PerkinElmer®

Instructions for PerkinElmer® New Coronavirus Nucleic Acid Detection Kit

v 7.0

For prescription use only. For in vitro diagnostic use only.
For Emergency Use Authorization only.

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Product Name

PerkinElmer® New Coronavirus Nucleic Acid Detection Kit

Kit Contents

48 Tests

Intended Use

The PerkinElmer® 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 human oropharyngeal swab 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 any individual, including individuals without symptoms or other reasons to suspect COVID-19 infection. 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 swab 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 upper respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial

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

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

The PerkinElmer® 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 PerkinElmer® 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 PerkinElmer® 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 PerkinElmer® 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-371) (chemagic™ MSM I software version 6.1.0.5).

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. Analytik Jena qTower^3 / qTower^3 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.

   f. Analytik Jena qTower^3 84 / qTower^3 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.

   g. 96-well PCR plate:

   - 0.1 mL for Applied Biosystems QuantStudio 3 and Applied Biosystems 7500 Fast Dx: 4344906 or equivalent
   - 0.2 mL for Applied Biosystems 7500 Standard: 4306737 or equivalent

- MicroAmp Optical Adhesive Film: 4311971

- Analytik Jena qTower^3 / qTower^3 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

h. 384-well PCR plate:

- Applied Biosystems QuantStudio 5: 4343370 or equivalent

    - MicroAmp Optical Adhesive Film: 4311971

- Analytik Jena qTower^3 84 / qTower^3 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**	**Chemagi 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	✓	

Storage & Handing Requirements

1. Store all reagents at -25 to -15°C.
2. Use the reagents within 30 days once opened.
3. 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.
4. Avoid excessive freeze/thaw cycles for reagents.

Warnings and Precautions

1. For *in vitro* diagnostic use. For prescription use only. For use under an Emergency Use Authorization only.
2. This test has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an Emergency Use Authorization (EUA) for use by authorized laboratories.
3. This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
4. The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
5. Positive results are indicative of the presence of SARS-CoV-2 RNA.
6. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
7. 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 when inappropriate handling of reference materials and specimens, which will cause inaccurate results. It is recommended to use sterile disposable filter-tips to aspirate reagents and specimens.
10. All specimen to be tested and the reference materials 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.

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 PerkinElmer New Coronavirus Nucleic Acid Detection Kit and decrease sensitivity.

Instruments

- PerkinElmer® 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
- Analytik Jena qTOWER^3 / qTower^3 G Real-Time PCR system
- Analytik Jena qTOWER^3 84 / qTower^3 84 G Real-Time PCR system

Collection, Storage & Shipment of Specimens

1. Specimen Collection

   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.

2. Storage

   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.

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 at 2-8°C and ship overnight to the lab on ice pack. If a specimen is frozen at -70°C ship overnight to the lab on dry ice. 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, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)

   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-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 PerkinElmer® 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, 70uL of each individual patient sample should be combined into one well of a deep-well plate for a total volume of 350uL 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

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 temperatures and follow the operations described in 1) for the controls.

3) Take the Magnetic Beads from the PerkinElmer® 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 µL of each specimen, Negative Control and Positive Control to the wells of a 96 deep-well plate, and add 5 µL nCoV Internal Control, 800 µL Lysis/Binding Buffer and 15 µL 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 µL elution buffer to elute DNA/RNA.
   - During elution, liquid handler pipettes/mixes PCR reagents to prepare a PCR mix and aliquot 20 µL to PCR tubes.
   - For each sample, 40 µL of eluted DNA/RNA is added to PCR mix in each tube, which is ready for amplification.

Extraction and PCR setup on chemagic 360

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;

2) Take a 2 mL deep-well-plate (riplate SW), add 300 µL Lysis buffer, 300 µL specimen, 5 µL 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 have to 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*: • 5uL for PCR reaction as 60uL per reaction • 10uL for PCR reaction as 30uL per reaction • 20uL for PCR reaction as 15uL per reaction 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]
Position6	Empty deep-well-plate (riplate SW) [purified water added automatically]
Position 7	Deep-well-plate (riplate SW) prefilled with 60 µL Elution Buffer 5

*: the recommended settings for reduced PCR reaction volumes. However, 5uL internal control is a valid setting for different PCR reaction volumes (15uL/30uL/60uL).

8) Double check the positions and directions of all consumables according to the tracking system.

