

Instructions for New Coronavirus Nucleic Acid Detection Kit

v 14.0

For Prescription Use Only.
For *In Vitro Diagnostic* Use.
For Use Under Emergency Use Authorization Only.

This manual is proprietary to Revvity, Inc., and intended only for customer use in connection with the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose without the prior written consent of Revvity. **Follow the protocol included with the kit.**

Table of Contents

Product Name	3
Kit Contents	3
Intended Use	3
Principles of the Assay	4
Kit Components and Packaging Specifications	4
Materials Required but Not Provided	5
Storage & Handling Requirements	7
Warnings and Precautions	7
Instruments	8
Collection, Storage & Shipment of Specimens	9
Assay Procedure	11
Nucleic Acid Extraction and PCR Setup	11
Extraction and PCR setup on Pre-NAT II	11
Extraction and PCR setup on chemagic 360.....	12
Extraction from saliva sample on chemagic 360	15
Extraction from transport media Mawi on chemagic 360.....	16
Extraction from saline on chemagic 360	16
Amplification	16
Interpretation of Results	23
Baseline and threshold setting for ABI 7500 Standard, ABI 7500 Fast Dx, QuantStudio 3 /5/7Flex/12K Flex	23
Baseline and threshold setting for qTower ³ / ³ G and qTower ³ 84 / ³ 84 G	24
Quality Control	26
Examination and Interpretation of Patient Specimen Results.....	27
Examination and Interpretation of Pooled Patient Specimen Results	27
Kit Limitations.....	28
Conditions of Authorization for the Laboratory	30
Assay Performance	31
Limit of Detection	31
LoD Using chemagic 360 for Extraction and Applied Biosystems 7500 PCR System	33
LoD Using chemagic 360 for Extraction and the Applied Biosystems Fast Dx, QuantStudio3, and QuantStudio 5 PCR Systems	35
LoD Using chemagic 360 for Extraction and the Analytik Jena PCR Systems	36
LoD study on saliva sample	37
LoD Using chemagic 360 for Extraction and the Applied Biosystems QuantStudio 12K Flex and QuantStudio 7 Flex PCR Systems with transport media Mawi and saline.....	38
Analytical Reactivity (Inclusivity).....	39
Analytical Specificity (Cross-reactivity)	41
Interfering Substances Studies	43
Clinical Study	44
Saliva Sample Stability Study	50
FDA SARS-CoV-2 Reference Panel Testing	50
References	51
Appendix A: Specimen Pooling Implementation and Monitoring Guidelines	53
Appendix B: Verification Recommendations	55
Appendix C: Additional Label	58

Product Name

New Coronavirus Nucleic Acid Detection Kit

Kit Contents

48 Tests

Intended Use

The New Coronavirus Nucleic Acid Detection Kit is a real-time RT-PCR *in vitro* diagnostic test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in oropharyngeal and nasopharyngeal swab specimens collected by a healthcare provider (HCP), and anterior nasal swab specimens collected by an HCP or self-collected under the supervision of an HCP from individuals suspected of COVID-19 by their HCP. This test is also for use with saliva specimens collected using the SalivaSecure Saliva Collection Kit either by an HCP or self-collected under the supervision of an HCP in a healthcare setting from individuals suspected of COVID-19 by their HCP. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

This test is also for the qualitative detection of nucleic acid from SARS-CoV-2 in pooled samples containing up to 5 individual upper respiratory swab specimens (i.e., oropharyngeal and nasopharyngeal swab specimens collected by an HCP and anterior nasal swab specimens collected by an HCP or self-collected under the supervision of an HCP) using individual vials containing transport media. Negative results from pooled testing should not be treated as definitive. If patient's clinical signs and symptoms are inconsistent with a negative result and results are necessary for patient management, then the patient should be considered for individual testing. Specimens included in pools with a positive 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. SARS-CoV-2 RNA is generally detectable in oropharyngeal and nasopharyngeal swab specimens and saliva 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 definitive 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. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.

The New Coronavirus Nucleic Acid Detection Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The New Coronavirus Nucleic Acid Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Principles of the Assay

The New Coronavirus Nucleic Acid Detection kit uses TaqMan- based real-time PCR technique to conduct *in vitro* reverse transcription of SARS-CoV-2 RNA, DNA amplification and fluorescence detection.

The assay targets specific genomic regions of SARS-CoV-2: nucleocapsid (N) gene and Open Reading Frame 1ab (ORF1ab) gene.

The TaqMan probes for the two amplicons are labeled with FAM and ROX fluorescent dyes respectively to generate target-specific signal.

The assay includes an RNA internal control (IC, bacteriophage MS2) to monitor the processes from nucleic acid extraction to fluorescence detection. The IC probe is labeled with VIC fluorescent dye to differentiate its fluorescent signal from SARS-CoV-2 targets.

The assay also uses a dUTP/UNG carryover prevention system to avoid contamination of PCR products and subsequent false positive results.

Kit Components and Packaging Specifications

Catalog Number: 2019-nCoV-PCR-AUS (48 tests/kit)

Component Name	Specifications & Loading		Main Ingredients	Storage Conditions
nCoV Reagent A	950 µL	×1 tube	Buffers, dNTPs, Mg ²⁺	-25 to -15°C
nCoV Reagent B	230 µL	×1 tube	TE buffer, primers, probes	-25 to -15°C
nCoV Enzyme Mix	170 µL	×1 tube	Taq DNA polymerase, MMLV, RNasin, UNG	-25 to -15°C

nCoV Internal Control	1.4 mL	×1 tube	TE buffer, bacteriophage MS2	-25 to -15°C
nCoV Positive Control	1.4 mL	×2 tubes	SARS-CoV-2 RNA fragments capsulated in bacteriophage	-25 to -15°C
nCoV Negative Control	1.4 mL	×2 tubes	TE buffer	-25 to -15°C

Notes: 1) The reference materials and other components in the kit should be treated as potential sources of infection. 2) The use of this kit should be strictly in accordance with the nucleic acid amplification guidelines to operate in compliance with the requirements of the appropriate laboratories. 3) The components in different batches of the kit cannot be used interchangeably.

Materials Required but Not Provided

1. RNA extraction reagents and instrument

The Nucleic Acid Extraction Kits (KN0212) and PreNAT II (SY61) (software version 1.00.06).

chemagic™ Viral DNA/RNA 300 Kit special H96 (CMG-1033, CMG-1033-S) and chemagic™ 360 (2024-0020) with chemagic™ Rod Head Set 96 (CMG-370) (software version 6.3.0.3).

2. PCR amplification instrument and software

- a. Applied Biosystems™ 7500 Real-Time PCR System: 4351104 with Laptop, 4351105 with desktop, software version 2.3.
- b. Applied Biosystems™ 7500 Fast Dx Real-Time PCR System: 4406984 with Laptop, 4406985 with desktop, software version 2.0.4.
- c. Applied Biosystems™ QuantStudio™ 3 Real-Time PCR System: 96-well block; REF: A28131, Design and Analysis Software v1.5.1.
- d. Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System: 384-well block; REF: A28135, Design and Analysis Software v1.5.1.
- e. Applied Biosystems™ QuantStudio™ 7 Flex Real-Time PCR System, 384-well (4485695 with Laptop, 4485693 with desktop), QuantStudio Real-time PCR Software v1.3 or Design and Analysis Software v2.4.3
- f. Applied Biosystems™ QuantStudio™ 12K Flex Real-Time PCR System, 384-well (4471081 with Laptop, 4471134 with desktop), QuantStudio 12K Flex software v1.4 or Design and Analysis Software v2.4.3
- g. Analytik Jena qTower³ / qTower³ G Real-Time PCR System: 844-00553-x, 844-00554-x, 844-00555-x, 844-00556-x, 844-00563-x, 844-00564-x, 844 00503-2, 844-00503-4, 844-00504-2 software version qPCRsoft 4.1.

h. Analytik Jena qTower³ 84 / qTower³ 84 G Real-Time PCR System: 844-00558-x, 844-00559-x, 844-00568-x, 844-00569-x, 844-00509-2, 844-00509-4 software version qPCRsoft384 1.2.

i. 96-well PCR plate:

- 0.1 mL for Applied Biosystems QuantStudio 3 and Applied Biosystems 7500 Fast Dx: 43464906 or equivalent
- 0.2 mL for Applied Biosystems 7500 Standard: 4306737 or equivalent
 - MicroAmp Optical Adhesive Film: 4311971
- Analytik Jena qTower³ / qTower³ G: Greiner: Bio-one Sapphire microplate (669285); Analytik Jena 96-well plate (844-70038-0); Eppendorf twin.tec real-time PCR Plate 96 (0030129644, 0030129636) or equivalent
 - VWR® Heat-Resistant Films for Real-Time qPCR, Ultra-Clear Polyester: 60941-078

j. 384-well PCR plate:

- Applied Biosystems QuantStudio 5: 4343370 or equivalent
 - MicroAmp Optical Adhesive Film: 4311971
- Analytik Jena qTower³ 84 / qTower³ 84 G: Greiner: 384 well full skirt PCR Plate, White (785235); SSI 384-well plate (3430-40) or equivalent.
 - VWR® Heat-Resistant Films for Real-Time qPCR, Ultra-Clear Polyester: 60941-078

3. Additional tools and consumables required for automatic nucleic acid extraction and PCR setup using Pre-NAT II and chemagic 360.

Items	Cat. No.	Pre-NAT II	Chemagic 360
Centrifuge	TDL-80-2B	✓	✓
Vortex mixer	XW-80A	✓	✓
900 µL conductive tip Sterilized	AF01MP-9-XS	✓	
175 µL conductive tip Sterilized	AF200P-9-XS	✓	
50 µL conductive tip Sterilized	ATO5OP-9- XS-LB	✓	
150 mL Reagent Trough	C3040016	✓	
33 mL Reagent Trough	CJ222161115	✓	
2 mL U type 96 deep-well plate	DP20UR-9-N	✓	
2 mL deep-well-plate (riplate SW)	CMG-555		✓
Low-well-plate	CMG-555-1		✓

Magnetic rods disposable tips	CMG-550	✓	✓
1.5 mL transparent centrifugal tube	MCT-150-C	✓	
0.2 mL PCR 8-trip tubes	PCR-0208-C	✓	
Caps for 0.2 mL PCR 8-trip tubes	PCR-2CP-RT-C	✓	
Deep-well plate sealing film	HY3020011	✓	

- An additional instrument to process saliva samples: Omni Bead Ruptor Elite (SKU: 19-040E), Hard Tissue Homogenizing Mix (2 mL Reinforced Tubes) Nuclease Free (SKU: 19-628)

Storage & Handling Requirements

- Store all reagents at -25 to -15°C.
- Use the reagents within 30 days once opened.
- Completely thaw the reagents before use. **Reagent A may precipitate upon thawing. Mix reagent A at room temperature until fully dissolved.** After thawed, store at 2-8 °C.
- Avoid excessive freeze/thaw cycles for reagents.
- Unopened Reagents are stable at -25 to -15°C for 24 months

Warnings and Precautions

- For *in vitro* diagnostic use. For prescription use only. For use under an Emergency Use Authorization only.
- This product has not been FDA cleared or approved but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories.
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- 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.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
- Keep the kit upright during storage and transportation.

8. Before using the kit, check tubes for leakage or damage. Each component in the kit should be thawed at room temperature, thoroughly mixed, and centrifuged before use.
9. Cross-contamination may occur if reference materials and specimens are handled inappropriately, which will cause inaccurate results. It is recommended to use sterile disposable filter-tips to aspirate reagents and specimens.
10. All specimen material to be tested and the reference material of the kits should be considered as infectious substances and processed strictly in accordance with laboratory biosafety requirements. Sterile centrifuge tubes and filter-tips should be used. After use, the tips should be disposed into a waste bin containing a 10% sodium hypochlorite solution. After the operation, the work area surface and the instrument surface should be disinfected with a freshly prepared 10% sodium hypochlorite solution, and then cleaned with 75% ethanol or pure water. Finally, turn on UV light to disinfect working surfaces for 30 minutes.
11. The PCR instrument used for this assay should be calibrated regularly according to instrument's instructions to eliminate cross-talks between channels. Refer to Appendix B.
12. This kit uses PCR-based technology and experiments should be conducted in three separate areas: reagent preparation area, specimen preparation area, amplification area. Protective equipment accessories (goggles, work clothes, hats, shoes, gloves, etc.) should be worn during operation and protective equipment accessories should be changed when entering and leaving different work areas. Protective equipment accessories in each work area are not interchangeable.
13. Contamination may occur if carryover of samples is not adequately controlled during sample pool preparation, handling, and processing.
14. Testing of pooled specimens may impact the detection capability of the New Coronavirus Nucleic Acid Detection Kit and decrease sensitivity.
15. Do not use the reagents beyond the expiration date.

Instruments

- PreNAT II Automated Workstation
- Chemagic 360
- Applied Biosystems® 7500 Real-Time PCR system
- Applied Biosystems® 7500 Fast Dx Real-Time PCR system

- QuantStudio™ 3 Real-Time PCR system
- QuantStudio™ 5 Real-Time PCR system
- QuantStudio™ 12K Flex
- QuantStudio™ 7 Flex
- Analytik Jena qTOWER³ / qTower³ G Real-Time PCR system
- Analytik Jena qTOWER³ 84 / qTower³ 84 G Real-Time PCR system

Collection, Storage & Shipment of Specimens

1. Specimen Collection

Swab Specimen

Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing 3 mL of viral transport media. For initial testing, nasopharyngeal swab specimens are recommended. Collection of oropharyngeal swabs is a lower priority and is acceptable if other swabs are not available.

- Nasopharyngeal swab (NP): Insert a swab into nostril parallel to the palate. Swab should reach depth equal to distance from nostrils to outer opening of the ear. Leave swab in place for several seconds to absorb secretions. Slowly remove swab while rotating it.
- Oropharyngeal swab (e.g., throat swab, OP): Swab the posterior pharynx, avoiding the tongue.
- Anterior Nasal Swab (NS): Using a flocked or spun polyester swab, insert the swab at least 1 cm (0.5 inch) inside the nostril (naris) and firmly sample the nasal membrane by rotating the swab and leaving in place for 10 to 15 seconds. Sample both nostrils with same swab.

Saliva Specimen

Use SalivaSecure collection kit catalog number SDX-56338. Follow SalivaSecure instruction for use for saliva collection.

