Gnomegen

COVID-19 RT-Digital PCR Detection Kit

Instructions for Use

For Emergency Use Authorization Only

Catalog # CV0202

48 reactions

For In-Vitro Diagnostic (IVD)

Rx Only

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1. Intended Use

The Gnomegen COVID-19 RT-Digital PCR Detection Kit is a real-time digital PCR *in vitro* diagnostic test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 virus in nasal, nasopharyngeal, and oropharyngeal 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, to perform high complexity tests.

Results are for the identification of SARS-COV-2 RNA. SARS-CoV-2 RNA is generally detectable in nasal, nasopharyngeal, and oropharyngeal swab 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 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 Gnomegen COVID-19 RT-Digital PCR Detection Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time digital PCR and *in vitro* diagnostic procedures. The Gnomegen COVID-19 RT-Digital PCR Detection kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Summary and Explanation

An outbreak of pneumonia caused by a novel coronavirus (SARS-CoV-2) in Wuhan City, Hubei Province, China was identified and reported to the WHO on December 31, 2019. The rapid spread of SARS-CoV-2 to numerous areas throughout the world necessitates preparedness and response in healthcare and lab facilities. The availability of specific and sensitive assays for the detection of the virus are essential for accurate diagnosis of cases, assessment of the extent of the outbreak, monitoring of intervention strategies, and surveillance studies.

The Gnomegen COVID-19 RT-Digital PCR Detection kit is a molecular *in vitro* diagnostic kit containing the reagents required to perform a reverse transcription digital polymerase chain reaction (RT-dPCR) test. The COVID-19 primer and probe set(s) are designed to detect RNA from the SARS-CoV-2 virus in upper respiratory nasal, nasopharyngeal, and oropharyngeal swab specimens from patients suspected of COVID-19 by their healthcare provider.

3. Principles of the Procedure

The Gnomegen COVID-19 RT-Digital PCR Detection kit consists of primers and Taqman probes for the detection of the N-gene of SARS-CoV-2. The reagents are for

use in a single microwell chip for real-time RT-digital PCR testing to directly detect RNA for the novel SARS-CoV-2 virus in human upper respiratory specimens.

The oligonucleotide primers and probes for the detection of SARS-CoV-2 were selected from regions of the virus nucleocapsid (N) gene. The N1 and N2 assays are designed for the specific detection of the SARS-CoV-2 virus. An additional primer/probe set is used as an internal reference to detect human RNase P (RP) mRNA in control samples and clinical specimens.

RNA isolated and purified from upper respiratory specimens is reverse transcribed to cDNA and subsequently amplified in the Gnomegen Real-Time Digital PCR Instrument or the QuantStudio™ 3D Digital PCR System. In the process, the probes anneal to their specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescent intensity is measured in real-time in the Gnomegen Real-Time Digital PCR System and fluorescence intensity is measured by the endpoint in the QuantStudio™ 3D Digital PCR System.

4. Required Materials (provided)

Component	Part No.	Description	Quantity/Tube	Reactions/Tube
COVID-19 Assay	CV02020001	An assay containing SARS-CoV-2 nucleocapsid (N gene) N1 and N2 primers, FAM labelled N1 and N2 probes, as well as RNase P (RP) primers and a VIC labelled reference RP probe. Formulated at 20X.	40 µL	48
COVID-19 Negative Control	CV02020002	A cDNA plasmid encoding the RP target. The COVID-19 Negative Control will yield a **negative** result with the viral SARS-CoV-2 targets (FAM), but a **positive** result for the RP (VIC).	20 µL	5
COVID-19 Positive Control	CV02020003	A combination of cDNA plasmids encoding the SARS-CoV-2 N gene and RP	20 µL	5

		target. The COVID-19 Positive Control will yield **positive** results for both the viral SARS-CoV-2 targets (FAM) and the RP (VIC).		

