. Each pool was tested, and agreement with the individual sample result was calculated. Since any pool that does not yield negative results is re-tested individually, the positive percent agreement includes all pools that were not negative (i.e., positive, inconclusive, and invalid). Of the 30 pools in the 1-3% positivity rate group, 100% (30/30, 95%CI 88.7-100%) were not negative (30/30 were positive). Of the 36 pools in the 3-6% prevalence group, 100% (36/36, 95%CI 90.4-100%) were not negative (36/36 were positive). Of the 35 pools in the 6-10% prevalence group, 100% (35/35, 95%CI 90.1-100%) were not negative (33/35 were positive, and 2/35 were inconclusive). Overall in the study, none of 101 positive specimens would have been determined to be negative when tested in a pool of 4 samples (0/101, 95% CI 0.0-3.7%).

Sensitivity for Pools with One Positive Sample and Three Negative Samples (n = 101)

Positivity Rate Group	n	Results of Pooled Specimens				% Positive Percent Agreement*
		Negative	Inconclusive	Positive	Invalid	
1-3%	30	0	0	30	0	100% (30/30) 95%CI: (88.7-100%)

3-6%	36	0	0	36	0	100% (36/36) 95%CI 90.4-100%
6-10%	35	0	2	33	0	100% (35/35) 95%CI 90.1-100%
total	101	0	2	99	0	100% (101/101) 95%CI 96.3-100%

* Since any pool that is not negative is re-tested as an individual sample, the % Agreement includes all pools that were not negative (i.e., positive, inconclusive and invalid).

17) Pooling Validation – Efficiency with Pooled Negative Specimens (n = 247)

Quest Diagnostics evaluated efficiency of sample pooling using negative samples collected from three different populations with the following positivity rates: 1-3% (n=103), 3-6% (n=107) and 6-10% (n=37).The samples were selected sequentially from de-identified specimen remnants that had been previously tested individually using the Quest Diagnostics SARS-2-CoV RT-PCR molecular assay. Each 4-sample pool contained four negative samples. Each pool was tested and the percent of negative results for these pools was calculated. Of the 103 4-sample pools in the 1-3% prevalence group, 99.0% (102/103, 95%CI 94.9-99.8%) were negative, and 1/103 was inconclusive. Of the 107 4-sample pools in the 3-6% prevalence group, 99.1% (106/107, 95%CI 94.9-99.8%) were negative, and 1/107 was invalid. Of the 37 4-sample pools in the 6-10% prevalence group, 100% (37/37, 95%CI 90.6-100%) were negative.

Overall in the study, 99.2% (245/247, 95% CI 97.1-99.8%) of 4-sample pools with 4 negative samples were negative. Two pools would have had to be subsequently deconvoluted, with each sample being tested individually.

Efficiency with Pooled Negative Specimens (n = 247)

Positivity Rate Group	n	Results of 4-sample Pools				% Negative Percent Agreement *
		Negative	Inconclusive	Positive	Invalid	
1-3%	103	102	1	0	0	99.0% (102/103) 95%CI 94.9-99.8%
3-6%	107	106	0	0	1	99.1% (106/107) 95%CI 94.9-99.8%
6-10%	37	37	0	0	0	100% (37/37) 95%CI 90.6-100%
Total	247	245	1	0	1	99.2% (245/247) 95% CI 97.1-99.8%

* Since any pool that is not negative (i.e., positive, inconclusive and invalid) is re-tested as an individual sample, the parameter NPA affects the efficiency of 4-sample pooling

18) Pooling Validation – *In Silico* Sensitivity in Population with Positivity Rate 1%-10%

Quest Diagnostics conducted an *in silico* analysis to evaluate the effect of 4-sample pooling on the clinical sensitivity of the SARS-CoV-2 assay. This analysis was conducted using Passing-Bablok regression analyses from the "Pooling Validation / Sensitivity for Pools with One Positive Sample and Three Negative Samples (n = 101)" data to calculate the Ct shift resulting from the dilution effect of 4-sample pools (1 positive sample combined with 3 negative samples). In the regression analysis, the X-axis displayed individual Ct values for positive samples and the Y-axis displayed Ct values for the corresponding pools with one positive sample and 3 negative samples. This analysis was conducted in three populations with different positivity rates: 1-3% (n=820), 3-6% (n=1,113) and 6-10% (n=1,158). The de-identified data were selected from sequentially tested positives based on the Quest Diagnostics SARS-2-CoV RT-PCR molecular assay. The regression analysis was used to calculate an interval of Ct values [X*, 40] where individual samples with Ct values within this interval would have negative results in 4-sample pools (1 positive and 3 negative) due to dilution effects. For each population, the percent of individual samples with Ct values ranging from [X*, 40] was calculated. The X* values for the N1 target in the three populations were 37.0 (1-3%), 38.3 (3-6%) and 37.65 (6-10%). The X* values for the N3 target in the three populations were 37.45 (1-3%), 38.7 (3-6%) and 38.1 (6-10%).