9) Click “Start” to start the extraction process.

10) 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 the procedures described below after nucleic acid extraction using chemagic 360.

PCR setup on ABI 7500 Standard (60uL 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 qTower^3 / qTower ^{3} G (30uL 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, qTower^3 84 / qTower^3 84 G (15uL 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 x (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.

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 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 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 then click Run and Start to initialize amplification.

Applied Biosystems QuantStudio3

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 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 then click Run and Start to initialize amplification.

QuantStudio™ 5

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™ 5 System
    - 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 then click Run and Start to initialize amplification.

Analytik Jena qTOWER^3 84 / qTower^3 84 G Real-Time PCR system

1) Set up and run the Analytik Jena qTOWER^3 84 / qTower^3 84 G Real-Time PCR instrument. Refer to Analytik Jena qTOWER^3 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: qTOWER^3 84 or qTower^3 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, 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 qTOWER^3 / ^{3}G Real-Time PCR system

1) Set up and run the Analytik Jena qTOWER^3 / ^{3}G Real-Time PCR instrument. Refer to Analytik Jena qTOWER^3 / ^{3}G 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: qTOWER^3 or qTower^3 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, 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

1. Baseline and threshold setting for ABI 7500 Standard, ABI 7500 Fast Dx, QuantStudio 3 and QuantStudio 5

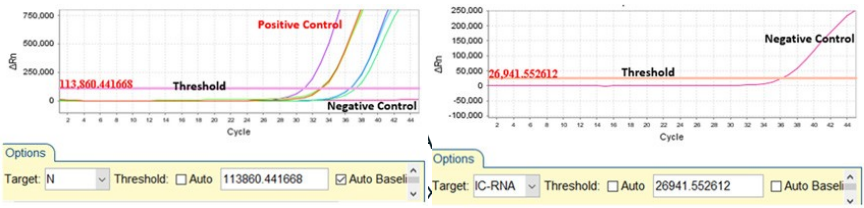
After the run completion, save and analyze the data according to PCR instrument instructions.

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.



5) Interpret the results based on the tables listed in “Quality Control” and

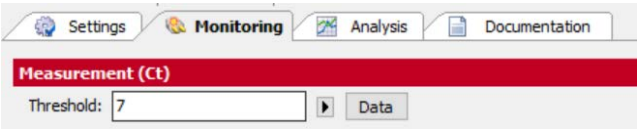
"Examination and Interpretation of Specimen Results".

2. Baseline and threshold setting for qTower^3 / ^{3} G and qTower^3 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 qTower^3 / ^{3} G (version 4.1) and qTower^3 84 / ^{3} 84 G.

Under **monitoring** tab, click "Calculate Ct", the following view shows up.

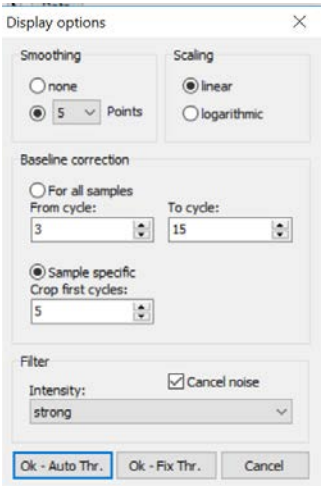


7) Set baseline for each target

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.

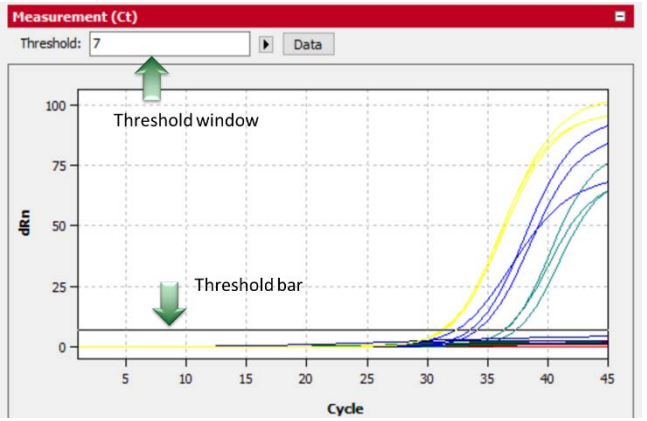


In order to switch back to the default setting, click "Sample specific Crop first cycles".

8) 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, it can either remove 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 5-15 as general.