5. Material Required but Not Provided:

RT-dPCR Enzymes and Master Mix

Reagent	Quantity	Catalog No.
SuperScript™ II Reverse Transcriptase	50 reactions	18064014
RNaseOUT™ Recombinant Ribonuclease Inhibitor	125 reactions	10777019
QuantStudio™ 3D Digital PCR Master Mix v2	200 reactions	A26358

RNA Extraction Kit

Manufacturer	Extraction Kit	Catalog No.
Qiagen	QIAamp® DSP Viral RNA Mini Kit	50 reactions: 61904

RT-dPCR Amplification Chip

Manufacturer	Chip Kit	Catalog No.
Thermofisher	QuantStudio™ 3D Digital PCR 20K Chip Kit v2	12 Chips: A26316

RT-dPCR Instrument

Instrument/Manufacturer	Description	Catalog No.
Gnomegen Real-Time Digital PCR Instrument	The Gnomegen Real-Time Digital PCR Instrument (Drt-PCR) is a bench top Real-Time Digital PCR Instrument that uses fluorescent-based polymerase chain reaction (PCR) reagents to detect target nucleic acid sequences (targets) using real-time analysis of digital PCR reactions performed on digital PCR chips. Instrument is compatible with the QuantStudio™ 3D Digital PCR 20K Chip Kit v2. The instrument is used with the Gnomegen Real-Time Digital PCR Instrument Software (v1) for data processing.	INS1

QuantStudio™ 3D Digital PCR System Package, with Master Mix, Chip Kit v2	The QuantStudio 3D Digital PCR System leverages a high-density nanofluidic chip technology to partition a sample into as many as 20,000 independent reaction wells. Samples and amplification products remain completely contained throughout the streamlined workflow, and an easily interpretable answer (target copies/µL) is produced—all of which minimizes the level of expertise required to quantify specific nucleic acid sequences of interest. Simply load the reaction mix onto the chip, amplify on the ProFlex™ 2 x Flat Block Thermal Cycler and read the target concentration on the QuantStudio 3D Digital PCR Instrument. The instrument is used with the QuantStudio 3D Digital PCR System software (Analysis Suite v3.1).	A29154

Human Specimen Control (HSC)

- Negative human specimen material: Laboratories may prepare a volume of human specimen material (e.g., human sera or pooled leftover negative respiratory specimens) to extract and run alongside clinical samples as an extraction control. This material should be prepared in sufficient volume to be used across multiple runs. Material should be tested prior to use as the extraction control to ensure it generates the expected results for the HSC listed in these instructions for use.
- Contrived human specimen material: Laboratories may prepare contrived human specimen materials by suspending any human cell line (e.g., A549, Hela or 293) in PBS. This material should be prepared in sufficient volume to be used across multiple runs. Material should be tested prior to use as the extraction control to ensure it generates the expected results for the HSC listed in these instructions for use.

6. Equipment and Consumables Required (but not provided)

- Vortex mixer
- Microcentrifuge
- Micropipettes (2 or 10 µL, 200 µL and 1000 µL)
- Racks for 1.5 mL microcentrifuge tubes
- 2 x 96-well -20°C cold blocks
- Molecular grade water, nuclease-free
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- DNAZap™ (Ambion, cat. #AM9890) or equivalent
- RNase Away™ (Fisher Scientific; cat. #21-236-21) or equivalent
- Disposable powder-free gloves and surgical gowns
- Aerosol barrier pipette tips

- 1.5 mL microcentrifuge tubes (DNase/RNase free)
- 0.2 mL PCR tubes (DNase/RNase free)

7. Warnings and Precautions

- For *in vitro* diagnostic use (IVD).
- For Emergency Use Authorization only.
- Positive results are indicative of the presence of SARS-CoV-2 RNA.
- Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.
- Specimen processing should be performed in accordance with national biological safety regulations.
- If infection with 2019-nCoV is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Performance characteristics have been determined with nasopharyngeal and oropharyngeal swab specimens. Testing other types of specimens may cause inaccurate results.
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplifications reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
    - Maintain separate areas for assay setup and handling of nucleic acids.
    - Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.
    - Change aerosol barrier pipette tips between all manual liquid transfers.
    - During preparation of samples, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of nucleases into

samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.
    - Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
    - Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
    - Change gloves between samples and whenever contamination is suspected.
    - Keep reagent and reaction tubes capped or covered as much as possible.
    - Primers, probes (including aliquots), and enzyme master mix must be thawed and maintained on cold block at all times during preparation and use.
    - Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNA*Zap*™" or "RNase AWAY®" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- RNA should be maintained on cold block or on ice during preparation and use to ensure stability.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

8. Reagent Storage, Handling, and Stability

- Store the assay and controls at -20°C.
- Store liquid HSC control materials at ≤ -20°C.
- Protect fluorogenic probes from light.
- Primers, probes (including aliquots), and enzyme master mix must be thawed and kept on a cold block at all times during preparation and use.
- Controls and aliquots of controls must be thawed and kept on ice at all times during preparation and use.