2. Storage

Swab Specimen in VTM

Store specimens at 2-8°C for up to 72 hours after collection. If a delay in testing or shipping is expected, store specimens at -70°C or below.

Swab Specimen in Mawi iSWAB Microbiome Collection Kit (Cat # ISWAB-

MB-1200)

Store specimens at Room Temperature for up to 7 days after collection.

Swab Specimen in saline

Store samples at Room Temperature for up to 48 hours after collection.

Saliva Specimen

Specimens stored in SalivaSecure can be shipped at 19°C – 23°C but must not exceed shipping times above 56 hours. Specimens stored in SalivaSecure can also be shipped at 2 – 8 °C for up to 24 hours.

3. Shipping

Specimens PUI's must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation External Icon. Store specimens as indicated above. Additional useful and detailed information on packing, shipping, and transporting specimens can be found at Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).

4. For more information, refer to:

Interim Guidelines for Collecting and Handling of Clinical Specimens for COVID-19 Testing <https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html>

Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19) <https://www.cdc.gov/coronavirus/2019-nCoV/lab/lab-biosafety-guidelines.html>

5. Specimen Pooling – Determining the Appropriate Strategy for Implementation and Monitoring:

When considering specimen pooling, laboratories should evaluate the appropriateness of a pooling strategy based on the positivity rate in the testing population and the efficiency of the pooling workflow. Refer to Appendix A of these Instructions for Use for additional information **prior** to implementation of specimen pooling.

6. Preparing Samples for Pooling:

The following upper respiratory tract specimens authorized under the Emergency Use Authorization of the New Coronavirus Nucleic Acid Detection Kit may be tested with sample pooling. This includes nasopharyngeal, oropharyngeal, mid-turbinate and nasal swab specimens collected into VTM. When pooling samples, 70 µL of each individual patient sample should be combined into one well of a deep-well plate for a total volume of 350 µL when using a pool size of 5 on the chemagic 360 nucleic

acid extraction platform.

Assay Procedure

Nucleic Acid Extraction and PCR Setup

Extraction and PCR setup on Pre-NAT II

Note: Pre-NAT II has not been validated with the saliva specimen type and nasopharyngeal, oropharyngeal, or anterior nasal samples collected in Mawi media or saline.

Pre-NAT II Automated Workstation is designed to process 1-96 samples for downstream molecular assays. It contains a liquid handling system which automatically pipettes and mixes reagents and samples, a purification module that extracts and purifies nucleic acids, and an automatic PCR setup function which is also conducted by the liquid handling system. The entire workflow is automatic without manual intervention. Detailed operation instructions of Pre-NAT II can be found in the Pre-NAT II Automated Workstation User Manual. A quick-start instruction for the SARS-CoV-2 assay is described as below.

- 1) Take the nCoV Internal Control, nCoV Positive Control and nCoV Negative Control out from freezer, place them in a biological safety cabinet and completely thaw them at room temperature. Vortex the tubes to mix the contents, then centrifuge the tubes briefly at 1000 rpm to collect the liquid to the bottom of the tubes.
- 2) Prepare specimens and place them in a biological safety cabinet. If the specimens are frozen, completely thaw them at room temperature and follow the operations described in 1) for the controls.
- 3) Take the Magnetic Beads from the Nucleic Acid Extraction Kits (KN0212) kit, vortex the tube for one minute to completely suspend the beads in the solution.
- 4) Turn on the PreNAT II instrument, double click the “Pre-NAT II” software icon, select username and enter password to start, then follow software guidance to initialize the instrument.
- 5) After initialization, click “Program Input” to choose an extraction protocol. For the SARS-CoV-2 assay, choose “2019-nCoV” from the protocol list.
- 6) In the same window, input the number of specimens that are going to be processed at the indicated box, positive control and negative control should not be counted, as they are pre-set in the 2019-nCoV protocol. After the sample number is entered, click “Set Complete” to proceed to the loading guidance for reagents and consumables.
- 7) Remove the lids from reagents, controls and specimens, load the

consumables, reagents, specimens, and controls according to software guidance, then double check to confirm that all items are at the positions indicated by software. Close instrument door after finish loading. Click “Run” to start the protocol, the procedures automatically performed by Pre-NAT II are described below.

- Add 400 μ L of each specimen, Negative Control and Positive Control to the wells of a 96 deep-well plate, and add 5 μ L nCoV Internal Control, 800 μ L Lysis/Binding Buffer and 15 μ L Magnetic Beads to each well.
- Magnetic rods take rod tips and rotate in 96 deep-well plate to mix (magnetic force off status), during which stage DNA/RNA is released through lysis and binds to magnetic beads.
- During lysis and binding, automatic liquid handler pipettes Wash Buffer A to a 96 deep-well plate.
- Magnetic force is turned on for magnetic rods and beads are collected from Lysis/Binding reaction to Wash Buffer A.
- Magnetic rods (magnetic force off) rotate to wash beads in Wash Buffer A and proceed in a same manner to wash beads in Wash Buffer B.
- Finally, the beads are collected and placed into 60 μ L elution buffer to elute DNA/RNA.
- During elution, liquid handler pipettes/mixes PCR reagents to prepare a PCR mix and aliquot 20 μ L to PCR tubes.
- For each sample, 40 μ L of eluted DNA/RNA is added to PCR mix in each tube, which is ready for amplification.

Extraction and PCR setup on chemagic 360

Note: Chemagic 360 has been validated with the saliva specimen type and nasopharyngeal, oropharyngeal, or anterior nasal samples collected with Mawi media and saline. If preparing saliva specimens or nasopharyngeal, oropharyngeal, or anterior nasal samples collected with Mawi media and saline, follow the procedures in section “Extraction from saliva sample on chemagic 360”, “Extraction from transport media Mawi on chemagic 360”, or “Extraction from transport media Saline on chemagic 360” respectively.

Please follow chemagic 360 User Manual for extraction setup. A quick-start instruction is described as below.

- 1) Place specimens in a biological safety cabinet. If the specimen is frozen, completely thaw it at room temperature before use; vortex the tubes to mix the contents, then centrifuge the tubes briefly at 1000 rpm to collect the liquid to the bottom of the tubes.
- 2) Take a 2 mL deep-well-plate (riplate SW), add 300 μ L Lysis buffer, 300 μ L

specimen, 5 μL (or 10 or 20 μL , refer to the following table) Internal Control, 4 μL Poly (A) RNA and 10 μL Proteinase K to each well in a sequential order.

Please note:

- i. Dissolve lyophilized Poly(A) RNA by adding 440 μL of the Poly(A) RNA Buffer to the Poly(A) RNA tube and mix thoroughly before use.
 - ii. Dissolve lyophilized Proteinase K in H_2O before use (volume is given on the label).
- 3) Take a low-well-plate, add 150 μL magnetic beads into each well.
 - 4) Take a new deep-well-plate (riplate SW), add 60 μL Elution Buffer 5 into each well.
 - 5) Turn on the chemagic 360, double click the software icon “chemagic_360”, select username and enter password to start. Follow the chemagic 360 User Manual to select the appropriate protocol.
 - 6) Load the magnetic rods disposable tips onto the tip rack according to the number of specimens, positive control and negative control being tested.
 - 7) Load the plates manually onto the tracking system (table) according to the instructions given by the chemagic software. The plates should be at the positions indicated in the below table.

Please note:

- i. Specimens and Magnetic Beads should be thoroughly vortex mixed before use.
- ii. Never move the tracking system (table) manually. All movements must be performed with the [Turn Table] function.

chemagic 360 layout:

Position 1	Magnetic rods disposable tips
Position 2	Low-well-plate (MICROTITER SYSTEM) prefilled with 150 μL Magnetic Beads

Position 3	Deep-well-plate (riplate SW) containing: 300 µL Lysis Buffer 1 300 µL specimen Internal Control*: <ul style="list-style-type: none"> • 5 µL for 60 µL PCR reactions • 10 µL for 30 µL PCR reactions • 20 µL for PCR 15 µL reactions 4 µL Poly(A) RNA 10 µL Proteinase K Binding Buffer 2 (added automatically)
Position 4	Empty deep-well-plate (riplate SW) [Wash Buffer 3 added automatically]
Position 5	Empty deep-well-plate (riplate SW) [Wash Buffer 4 added automatically]
Position 6	Empty deep-well-plate (riplate SW) [purified water added automatically]
Position 7	Deep-well-plate (riplate SW) prefilled with 60 µL Elution Buffer 5

*: Increasing the IC volume added to each specimen holds the copy number constant as the PCR reaction/RNA input volume decreases. Double check the positions and directions of all consumables according to the tracking system.

- 8) Click "Start" to start the extraction process.
- 9) Proceed to downstream assay with the extracted nucleic acids or store the nucleic acids at -25°C to -15°C.

Setup PCR Manually

Setup PCR manually according to the procedures described below after nucleic acid extraction using chemagic 360.

PCR setup on ABI 7500 Standard (60 µL per reaction)

- 1) Prepare PCR mix in Reagent Preparation Area according to the following table. It is recommended to prepare 110% of the calculated amount of PCR mix to account for pipetting carryovers.

Component	Volume/ test	Volume for N Samples and 2 Controls	110% of volume
nCoV Reagent A	15 µL	15 x (n + 2) µL	16.5 x (n + 2) µL
nCoV Reagent B	3 µL	3 x (n + 2) µL	3.3 x (n + 2) µL
nCoV Enzyme mix	2 µL	2 x (n + 2) µL	2.2 x (n + 2) µL

- 2) Completely vortex the prepared PCR mix, aliquot 20 µL into each PCR tube or each well of a 96-well PCR plate.
- 3) Add 40 µL of extracted nucleic acid into each tube or well containing PCR mix, close lids for the PCR tubes or seal PCR plates with an appropriate film, slightly vortex the tubes and briefly centrifuge them to get rid of bubbles.

PCR setup on Applied Biosystems QuantStudio 3, Applied Biosystems 7500 Fast Dx, Analytik Jena qTower³ / qTower³ G (30 µL per reaction)

- 1) Prepare PCR mix in Reagent Preparation Area according to the following table. It is recommended to prepare 110% of the calculated amount of PCR mix to account for pipetting carryovers.

Component	Volume/ test	Volume for N Samples and 2 Controls	110% of volume
nCoV Reagent A	7.5 µL	7.5 x (n + 2) µL	8.25 x (n + 2) µL
nCoV Reagent B	1.5 µL	1.5 x (n + 2) µL	1.65 x (n + 2) µL
nCoV Enzyme mix	1 µL	1 x (n + 2) µL	1.1 x (n + 2) µL

- 2) Completely vortex the prepared PCR mix, aliquot 10 µL into each PCR tube or each well of a 96-well PCR plate.
- 3) Add 20 µL of extracted nucleic acid into each tube or well containing PCR mix, close lids for the PCR tubes or seal PCR plates with an appropriate film, slightly vortex the tubes and briefly centrifuge them to get rid of bubbles.

PCR setup on QuantStudio™ 5, QuantStudio™ 7 Flex, QuantStudio™ 12K Flex, qTower³ 84 / qTower³ 84 G (15 µL per reaction)

- 1) Prepare PCR mix in Reagent Preparation Area according to the following table. It is recommended to prepare 110% of the calculated amount of PCR mix to account for pipetting carryovers.

Component	Volume/ test	Volume for N Samples and 2 Controls	110% of volume
nCoV Reagent A	3.75 µL	3.75 x (n + 2) µL	4.125 x (n + 2) µL
nCoV Reagent B	0.75 µL	0.75 x (n + 2) µL	0.825 x (n + 2) µL
nCoV Enzyme mix	0.5 µL	0.5 x (n + 2) µL	0.55 (n + 2) µL

- 2) Completely vortex the prepared PCR mix, aliquot 5 µL into each PCR tube or each well of a 96-well PCR plate.
- 3) Add 10 µL of extracted nucleic acid into each tube or well containing PCR mix, close lids for the PCR tubes or seal PCR plates with an appropriate film, slightly vortex the tubes and briefly centrifuge them to get rid of bubbles.

Extraction from saliva sample on chemagic 360

Additional simple processing steps were added for saliva samples before Chemagen extraction and PCR setup.

1. Transfer saliva samples from collection tube into Omni tubes
2. Place Omni tubes in Omni bead mill homogenizer homogenized at 4.5 m/s for 30 seconds
3. 300µl homogenized saliva samples are transferred to the plate for Chemagen extraction.
4. Follow the steps described in “Extraction and PCR setup on chemagic™” for the downstream process. The leftover samples can be store at -70 °C freezer for further analysis.

Extraction from transport media Mawi on chemagic 360

Additional simple processing steps were added before Chemagen extraction and PCR setup.

1. Place and clamp Mawi samples in a specimen container/purprack on a microplate shaker, shaking for 1 minute at 1550 rpm.
2. Remove the shaken samples from the shaker, centrifuge the barcoded specimen racks as follows: 4 minutes at 4200 X g in a centrifuge with rotors for Mawi tubes.
3. 300µl of the samples are transferred to the plate for Chemagen extraction.
4. Follow the steps described in “Extraction and PCR setup on chemagic™” for the downstream process.

Extraction from saline on chemagic 360

Additional simple processing steps were added before Chemagen extraction and PCR setup.

1. Put samples on a plate orbital shaker, shake for 1 minute at 750 rpm.
2. Remove the shaken samples from the shaker, centrifuge for 30 seconds at 200 X RCF in centrifuge with plate rotor.
3. 300µl of the samples are transferred to the plate for Chemagen extraction.
4. Follow the steps described in “Extraction and PCR setup on chemagic™” for the downstream process.

Amplification

Applied Biosystems™ 7500 Standard

- 1) Set up and run the Applied Biosystems™ 7500 Real-Time PCR instrument. Refer to Applied Biosystems™ 7500 Real-Time PCR Instrument Reference Guide for detailed instructions. In general, double-click 7500 software 2.3 > New experiments > Setup Experiment Properties > Setup the Targets and Samples in Plate Setup > Setup Run Method, then click Run and Start.
- 2) When setup Experiment Properties, please check the following run settings

and choose the correct settings.

- Instrument: 7500 (96 wells)
 - Run type: Quantitation – Standard Curve
 - Run reagent: TaqMan reagents
 - Run mode: Standard
- 3) When setting up the Targets and Samples, create the following detectors with the quencher set as none. The passive reference must be set as None.