3. Quality Control

The 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 60uL reaction:

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

/: No requirements on the Ct value.

Undet: Undetermined

Result Interpretation of Test Controls for 30uL reaction:

Control type	Ct		
	N (FAM)	ORF1ab (ROX)	IC (HEX/VIC)
Negative	Undet or > 42	Undet or > 42	Ct ≤ 40

Positive	≤ 36	≤ 36	/

/: No requirements on the Ct value.

Undet: Undetermined

Result Interpretation of Test Controls for 15uL 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.

4. 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.

5. 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 assay 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, to perform high complexity tests.
2. This kit is used for qualitative detection of SARS-CoV-2 RNA from human oropharyngeal swab, nasopharyngeal swab, and anterior nasal swab. The results cannot directly reflect the viral load in the original specimens.
3. Nasal swab specimens self-collected under the supervision of or collected by a healthcare provider can be tested with the PerkinElmer New Coronavirus Nucleic Acid Detection Kit; however, performance with this specimen type has not been determined.
4. Sample pooling has only been validated using nasopharyngeal and oropharyngeal swab specimens.
5. Samples should only be pooled when testing demand exceeds laboratory capacity and/or when testing reagents are in short supply.

6. The PerkinElmer New Coronavirus Nucleic Acid Detection Kit may be used to test asymptomatic individuals, although performance has not been demonstrated in an asymptomatic population. This assay has been shown to exhibit high sensitivity when tested with the FDA reference panel.

7. Use of the PerkinElmer New Coronavirus Nucleic Acid Detection Kit in a general, asymptomatic screening population is intended to be used as part of an infection control plan, that may include additional preventative measures, such as a predefined serial testing plan or directed testing of high-risk individuals. Negative results should not be treated as definitive and do not preclude current or future infection obtained through community transmission or other exposures. Negative results must be considered in the context of an individual's recent exposures, history, and presence of clinical signs and symptoms consistent with COVID-19.

8. In the absence of symptoms, it is difficult to determine if asymptomatic individuals have been tested too late or too early. Therefore, negative results in asymptomatic individuals may include individuals who were tested too early and may become positive later, individuals who were tested too late and may have serological evidence of infection, or individuals who were never infected.

9. The PerkinElmer® New Coronavirus Nucleic Acid Detection Kit performance has only been established with nasopharyngeal swab and oropharyngeal swab specimens. Anterior nasal swabs are acceptable upper respiratory specimens; however, performance with this specimen type has not been determined.

10. 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.

11. 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.

12. Amplification and detection of SARS-CoV-2 with the PerkinElmer® 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.

13. 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.

14. 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.

15. 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.
16. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.
17. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics or immunosuppressant drugs have not been evaluated.
18. Laboratories are required to report all results to the appropriate public health authorities.

Conditions of Authorization for the Laboratory

The PerkinElmer® 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/vitro- diagnostics-euas.

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

A. Authorized laboratories[1] 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 Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to 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-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and PerkinElmer (via email: COVID-19.TechnicalSupport@PerkinElmer.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. PerkinElmer, its authorized distributor(s) and authorized laboratories using the PerkinElmer 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 PerkinElmer® New Coronavirus Nucleic Acid Detection Kit can

detect at a ≥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 PerkinElmer® 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 PerkinElmer® 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 Pre-NAT II.

Dilution Fold	N		ORF1ab		Mean Ct		
	Conc. (copies/ml)	Detection rate	Conc. (copies/ml)	Detection 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 Pre-NAT II.

Dilution Fold	N		ORF1ab		Mean Ct		
	Conc. (copies/ml)	Detection rate	Conc. (copies/ml)	Detection 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 PerkinElmer® Nucleic Acid Extraction Kit (KN0212) on Pre-NAT II and tested using the PerkinElmer® 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 PerkinElmer® 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 PerkinElmer® 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		
	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 PerkinElmer 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 PerkinElmer New Coronavirus Nucleic Acid Detection Kit for use with the Analytik Jena qTower^3/qTower^3 G and qTower^3 84/qTower^3 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)	

Analytical Reactivity (Inclusivity)

The inclusivity of the SARS-CoV-2 specific PerkinElmer New Coronavirus Nucleic Acid Detection kit primers/probes have been routinely evaluated using full length genomic sequences available in the NCBI and GISAID databases. The most recent in silico evaluation was completed in November 2020. More than 26,343 sequences in from the NCBI database, and 143,701 sequences in the GISAID database at this time were classified as full length (complete sequences), and met the other inclusion criteria for the analysis (high quality and high coverage, low N base pair counts) described below.