9. Specimen Collection, Handling, and Storage

Adequate, appropriate specimen collection, storage, and transport are important in order to obtain sensitive and accurate test results. Training in correct specimen collection procedures is highly recommended to assure good quality specimens and results. CLSI MM13-A may be referenced as an appropriate resource.

- Sample acceptance criteria
    - Samples should be collected into sterile, labeled tubes, and shipped at 2°C to 8°C on frozen gel packs.
- Specimen rejection criteria
    - Samples that have not been pre-approved for testing and those that are labeled improperly will not be tested until the required information is obtained.
- Collecting the Specimen

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

10. General Preparation

Equipment Preparation:

- Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used including 5% bleach, 70% ethanol, and DNAzap™ or RNase AWAY® to minimize the risk of nucleic acid contamination.

11. Nucleic Acid Extraction

All necessary safety precautions should be taken according to the Laboratory guidelines. Precautions must also be taken to prevent cross contamination of samples. Separate work areas should be used for:

- Nucleic acid extraction
- Reagent preparation (e.g., preparation of RT-PCR master mix; NO amplified reactions, target solutions, or clinical specimens should be brought into this area. After working in this area, laboratory coat and gloves should be changed before moving into the nucleic acid addition area)
- Nucleic acid addition
- Instrumentation (e.g., thermocyclers)

General Handling: Proper microbiological, aseptic technique should always be used when working with RNA. Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination. Always wear powder-free latex,

vinyl, or nitrile gloves while handling reagents, tubes and RNA samples to prevent RNase contamination from the surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed. During the procedure, work quickly and keep everything on cold blocks when possible to avoid degradation of RNA by endogenous or residual RNases. Clean working surfaces, pipettes, etc. with 20% bleach or other solution that can destroy nucleic acids and RNases. To eliminate accelerated deterioration of any plastics and metals, wipe down with 70% ethanol after using 20% bleach. Make sure all bleach is removed to eliminate possible chemical reactions between bleach and guanidine thiocyanate which is present in the extraction reagents.

Performance of the Gnomegen COVID-19 RT-Digital PCR Detection Kit is dependent upon the amount and quality of template RNA purified from human specimens. The following commercially available RNA extraction kits and procedures have been qualified and validated for recovery and purity of RNA for use with the panel:

- Qiagen QIAamp® DSP Viral RNA Mini Kit: Concentrate patient samples to a final volume of 140 µL following manufacturer's instructions. Utilize 140 µL of concentrated sample for extraction and elute with 60 µL of buffer.
- Extracted nucleic acid should be stored at 4°C if it is to be used within 4 hours, or at -70°C if stored longer than 4 hours.

*Manufacturer's recommended procedures (except as noted in recommendations above) are to be followed for sample extraction. No template control (NTC) and HSC must be included in each extraction batch.

12. Assay Setup

Note: Run configuration can vary with the number of specimens and work-day organization. One NTC, and one positive and negative control must be included in each run. NOTE: Per CLIA regulations, HSC must be tested at least once per day.