Target Name or Detector	Reporter	Quencher
N	FAM	None
ORF1ab	ROX	None
IC	VIC/HEX	None

- 4) Set up the plate layout by assigning a unique sample name to each well.
- 5) Assign a Task to each well.
- Unknown: for patient samples
 - Standard: for Positive Control
 - NTC: for Negative Control
- 6) Set Run method as following for PCR amplification and fluorescence detection, set the sample volume at 60 µL.

Step	Temperature	Time	Number of Cycles
1	37°C	2 minutes	1
2	50°C	5 minutes	1
3	42°C	35 minutes	1
4	94°C	10 minutes	1
5	94°C	10 seconds	45
	55°C	15 seconds	
	65°C*	45 seconds	

* Collect fluorescence signal during the final 65°C step.

- 7) Double check all settings, load the plate, then click Run and Start to initialize amplification.

Applied Biosystems™ 7500 Fast Dx

- 1) Set up and run the Applied Biosystems 7500 Real-Time PCR instrument. Refer to Applied Biosystems 7500 Real-Time PCR Instrument Reference Guide for detailed instructions. In general, double-click 7500 software 2.3 › New experiments › Setup Experiment Properties › Setup the Targets and Samples in Plate Setup › Setup Run Method, then click Run and Start.
- 2) When setup Experiment Properties, please check the following run settings and choose the correct settings.
- Instrument: 7500 Fast (96 wells)
 - Run type: Quantitation – Standard Curve

- Run reagent: TaqMan reagents
 - Run mode: Default setting of 7500 Fast (Fast)
- 3) When setting up the Targets and Samples, create the following detectors with the quencher set as none. The passive reference must be set as None.

Target Name or Detector	Reporter	Quencher
N	FAM	None
ORF1ab	ROX	None
IC	VIC/HEX	None

- 4) Set up the plate layout by assigning a unique sample name to each well.
- 5) Assign a Task to each well.
- Unknown: for patient samples
 - Standard: for Positive Control
 - NTC: for Negative Control
- 6) Set Run method as following for PCR amplification and fluorescence detection, set the sample volume at 30 μ L.

Step	Temperature	Time	Number of Cycles
1	37°C	2 minutes	1
2	50°C	5 minutes	1
3	42°C	35 minutes	1
4	94°C	10 minutes	1
5	94°C	10 seconds	45
	55°C	15 seconds	
	65°C*	45 seconds	

* Collect fluorescence signal during the final 65°C step.

- 7) Double check all settings, load the plate, then click Run and Start to initialize amplification.

Applied Biosystems QuantStudio™ 3

- 1) Set up and run the QuantStudio™ Real-Time PCR instrument refer to the Instrument Reference Guide for detailed instructions. In general, double-click QuantStudio™ Design and Analysis Desktop Software v1.5.1 › New experiments › Setup Experiment Properties › Setup the Targets and Samples in Plate Setup › Setup Run Method, then click Run and Start.
- 2) When setup Experiment Properties, please check the following run settings and choose the correct settings.
- Instrument type: QuantStudio™ 3 System
 - Block type: 96-Well 0.2-mL Block or 96-Well 0.1-mL Block
 - Experiment type: Standard Curve
 - Chemistry: TaqMan reagents
 - Run mode: Standard

- 3) When setting up the Targets and Samples, create the following detectors with the quencher set as none. The passive reference must be set as None.

Target Name or Detector	Reporter	Quencher
N	FAM	None
ORF1ab	ROX	None
IC	VIC/HEX	None

- 4) Set up the plate layout by assigning a unique sample name to each well.
- 5) Assign a Task to each well.
- Unknown: for patient samples
 - Standard: for Positive Control
 - NTC: for Negative Control
- 6) Set Run method as following for PCR amplification and fluorescence detection, set the sample volume at 30 μ L.

Step	Temperature	Time	Number of Cycles
1	37°C	2 minutes	1
2	50°C	5 minutes	1
3	42°C	35 minutes	1
4	94°C	10 minutes	1
5	94°C	10 seconds	45
	55°C	15 seconds	
	65°C*	45 seconds	

* Collect fluorescence signal during the final 65°C step.

- 7) Double check all settings, load the plate, then click Run and Start to initialize amplification.

QuantStudio™ 5, 7 Flex and 12K Flex

- 1) Set up and run the QuantStudio™ Real-Time PCR instrument refer to the Instrument Reference Guide for detailed instructions. In general, double-click QuantStudio™ Design and Analysis Desktop Software v1.5.1 for QuantStudio™ 5, or QuantStudio™ Real-time PCR software v1.3 for QuantStudio™ 7 Flex, or QuantStudio™ 12K Flex software v1.4 for QuantStudio™ 12K Flex › New experiments › Setup Experiment Properties › Setup the Targets and Samples in Plate Setup › Setup Run Method, then click Run and Start.
- 2) When setup Experiment Properties, please check the following run settings and choose the correct settings.

- Instrument type: QuantStudio™ 5 System for QuantStudio™ 5, or QuantStudio™ 7 Flex System for QuantStudio™ 7 Flex, or not applicable for QuantStudio™ 12K Flex
 - Block type: 384-well Block
 - Experiment type: Standard Curve
 - Chemistry: TaqMan reagents
 - Run mode: Standard
- 3) When setting up the Targets and Samples, create the following detectors with the quencher set as none. The passive reference must be set as None.

Target Name or Detector	Reporter	Quencher
N	FAM	None
ORF1ab	ROX	None
IC	VIC/HEX	None

- 4) Set up the plate layout by assigning a unique sample name to each well.
- 5) Assign a Task to each well.
- Unknown: for patient samples
 - Standard: for Positive Control
 - NTC: for Negative Control
- 6) Set Run method as following for PCR amplification and fluorescence detection, set the sample volume at 15 µL.

Step	Temperature	Time	Number of Cycles
1	37°C	2 minutes	1
2	50°C	5 minutes	1
3	42°C	35 minutes	1
4	94°C	10 minutes	1
5	94°C	10 seconds	45
	55°C	15 seconds	
	65°C*	45 seconds	

* Collect fluorescence signal during the final 65°C step.

- 7) Double check all settings, load the plate, then click Run and Start to initialize amplification.

Analytik Jena qTOWER³ 84 / qTower³ 84 G Real-Time PCR system

- 1) Set up and run the Analytik Jena qTOWER³ 84 / qTower³ 84 G Real-Time PCR instrument. Refer to Analytik Jena qTOWER³ 84 / 84 G Real-Time PCR Operating Manual for detailed instructions. In general, double-click

qPCRsoft384 software 1.2 › File | New › Settings › Thermal Cycler | Scan | Samples, then click *Start qPCR run*.

NOTE – the settings described below can be saved and recalled by use of a project template file (.rts384 or *.rtsx384 file)*

2) Settings | General:

- Title: as appropriate for this run
- Operator: appropriate operator designation
- Start and End: populated automatically as part of the run
- Comment: any additional information regarding the run

3) Settings | Thermal Cycler:

- Set Run method as following for PCR amplification and fluorescence detection, using default ramping rate.
- Lid Temp: 100 °C (or 105 °C), Preheat lid.
- Device: qTOWER³ 84 or qTower³ 84 G, depending on instrument type

Step	Scan	Temperature	Time (m:s)	GoTo	Loops
1		37°C	02:00	-	-
2		50°C	05:00	-	-
3		42°C	35:00	-	-
4		94°C	10:00	-	-
5		94°C	00:10	-	-
6		55°C	00:15	-	-
7	X	65°C*	00:45	5	44

4) Settings | Scan:

- Activate the following measurement detectors. The passive reference (Pass. Ref.) cells/column must be left empty.

Pos.	Channel	Dye	Gain	Measurement
1	Blue	FAM	5	X
2	Green	JOE	5	
3	Yellow	HEX 3 or TAMRA	5	X
4	Orange	ROX	5	X
5	Red	Cy5	5	
6	NIR1	Cy5.5	5	

- Color compensation: Standard1

NOTE – all six Pos. and Channel options must be activated in Edit color modules before opening any new project files (on software main page, click Extras>Edit color modules). Otherwise, corresponding Pos. and Channel

options may not show up in Scan setting.

5) Settings | Samples:

- Set up the plate layout by assigning a unique sample name to each well.
- Assign a sample type to each well:
 - Positive control
 - Negative control
 - Unknown (patient sample)

6) Double check all settings, save the project, load the plate and then click *Start qPCR* run to initialize amplification.

Note – Device selection must match the specific device in thermal cycler setting, otherwise qPCR run will not start, and error message may pop up.

Analytik Jena qTOWER³ / ³G Real-Time PCR system

1) Set up and run the Analytik Jena qTOWER³ / ³G Real-Time PCR instrument. Refer to Analytik Jena qTOWER³ / ³G Real-Time PCR Operating Manual for detailed instructions. In general, double-click qPCRsoft software 4.1 › File | New › Settings › Thermal Cycler | Scan | Samples, then click *Start qPCR run*.

NOTE – the settings described below can be saved and recalled by use of a project template file (.rts or *.rtsx file)*

2) Settings | General:

- Title: as appropriate for this run
- Operator: appropriate operator designation
- Start and End: populated automatically as part of the run
- Comment: any additional information regarding the run

3) Settings | Thermal Cycler:

- Set Run method as following for PCR amplification and fluorescence detection, using default ramping rate.
- Lid Temp: 100 °C (or 105 °C), Preheat lid.
- Device: qTOWER³ or qTower³ G, depending on instrument type

Step	Scan	Temperature	Time (m:s)	GoTo	Loops
1		37°C	02:00	-	-
2		50°C	05:00	-	-

3		42°C	35:00	-	-
4		94°C	10:00	-	-
5		94°C	00:10	-	-
6		55°C	00:15	-	-
7	X	65°C*	00:45	5	44

4) Settings | Scan:

- Activate the following measurement detectors. The passive reference (Pass. Ref.) cells/column must be left empty.

Pos.	Channel	Dye	Gain	Measurement
1	Blue	FAM	5	X
2	Green	JOE	5	
3	Yellow	HEX_3 or TAMRA	5	X
4	Orange	ROX	5	X
5	Red	Cy5	5	
6	NIR1	Cy5.5	5	

- Color compensation: Standard 1 for 4.1

NOTE – all six Pos. and Channel options must be activated in Edit color modules before opening any new project files (on software main page, click Extras>Edit color modules). Otherwise, corresponding Pos. and Channel options may not show up in Scan setting.

5) Settings | Samples:

- Set up the plate layout by assigning a unique sample name to each well.
- Assign a sample type to each well:
 - Positive control
 - Negative control
 - Unknown (patient sample)

6) Double check all settings, save the project, load the plate and then click *Start qPCR* run to initialize amplification.

Note – Device selection must match the specific device in thermal cycler setting, otherwise qPCR run will not start, and error message may pop up.

Interpretation of Results

Baseline and threshold setting for ABI 7500 Standard, ABI 7500 Fast Dx, QuantStudio 3 /5/7Flex/12K Flex

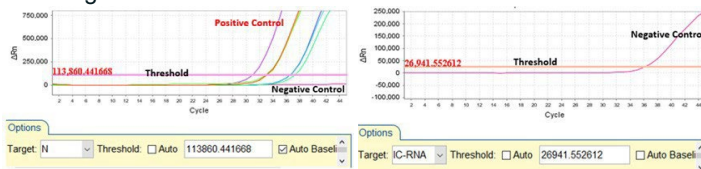
After the run completion, save and analyze the data according to PCR instrument instructions.

- 1) Set baseline for each target

View the baseline values, in the Graph Type drop-down list, select Linear. Select the Baseline check box to show the start cycle and end cycle. The horizontal part of the baseline is used for the baseline range, which normally starts from 3-5 cycles and ends at 15-20 cycles. Baseline setting is normally automatically done by instrument. It can also be manually adjusted to choose the horizontal part of the curve.

- 2) Set threshold for each target

View the threshold values, In the Graph Type drop-down list, select Linear. In the Target drop-down list, select N, ORF1ab or IC. Select the Threshold check box to show the threshold. Thresholds should be adjusted to fall within exponential phase of the fluorescence curves and above any background signal (refer to figures below). The threshold value for different instruments varies due to different signal intensities.



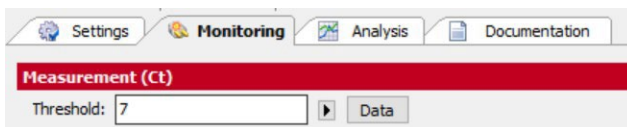
- 3) Perform data analysis by clicking “Analyze” button of the software.
- 4) Output the data to csv file by the “export” function of the software.
- 5) Interpret the results based on the tables listed in “Quality Control” and “Examination and Interpretation of Specimen Results”.

Baseline and threshold setting for qTower³ / 3 G and qTower³ 84 / 3 84 G

After the run completion, save and analyze the data according to PCR instrument instructions.

Under Settings tab, for color compensation configuration, select “Standard1” for qTower3 / 3 G (version 4.1) and qTower3 84 / 3 84 G.

Under **Monitoring** tab, click “Calculate Ct”, the following view shows up.



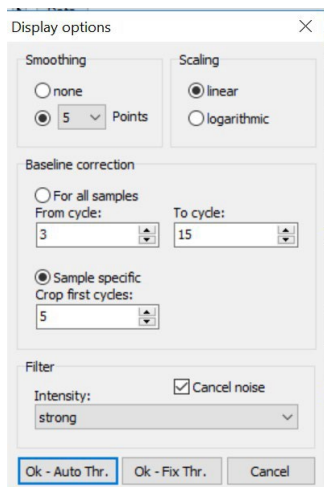
- 7) Set baseline for each target

In most of the cases, the default baseline can be used. In order to adjust

baseline, click  icon

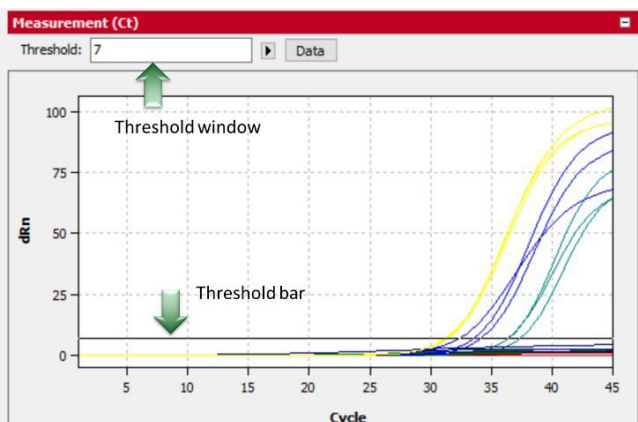
The default setting is “Sample specific crop first cycles”, which is good for most of the cases. The default is 5, which can be adjusted, for example 10, 15 to minimize background noises in some cases.