Of the 820 samples in the 1-3% prevalence group, 100% (820/820, 95% CI 99.5-100%) of the samples would not have negative results in 4-sample pools: 97.3% were positive (798/820, 95% CI 96.0-98.3%), 2.7% were inconclusive (22/820, 95% CI 1.7-4.0%), and none were negative.

Of the 1,113 samples in the 3-6% prevalence group, 100% (1,113/1,113, 95% CI 99.7-100%) of the samples would not have negative results in 4-sample pools: 99.4% were positive (1,106/1,113, 99.4-99.8%), 0.6% were inconclusive (7/1,113, 95% CI 0.3-1.3%), and none were negative.

Of the 1,158 samples in the 6-10% prevalence group, 100% (1,158/1,158, 95% CI 99.7-100%) of the samples would not have negative results in 4-sample pools: 98.5% were positive (1,141/1,158, 95% CI 97.7-99.1%), 1.5% were inconclusive (17/1,158, 95% CI 0.9-2.3%), and none were negative.

Overall in the study, none of the 3,091 samples, if pooled, would have been incorrectly determined to be negative (0/3,091, 95%CI 0.0-0.12%).

In Silico Sensitivity in Population with Positivity Rate 1-10% (n = 3,091)

Group #	n	Interval [X*, 40] for N1	Number of samples with N1 Ct values in the interva	Interval [X*, 40] for N3	Number of samples with N3 Ct values in the interval	# above both shifted Thresholds	Neg	Inc	Pos	% Positive Percent Agreement*
1-3%	820	[37.0, 40]	22	[37.45, 40]	0	0	0	22	798	100% (820/820) 95% CI 99.5-100%
3-6%	1,113	[38.3, 40]	7	[38.7, 40]	0	0	0	7	1106	100% (1,113/1,113) 95% CI 99.7-100%
6-10%	1,158	[37.65, 40]	17	[38.1, 40]	0	0	0	17	1141	100% (1,158/1,158) 95% CI 99.7-100%
total	3,091	NA	46	NA	0	0	0	46	3045	100% (3,091/3,091) 95% CI 99.9-100%

* Since any pool that is not negative (i.e., positive, inconclusive, and invalid) is re-tested as individual samples, the Positive Percent Agreement includes all pools that were not negative.

19) Omega Extraction Validation - Limit of Detection

Remnant respiratory specimens that were previously negative for SARS-CoV-2 RNA using the Quest Diagnostics SARS-CoV-2 Assay and the Magna Pure Method in four different types of transport media were used in the Limit of Detection study. The four types of transport media were VCM/UTM, UTM-RT, saline (0.9% NaCl), PBS and Eswabs. Each pool was spiked with a positive specimen and serially diluted with corresponding transport media. Ten aliquots were extracted and amplified in duplicate. One run was repeated because the instrument malfunctioned. The Omega extraction LOD (95% detection rate) is summarized in the table below. The previously determined LOD for the Magna Pure Method is included for comparison.

Limit of Detection - Omega Method vs Magna Pure Method

Transport Media	SARS-CoV-2 RNA LoD			
	MagnaPure Method LOD (previously determined)		Omega LOD	
	≥95% Detection (c/mL)	Probit Analysis, 95% Detection (c/mL)	≥95% Detection (c/mL)	Probit Analysis, 95% Detection (c/mL)
VCM/UTM	100	100	250	184
UTM-RT	250	327	250	215
Saline	500	286	250	124
PBS	250	246	250	195
Eswab	500	368	500	394

20) Omega Extraction Validation - Agreement with the MagnaPure Extraction Method (n = 168)

Quest selected randomly 168 specimens that had been previously tested for SARS-CoV-2 RNA using the Quest Diagnostics SARS-CoV-2 Assay and the Magna Pure Method. Of the 168 specimens selected 78 were positive previously and 90 were negative previously. Specimens included a range of transport media types (VCM/UTM, UTM-RT, PBS, Saline (0.9% NaCl) and Eswabs), and Ct values. Results using the Omega method were compared to the previous results using the Magna Pure Method and percent agreement was calculated. The agreement was 98.7% (77/78, 95%CI 93.1-100%) with positive specimens, and 93.3% (84/90, 95%CI 86.0-97.5%) with negative specimens.