Reaction Master Mix:

1) Thaw RT-Digital PCR Master Mix, SuperScript II™ and RNaseOut™ enzymes, and COVID-19 assay on ice or cold-block. Keep cold during preparation and use.
2) Mix master mix, enzyme, and assay tubes by inverting each tube 5 times.
3) Centrifuge master mix, enzyme, and assay tubes for 5 seconds to collect contents at the bottom of the tube, and then place the tube in on ice or in a cold rack.
4) Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction mix for the NTC, Positive Control, Negative Control, human specimen control (as applicable), and pipetting errors. Use the following guide to determine N:
    a. N = number of samples (n) + 4 controls + 1 (excess reaction for pipetting error)
5) Mix the reaction according to table 1 below:

Table 1 One-step RTdPCR Reaction

Reagent	Amount (µL) 1 Reaction	Amount (µL) N Reactions
Superscript II	0.20	N x .20
RNaseOUT	0.20	N x .20
Digital PCR Master Mix	7.25	N x 7.25
COVID-19 Assay	0.725	N x 0.725
Molecular Grade Nuclease Free Water	2.125	N x 2.125
Total	10.5	N x 10.5

6) Dispense reagents into a 1.5 mL microcentrifuge tube. After addition of the reagents, mix reaction mixtures by pipetting up and down. Do not vortex.
7) Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube on ice or in a cold rack.
8) Label 0.2 mL DNase/RNase Free tubes for n samples and 4 controls (positive, negative, no template, human specimen control).
9) Aliquot 10.5 µL of the reaction master mix into a 0.2 mL DNase/RNase Free tube.
10) Prior to moving to the nucleic acid handling area, prepare the No Template Control (NTC) reactions in the assay preparation area.
    a. Pipette 4 µL of nuclease-free water that has been run through the extraction process into the NTC sample tube.
11) Close the tube and move to the specimen nucleic acid handling area.

Nucleic Acid Template Addition:

1) Gently vortex nucleic acid sample tubes for approximately 5 seconds.
2) Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube on ice or in a cold rack.
3) Carefully pipette 4 µL of each sample into each sample tube. Keep other sample tubes closed during addition. Change pipette tips after each addition.
4) Change gloves often and when necessary to avoid contamination.
5) Repeat step 3 for the remaining samples.
6) Add 4 µL of Human Specimen Control (HSC) extracted sample to the HSC tube. NOTE: Per CLIA regulations, HSC must be tested at least once per day.
7) Ensure all tubes are closed and move the reaction plate to the positive template control handling area.

Assay Control Addition:

1) Gently vortex the positive and negative controls for approximately 5 seconds.
2) Centrifuge for 5 seconds to collect contents at the bottom of the tube and then place on ice or in a cold rack.
3) Carefully pipette 4 µL of the COVID-19 Positive Control into the positive control tube. Change pipette tips and pipette 2.125 µL of molecular grade nuclease-free water into the positive control tube to bring the reaction up to 14.5 µL.

4) Carefully pipette 4 µL of the COVID-19 Negative Control into the negative control tube. Change pipette tips and pipette 2.125 µL of molecular grade nuclease-free water into the positive control tube to bring the reaction up to 14.5 µL.

Chip Preparation:

1) Prepare and load the 14.5 µL to the digital PCR chips according to the QuantStudio™ 3D Digital PCR System User Manual.

*Manufacturer's recommended procedures (except as noted in recommendations above) are to be followed for chip sample preparation.

Thermocycling Conditions

1) Load the chips onto the one of the acceptable thermocyclers for use with the Gnomegen COVID-19 RT-Digital PCR Detection Kit and cycle the chips according to the cycling conditions in table 2 below:

Table 2 Cycling for One-step RTdPCR

Temperature (°C)	Time	No. Cycles
42	20 min	1X
96	10 min	
60	2 min	
98	30 sec	39X
60	2 min	
10	Infinite hold	1X

2) Once the chips have finished cycling, remove the chips from the thermocycler and let them incubate at room temperature for 15 minutes.
3) Image and analyze the chip results.

* Manufacturer's recommended procedures (except as noted in recommendations above) are to be followed for setting up thermocycling conditions.