In order to set up different baseline, click “For all samples”, from cycle X (default 3) to Y (default 15) as the following window.



In order to switch back to the default setting, click “Sample specific Crop first cycles”.

Set threshold for each target: Under **Monitoring** tab, view the threshold values under “linear” scaling (showed in above figure) for each target. Thresholds should be adjusted to fall within exponential phase of the fluorescence curves and above any background signal. The threshold value for different instruments varies due to different signal intensities. It is recommended to setup threshold manually instead of default settings. For manual threshold setup, either move threshold bar up and down, or manually input threshold number to the “Threshold” window, shown in the following figure. It is recommended to setup the threshold in the range of 5-15 as general.



Quality Control

The kit product provides negative control, positive control, and internal control to monitor the reliability of the results for the entire batch of specimens from sample extraction to PCR amplification. All test controls should be examined prior to interpretation of patient results. Positive control, negative control and IC in positive and negative control should meet the requirements listed in the below table to ensure valid results. If the controls are not valid, the patient results cannot be interpreted.

Result Interpretation of Test Controls for 60 μ L reaction:

Control type	Ct		
	N (FAM)	ORF1ab (ROX)	IC (HEX/VIC)
Negative	Undet or > 42	Undet or > 42	Ct \leq 40
Positive	\leq 35	\leq 35	/

/: No requirements on the Ct value.

Undet: Undetermined

Result Interpretation of Test Controls for 30 μ L reaction:

Control type	Ct		
	N (FAM)	ORF1ab (ROX)	IC (HEX/VIC)
Negative	Undet or > 42	Undet or > 42	Ct \leq 40
Positive	\leq 36	\leq 36	/

/: No requirements on the Ct value.

Undet: Undetermined

Result Interpretation of Test Controls for 15 μ L reaction:

Control type	Ct		
	N (FAM)	ORF1ab (ROX)	IC (HEX/VIC)

Negative	Undet or > 42	Undet or > 42	Ct ≤ 40
Positive	≤ 37	≤ 37	/

/: No requirements on the Ct value.

Undet: Undetermined

- 1) Negative Control: both ORF1ab and N of SARS-CoV-2 must be not detected, and the Ct value of internal control should be ≤ 40.
- 2) Positive Control: both ORF1ab and N of SARS-CoV-2 must be detected, and their Ct values should fall within the ranges described in the above tables, the Ct value of internal control does not have to be ≤ 40 for positive control.

Examination and Interpretation of Patient Specimen Results

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and confirmed to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

The table below lists the expected results for the kit with valid positive control and negative control:

Ct		Result interpretation
IC (VIC/HEX)	N(FAM), ORF1ab (ROX)	
≤40	Both targets Undet or >42	SARS-CoV-2 not detected
/	Both targets ≤ 42	SARS-CoV-2 detected
/	One of the targets ≤ 42	SARS-CoV-2 detected
>40 or Undet	Both targets Undet or >42	Invalid result, specimen needs to be re-tested from re-extraction or re-collected from patient for test.

Undet: Undetermined

/: No requirements on the Ct value.

- 1) If the result for a specimen is SARS-CoV-2 RNA not detected, the Ct value of the internal control must be ≤40, otherwise the result of that specimen is invalid.
- 2) If the result for a specimen is SARS-CoV-2 RNA detected, the Ct value of the internal control is not required to be considered valid.

Examination and Interpretation of Pooled Patient Specimen Results

Negative—Negative results from pooled sample testing should not be treated as definitive. If the patient's clinical signs and symptoms are inconsistent with a negative result and if results are necessary for

patient management, then the patient should be considered for individual testing. The utilization of sample pooling should be indicated for any specimens with reported negative results.

Positive—Specimens with a positive sample pool 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.

Invalid—Specimens with an invalid pool result must be tested individually prior to reporting a result. However, in instances of an invalid run, repeat testing of pooled specimens may be appropriate depending on laboratory workflow and required result reporting time.

Kit Limitations

1. The use of this product as an *in vitro* diagnostic under FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high complexity tests.
2. The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.
3. This kit is used for qualitative detection of SARS-CoV-2 RNA from human oropharyngeal swab, nasopharyngeal swab, anterior nasal swab and saliva specimens. The results cannot directly reflect the viral load in the original specimens.
4. Anterior nasal swab specimens self-collected under the supervision of or collected by a healthcare provider can be tested with the New Coronavirus Nucleic Acid Detection Kit; however, performance with this specimen type has not been determined.
5. Testing of saliva specimens is limited to the QuantStudio 5, qTower³ G, ABI 7500 Fast Dx, and ABI 7500 Standard RT-PCR instruments.
6. The saliva specimen type has not been validated with the Pre-NAT II Automated Workstation.
7. Sample pooling has only been validated using nasopharyngeal and oropharyngeal swab specimens. Pooling of saliva specimens has not been

validated.

8. Samples should only be pooled when testing demand exceeds laboratory capacity and/or when testing reagents are in short supply.
9. Negative results for SARS-CoV-2 RNA from saliva should be confirmed by testing of an alternative specimen type if clinically indicated.
10. The New Coronavirus Nucleic Acid Detection Kit performance has only been established with nasopharyngeal swab and oropharyngeal swab specimens, and with saliva specimens collected using the SalivaSecure collection kit. Anterior nasal swabs are acceptable upper respiratory specimens; however, performance with this specimen type has not been determined.
11. The specimens to be tested shall be collected, processed, stored and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.
12. Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
13. Amplification and detection of SARS-CoV-2 with the New Coronavirus Nucleic Acid Detection Kit has only been validated with the instruments specified in the instructions. Use of other instrument systems may cause inaccurate results.
14. The limit of detection (LoD) is determined based on a 95% confidence of detection. When SARS-CoV-2 presents at or above the LoD concentration in the test specimen, there will be a low probability that SARS-CoV-2 is not detected. When SARS-CoV-2 presents below the LoD concentration in the test specimen, there will also be certain probability that SARS-CoV-2 can be detected.
15. Primers and probes for this kit target highly conserved regions within the genome of SARS-CoV-2. Mutations occurred in these highly conserved regions (although rare) may result in RNA being undetectable.
16. This kit uses an UNG/dUTP PCR products carryover prevention system which can prevent contamination caused by PCR products. However, in the actual operation process, the amplicon contamination can be avoided only by strictly following the instructions of PCR laboratories.
17. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.

18. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics or immunosuppressant drugs have not been evaluated.
19. Laboratories are required to report all results to the appropriate public health authorities.

Conditions of Authorization for the Laboratory

The New Coronavirus Nucleic Acid Detection Kit 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/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas>

However, to assist clinical laboratories using the New Coronavirus Nucleic Acid Detection Kit (“your product” in the conditions below), the relevant Conditions of Authorization are listed below:

- A. Authorized laboratories¹ using your product 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.
- B. Authorized laboratories using specimen pooling strategies when testing patient specimens with your product must include with test result reports for specific patients whose specimen(s) were the subject of pooling, a notice that pooling was used during testing and that “Patient specimens with low viral loads may not be detected in sample pools due to the decreased sensitivity of pooled testing.”
- C. Authorized laboratories using your product must use your product 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 your product are not permitted.
- D. Authorized laboratories implementing pooling strategies for testing patient specimens must use the “Specimen Pooling Implementation and Monitoring Guidelines” provided in the authorized labeling to evaluate the appropriateness of continuing to use such strategies based on the recommendations in the protocol.
- E. Authorized laboratories that receive your product must notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- F. Authorized laboratories using your product must have a process in place

for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

- G. 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 Specimen Pooling Implementation and Monitoring Guidelines. For the first 12 months from the date of their creation, such records must be made available to FDA within 48 business hours for inspection upon request, and must be made available within a reasonable time after 12 months from the date of their creation.
- H. Authorized laboratories must collect information on the performance of your product and report to DMD/OHT7/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Revvity (via email: COVID-19.TechnicalSupport@Revvity.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.
- I. All laboratory personnel using your product must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.
- J. Revvity, its authorized distributor(s) and authorized laboratories using the New Coronavirus Nucleic Acid Detection Kit 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.

¹ The letter of authorization refers to, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests" as "authorized laboratories."

Assay Performance

Limit of Detection

LoD Using Pre-NAT II for Extraction and Applied Biosystems 7500 PCR System

Limit of detection (LoD) was determined as the lowest concentration of SARS-CoV-2 that at which the New Coronavirus Nucleic Acid Detection Kit can detect at a $\geq 95\%$ positive rate. Samples were prepared using pooled clinical oropharyngeal swab specimen matrix collected from 12 individuals at 4 different time points giving. The pooled oropharyngeal swab matrix was tested using New Coronavirus Nucleic Acid Detection Kit and confirmed to be negative. In the first part of the study, a total of six 10-fold dilutions of known concentrations of

inactivated SARS-CoV-2 virus (Isolate 2/231/human/2020/CHN) were prepared in negative clinical matrix and processed using the Nucleic Acid Extraction kit on the PreNAT II Automated Workstation. Four PCR replicates per concentration were tested on the Applied Biosystems 7500 Real-Time PCR System. The results are summarized in the following tables.

Table: Preliminary LoD study results from 10-fold dilution of virus stock on PreNAT II.

Dilution Fold	N		ORF1ab		Mean Ct		
	Conc. (copies/ml)	Detecti on rate	Conc. (copies/ml)	Detecti on rate	N	ORF1ab	IC
1.0E+04	274	4/4	83.7	4/4	34.88	34.29	33.17
1.0E+05	27.4	4/4	8.37	3/4	38.74	37.67	33.27
1.0E+06	2.74	2/4	0.837	2/4	39.57	38.71	33.11
1.0E+07	0.274	1/4	0.0837	1/4	40.11	38.75	33.44
1.0E+08	0.0274	0/4	0.00837	0/4	/	/	32.68
1.0E+09	0.00274	0/4	0.000837	0/4	/	/	33.02
Negative	0	0/4	0.00	0/4	/	/	32.83

Based on the previous results, an additional eight 2-fold dilutions of known concentrations of genomic RNA were prepared in negative clinical matrix. Twenty individual extraction replicates per dilution were tested. The results are summarized in the following table.

Table: Preliminary LoD study results from 2-fold dilution of virus stock on PreNAT II.

Dilution Fold	N		ORF1ab		Mean Ct		
	Conc. (copies/ml)	Detecti on rate	Conc. (copies/ml)	Detecti on rate	N	ORF1ab	IC
1.0E+04	274	20/20	83.7	20/20	34.95	35.48	31.55
2.0E+04	137	20/20	41.85	20/20	35.93	36.23	31.65
4.0E+04	68.5	20/20	20.93	20/20	36.91	37.10	31.70
8.0E+04	34.25	19/20	10.46	19/20	38.15	38.64	31.61
1.6E+05	17.13	18/20	5.23	13/20	38.80	39.48	31.60
3.2E+05	8.56	11/20	2.62	11/20	39.44	39.93	31.28
6.4E+05	4.28	8/20	1.31	7/20	40.26	40.44	31.41
1.28E+06	2.14	5/20	0.65	3/20	40.10	40.65	31.16
Negative	0	0/20	0	0/20	/	/	31.15

Probit analysis predicted 95% detection rate is presented in the below table.

Table: Probit predicted 95% detection rate using inactivated cultured SARS-CoV-2 (Isolate 2/231/human/2020/CHN).

Probit predicted 95% detection rate (copies/mL)	
N	ORF1ab
24.884 (95% CI: 17.032 – 57.917)	9.307 (95% CI: 7.428 – 13.003)

The probit-predicted LoD is estimated to result in approximately 3 copies/reaction.

Verification of LoD Using Pre-NAT II for Extraction and PCR Setup

The 95% probit LoD was further verified by testing 20 extraction replicates of oropharyngeal swab matrix spiked with inactivated virus (Isolate 2/231/human/2020/CHN) at a concentration containing 9.307 copies/mL of the ORF1ab target and 30.467 copies/mL of the N gene target. Each replicate was extracted using the Nucleic Acid Extraction Kit (KN0212) on Pre- NAT II and tested using the New Coronavirus Nucleic Acid Detection Kit. The results are summarized in the following table.

Table: Pre-NAT II LoD verification results.

Concentration (copies/ml)			Detection rate		Mean Ct		
LoD	N	ORF1ab	N	ORF1ab	N	ORF1ab	IC
1X	30.467	9.307	100% (20/20)	95% (19/20)	38.39	38.11	31.18

The results confirm an LoD of 9.307 copies/mL for the ORF1ab target and 30.467 copies/mL for the N target.

LoD Using chemagic 360 for Extraction and Applied Biosystems 7500 PCR System

Samples were prepared using pooled clinical oropharyngeal swabs or nasopharyngeal swabs specimen matrix. The pooled matrix was tested using New Coronavirus Nucleic Acid Detection Kit and confirmed to be negative. A total of six 2-fold dilutions of known concentrations of inactivated SARS-CoV-2 virus (Isolate 2/231/human/2020/CHN) were prepared in the negative clinical matrix and processed using chemagic Viral DNA/RNA 300 Kit special H96 (CMG-1033) on chemagic 360 instrument. Six individual extraction replicates per dilution were tested on the Applied Biosystems 7500 Real-Time PCR System. The results are summarized in the following tables.

Table: Preliminary LoD study using oropharyngeal swabs on chemagic™ 360

Oropharyngeal swab							
Dilution fold	N		ORF1ab		Mean Ct		
	Conc. (copies/ml)	Detection rate	Conc. (copies/ ml)	Detection rate	N	ORF1ab	IC
2.0E+04	137	6/6	41.85	6/6	36.48	36.82	32.18
4.0E+04	68.5	6/6	20.93	6/6	37.04	37.98	32.14
8.0E+04	34.25	6/6	10.46	6/6	39.10	38.88	32.21
1.6E+05	17.13	5/6	5.23	4/6	38.89	39.77	32.35
3.2E+05	8.56	3/6	2.62	2/6	39.35	39.85	32.28
6.4E+05	4.28	0/6	1.31	0/6	/	/	32.41
Negative	0	0/6	0	0/6	/	/	32.23

Table: Probit predicted 95% detection rate using oropharyngeal swabs spiked with SARS-CoV-2 (Isolate 2/231/human/2020/CHN) on chemagic 360.

