Instructions for PerkinElmer® New Coronavirus Nucleic Acid Detection Kit

v 4.0

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 the SARS-CoV-2 virus in human oropharyngeal swab, nasopharyngeal swab, and anterior nasal swab specimens collected from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. SARS-CoV-2 RNA is generally detectable in human respiratory specimens during the acute phase of infection. Positive results are indicative of presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infection status. Positive results do not rule out bacterial infection or co-infection with other viruses. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

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

The PerkinElmer® New Coronavirus Nucleic Acid Detection Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The 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 ORF1ab.

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

   Applied Biosystems TM 7500 Real-Time PCR System (4351104 with Laptop, 4351105 with desktop) (software version 2.3).

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	✓	

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.
4. Avoid excessive freeze/thaw cycles for reagents.

Warnings and Precautions

1. For *in vitro* diagnostic use under Emergency Use Authorization only.
2. Positive results are indicative of the presence of SARS-CoV-2 RNA.
3. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
4. Keep the kit upright during storage and transportation.

5. 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.

6. 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.

7. 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.

8. The PCR instrument used for this assay should be calibrated regularly according to instrument's instructions to eliminate cross-talks between channels.

9. 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.

Instruments

PerkinElmer® PreNAT II Automated Workstation

chemagic™ 360

Applied Biosystems® 7500 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

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 5 µL Internal Control 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

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.

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.

Amplification

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.

Interpretation of Results

1. Baseline and threshold setting

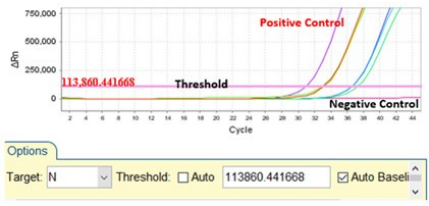
After the run completion, save and analyze the data according to PCR instrument instructions.

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”.

2. 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.

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

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 be ≤35, the Ct value of internal control does not have to be ≤40 for positive control.

3. 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

1) /: No requirements on the Ct value. 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 iinvalid;

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.

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. The PerkinElmer® New Coronavirus Nucleic Acid Detection Kit performance has only been established with the specimen types described in the Intended Use section. Testing other types of specimen may cause inaccurate results.
4. 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.

5. 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.
6. Amplification and detection of SARS-CoV-2 with the PerkinElmer® New Coronavirus Nucleic Acid Detection Kit has only been validated with the Applied Biosystems® 7500 Real-Time PCR instrument. Use of other instrument systems may cause inaccurate results.
7. 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.
8. 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.
9. 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.
10. Negative results do not preclude SARS-CoV-2 infections and should not be used as the sole basis for treatment or other management decisions.
11. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutics or immunosuppressant drugs have not been evaluated.
12. Laboratories are required to report all positive 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 will include with result reports of your product, 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 your product will 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.

C. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will 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 You (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.

F. 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.

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 PCR Setup

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. 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 PCR Setup

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. 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.

Analytical Reactivity (Inclusivity)

BLASTn analysis queries alignments were performed with the SARS-CoV-2 ORF1ab and N oligonucleotide primer and probe sequences with all publicly available nucleic acid sequences for 2019-nCoV in GenBank to demonstrate the predicted inclusivity of the PerkinElmer® New Coronavirus Nucleic Acid Detection Kit.

All the alignments show 100% identity to the available 2019-nCoV sequences.

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	$TCID_{50}$/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	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™	$5.0 \times 10^{3.5}$	$TCID_{50}$/mL
Rhinovirus B17	ATCC VR-1663™	2.0×10^{6}	PFU/mL
Rhinovirus A2	ATCC VR-482™	8.9×10^{4}	$TCID_{50}$/mL
Chlamydia pneumoniae	ATCC 53592™	2.9×10^{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™	3.5×10^{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

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.

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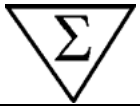
Revision history: Publication Number v1.0

Revision	Date	Description
1.0	March 20, 2020	New document
2.**0**	March 30, 2020	Added chemagic
3.0	April 3, 2020	Added CMG-1033-S
4.0	July 28, 2020	Added Nasal Swab

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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