13. Analyze the data

Threshold determination: the thresholds for negative/positive determination of fluorescent signals should be based off the controls

1) For each run, the viral target FAM positive threshold is ≥ 3 FAM signals.
2) Use viral target FAM positive threshold for the positive/negative determination of clinical test samples

14. Interpretation of Results

Controls

1) **No template control (NTC)**: should be negative and does not exhibit fluorescent signals for either the SARS-CoV-2 FAM-labelled targets or the human RP VIC-labeled target.
    i. If the no template control contains positive FAM (≥ 3) or VIC (≥ 60) signals that meet the signal threshold, sample contamination may have occurred. Invalidate the run and repeat the digital PCR process using residual extracted nucleic acid following strict adherence to the guidelines.
2) **Negative template control**: should be negative for the SARS-CoV-2 targets demonstrating ≤ 2 FAM fluorescent signals, and positive for human RP reference target demonstrating ≥ 60 VIC fluorescent signals.
3) **Positive template control**: should be positive for the SARS-CoV-2 targets demonstrating ≥ 3 FAM fluorescent signals, and positive for the human RP reference target demonstrating ≥ 60 VIC fluorescent signals.
4) **HSC**: should be negative for the SARS-CoV-2 targets demonstrating ≤ 2 FAM fluorescent signals, and positive for human RP reference target demonstrating ≥ 60 VIC fluorescent signals.

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the assay may have been set up and/or executed improperly, or reagents or equipment malfunction may have occurred. Repeat the test run using residual extraction material.

Table 3 Expected Results for Test Controls

Control Type	Control Name	Used to Monitor	2019 nCoV N1/N2 (FAM Signals)	Human RNase P (VIC Signals)
No Template Control	NTC	Reagent and/or environmental contamination	0-2	0-2
Negative Template Control	Negative Control	Proper assay set-up, and PCR reagent failure	0-2	≥ 60
Positive Template Control	Positive Control	SARS-CoV-2 specific PCR reagent failure including primer and probe integrity	≥ 3	≥ 60
Human Specimen Control	HSC	Successful nucleic acid extraction and reverse transcription	0-2	≥60

Patient Samples

Assessment of clinical specimen test results should be performed after the test controls have been examined and determined to be valid and acceptable. Interpretation of patient sample results is as follows:

1) **Positive:** if the sample is positive for the SARS-CoV-2 targets (≥ 3 FAM) and the human RP reference target has a VIC signal ≥ 0, the sample is positive for SARS-CoV-2 RNA.
2) **Negative:** if the sample is negative for the SARS-CoV-2 targets (≤ 2 FAM) and positive for the human RP reference target (≥ 60 VIC), the sample is negative for SARS-CoV-2 RNA.
3) **Inconclusive:** if the sample is negative for the SARS-CoV-2 targets (⩽ 2 FAM) and negative for the human RP reference target (⩽60 VIC), the result for the sample is inconclusive.

Table 4 Gnomegen COVID-19 RT-Digital PCR Detection kit Results Interpretation

FAM Positive	VIC Positive	Result Interpretation	Actions
≥3	≥0	SARS-CoV-2 detected	Report positive results to healthcare provider and appropriate public health authorities
≤ 2	≥ 60	SARS-CoV-2 not detected	Report negative results to healthcare provider. Consider testing for other pathogens
≤ 2	≤ 60	Inconclusive	Repeat testing of nucleic acid and/or re-extract and repeat RT-Digital PCR.

15. Quality Control

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1256.
- Quality control procedures are intended to monitor reagent and assay performance.
- Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.
- Good laboratory practice (cGLP) recommends including a positive extraction control in each nucleic acid isolation batch.
- Always include a NTC, negative control, and positive control in each amplification and detection run. Per CLIA regulations, HSC must be tested at least once per day.
- All clinical samples should use VIC positive numbers to control for specimen quality and extraction.

16. Limitations

- The use of this assay as in 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.
- The Gnomegen COVID-19 RT-Digital PCR Detection Kit is intended for the qualitative detection of SARS-CoV-2 RNA in patient samples collected from individuals suspected of COVID-19 infection by their healthcare provider.
- Nasal swabs and mid-turbinate swabs are considered acceptable specimen types for use with the Gnomegen COVID-19 RT-Digital PCR Detection Kit but performance with these specimen types has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected under supervision of or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.
- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.
- 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 digital PCR systems have not been evaluated.
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is moderate to low.
- Do not use any reagent past the expiration date.
- If the virus mutates in the rRT-PCR target region, 2019-nCoV may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result. An interference study evaluating the effect of common cold medications was not performed.