Probit predicted 95% detection rate (copies/mL)	
N	ORF1ab
19.077 (95% CI: 14.498 – 37.122)	7.142 (95% CI: 5.341 – 23.998)

Table: Preliminary LoD study using nasopharyngeal swabs on chemagic 360.

Nasopharyngeal swab							
Dilution fold	N		ORF1ab		Mean Ct		
	Conc. (copies/ml)	Detection rate	Conc. (copies/ ml)	Detection rate	N	ORF1ab	IC
2.0E+04	137	6/6	41.85	6/6	36.65	36.55	32.32
4.0E+04	68.5	6/6	20.93	6/6	38.17	36.78	32.38
8.0E+04	34.25	6/6	10.46	6/6	38.55	38.24	32.60
1.6E+05	17.13	4/6	5.23	6/6	39.40	40.50	32.59
3.2E+05	8.56	2/6	2.62	1/6	39.59	40.53	32.86
6.4E+05	4.28	2/6	1.31	2/6	39.50	39.70	32.28
Negative	0	0/6	0	0/6	/	/	32.33

Table: Probit predicted 95% detection rate using nasopharyngeal swabs spiked with SARS-CoV-2 (Isolate 2/231/human/2020/CHN) on chemagic 360.

Probit predicted 95% detection rate (copies/mL)	
N	ORF1ab
26.44 (95% CI: 18.338 – 69.511)	8.323 (95% CI: 5.833 – 20.685)

Verification of LoD Using chemagic 360 for Extraction and PCR Setup

For the LoD verification study, pooled negative oropharyngeal swab matrix and pooled negative nasopharyngeal swab matrix was spiked with inactivated SARS-CoV-2 virus at the lowest tentative LoD that was predicted among the two SARS-CoV-2 targets for each matrix (7.142 copies/mL of ORF1ab for oropharyngeal swab matrix and 8.323 copies/mL of ORF1ab for nasopharyngeal swab matrix). Twenty replicates per specimen matrix were prepared and extracted using the chemagic Viral DNA/RNA 300 Kit special H96 (CMG-1033) on the chemagic 360 instrument and tested using the New Coronavirus Nucleic Acid Detection Kit. Twenty additional replicates prepared at 1.5x the tentative LoD were also tested. The results are summarized in the following tables. For both sample types tested at 1× 95% probit LoD, one replicate was negative for the N target and one replicate was negative for the ORF1ab target, producing a detection rate for N and ORF1ab of 95% (19/20). At 1.5× 95% probit LoD, both sample types gave a detection rate of 100% for both targets.

Table: chemagic 360 LoD verification results for oropharyngeal swab.

Concentration (copies/ml)			Detection rate		Mean Ct		
LoD	N	ORF1ab	N	ORF1ab	N	ORF1ab	IC
1X	23.380	7.142	95% (19/20)	95% (19/20)	38.44	38.76	33.13
1.5X	35.070	10.713	100% (20/20)	100% (20/20)	38.74	38.11	33.09

The results confirm an LoD of 7.142 copies/mL for the ORF1ab target and 23.380 copies/mL for the N target in oropharyngeal swab matrix using the Chemagic 360 platform.

Table: chemagic 360 LoD verification results for nasopharyngeal swab.

Concentration (copies/ml)			Detection rate		Mean Ct		
LoD	N	ORF1ab	N	ORF1ab	N	ORF1ab	IC
1X	27.246	8.323	95% (19/20)	95% (19/20)	38.53	38.44	32.81
1.5X	40.871	12.485	100% (20/20)	100% (20/20)	38.50	37.79	32.72

The results confirm an LoD of 8.323 copies/mL for the ORF1ab target and 27.246 copies/mL for the N target in nasopharyngeal swab matrix using the chemagic 360 platform.

LoD Using chemagic 360 for Extraction and the Applied Biosystems Fast Dx, QuantStudio3, and QuantStudio 5 PCR Systems

To expand the use of the New Coronavirus Nucleic Acid Detection Kit for use with the Applied Biosystems Fast 7500 Fast Dx, QuantStudio 3 and

QuantStudio 5 Real-Time PCR Systems, a validation study was conducted using negative clinical nasopharyngeal swab specimens. Pooled nasopharyngeal swab specimens were spiked with two or three known concentrations of SeraCare RNA reference material encoding the entire SARS-CoV-2 viral genome (0505-0159). Nucleic acid was extracted using the chemagic Viral DNA/RNA 300 Kit special H96 (CMG-1033) on chemagic 360 instrument and up to 20 individual extraction replicates were tested on each of the PCR instrument platforms according to the instructions for use. Testing on the original Applied Biosystems 7500 PCR System was included in this study for comparison. The results are summarized in the following tables. The LoD was confirmed to be 20 copies/mL on all four instruments.

Table: LoD verification on alternate Applied Biosystems PCR platforms

Instrument	Concentration (copies/mL)	Target Gene	Mean Ct	Detection Rate for Target Gene	Overall Detection Rate for Algorithm
ABI 7500	6.7	N	40.2	80% (16/20)	90% (18/20)
		ORF	39.4	75% (15/20)	
	20	N	37.8	95% (19/20)	100% (20/20)
		ORF	37.5	95% (19/20)	
ABI 7500 Fast Dx	6.7	N	38.1	45% (9/20)	90% (18/20)
		ORF	39.0	85% (17/20)	
	20	N	37.7	75% (15/20)	100% (20/20)
		ORF	37.5	100% (20/20)	
QS3	12	N	ND	0% (0/3)	67% (2/3)
		ORF	34.1	67% (2/3)	
	20	N	35.7	30% (6/20)	100% (20/20)
		ORF	35.3	95% (19/20)	
	60	N	35.8	45% (9/20)	95% (19/20)
		ORF	33.0	95% (19/20)	
QS5	12	N	ND	0% (0/3)	0% (0/3)
		ORF	ND	0% (0/3)	
	20	N	35.8	25% (5/20)	95% (19/20)
		ORF	37.0	95% (19/20)	
	60	N	36.3	55% (11/20)	100% (20/20)
		ORF	35.1	100% (20/20)	

LoD Using chemagic 360 for Extraction and the Analytik Jena PCR Systems

To expand the use of the New Coronavirus Nucleic Acid Detection Kit for use with the Analytik Jena qTower³/qTower³ G and qTower³ 84/qTower³ 84 G PCR Systems, a validation study was conducted using negative clinical nasopharyngeal swab specimens. Pooled nasopharyngeal swab specimens were spiked with three or four known concentrations of SeraCare RNA reference material encoding the entire SARS-CoV-2 viral genome (0505-0159). Nucleic acid was extracted using the chemagic Viral DNA/RNA 300 Kit special H96 (CMG-1033) on chemagic 360 instrument and up to 20 individual extraction

replicates were tested on each of the PCR instrument platforms according to the instructions for use. Testing on the original Applied Biosystems 7500 PCR System was included in this study for comparison (see table above). The LoD was confirmed to be 10-20 copies/mL on all three instruments. Results for the Analytik Jena PCR Systems are shown below.

Table: LoD verification on alternate Analytik Jena PCR platforms

Instrument	Concentration (copies/mL)	Target Gene	Mean Ct	Detection Rate for Target Gene	Overall Detection Rate for Algorithm
qTower ³	6.7	N	39.3	30% (6/20)	75% (15/20)
		ORF	39.7	65% (13/20)	
	10	N	38.2	65% (13/20)	100% (20/20)
		ORF	37.8	95% (19/20)	
	20	N	38.5	75% (15/20)	100% (20/20)
		ORF	36.9	100% (20/20)	
40	N	37.9	95% (19/20)	100% (20/20)	
	ORF	36.1	100% (20/20)		
qTower ³ 84	10	N	38.5	35% (7/20)	90% (18/20)
		ORF	38.4	80% (16/20)	
	20	N	39.0	55% (11/20)	95% (19/20)
		ORF	37.3	85% (17/20)	
	40	N	38.0	80% (16/20)	100% (20/20)
		ORF	36.7	100% (20/20)	

LoD study on saliva sample

This study established the LoD using inactivated SARS-CoV-2 virus (BEI NR-52286, Isolate USA-WA1/2020, Heat Inactivated). A panel of contrived saliva samples was prepared at 0.43 and 0.13 TCID₅₀/mL. Each panel member was then tested in replicates of 20 using the QuantStudio 5, qTower³, ABI 7500 Standard, and ABI 7500 Fast Dx RT-PCR instruments according the instructions for use. The results, summarized below, established an LoD for all RT-PCR platforms of 0.13 TCID₅₀/mL.

Table: Saliva LoD study summary with inactivated SARS-CoV-2 virus

Instrument	Concentration (TCID ₅₀ /mL)	Target Gene	Mean Ct	Detection Rate for Target Gene	Overall Detection Rate for Algorithm
QuantStudio TM 5	0.13	N	37.85	95% (19/20)	100% (20/20)
		ORF	36.68	95% (19/20)	
		N	38.43	80% (16/20)	80%

	0.043	ORF	37.56	55% (11/20)	(16/20)
qTower ³	0.13	N	35.89	100% (20/20)	100%
		ORF	36.94	100% (20/20)	(20/20)
	0.043	N	37.44	85% (17/20)	90%
		ORF	37.75	75% (15/20)	(18/20)
ABI 7500 Fast Dx	0.13	N	35.89	100% (20/20)	100%
		ORF	38.04	95% (19/20)	(20/20)
	0.043	N	37.24	75% (15/20)	90%
		ORF	38.96	65% (13/20)	(18/20)
ABI 7500 Standard	0.13	N	39.75	100% (20/20)	100%
		ORF	38.99	100% (20/20)	(20/20)
	0.043	N	40.76	55% (11/20)	80%
		ORF	40.42	70% (14/20)	(16/20)

LoD Using chemagic 360 for Extraction and the Applied Biosystems QuantStudio 12K Flex and QuantStudio 7 Flex PCR Systems with transport media Mawi and saline

To expand the use of the New Coronavirus Nucleic Acid Detection Kit for use with the Applied Biosystems QuantStudio™ 12K and QuantStudio™ 7 Flex Real-Time PCR Systems and transport media Mawi (Cat# ISWAB-MB- 1200 from Mawi company) and saline, a validation study was conducted using negative clinical nasopharyngeal swab specimens. Pooled negative clinical Mawi or saline swab specimens (and negative clinical VTM swab specimens for reference) were spiked with inactivated SARS-CoV-2 virus (Zeptomatrix, strain USA-WA1/2020, Cat #: 0810587CFHI) at known concentrations and nucleic acid was extracted using the chemagic Viral DNA/RNA 300 Kit special H96 (CMG-1033) on chemagic 360 instrument. Up to 20 individual extraction replicates for each concentration in each media type were tested on the QuantStudio 12K Flex, QuantStudio 7 Flex PCR instrument platforms according to the instructions for use. Testing on the original Applied Biosystems QuantStudio™ 5 with pooled negative clinical VTM matrix spiked with SARS-CoV-2 virus was included in this study for comparison/reference. The results are summarized in the following tables. The LoD was confirmed to be 1 TCID₅₀/mL on all three instruments with all three transport media types.

Table: LoD verification of Mawi and saline collection medium on Quant Studio 12K Flex and 7 Flex PCR Systems

Instrument	Concentration	VTM ((Reference)	Mawi	Saline
------------	---------------	------------------	------	--------

	(TCID ₅₀ /mL)	N Ct Mean	ORF1a b Ct Mean	Hit Rate	N Ct Mean	ORF1ab Ct Mean	Hit Rate	N Ct Mean	ORF1ab Ct Mean	Hit Rate
	QuantStudio™ 5 (Reference)	30	32.61	33.96	20/20	31.77	33.00	20/20	31.61	34.07
10		34.20	35.67	20/20	33.48	34.67	20/20	33.12	35.49	20/20
3.33		35.56	37.25	20/20	35.25	36.76	20/20	34.73	37.15	20/20
1		36.68	37.81	20/20	37.26	37.89	20/20	37.33	38.60	20/20
0.33		38.07	39.10	17/20	38.03	38.95	16/20	38.08	37.66	17/20
0.111		38.89	38.91	8/20	38.67	39.29	11/20	38.78	39.72	6/20
QuantStudio™ 12K Flex	30	32.55	33.29	20/20	31.71	32.16	20/20	31.63	33.17	20/20
	10	34.20	34.87	20/20	33.40	33.82	20/20	33.13	34.86	20/20
	3.33	35.76	37.09	20/20	34.92	35.45	20/20	34.88	36.54	20/20
	1	37.17	38.42	20/20	36.93	37.62	20/20	37.21	37.90	19/20
	0.33	38.67	38.87	17/20	38.19	38.91	17/20	38.03	38.63	16/20
	0.111	38.85	39.04	8/20	38.17	39.00	6/20	37.82	38.98	4/20
QuantStudio™ 7 Flex	30	32.59	33.29	20/20	31.73	32.16	20/20	31.64	33.34	20/20
	10	34.10	34.98	20/20	33.33	33.96	20/20	33.05	34.94	20/20
	3.33	35.44	36.75	20/20	35.21	35.75	20/20	34.75	36.51	20/20
	1	37.00	38.17	19/20	36.91	37.44	20/20	36.66	38.91	20/20
	0.33	38.23	38.39	14/20	37.55	38.20	18/20	37.09	38.69	16/20
	0.111	38.45	39.24	9/20	38.12	39.34	11/20	37.79	39.49	6/20

Analytical Reactivity (Inclusivity)

The inclusivity of the SARS-CoV-2 primers/probes of the New Coronavirus Nucleic Acid Detection Kit was evaluated *in silico* using sequences from NCBI and GISAID databases. The most recent evaluation was in February 2023.

Three filters were applied on the database browsers before downloading the sequences in order to avoid a misleading analysis. The filter definitions included the following:

- Complete sequences (full length): Genomes with >29,000bp
- High quality inclusion: Entries with <1% Ns (unknown bases) and <0.05% unique amino acid mutations (not seen in other sequences in the database) and no indels unless verified by the submitter
- Low coverage exclusion: Excludes entries with >5% Ns

Primer sequence mutations that were predicted to impact gene detection

included the following: (1) primer sequence has at least one non-ambiguous nucleotide mismatch to the genome in the last three base pairs from the primer's 3' end, or (2) primer sequence has no match to the genome. Mutations in the probe sequence that were predicted to impact detection include the following: (1) probe sequence has greater than two non-ambiguous nucleotide mismatches to the genome, or (2) probe sequence has no match to the genome.