- Complete sequences: Genomes with >29000bp
- High quality: Entries with <1% N base pair and <0.05% unique amino acid mutations (not seen in other sequences in the database) and no indels unless verified by the submitter
- High coverage: Entries with >5% N base pairs were excluded

The PerkinElmer New Coronavirus Nucleic Acid Detection Kit SARS-COV-2

ORF1ab primer and probe sequences were an identical match to over 99% of the 170,044 sequences included in the analysis. This percentage was reduced to 92% when the SARS-CoV-2 N primer and probe sequences were also considered. A breakdown of the N primer and probe sequences shows that the reverse primer and probe were an identical match to over 97% and 99% of the available sequences, respectively, while the forward primer was identical to >56% of the available sequences.

The main reason for the reduction in overall homology between the PerkinElmer New Coronavirus Nucleic Acid Detection Kit primer/probe sequences with strains from the NCBI and GISAID databases is a recent emergence of circulating SARS-CoV-2 strains with a three base pair substitution at the 5' end of the PerkinElmer New Coronavirus Nucleic Acid Detection Kit N forward primer (GGG -> AAC). The percentage of sequences in the NCBI and GISAID databases with this mutation was 29% and 37% respectively. 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 the each of the mutations identified. The criteria for primer sequences predicted to be impacted are as follows: (1) primer sequence has at least one mismatch to the genome in the last five base pairs from the primer's 3' end, (2) primer sequence has multiple mismatches to the genome with at least one mismatch landing in the 3' half of the primer, or (3) 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 mismatches to the genome, or (2) probe sequence has no match to the genome. For all criteria regarding impact, any mismatches caused by N base pairs or other ambiguous nucleotide nomenclature are ignored.

Table: Summary of individual oligo mutations

Target	**N gene**						**ORF1ab gene**					
Database	**NCBI**			**GISAID**			**NCBI**			**GISAID**		
Oligo	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe	For	Rev	Probe
Total Sequences	26343	26343	26343	143701	143701	143701	26343	26343	26343	143701	143701	143701
Sequences with mutations	11559	631	31	55476	3952	218	19	43	83	182	388	398
1 mismatch	518	624	31	1970	3863	162	18	36	15	175	359	280
2 mismatches	14	6	0	35	27	3	0	0	1	1	2	7
3 or more mismatches	10963	1	0	53348	62	3	0	7	4	2	2	36
Other mutations	64	0	0	123	0	49	1	0	63	4	25	75
Sequences predicted to impact	211	265	0	672	454	49	63	14	1	12	57	75

For: Forward primer, Rev: Reverse primer.

Several other factors were considered when determining what impact the 5' substitutions in the N forward primer of the PerkinElmer New Coronavirus Nucleic Acid Detection Kit is expected to have on overall sensitivity of the assay. First, a Tm analysis of both the changed and unchanged N primer sequence

was conducted and compared against the PCR annealing temperature parameters used in the assay workflow. The result of this analysis indicated that the likelihood of the identified mutations at the 5' end of N forward primer sequence impacting the sensitivity of N target detection is low. Second, the algorithm used by the PerkinElmer New Coronavirus Nucleic Acid Detection Kit to determine a positive or negative result states that if either the N or ORF1ab target gene is detected, then the result is reported as SARS-CoV-2 detected. Since the emerging mutations appear to be localized to just one of the assay targets, the algorithm also suggests that the overall sensitivity of the device is not likely to be impacted.

Analytical Specificity (Cross-reactivity)

Cross-reactivity of the PerkinElmer® 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 PerkinElmer® 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 PerkinElmer® 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 PerkinElmer® 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 PerkinElmer® New Coronavirus Nucleic Acid Detection Kit