17. Conditions of Authorization for the Laboratory

The Gnomegen COVID-19 RT-Digital PCR Detection kit test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-useauthorizations#coronavirus2019

However, to assist clinical laboratories using the Gnomegen COVID-19 RT-Digital PCR Detection Kit, the relevant Conditions of Authorization are listed below:

- Authorized laboratories[1] using the Gnomegen COVID-19 RT-Digital PCR Detection Kit 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.
- Authorized laboratories using the Gnomegen COVID-19 RT-Digital PCR Detection Kit 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.
- Authorized laboratories that receive the Gnomegen COVID-19 RT-Digital PCR Detection Kit will notify the relevant public health authorities of their intent to run your product prior to initiating testing.
- Authorized laboratories using the Gnomegen COVID-19 RT-Digital PCR Detection Kit will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.
- Authorized laboratories will collect information on the performance of the Gnomegen COVID-19 RT-Digital PCR Detection Kit and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Gnomegen (via email: info@gnomegendx.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.
- All laboratory personnel using the Gnomegen COVID-19 RT-Digital PCR Detection Kit 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.
- Gnomegen LLC, authorized distributors, and authorized laboratories using Gnomegen COVID-19 RT-Digital PCR Detection Kit will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, “United States (U. S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests” as “authorized laboratories.”

18. Performance Characteristics

Analytical performance of the Gnomegen COVID-19 RT-Digital PCR Detection kit was evaluated by determining limit of detection (LoD), characterizing the impact of interfering substances and cross-reactivity, as described in the following sections.

1) Limit of Detection

The LoD studies will determine the lowest detectable concentration of the SARS-CoV-2 at which approximately 95% of replicates test positive using the Gnomegen COVID-19 RT Digital PCR Detection kit. A two-phase approach was used to determine the LoD for the upper respiratory specimen type. In phase I, the preliminary LoD was established and confirmed in phase II by testing 20 replicates. Using this two-phase approach, the LoD was determined to be 8 genomic copies per reaction.

To determine the LoD, 5 oropharyngeal swabs specimens (Copan Collection, Transport and Processing Kit UTM 306) were pooled and individually spiked with serially diluted quantified whole viral genomic RNA extracted from cells infected with SARS-CoV-2 obtained from a COVID-19 positive specimen in Hefei, China (COV20851-2) to prepare a simulated, contrived clinical matrix. Contrived clinical matrix with viral spike-ins were individually processed using the QIAGEN QIAamp® DSP Viral RNA Mini Kit. Following the QIAGEN QIAamp® DSP Viral RNA Mini Kit user manual instructions, SARS-CoV-2 spiked sample matrix was concentrated to 140 ul volume prior to nucleic acid extraction. Three replicates per concentration were extracted and tested with the Gnomegen COVID-19 RT-Digital PCR Detection Kit on the QuantStudio 3D digital PCR System. Negative samples containing no SARS-CoV-2 viral RNA were also tested. The lowest concentration tested that yielded positive results (FAM ≥3) for all three replicates was 8 genomic copies/reaction. Data shown in Table 5.

Table 5: LoD determination in upper respiratory specimen – Tentative

2019-nCoV Strain Tested	Stock Concentration	Concentration in Dilution Tested [copies of viral RNA /reaction]	Replicate 1 C_t (FAM positive wells)	Replicate 2 C_t (FAM positive wells)	Replicate 3 C_t (FAM positive wells)	# of positives samples	Percent positive	Lowest Concentration with Uniform Positivity per Analyte
COVID-19 Virus	$5.8x10^5$ copies/ µl	16	14 (positive)	7 (positive)	24 (positive)	3/3	100%	8 copies/ reaction
		8	4 (positive)	7 (positive)	10 (positive)	3/3	100%	
		4	6 (positive)	2 (negative)	4 (positive)	2/3	67%	
		2	2 (negative)	4 (positive)	1 (negative)	1/3	33%	
		1	2 (negative)	4 (positive)	3 (positive)	2/3	67%	
		0	0 (negative)	1 (negative)	1 (negative)	0/3	0%	

From there, the approximate LoD was confirmed by testing 20 additional extraction replicates of LoD contrived samples. The contrived samples were prepared and extracted following the same procedure above. The LoD was confirmed to be 8

copies of viral RNA per reaction as (19/20) of the replicates were positive. Data shown in Table 5.