More than 5 million sequences in the GISAID database and 1.6 million sequences in the NCBI database met the criteria for inclusivity analysis (i.e., "complete," "high quality inclusion," "low coverage exclusion"). The primers/probe for the ORF1ab region were 100% homologous to more than 99% of the sequences. For the N gene probe, more than 99% of the analyzed sequences were 100% homologous; however, for the N gene forward primer, approximately 90% of sequences exhibited one or more mismatches. The main reason for the reduction in overall homology between the N gene forward primer sequence with strains from the NCBI and GISAID databases is due to emergent SARS-CoV-2 strains with mutations at the 5' end of the N forward primer (GGG -> AAC). However, the observed mismatches are not predicted to have an adverse effect on clinical sensitivity. A summary of the findings is shown in the table below. Also included in the table below is the number of sequences that were predicted to be impacted by each of the mutations identified.

Table: *In silico* Analysis of SARS-CoV-2 Assay Oligonucleotides as of November 2022

Target Database	N gene						ORF1ab gene					
	NCBI			GISAID			NCBI			GISAID		
Oligo	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe
Total Sequences	1642361	1642361	1642361	5555629	5555629	5555629	1642361	1642361	1642361	5555629	5555629	5555629
Sequences with mutations	1462724	238956	5923	4980488	1107607	19705	3991	5484	7364	16408	12294	21081
1 mismatch	350107	237081	5756	3209099	1100315	19521	3616	5234	4585	15407	12224	17900
2 mismatches	9895	1667	23	66543	6577	55	49	48	140	918	28	426
3 or more mismatches	1102722	208	144	1704846	715	129	326	202	2639	83	42	2755
No match to genome[^]	6853	6281	5772	7001	9143	3745	3704	13985	13425	13801	2313	3692
Sequences predicted to impact (%)[*]	7525 (0.46)	6526 (0.40)	5772 (0.35)	10307 (0.19)	10374 (0.19)	3752 (0.07)	3704 (0.23)	13989 (0.85)	13431 (0.82)	13807 (0.25)	2317 (0.04)	3830 (0.07)

For: Forward primer, Rev: Reverse primer

Sequence with mutations includes 1 mismatch, 2 mismatches and 3 or more mismatches. It does not include "No match to genome".

[^] Sequences that did not align to any region of interest with the BLAST search

^{*} "Sequences predicted to impact" includes those that fall under any of the categories below. Contrary to other categories in the table, this count excludes sequences with mismatches due to ambiguous nucleotides:

- The criteria for primer sequences predicted to be impacted are as follows:
 - (1) primer sequence has at least one non-ambiguous nucleotide mismatch to the genome in the last three base pairs from the primer's 3' end, or
 - (2) primer sequence has no match to the genome.
- The criteria for probe sequences predicted to be impacted are as follows:
 - (1) probe sequence has greater than two non-ambiguous nucleotide mismatches to the genome, or
 - (2) probe sequence has no match to the genome.

based on amplification/detection of either the N or ORF1ab region sequences. Therefore, the identified sequence variants are not predicted to affect inclusivity for SARS-CoV- 2.

In an independent analysis of Omicron, including 759,473 sequences from NCBI and 442,516 sequences from GISAID, showed that the detection of both the N and the ORF1ab target are not predicted to be significantly impacted by the observed mutations. The New Coronavirus Nucleic Acid Detection Kit is predicted to detect the majority of circulating SARS-CoV-2 Omicron variant strains.

Table: *In silico* Analysis of SARS-CoV-2 Assay Oligonucleotides Against Omicron Variant Sequences

Target Database	N gene						ORF1ab gene					
	NCBI		GISAID				NCBI			GISAID		
Oligo	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe
Total Sequences	759473	759473	759473	442516	442516	442516	759473	759473	759473	442516	442516	442516
Sequences with mutations	758422	1213	2905	441376	3932	1093	761	1275	1803	1680	391	1457
1 mismatch	161	1187	2888	1172	3226	1077	707	1216	1531	764	377	1208
2 mismatches	127	11	3	264	690	1	25	12	25	876	6	71
3 or more mismatches	758134	15	14	439940	16	15	29	47	247	40	8	178
No match to genome*	596	354	328	466	4156	448	1080	1386	1041	1254	598	578
Sequences predicted to impact (%)*	772 (0.10)	354 (0.05)	328 (0.04)	604 (0.14)	4157 (0.94)	448 (0.10)	1080 (0.14)	1386 (0.18)	1046 (0.14)	1259 (0.28)	599 (0.14)	607 (0.14)

For: Forward primer, Rev: Reverse primer

Sequence with mutations includes 1 mismatch, 2 mismatches and 3 or more mismatches. It does not include "No match to genome".

^ Sequences that did not align to any region of interest with the BLAST search

* "Sequences predicted to impact" includes those that fall under any of the categories below. Contrary to other categories in the table, this count excludes sequences with mismatches due to ambiguous nucleotides:

- The criteria for primer sequences predicted to be impacted are as follows:
 - (1) primer sequence has at least one non-ambiguous nucleotide mismatch to the genome in the last three base pairs from the primer's 3' end, or
 - (2) primer sequence has no match to the genome.
- The criteria for probe sequences predicted to be impacted are as follows:
 - (1) probe sequence has greater than two non-ambiguous nucleotide mismatches to the genome, or
 - (2) probe sequence has no match to the genome.

Analytical Specificity (Cross-reactivity)

Cross-reactivity of the New Coronavirus Nucleic Acid Detection Kit was evaluated using both *in silico* analysis and wet testing against normal and pathogenic organisms found in the respiratory tract.

BLASTn analysis queries of the New Coronavirus Nucleic Acid Detection Kit primers and probes were performed against public domain nucleotide sequences with default settings. The database search parameters were as follows:

- The match and mismatch scores were 1 and -3, respectively.

- The penalty to create and extend a gap in an alignment was 5 and 2, respectively.
- The search parameters automatically adjusted for short input sequences and the expected threshold was 1000.

In summary no organisms, including other related SARS-coronaviruses, exhibited >80% homology to the forward primer, reverse primer, and probe for either the ORF1ab or N target. The results of the *in silico* analysis suggest the New Coronavirus Nucleic Acid Detection kit is designed for the specific detection of SARS-CoV-2, with no expected cross reactivity to the human genome, other coronaviruses, or human microflora that would predict potential false positive RT-PCR results.

Wet testing against normal and pathogenic organisms of the respiratory tract was performed to confirm the results of the *in silico* analysis. Each organism identified in the table below was tested in triplicate with the New Coronavirus Nucleic Acid Detection kit at the concentrations indicated. Each replicate was tested with a different reagent lot. All results were negative.

Table: Organisms tested for cross-reactivity with the New Coronavirus Nucleic Acid Detection Kit

Pathogen	Source	Concentration	
		Evaluation	Unit
Human coronavirus 229E	ATCC VR-740™	2.8×10^2	TCID ₅₀ /mL
Human coronavirus OC43	ATCC VR-1558™	2.8×10^3	TCID ₅₀ /mL
Adenovirus type 3	ATCC VR-847™	$5.0 \times 10^{5.5}$	TCID ₅₀ /mL
Adenovirus type 2	ATCC VR-846™	5.6×10^4	TCID ₅₀ /mL
Adenovirus type 31	ATCC VR-1109™	1.6×10^6	TCID ₅₀ /mL
Adenovirus type 37	ATCC VR-929™	1.8×10^4	TCID ₅₀ /mL
Adenovirus type 51	ATCC VR-1603™	2.3×10^6	TCID ₅₀ /mL
Parainfluenza virus type 1	ATCC VR-94™	2.8×10^4	TCID ₅₀ /mL
Parainfluenza virus type 2	ATCC VR-92D™	0.303	ng/μL
Parainfluenza virus type 3	ATCC VR-93™	$5.0 \times 10^{4.5}$	TCID ₅₀ /mL
Parainfluenza virus type 4a	ATCC VR-1378™	2.8×10^4	TCID ₅₀ /mL
Parainfluenza virus type 4b	ATCC VR-1377™	1.6×10^3	TCID ₅₀ /mL
Influenza A virus (H1N1pdm09)	ATCC VR-1736™	2.6×10^3	PFU/mL
Influenza A virus (seasonal H1N1)	ATCC VR-1520™	$5.0 \times 10^{4.5}$	TCID ₅₀ /mL
Influenza A virus (H3N2)	ATCC VR-1679™	$5.0 \times 10^{3.5}$	TCID ₅₀ /mL

Influenza B virus	ATCC VR-1807™	7.6 x 10 ²	PFU/mL
Enterovirus A71	ATCC VR-1432™	5.0 x 10 ^{5.5}	TCID ₅₀ /mL
Enterovirus D68	ATCC VR-1823™	1.6 x 10 ⁶	TCID ₅₀ /mL
Respiratory syncytial virus	ATCC VR-1400™	5.0 x 10 ^{3.5}	TCID ₅₀ /mL
Rhinovirus B17	ATCC VR-1663™	2.0 x 10 ⁶	PFU/mL
Rhinovirus A2	ATCC VR-482™	8.9 x 10 ⁴	TCID ₅₀ /mL
<i>Chlamydia pneumoniae</i>	ATCC 53592™	2.9 x 10 ⁵	IFU/mL
<i>Haemophilus influenzae</i>	ATCC 51907D™	10	µg/mL
<i>Streptococcus pyogenes</i>	ATCC 700294D-5TM	7	µg/ml
<i>Streptococcus salivarius</i>	ATCC BAA-250D-5TM	5.2	µg/ml
<i>Bordetella pertussis</i>	ZeptoMetrix Panel	Unknown	
Measles virus	National Standard for Influenza A/B Viral Nucleic Acids Detection Kit	Unknown	
Mumps virus		Unknown	
<i>Staphylococcus aureus</i>		Unknown	
Influenza A virus (H7N9)		Unknown	
<i>Mycoplasma pneumoniae</i>		ATCC 15531TM	3.5 x 10 ⁶
Human cytomegalovirus	Clinical specimen	Unknown	
Hepatitis A virus	Clinical specimen	1.84 x 10 ⁵	copies/mL
Hepatitis B virus	WHO NIBSC 10/266	9.55 x 10 ⁵	IU/mL
Hepatitis C virus	WHO NIBSC 14/150	1.00 x 10 ⁵	IU/mL
Human immunodeficiency virus type I (HIV-1)	WHO NIBSC 16/194	1.26 x 10 ⁵	IU/mL
Human immunodeficiency virus type II (HIV-2)	WHO NIBSC 08/150	1.00 x 10 ³	IU/mL
Epstein-barr virus	Clinical specimen	1.46 x 10 ⁵	copies/mL
Cytomegalovirus	Clinical specimen	1.15 x 10 ⁴	copies/mL

Interfering Substances Studies

The potential interference of the substances listed below were tested in both the presence and absence of SARS-CoV-2 RNA with the New Coronavirus Nucleic Acid Detection Kit. SARS-CoV-2 positive samples were prepared by mixing each of the potentially interfering substances with the assay positive control (synthetic SARS-CoV-2 ORF1ab and N RNA template encapsulated in MS2 bacteriophage) at approximately 3× the LoD. All positive and negative

samples yielded expected results.

Table: Substances tested for interference with the New Coronavirus Nucleic Acid Detection Kit

Substance	Concentration Tested	Substance	Concentration Tested
Valacyclovir	3.6 mg/mL	Saline	1 mg/mL
Entecavir	24.6 ng/mL	Beclomethasone dipropionate	22.5 µg/mL
Adefovir	90 ng/mL	Dexamethasone acetate	375 µg/mL
Ribavirin	5 mg/mL	Triamcinolone tablets	25 µg/mL
Acyclovir	3.6 mg/mL	Mometasone furoate	41.7 µg/mL
Azithromycin	1.35 mg/mL	Fluticasone propionate	1 mg/mL
Clarithromycin	30 µg/mL	Oxymetazoline hydrochloride	15% v/v
Ciprofloxacin	7.5 µg/mL	Sulfur ointment	0.05% v/v
Telbivudine	15 µg/mL	Pharyngitis lozenges	0.05% v/v
Efavirenz	12.2 µg/mL	Chlorhexidine benzocaine	1.25 mg/mL
Tenofovir	1335 ng/mL	Menthol	5% v/v
Zanamivir	5 mg/mL	Rheumatoid factor	/
Mupirocin	0.02% w/v	Systemic Lupus Erythematosus	/
Tobramycin	0.6 mg/mL	Antinuclear antibody	/
Flunisolide	20 mg/mL	Hemoglobin	5 mg/mL
Budesonide	16.7 µg/mL	Human serum albumin	60 mg/mL
Bilirubin	0.6 mg/mL	Triglycerides	25 mg/mL
		Human genomic DNA	3 mg/mL

Clinical Study

Contrived Specimens

The performance of the New Coronavirus Nucleic Acid Detection Kit was evaluated using contrived clinical oropharyngeal swabs and nasopharyngeal swabs. In total, 141 healthy individuals with no COVID-19 infection history, no COVID-19 symptoms and no contact with SARS-CoV-2 infected patients within in 14 days were recruited for the study. Both oropharyngeal swabs and nasopharyngeal swabs were collected from the 141 healthy individuals by trained personnel. Samples were immediately screened with The New Coronavirus Nucleic Acid Detection Kit and stored frozen until use.

The inactivated cultured virus (Isolate 2/231/human/2020/CHN) was spiked into 47 of the oropharyngeal swabs and 47 of the nasopharyngeal swabs at various concentrations (2×LoD, 4×LoD, 10×LoD, 20×LoD, 50×LoD, 100×LoD, 200×LoD, 250×LoD and 500×LoD, according to the LoD of target ORF1ab on Pre-NAT II). Of the 47 contrived positive samples, 20 were spiked at concentrations equivalent to 2× the LoD, 20 were spiked with concentrations equivalent to 4× the LoD, and 7 were spiked with concentrations ranging from 10× LoD to 500× LoD. The remaining 94 oropharyngeal swabs and 94 nasopharyngeal swabs were tested as negative clinical samples.

The 141 oropharyngeal samples and 141 nasopharyngeal samples were tested in a blinded fashion (samples were prepared and capped, then all the tubes were mixed in a box and extracted using the Nucleic Acid Extraction Kit (KN0212) and Pre-NAT II Automated Workstation in a random order). Testing was performed in a total of four RT-PCR runs with one positive and one negative control included per run. Results of the study are summarized below.

Table: Positive and negative control results from clinical evaluation.