Pathogen	Source	Concentration	
		Evaluation	Unit
Human coronavirus 229E	ATCC VR-740™	2.8×10^2	TCID50/mL
Human coronavirus OC43	ATCC VR-1558™	2.8×10^3	TCID50/mL
Adenovirus type 3	ATCC VR-847™	$5.0 \times 10^{5.5}$	TCID50/mL
Adenovirus type 2	ATCC VR-846™	5.6×10^4	TCID50/mL
Adenovirus type 31	ATCC VR-1109™	1.6×10^6	TCID50/mL
Adenovirus type 37	ATCC VR-929™	1.8×10^4	TCID50/mL
Adenovirus type 51	ATCC VR-1603™	2.3×10^6	TCID50/mL
Parainfluenza virus type 1	ATCC VR-94™	2.8×10^4	TCID50/mL
Parainfluenza virus type 2	ATCC VR-92D™	0.303	ng/µL
Parainfluenza virus type 3	ATCC VR-93™	$5.0 \times 104.^5$	$TCID_{50}$/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™	7.6×10^2	PFU/mL
Enterovirus A71	ATCC VR-1432™	$5.0 \times 10^{5.5}$	$TCID_{50}$/mL
Enterovirus D68	ATCC VR-1823™	1.6×10^6	$TCID_{50}$/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
Chlamydia pneumoniae	ATCC 53592™	2.9 x 10^{5}	IFU/mL
Haemophilus influenzae	ATCC 51907D™	10	µg/mL
Streptococcus pyogenes	ATCC 700294D-5™	7	µg/ml
Streptococcus salivarius	ATCC BAA-250D-5™	5.2	µg/ml
Bordetella pertussis	ZeptoMetrix Panel	Unknown	
Measles virus	National Standard for Influenza A/B Viral Nucleic Acids Detection Kit	Unknown	
Mumps virus		Unknown	
Staphylococcus aureus		Unknown	
Influenza A virus (H7N9)		Unknown	
Mycoplasma pneumoniae	ATCC 15531™	3.5 x 10^{6}	
Human cytomegalovirus	Clinical specimen	Unknown	
Hepatitis A virus	Clinical specimen	1.84E+05	copies/mL
Hepatitis B virus	WHO NIBSC 10/266	9.55E+05	IU/mL
Hepatitis C virus	WHO NIBSC 14/150	1.00E+05	IU/mL
Human immunodeficiency virus type I (HIV-1)	WHO NIBSC 16/194	1.26E+05	IU/mL
Human immunodeficiency virus type II (HIV-2)	WHO NIBSC 08/150	1.00E+03	IU/mL
Epstein-barr virus	Clinical specimen	1.46E+05	copies/mL
Cytomegalovirus	Clinical specimen	1.15E+04	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 PerkinElmer® 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 PerkinElmer® 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 ug/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 PerkinElmer® 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 PerkinElmer® 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 PerkinElmer® 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	Number of samples	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 PerkinElmer 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 PerkinElmer New Coronavirus Nucleic Acid Detection Kit. A summary of the clinical study is shown below.

Table: Summary of clinical study and comparator analysis

		FDA EUA Comparator Method	
PerkinElmer® New Coronavirus Nucleic Acid Detection Kit	Positive	32	0
	Negative	0	30

PPA: 100.0 %, 95% CI (89.3% - 100.0%)
NPA: 100.0%, 95% CI (88.7 -100.0%)

Clinical Performance of Pooling for up to 5 Specimens

The clinical performance of the PerkinElmer 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 PerkinElmer 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.

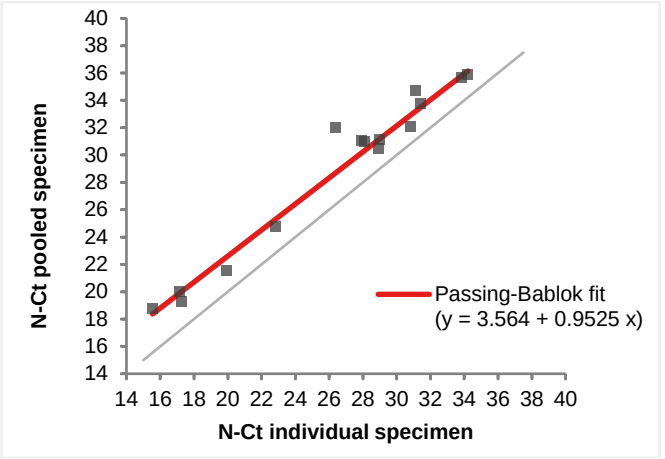
The positive percent agreement (PPA) and negative percent agreement (NPA) were calculated in relation to the expected (individual) result for the positive and negative sample pools, respectively. The study results are summarized in the following table.