Table 6: LoD determination in upper respiratory specimen - confirmation

Effective Concentration	Replicate	FAM Positives	Interpretation	# of Positive Samples	Percentage
LoD (8 copies/reaction)	1	8	Positive	19/20 positive	95% Positive
	2	10	Positive		
	3	11	Positive		
	4	8	Positive		
	5	6	Positive		
	6	7	Positive		
	7	2	Negative		
	8	8	Positive		
	9	4	Positive		
	10	4	Positive		
	11	4	Positive		
	12	7	Positive		
	13	11	Positive		
	14	4	Positive		
	15	10	Positive		
	16	7	Positive		
	17	9	Positive		
	18	10	Positive		
	19	13	Positive		
	20	8	Positive		

2) Limit of Detection: Gnomegen Real-Time Digital PCR Instrument and QuantStudio™ 3D Digital PCR System

To expand the use of the Gnomegen COVID-19 RT-Digital PCR Detection Kit for use with the Gnomegen Real-Time Digital PCR Instrument, a bridging and validation study was conducted on contrived clinical oropharyngeal specimens. Pooled oropharyngeal swabs specimen matrix was (Copan Collection, Transport and Processing Kit UTM 306) spiked with serially diluted quantified whole viral genomic RNA extracted from cells infected with SARS-CoV-2 obtained from a COVID-19 positive specimen in Hefei, China (COV20851-2) to prepare a simulated, contrived clinical matrix. Three RNA concentrations (3X, 1X and 0.3X the LoD as previously established on the QuantStudio 3D Digital PCR System) were tested. Contrived clinical matrix with viral spike-ins were individually processed using the QIAGEN QIAamp® DSP Viral RNA Mini Kit. Following the QIAGEN QIAamp® DSP Viral RNA Mini Kit user manual instructions, SARS-CoV-2 spiked sample matrix was concentrated to 140 ul volume prior to nucleic acid extraction. Three replicates per concentration were extracted and tested with the Gnomegen COVID-19 RT-Digital PCR Detection Kit on the Gnomegen Real-Time Digital PCR Instrument and the QuantStudio™ 3D Digital PCR System. A total of 18 samples were tested (3 dilutions X 3 replicates X 2 system= 18 samples). Data is shown in table 7 below:

Table 7: Comparison Gnomegen Real-Time Digital PCR Instrument vs. QuantStudio™ 3D Digital PCR System

2019-nCoV Strain Tested	Stock Concentration	Instrument System	Concentration in Dilution Tested [copies of viral RNA /reaction]	Replicate 1 (FAM positive wells)	Replicate (FAM positive wells)	Replicate 3 (FAM positive wells)	# of Positive samples	Percent positive	Lowest Concentration with Uniform Positivity per Analyte
COVID-19 Isolate COV20851-2	$5.8x10^5$ copies/ ul	Gnomegen Real-Time Digital PCR Detection Kit	3X LoD	13 (positive)	17 (positive)	13 (positive)	3/3	100%	8 copies/ reaction
			1X LoD	9 (positive)	5 (positive)	7 (positive)	3/3	100%	
			0.3X LoD	2 (negative)	1 (negative)	1 (negative)	0/3	0%	
		QuantStudio 3D Digital PCR System	3X LoD	13 (positive)	16 (positive)	15 (positive)	3/3	100%	8 copies/ reaction
			1X LoD	5 (positive)	5 (positive)	4 (positive)	3/3	100%	
			0.3X LoD	1 (negative)	1 (negative)	0 (negative)	0/3	0%	

Results indicated that for the samples tested at 3X LoD, 1X LoD, and 0.3X LoD, the lowest concentration whereby 100% of the samples were positive for both systems was at 1X LoD which corresponds to 8 copies/reaction. This indicates that the Gnomegen Real-Time Digital PCR Instrument is comparable to the QuantStudio™ 3D Digital PCR System.