Agreement with the Magna Pure Extraction Method (n = 168)

Magna Pure Method	n	Omega Method				% Agreement
		Negative	Inconclusive	Positive	Invalid	
Positive	78	1	0	77	0	98.7% (77/78) 95%CI 93.1-100%
Negative	90	84	0	6*	0	93.3% (84/90) 95%CI 86.0-97.5%

* Quest Diagnostics performed an ad hoc discrepant analysis after the study and determined that 6/6 discordant specimens were low positives with the Omega extraction method.

References

Contact Information, Laboratory Service Ordering, and Support

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Attachment 1 – Protocol for Monitoring of Specimen Pooling Testing Strategies

Monitoring plan for use of pooling

Laboratories should evaluate the appropriateness of the pooling and pool size using the FDA recommended monitoring procedure described below. Laboratories may also consider the sensitivity of pooled testing based on the assay's Limit of Detection.

Ongoing assessment of positivity rate during application of the initial selected n-sample pooling strategy:

a. If historical data on testing individual samples from the laboratory is <u>available</u>:
   - The percent positivity rate, P_{pools}, should be updated daily using a moving average of the data from pooled samples from the previous 7-10* days. If P_{pools} is less than 85% of $P_{individual}$ ($P_{pools} < 0.85 \cdot P_{individual}$), then it is recommended that the pool size be adjusted to maximize pooling efficiency, according to the criteria in Table 1 below.
   - It is recommended that $n_{maxefficiency}$, using P_{pools} and Table 1 be re-assessed periodically while sample pooling is implemented by the laboratory to ensure maximum pooling efficiency

b. If historical data on testing individual samples from the laboratory is <u>unavailable</u>:
   - After initiating the pooling strategy, calculate the initial pooling positivity rate ($P_{pools\text{-}initial}$) for the first 7-10* days using a moving average of the data from n pool testing results.
   - If $P_{pools\text{-}initial}$ is greater than 25%, then Dorfman pooling of patient specimens is not efficient and should cease.
   - Following the first 7-10* day period of sample pooling, calculate the pooling positivity rate ($P_{pools\text{-}x}$) for the next 7-10* day period based on n pool testing results.
   - If $P_{pools\text{-}x}$ is less than 90% of $P_{pool\text{-}initial}$ ($P_{pools\text{-}x} < 0.90 \cdot P_{pools\text{-}initial}$), it is recommended that the pool size be adjusted to maximize pooling efficiency, according to the criteria in Table 1.
   - It is recommended that $n_{maxefficiency}$, using $P_{pools\text{-}x}$ and Table 14 be re-assessed periodically while sample pooling is implemented by the laboratory to ensure maximum pooling efficiency.

***** It is recommended that $P_{individual}$ be calculated from the previous 7-10 days, while P_{pools} and $P_{pools\text{-}x}$ are calculated from data collected during a 7-10 day time frame. However, when determining if 7-10 days is appropriate, take into consideration the laboratory testing volume and percent positivity, among other factors. Note that if the number of individual or pooled positive results collected during a given time frame is less than 10, $P_{individual}$, P_{pools} and $P_{pools\text{-}x}$ may not be representative of the percent positivity in the testing population and the laboratory may want to consider extending the testing time period to increase the chance of capturing positives.

Table 1 Efficiency of pooling based on prevalence

P, percent of positive subjects in the tested population	***$n_{maxefficiency}$ (n corresponding to the maximal efficiency)***	***Efficiency (F) of n-sample pooling (a maximum increase in the number of tested patients when Dorfman n- pooling strategy used)***
1% - 4%	6	4.44 - 2.60
5% - 6%	6	2.32 - 2.10
7% - 12%	6	1.92 - 1.42
13% - 25%	6	1.36 - 1.01
1% - 4%	5	4.02 - 2.60
5% - 6%	5	2.35 - 2.15
7% - 12%	5	1.98 - 1.49
13% - 25%	5	1.43 - 1.04
1% - 4%	4	3.46 - 2.50
5% - 6%	4	2.30 - 2.13
7% - 12%	4	1.99 - 1.54
13% - 25%	4	1.48 - 1.07
1% - 4%	3	2.75 - 2.23
5% - 6%	3	2.10 - 1.99
7% - 12%	3	1.89 - 1.53
13% - 25%	3	1.48 - 1.10
1% - 4%	2	1.92 - 1.73
5% - 6%	2	1.67 - 1.62
7% - 12%	2	1.57 - 1.38
13% - 25%	2	1.35 - 1.07