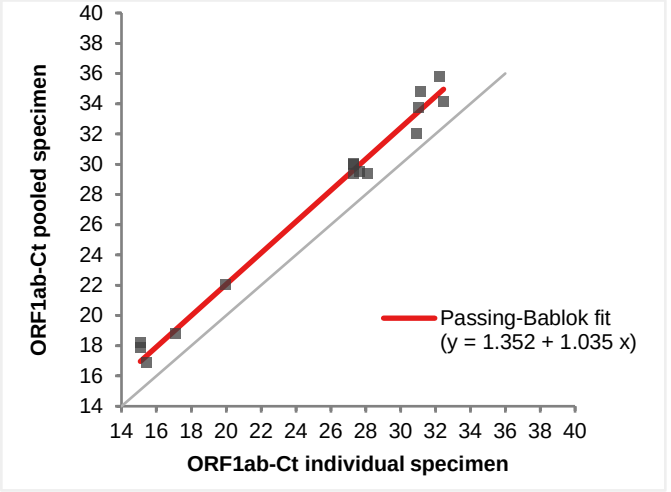
Table: Individual and Pooled Specimen Agreement for Pool Sizes of 5

		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	

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

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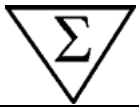
3. *Mahony JB. Detection of Respiratory Viruses by Molecular Methods. Clinical Microbiology Reviews. 2008; 21 (4): 716-747.*

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, 2020	Added Asymptomatic data and updated inclusivity analysis

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

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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 PerkinElmer 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 <u>available</u>:

- If historical data for individual specimens from the previous 7-10* days is available, estimate the percent positivity rate ($P_{individual}$) based on individual testing results.
    - $P_{individual}$ = (Number of positive specimens over chosen date range ÷ Total number of specimens tested over chosen date range)*100
- Using the calculated $P_{individual}$ and **Table 1**, identify the appropriate *n* number of samples to pool.
    - If $P_{individual}$ 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 $P_{individual}$ 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 <u>unavailable</u>:

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

Table 1

P, percent of positive subjects in the tested population	$n_{maxefficiency}$ ***(n corresponding to the maximal efficiency)***	***Efficiency of n-sample pooling*** ***(a maximum increase in the number of tested patients when Dorfman n-pooling strategy used)***
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:

- 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.

- 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_{pools} = (Number of patient specimens with a positive result as determined by individual specimen reflex testing of positive pools over chosen date range ÷ Total number of patient specimens tested in pools over chosen date range)*100

- Compare P_{pools} to $P_{individual}$. If P_{pools} is less than 85% of $P_{individual}$ ($P_{pools} < 0.85 \times P_{individual}$), it is recommended that the pool size be reassessed and adjusted to maximize pooling efficiency (if necessary), according to the criteria in Table 1.

- To ensure maximum pooling efficiency, it is recommended that *nmaxefficiency,* be re-assessed periodically while sample pooling is implemented by the laboratory.

If historical laboratory data for individual specimens is **<u>unavailable</u>:**

- After initiating a pooling strategy, evaluate the performance of pooled testing by calculating the initial percent positivity rate for pooled specimens ($P_{pools\text{-}initial}$). $P_{pools\text{-}initial}$ 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 ($P_{pools\text{-}initial}$) from the first 7-10* days of testing.
    - $P_{pools\text{-}initial}$ = (Number of patient specimens with a positive result as determined by individual specimen reflex testing of positive pools in first 7-10* days ÷ Total number of patient specimens tested in pools in the first 7-10* days)*100
    - If $P_{pools\text{-}initial}$ is greater than 25%, pooling of patient specimens is not efficient and should be discontinued until the percent positivity rate decreases.
    - If $P_{pools\text{-}initial}$ 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 ($P_{pools\text{-}x}$) for subsequent 7-10* day periods. $P_{pools\text{-}x}$ should be updated daily using a moving average.

- Compare $P_{pools\text{-}x}$ to $P_{pools\text{-}initial}$. If $P_{pools\text{-}x}$ is less than 90% of $P_{pools\text{-}initial}$ ($P_{pools\text{-}x} < 0.90 \times P_{pools\text{-}initial}$), 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 *nmaxefficiency,* be re-assessed periodically while sample pooling is implemented by the laboratory.

*****7-10 days is recommended for calculating $P_{individual}$, P_{pools}, $P_{pools\text{-}initial}$, and $P_{pools\text{-}x}$. 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, $P_{individual}$, P_{pools} $P_{pools\text{-}initial}$, and $P_{pools\text{-}x}$ 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.