3) Inclusivity

The primers and probes were analyzed *in silico* with all the known strains/isolates in the NCBI Severe acute respiratory syndrome coronavirus 2 data hub as of April 01, 2020. The sequences were aligned with 320 complete sequences using the nBLAST to the database: Severe acute respiratory syndrome coronavirus 2 (taxid:2697049) which contains 320 complete sequences and 436 total sequences. The search parameters were adjusted to search for a short input sequence.

Table 8 nBLAST results of Gnomegen COVID-19 RT-digital PCR Detection kit primers and probes

(Request ID) RID*	Sequence	Sequences producing significant alignment	% Homology
8A58SXD6114	N1 fwd	320	100%
	N1 rev	320	100%
	N1 probe	320	100%
	N2 fwd	320	100%
	N2 rev	320	100%
	N2 probe	320	100%

*All RIDs and search results were saved and exported into an excel files and kept in a repository. The files can be made available upon request.

For isolate inclusivity, all the alignments for the N1 and N2 targets (primers and probes) show 100% identity of the kit to the 320 available COVID-19 complete sequences from known strains/isolates in the NCBI Severe acute respiratory syndrome coronavirus 2 data hub as of April 01, 2020 (Table 8).

4) Exclusivity/Cross-reactivity

The Gnomegen COVID-19 RT-Digital PCR Detection Kit utilizes identical oligonucleotide sequences for the N1 and N2 SARS-CoV-2 target genes as those used in the CDC 2019-Novel Coronavirus (2019-CoV) Real-Time RT-PCR Diagnostic Panel.

- As reported under the CDC EUA, the *in silico* analysis for the N1 primer/probe set showed high sequence homology of the N1 probe with SARS coronavirus and Bat SARS-like coronavirus genome. However, forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining the primers and probe results, there is no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive RT-PCR results.

- Analysis of the forward primer of the N2 target showed high homology to Bat SARS-like coronaviruses. However, the reverse primer and probe sequences showed no significant homology with human genome, other coronaviruses or human microflora was observed. that would predict potential false positive rRT-PCR results. Combining the primers and probe results, there is no prediction of potential false positive RT-PCR results.

5) Clinical Evaluation

Performance of the Gnomegen COVID-19 RT-Digital PCR Detection Kit on the QuantStudio 3D digital PCR System was evaluated using contrived clinical oropharyngeal swab matrix from specimens collected with the Copan Collection, Transport and Processing Kit (UTM 306C).

Oropharyngeal swab samples were collected and handled by qualified personnel according to the package insert of the collection device. Low positive and moderate positive contrived clinical samples were prepared by spiking quantified whole viral RNA (COV20851-2) into individual negative clinical samples to approximately 1-2 X LoD (20 samples) and at 3-5X LoD (10 samples), respectively. The 30 contrived positive samples, and 30 negative samples consisting of oropharyngeal swab specimen matrix only, were extracted manually using the QIAGEN DSP Viral RNA Mini Kit and tested using the Gnomegen COVID-19 RT PCR Detection Kit on the QuantStudio 3D Digital PCR System. Samples were blinded and randomized prior to testing. Results are summarized below.

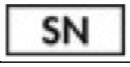
Table 9: Limit of Detection Confirmation of the Gnomegen COVID-19 RT-Digital PCR Detection kit upper respiratory specimen

Sample Type	Sample Concentration	Number of Samples	% Positive	Mean FAM Positives
Upper Respiratory Specimen	Negative	30	0	1
	1-2X LoD	20	100%	11
	3-5X LoD	10	100%	18

PPA: 100% (88.65-100%)
NPA:100% (88.65-100%)

As shown in Table 9 all low positive and moderate positive samples were positive and all negative samples were negative in the background of individual clinical sample matrix.

19. Symbols

	Contains reagents sufficient for <N> number of reactions
	Use by
	In vitro Diagnostic Device
	Catalog Number
	Lot Number
	Serial Number
	Prescription Use Only
	Temperature Limitations
	Manufacturer
	Keep away from sunlight
	Consult Instructions for use

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Revision history: Pub. No. CV02020004

Revision	Date	Description
01	02 April 2020	New Document
02	23 April 2020	Add indications for use with the Gnomegen Real-Time Digital PCR Instrument