Run	Control	N Ct	ORF1ab Ct	IC Ct	Pass
Run 1	Positive control	30.34	29.08	29.43	Yes
	Negative control	Undetermined	Undetermined	32.59	Yes
Run 2	Positive control	31.59	30.99	32.47	Yes
	Negative control	Undetermined	Undetermined	33.97	Yes
Run 3	Positive control	31.79	30.98	31.27	Yes
	Negative control	Undetermined	Undetermined	31.35	Yes
Run 4	Positive control	30.92	30.28	28.72	Yes
	Negative control	Undetermined	Undetermined	34.54	Yes

Table: Clinical evaluation with oropharyngeal samples.

SARS-CoV-2 concentration	Samples (N)	Detection rate		Mean Ct		
		N	ORF1ab	N	ORF1ab	IC
2×LoD	20	20/20	20/20	37.05	37.03	31.90
4×LoD	20	20/20	20/20	35.48	35.56	32.58
10×LoD	1	1/1	1/1	34.93	35.58	33.98
20×LoD	1	1/1	1/1	34.94	34.38	30.72
50×LoD	1	1/1	1/1	34.53	34.17	34.44
100×LoD	1	1/1	1/1	32.17	31.48	31.33

200×LoD	1	1/1	1/1	33.38	32.33	34.94
250×LoD	1	1/1	1/1	32.15	31.44	34.73
500×LoD	1	1/1	1/1	30.32	30.27	33.38
Negative	94*	0/94	0/94	/	/	32.63

*Three of the negative samples initially yielded undetermined Ct values for the IC and were reported as invalid. Repeat results were valid and negative.

Table: Clinical evaluation with nasopharyngeal samples.

SARS-CoV-2 concentration	Samples (N)	Detection rate		Mean Ct		
		N	ORF1ab	N	ORF1ab	IC
2×LoD	20	20/20	20/20	38.01	37.77	31.98
4×LoD	20	20/20	20/20	37.12	36.32	32.11
10×LoD	1	1/1	1/1	35.46	34.72	31.64
20×LoD	1	1/1	1/1	35.46	34.23	32.13
50×LoD	1	1/1	1/1	33.27	32.92	29.86
100×LoD	1	1/1	1/1	31.78	31.43	30.46
200×LoD	1	1/1	1/1	32.95	31.49	32.08
250×LoD	1	1/1	1/1	31.85	30.49	32.04
500×LoD	1	1/1	1/1	30.40	29.73	30.24
Negative	94*	0/94	0/94	/	/	31.78

*One of the negative samples initially yielded undetermined Ct values for the IC and was reported as invalid. The repeat result was valid and negative.

As shown all positive samples at 2×LoD, 4×LoD, 10×LoD, 20×LoD, 50×LoD, 100×LoD, 200×LoD, 250×LoD and 500×LoD were positive and all negative samples were negative in the background of individual oropharyngeal swab and nasopharyngeal swab matrix.

Clinical Specimens

In addition to testing contrived specimens in clinical matrix, the New Coronavirus Nucleic Acid Detection Kit was also evaluated using nasopharyngeal swab specimens collected from individuals suspected of COVID-19 infection. The clinical study included 32 positive and 30 negative NP samples, collected in VTM. For this study, 300 µL of each sample was extracted using the Chemagic 360 with the Chemagic Viral DNA/RNA 300 H96 Kit, eluting with 60µL elution buffer. A 40µL aliquot of the eluted nucleic acid was then used as input for the PCR reactions on the ABI 7500 standard PCR instrument as described in the instructions for use. Each of the patient samples were also tested with a highly sensitive RT-PCR comparator method.

Among the 32 positive samples, 23 samples (72% of positives) were considered to be low positives. There was 100% positive and negative percent agreement between the comparator method and the New Coronavirus Nucleic Acid

Detection Kit. A summary of the clinical study is shown below.

Table: Summary of clinical study and comparator analysis

New Coronavirus Nucleic Acid Detection Kit		FDA EUA Comparator Method	
		Positive	0
		Negative	30
		0	

PPA: 100.0 %, 95% CI (89.3% - 100.0%)

NPA: 100.0%, 95% CI (88.7 -100.0%)

Calculated with the Wilson score method

Clinical Performance of Pooling for up to 5 Specimens

The clinical performance of the New Coronavirus Nucleic Acid Detection Kit was evaluated in pools consisting of up to 5 specimens. Testing included 20 positive and 20 negative specimen pools. Each positive specimen pool consisted of one positive specimen with the remaining specimens being negative, whereas the negative specimen pools consisted only of negative specimens. The positive specimens used in the study covered the detectable range of the assay (see Table below) and included low positive specimens (defined as within 2 Ct of the assay LoD).

Table: Ct Range of SARS-CoV-2 Positive Specimens

Expected Ct value	Number of samples
[36, 40]	5
[30, 36)	5
[25, 30)	5
[20, 25)	5

The pooling study was performed on both pooled and individual nasopharyngeal and oropharyngeal specimens with the New Coronavirus Nucleic Acid Detection Kit using the Chemagic 360 nucleic acid extraction platform and QuantStudio™ 5 (384-well block) PCR System, as a blinded test.

The positive percent agreement (PPA) and negative percent agreement (NPA) were calculated in relation to the expected (individual) result for the positive and negative sample pools, respectively. The study results are summarized in the following table.

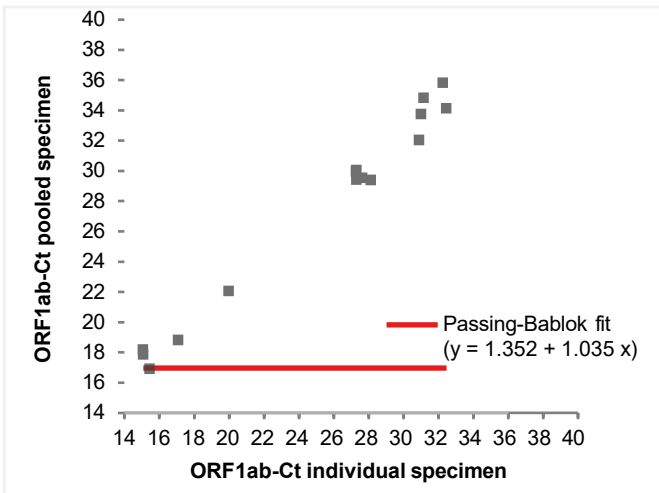
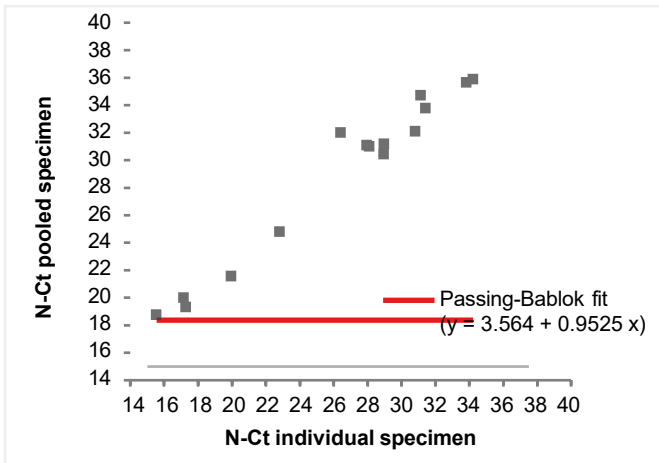
Table: Individual and Pooled Specimen Agreement for Pool Sizes of 5

		Individual Specimen Result		
		Positive	Negative	Total
Pooled Specimen Result	Positive	20	0	20
	Negative	0	20	20
	Total	20	20	40

PPA = 100% (83.9-100%)

NPA = 100% (83.9-100%)

Scatter plots for the observed individual specimen and pooled specimen Ct values for a pool size of 5 are shown in the figures below, stratified by SARS-CoV-2 target gene. For a pool size of 5, a linear relationship was observed for N target and ORF1ab targets up to individual Ct values of 35.7 and 32.5, respectively. An average shift in Ct of 1.7 for the N target and 2.7 for the ORF1ab target between the individual specimens and 5-specimen pools was observed. With a pool size of 5, individual specimens with Ct values ≥ 35.7 for the N target and ≥ 32.5 for the ORF1ab did not show consistent linearity between the individual and pooled specimen Ct values. See graphs below.



Based on the data above, it is estimated that for a pool size of 5, 100% of specimens with an individual test Ct value <35.7 for the N target and <32.5 for the ORF1ab target are not expected to be missed, and 95% of specimens with an individual test Ct between 35.7 and 40.3 for the N target and between 32.5 and 39.2 for the ORF1ab target are not expected to be missed. Specimens with individual test Ct values above 40.3 for the N target and above 39.2 for the ORF1ab target are expected to be missed 100% of the time. The results of this analysis are shown in the table below.

Table: Estimated Percent Detection Across Ct Ranges

	Ct shift	Zone 1	Percent Detection	Zone 2	Percent Detection	Zone 3	Percent Detection
N Target	1.7	<35.7	100%	35.7-40.3	95%	40.3-42	0%
ORF Target	2.7	<32.5		32.5-39.2		39.2-42	

Clinical study on saliva sample and viral transport medium

A clinical study was conducted to compare the performance of the New Coronavirus Nucleic Acid Detection Kit with saliva specimens to the performance of the New Coronavirus Nucleic Acid Detection Kit with paired NP swab specimens.

A total of 60 paired clinical specimens (30 saliva specimens and 30 NPS specimens) was collected from individuals suspected of COVID-19, in a healthcare setting.

The saliva samples were processed using the protocol described in the section “Extraction from saliva sample on chemagic 360”, the NPS samples were processed using the protocol described in the section “Extraction and PCR setup on chemagic 360”. The study was conducted using the QuantStudio™ 5 instrument.

The positive percent agreement (PPA) and negative percent agreement (NPA) were calculated in relation to paired NPS specimen. The performance of the New Coronavirus Nucleic Acid Detection Kit with saliva specimens is summarized in the table below.

Table: Clinical study of NPS samples vs. saliva samples

		NPS sample		
		Negative	Positive	Total
Saliva sample	Positive	0	30	30
	Negative	30	0	30

	Total	30	30	60
--	-------	----	----	----

PPA = 100% (88.7-100%)

NPA = 100% (88.7-100%)

The statistical summary is listed in the following table.

Table: Ct value statistical analysis of NPS samples vs. saliva samples.

Clinical sample	Sample type	IC			N			ORF1ab		
		Mean	SD	N	Mean	SD	N	Mean	SD	N
Negative	NPS	32.24	3.32	30	ND			ND		
	Saliva	31.70	3.01	30						
Positive	NPS	31.32	3.52	29	26.30	8.07	28	28.01	9.32	29
	Saliva	32.18	3.98	28	26.70	7.81	30	28.27	6.68	26

ND = Not Detected; SD: Standard Deviation

Saliva Sample Stability Study

A study was conducted to establish the stability of saliva specimens collected using the SalivaSecure collection kit. A panel, consisting of a low positive member, at 2x LoD, and a negative member, was tested.

All panel members tested at each storage temperature/condition returned the expected results. The study data support the recommended specimen storage condition for shipping at 19 - 23°C for up to 56 hours or 2 – 8 °C for up to 24 hours.

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD on ABI 7500 Standard based on Chemagic™ 360 extraction. Blinded sample testing was used to establish specificity and to confirm the LoD. The results are summarized in the Table below.

Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	NP	180 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL N/A:

Not applicable

ND/D: Not detected/Detected









References

1. *Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases: World Health Organization; 2020.*
2. *Innis, MA, et Al, and the PCR Protocols Applications: A Laboratory Manual , Academic, New York, 1989.*
3. *Mahony JB. Detection of Respiratory Viruses by Molecular Methods. Clinical Microbiology Reviews. 2008; 21 (4): 716-747.*
4. *China CDC Virus Disease Control and Prevention. Novel coronavirus nucleic acid detection primer and probe sequences (Specific Primers and Probes for Detection Novel coronavirus 2019) [EB / OL]., 2020-01-21.*

Revision history: Publication Number v1.0

Revision	Date	Description
1.0	March 20, 2020	New document
2.0	March 30, 2020	Added chemagic 360
3.0	April 3, 2020	Added CMG-1033-S
4.0	July 28, 2020	Added Nasal Swab
5.0	September 16, 2020	FDA Panel Results
6.0	September 21, 2020	Added Pooling & Applied Biosystems QuantStudio 3/5, 7500 Fast Dx, qTower 96 and 384.
7.0	January 6, 2021	Added Asymptomatic data and updated inclusivity analysis
8.0	February 23, 2021	Added Appendix B & C
9.0	June 7, 2021	Added saliva sample
10.0	Dec 10, 2021	Added post authorization sample saliva study
11.0	February 02, 2022	Added Quantstudio 12K Flex, QuanstStudio 7 Flex and transport media (Mawi and saline)
12.0	July 21, 2022	Updated to the reagent stability claim from 12 to 24 months
13.0	March 23, 2023	Removed the asymptomatic testing population and updated in silico inclusivity analysis. Added additional software (Design and Analysis Software v2.4.3) for Quantstudio 12K Flex, QuanstStudio 7 Flex data analysis
14.0	January 31, 2024	Update to the product name, company name and branding, legal manufacture address, and technical support contact information.

Key to symbols used

Symbol	Symbol Title and Reference Number
	Batch number
	Use-by date
	Temperature limit
	Contains sufficient for <n> tests
	Consult instructions for use
	Manufacturer
	This way up
	Fragile

For more information contact: COVID-19.TechnicalSupport@Revvity.com



Suzhou Sym-Bio Lifescience Co., Ltd.
No.115, North Tai Ping Road
Taicang Economic Development Zone
215400 Jiangsu Province, PRC
P: (800) 762-4000 or (+1)203-925-4602
www.revvity.com

The word "revvity" in a lowercase, rounded, sans-serif font.

For a complete listing of our global offices, visit www.revvity.com/ContactUs

Copyright ©2023, Revvity, Inc. All rights reserved. Revvity® is a registered trademark of Revvity, Inc. All other trademarks are the property of their respective owners.

Appendix A: Specimen Pooling Implementation and Monitoring Guidelines

Before Implementation of Pooling: Determine Appropriate Pool Size

Before a pooling strategy is implemented, a laboratory should determine the appropriate pool size based on percent positivity rate and desired testing efficiency. The New Coronavirus Nucleic Acid Detection Kit has been validated for n-sample pool sizes up to five samples per pool.

If historical laboratory data for individual specimens is available:

- 1) If historical data for individual specimens from the previous 7-10* days is available, estimate the percent positivity rate ($P_{\text{individual}}$) based on individual testing results.
 - $P_{\text{individual}} = (\text{Number of positive specimens over chosen date range} \div \text{Total number of specimens tested over chosen date range}) * 100.$
- 2) Using the calculated $P_{\text{individual}}$ and **Table 1**, identify the appropriate n number of samples to pool.
 - If $P_{\text{individual}}$ is less than 5%, the maximum pool size validated, ($n=5$), should be selected to maximize the efficiency of specimen pooling. Pooling with greater than 5 samples has not been validated and should not be performed.
 - If $P_{\text{individual}}$ is greater than 25%, Dorfman pooling of patient specimens is not efficient and should not be implemented.

If historical laboratory data for individual specimens is unavailable:

- 3) If historical data from the previous 7-10* days is unavailable, 5, 4 or 3-specimen pooling may still be implemented as the New Coronavirus Nucleic Acid Detection Kit has been validated for 5-specimen pooling.
- 4) Note: without calculating $P_{\text{individual}}$, the pooling size implemented may not maximize pooling efficiency.

Table 1

<i>P, percent of positive subjects in the tested population</i>	<i>$n_{\text{maxefficiency}}$ (n corresponding to the maximal efficiency)</i>	<i>Efficiency of n-sample pooling (a maximum increase in the number of tested patients when Dorfman n- pooling strategy used)</i>
5-6%	5	2.15-2.35
7-12%	4	1.54-1.99
13-25%	3	1.10-1.48

Because a positive pool requires individual retesting of each sample in the pool, the efficiency of any pooling strategy depends on the positivity rate. The efficiency (F) of n-sample pooling for positivity rate (P) can be calculated with the following formula $F = 1 / (1 + 1/n - (1 - P)^n)$. The efficiency (F) indicates how many more patients can be tested with n-sample pools compared to individual testing. For example, a 5-sample pooling strategy increases the number of tested patients by 2.15 times for positivity rate P of 6% (F=2.15). At F=2.15, 1,000 tests can on average cover testing of 2,150 patients.

Implementation of Pooling

See above section titled: Preparing Samples for Pooling and perform pooling procedure as outlined.

After Implementation of Pooling: Ongoing Monitoring of Pooling Strategy

If historical laboratory data for individual specimens is available:

- 5) After implementing a pooling strategy, evaluate the performance of pooled testing by comparing the percent positivity rate of pooled testing to that of individual testing.
- 6) Calculate the percent positivity rate among patient specimens during specimen pooling (P_{pools}) on a daily basis using a moving average of the data from the previous 7-10* days of testing.
 - $P_{\text{pools}} = (\text{Number of patient specimens with a positive result as determined by individual specimen reflex testing of positive pools over chosen date range} \div \text{Total number of patient specimens tested in pools over chosen date range}) * 100.$
- 7) Compare P_{pools} to $P_{\text{individual}}$. If P_{pools} is less than 85% of $P_{\text{individual}}$ ($P_{\text{pools}} < 0.85 \times P_{\text{individual}}$), it is recommended that the pool size be reassessed and adjusted to maximize pooling efficiency (if necessary), according to the criteria in Table 1.
- 8) To ensure maximum pooling efficiency, it is recommended that $n_{\text{maxefficiency}}$, be re-assessed periodically while sample pooling is implemented by the laboratory.

If historical laboratory data for individual specimens is unavailable:

- After initiating a pooling strategy, evaluate the performance of pooled testing by calculating the initial percent positivity rate for pooled specimens ($P_{\text{pools-initial}}$). $P_{\text{pools-initial}}$ is the percent positivity rate for pooled specimens for the first 7-10* days of pooled testing.
- Calculate the initial percent positivity rate for individual specimens from pool testing ($P_{\text{pools-initial}}$) from the first 7-10* days of testing.
 - $P_{\text{pools-initial}} = (\text{Number of patient specimens with a positive result as determined by individual specimen reflex testing of positive pools in first 7-10* days} \div \text{Total number of patient specimens tested in pools in the first 7-10* days}) * 100.$
 - If $P_{\text{pools-initial}}$ is greater than 25%, pooling of patient specimens is

not efficient and should be discontinued until the percent positivity rate decreases.

- If $P_{\text{pools-initial}}$ is less than or equal to 25%, pooling of patient specimens can be continued.
- Continue to monitor pooling strategy by calculating the percent positivity rate among patient specimens during specimen pooling ($P_{\text{pools-x}}$) for subsequent 7-10* day periods. $P_{\text{pools-x}}$ should be updated daily using a moving average.
- Compare $P_{\text{pools-x}}$ to $P_{\text{pools-initial}}$. If $P_{\text{pools-x}}$ is less than 90% of $P_{\text{pools-initial}}$ ($P_{\text{pools-x}} < 0.90 \times P_{\text{pools-initial}}$), it is recommended that the pool size be reassessed and potentially adjusted to maximize pooling efficiency.
- In order to ensure maximum pooling efficiency, it is recommended that $n_{\text{maxefficiency}}$, be re-assessed periodically while sample pooling is implemented by the laboratory.

*7-10 days is recommended for calculating $P_{\text{individual}}$, P_{pools} , $P_{\text{pools-initial}}$, and $P_{\text{pools-x}}$. Laboratories should determine if 7-10 days is appropriate by taking into consideration laboratory testing volume and percent positivity. If the number of individual or pooled positive results collected during a given time frame is less than 10, $P_{\text{individual}}$, P_{pools} , $P_{\text{pools-initial}}$, and $P_{\text{pools-x}}$ may not be representative of the percent positivity in the testing population. Consider extending the data collection time period to increase the number of positives evaluated.

Appendix B: Verification Recommendations

Purpose

This appendix is intended to provide a qualification procedure for verifying the performance of the real-time PCR instruments, specifically the RUO instruments, authorized for use with the New Coronavirus Nucleic Acid Detection Kit. The RUO instruments Analytik Jena qTower3/qTower3 G and qTower3 84/qTower3 84 G Real Time PCR Systems. Qualification of the RUO PCR system with the New Coronavirus Nucleic Acid Detection kit must be achieved prior to usage for diagnostic testing.

Required Materials

Description	Quantity	Provided in the Kit
nCoV Positive Control	1	Yes
nCoV Negative Control	1	Yes

SeraCare full genome (0505-0159)	1	No. Order through SeraCare. https://www.seracare.com/
----------------------------------	---	---

The kit positive control (virus-like particle) consists of SARS-CoV-2 RNA fragments capsulated in bacteriophage. It is extracted before use and ensures RNA is correctly released in the extraction process. It will yield a positive result for SARS- CoV-2. The kit positive control should be prepared according to the Instructions for Use.

The SeraCare full genome reference material is used to prepare evaluation panel which is around 5xLoD. It will yield a positive result for SARS-CoV-2.

Negative samples can be prepared from viral transport medium, or TE buffer (nCoV Negative Control) or nuclease-free water or known negative clinical samples.

Refer to the Instructions for Use for additional reagents, materials, and instructions.

Instruments installation and service

It is required to calibrate and verify the real-time PCR instrument according to the instrument’s user manual. The links to the calibration reagent order information, user guides and OQ/PQ (“Operational Qualification”/“Performance Qualification) procedures for the instruments are provided in the following table below.

Instrument	Calibration reagent order information (part number)	Calibration and OQ or PQ procedure
qTower 96	NA	Schedule install service (OQ): Analytik Jena US Phone: (909) 946-3197 Email: covid19support@us.analytik-jena.com
qTower 384	NA	Schedule install service (OQ): Analytik Jena US Phone: (909) 946-3197 Email: covid19support@us.analytik-jena.com

After instrument OQ and/or PQ, follow the procedure for verification.

Procedure

Follow kit IFU, perform tests on 6 positive samples (kit positive control: sample 1- 3; Seracare panel: sample 4-6), 3 negative samples with extraction process (sample 7-9) and 3 NTC (non-template control, sample 10-12) without extraction process, and run on the same real-time PCR plate.

The detail procedure is listed below.

- 1) Take the nCoV Internal Control, nCoV Positive Control and nCoV Negative Control, or Negative samples out from freezer, place them in a biological safety cabinet and completely thaw them at room temperature. Vortex the tubes to mix the contents, then centrifuge the tubes briefly at 1000 rpm to collect the liquid to the bottom of the tubes.
- 2) Prepare SeraCare reference material, dilute stock to 100 cp/mL with TE buffer, named as “SeraCare panel”. For example, if stock concentration is 5000 cp/mL, 20uL stock material will be diluted with 980 uL TE buffer (or VTM) to make evaluation concentration as 100 cp/mL.
- 3) Prepare the evaluation panel for sample extraction as following:
 - Sample 1-3: 300 µL nCoV Positive Control per sample
 - Sample 4-6: 300uL SeraCare panel per sample
 - Sample 7-9: 300 µL nCoV Negative Control (or Negative Sample) per sample
- 4) Perform sample extraction on sample 1-9, following the procedure in section “Extraction and PCR setup on chemagic 360”, page 11-13.
- 5) Prepare PCR master mix and plate, following the procedure in section “Extraction and PCR setup on chemagic 360” according to the corresponding verification instrument, page 13-14.
 - Sample 1-3: Add 40/20/10 (depending on the PCR instrument) of extracted nCoV Positive Control to PCR plate position 1-3.
 - Sample 4-6: Add 40/20/10 uL (depending on the PCR instrument) of extracted SeraCare panel to PCR plate position 4-6.
 - Sample 7-9: Add 40/20/10 uL (depending on the PCR instrument) of extracted nCoV Negative Control (or Negative Sample) to PCR plate position 7-9.
 - Sample 10-12: Add 40/20/10 uL (depending on the PCR instrument) of nCoV Negative Control directly to PCR plate position 10-12.
- 6) Run the PCR program according to the settings for the corresponding verification instrument, following the procedure in section “Amplification”, page 15-22

Acceptance criteria

All evaluated 12 samples must meet the criteria for “PASS” based on the following table prior to usage of the New Coronavirus Nucleic Acid Detection Kit for diagnostic testing.

Result Interpretation for 60 µL reaction:

Sample type	Ct			Result interpretation
	N (FAM)	ORF1ab (ROX)	IC (HEX/VIC)	
NTC	Undet or > 42	Undet or > 42	Ct > 40	PASS
Negative	Undet or > 42	Undet or > 42	Ct ≤ 40	PASS
Positive	≤ 35	≤ 35	/	PASS
SeraCare panel	Either FAM or ROX with Ct ≤ 42		/	PASS

/: No requirements on the Ct value.

Undet: Undetermined

Result Interpretation for 30 µL reaction:

Sample type	Ct			Result interpretation
	N (FAM)	ORF1ab (ROX)	IC (HEX/VIC)	
NTC	Undet or > 42	Undet or > 42	Ct > 40	PASS
Negative	Undet or > 42	Undet or > 42	Ct ≤ 40	PASS
Positive	≤ 36	≤ 36	/	PASS
SeraCare panel	Either FAM or ROX with Ct ≤ 42		/	PASS

/: No requirements on the Ct value.

Undet: Undetermined

Result Interpretation for 15 µL reaction:

Sample type	Ct			Result interpretation
	N (FAM)	ORF1ab (ROX)	IC (HEX/VIC)	
NTC	Undet or > 42	Undet or > 42	Ct > 40	PASS
Negative	Undet or > 42	Undet or > 42	Ct ≤ 40	PASS
Positive	≤ 37	≤ 37	/	PASS
SeraCare panel	Either FAM or ROX with Ct ≤ 42		/	PASS

Appendix C: Additional Label
For qTower 96 and qTower 384 Instruments

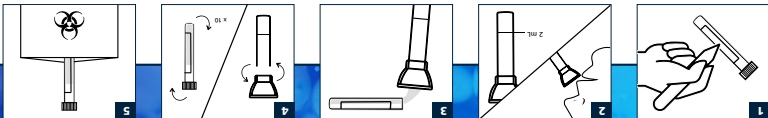
Please print the below label and place the label on the front panel of the instrument. If the instrument includes labeling indicating “For Research Use Only”, please cover with the below “Emergency Use Only” labeling. The instrument should retain this labeling throughout the EUA use of the New Coronavirus Nucleic Acid Detection Kit.

Emergency Use Only

**This instrument is authorized for use with the
New Coronavirus Nucleic Acid Detection Kit**

room temperature. No cold chain required. Specimens preserved in the SalivaSecure™ Saliva Collection Kit are stable and can be **transported and stored at**

1. Write on the collection tube (the one with the funnel adapter) your ID number and the date of collection
2. Spit approximately 2 mL sample into the collection device.
3. Pour all of the DNA/RNA Shield™ solution into the collection device. **Warning: DO NOT DRINK**
4. Remove and discard the funnel portion of the device and close the tube containing the samples with the cap of the solution bottle. Mix the sample by vigorously inverting it 10 times.
5. Place the tube containing the sample into a biohazard bag for shipping.
6. The specimen should be sent to the testing lab as instructed by the test provider.



SDX-56338-MW
Rev 2.0

SAMPLE COLLECTION INSTRUCTIONS

Cat.#: SDX-56338

SalivaSecure™

SALIVA COLLECTION KIT

For in vitro diagnostic use | Rx only
For use under Emergency Use Authorization (EUA) only

- This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories;
- This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
- 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.

Warnings and Precautions

FOR EXTERNAL USE ONLY.

DO NOT drink, touch or remove the reagent from the vial.

- The DNA/RNA Shield™ reagent can be harmful if ingested and may cause irritation if exposed to the skin and eyes.
- If the contents of the tube contact your skin, wash the affected area with soap and water. If the contents of the tube are splashed in your eyes, immediately flush eyes with water. Notify your healthcare provider if irritation develops. If the contents of the vial are spilled, use a new collection kit.
- This product is intended for professional use or for use in POC. DO NOT use if the product is visibly damaged.
- Avoid mixing the reagent and sodium hypochlorite (bleach) or other strong acids and bases. These mixtures could release noxious gases. For specifics, consult product Safety Data Sheet (SDS)



H302: Harmful if Swallowed



H315: Causes Skin Irritation



H319: Causes Eye Irritation

Label Legend



Collect Sample by



Storage Instructions



Lot Number



Catalog Number



Harmful



See Instruction Manual



Manufacturer



No.115, North Tai Ping Road
Taicang Economic Development
Zone
215400 Jiangsu Province, PRC
P: (800) 762-4000 or
(+1)203-925-4602
www.revivity.com