2020

GeneOne Diagnostics Corporation

COVID-19 Nucleic Acid Diagnostic Kit

GFYV103-001

Innovation

Performance

Integrity

Teamwork

GeneOne

COVID-19 Nucleic Acid Diagnostic Kit

(100 rxns)

For use under the Emergency Use Authorization (EUA) only

℞ Only

Instructions for Use

GeneOne Diagnostics Corporation

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1 Intended use

The GeneOne COVID-19 Nucleic Acid Diagnostic Kit is an *in vitro* diagnostic test, based on real-time reverse transcription PCR (RT-PCR) technology, for the detection of the RNA from SARS-CoV-2 in upper respiratory tract (e.g. oropharyngeal swab) from patients who meet the clinical criteria (e.g. signs and symptoms) for Coronavirus disease 2019 (COVID-19) as established by WHO (WHO, 2020) and the US CDC (CDC, 2020) (e.g. fever, cough, shortness of breath).

2 Kit contents

Lid/ Tube Color	Component	Cat. No.	Specification
Green/ Brown	Deoxy+ Fast One-Step qRT-PCR Premix	GHYT535-A01	0.625 ml **x** 2 tubes
Red/ Transparent	Sterilized ddH_2O	GHYT535-A02	1 ml **x** 2 tubes
Green/ Brown	G1C Primer Mix	GHYV103-A01	0.1 ml **x** 2 tubes
Yellow/ Brown	G1I Primer Mix	GHYV101-A02	0.05 ml **x** 1 tube
Green/ Transparent	G1C Positive Control	GHYV103-A02	0.5 ml **x** 1 tube
Transparent / Transparent	G1 Internal Control	GHYV103-A03	0.5 ml **x** 1 tube

➢ Kit Catalog Number is GFYV103-001. Contact Sales at +886-2-2377-6331 to order.

3 GeneOne COVID-19 Nucleic Acid Diagnostic kit storage, handling, & disposal

- The GeneOne COVID-19 Nucleic Acid Diagnostic Kit is shipped on dry ice. The components of the kit should arrive frozen. If one or more of the components are not frozen upon receipt, or are compromised during shipment, please contact your distributor for assistance.
- Upon receipt of kit, laboratory should follow internal procedures for quality control.
- Storage and transportation:

  During transportation this product must be kept frozen at low temperature (-20 °C). Large temperature variation or frequent freeze-thaw cycles deteriorate the quality of reagent components and should be carefully avoided. All components should be stored below -20˚C upon arrival to prevent degradation of reagents.
- Repeated thawing and freezing (more than four times) of components, specifically the master mix, should be avoided, as this might affect the performance of the assay. The reagents should be frozen in multiple aliquots if they are to be used intermittently.
- GeneOne Diagnostic of experiment between +2°C and +8°C should not exceed a period of 4 hours.
- If you work in an area prone to power outages, it is recommended that you should have a back-up generator for freezer as well as a temperature data log system to ensure the GeneOne COVID-19 Nucleic Acid Diagnostic Kit remains frozen at -20°C.
- Protect Deoxy+ Fast One-Step RT-PCR Premix from light exposure.
- Expired products should not be used, as the integrity of the components cannot be guaranteed.
- The product is not a biomedical waste. See Safety Data Sheets for hazard classification. Disposal should be in accordance with applicable regional, national, and local laws and regulations.

4 Warnings and precautions

Read this Instructions for Use carefully before using the product. Before first use check the components for:

- Integrity
- Correct labelling
- Frozenness upon arrival

Users should pay attention to the following:

- The RT-PCR tests included in this kit serve only as an auxiliary diagnosis method in a laboratory or hospital; the results of clinical diagnosis must be determined by professional personnel.
- Use of this product should be limited to personnel instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures.
- Patient samples should always be treated as infectious and/or biohazardous. Use standard precautions.
- Wear protective gloves, lab coat, and eye protection when handling patient samples. Always wear gloves when handling kit components.
- Always uses DNase/RNase-free disposable pipette tips with filters.
- Use segregated working areas for sample preparation, reaction setup, and amplification/detection activities. The workflow in the laboratory should proceed in a unidirectional workflow. To prevent contamination, change PPE between areas.
- Store and extract positive materials (specimen, controls, and amplicons) separately from other reagents. Dedicate supplies and equipment to separate working areas and do not move them from one area to another.
- Consult appropriate Safety Data Sheets (SDS) for safety. The SDS for the GeneOne COVID-19 Nucleic Acid Diagnostic Kit is provided with the shipment.
- Do not collect samples for nucleic acid PCR assays in Heparin (green top tube) or EDTA (purple top) tubes as these components are well-known PCR inhibitors. Preferably collect whole blood in serum separator tubes.
- Do not open the reaction tubes/plates after amplification.
- Do not autoclave reaction tubes/plates after the PCR, because this will not degrade the amplified nucleic acid and may pose a contamination risk to the laboratory area.
- Do not use components of the kit that have passed expiration date.

- Waste disposal:
  - Highly infectious specimen or samples must be manipulated in a laboratory that has appropriate safety equipment. Any waste product must be collected, sterilized, and then disposed in accordance with relevant regulations.
  - After amplification, the PCR waste and any waste generated during the experimental process must be collected and sterilized prior to disposal.
  - Discard sample and assay waste according to your local safety regulations.

5 Background information

5.1 Coronavirus disease 2019 (COVID-19)

- **About:** is a contagious, zoonotic disease that causes respiratory infection varying from common cold symptoms to severe pneumonia and occasionally death. The disease was first reported in 31-Dec-2019 by the Chinese government to the World Health Organization (WHO) after a cluster of pneumonia of unknown cause was identified in the city of Wuhan, Hubei province, China. This virus was found genetically similar to SARS-CoV, responsible for the 2002-2003 outbreak of severe acute respiratory syndrome.

- **The virus:** is a positive-sense, single-stranded RNA virus, from the *Coronaviridae* family, genus *Betacoronavirus*. Comparisons of genetic sequences between the novel strain and other coronaviruses have shown that the SARS-CoV-2 have similarities to SARS-CoV (79.5%) and bat coronaviruses (96%). The reason why a likely origin in bats have been theorized.

- **Transmission:** Person-to person transmission, especially close contact, has been confirmed in asymptomatic and symptomatic phases of the disease. The degree of transmission on each phase has not been established yet. The incubation period has shown to be from 1 to 12.5 days, however, because SARS has a 14 days incubation period, the CDC and WHO recommend considering 14 days incubation period within which self-isolation and quarantine is recommended. There have been reported outliers of 24 days incubation period.

- **Signs and Symptoms:** include fever, fatigue, dry cough, and shortness of breath. Cases of severe infection can result in pneumonia, acute respiratory distress syndrome (ARDS), and kidney failure leading to death in some cases. Based on early evidence, many of those who died had pre-existing medical conditions such as hypertension, diabetes, or cardiovascular disease that impaired their immune system.

- **Detection:** the infection can be confirmed by laboratory testing of sputum, nasopharyngeal and oropharyngeal swabs, bronchoalveolar lavage (BAL), nasopharyngeal wash/aspirate or nasal aspirate or serum. Detection by means of real-time RT-PCR should be done from the first day of onset of symptoms. There are no data indicating how long the coronavirus continues being excreted after

symptoms disappear.

5.2 Patient Sample Selection, Collection, Storage, and Handling Recommendations

The sample selection, collection, storage, and handling play an essential part in the performance of nucleic acid assays. Thus, valuable information was summarized from the CDC and WHO guidelines to help laboratories develop better procedures for the analysis of results and troubleshooting of other problems. For more information, visit the CDC and WHO websites in the following addresses:

- ✧ CDC – Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons Under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) - https://www.cdc.gov/coronavirus/2019-nCoV/lab/guidelines-clinical-specimens.html.
- ✧ WHO – Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases – Interim guidance of 17 January 2020 - https://www.who.int/publications-detail/laboratorytesting-for-2019-novel-coronavirus-in-suspected-human-cases-20200117

5.2.1 Sample Selection for:

5.2.1.1 COVID-19:

- ➢ **Upper respiratory tract:**
  Oropharyngeal swab (OP swab)-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 2-3 mL of viral transport media. OP specimens should be kept in separate vials. Refrigerate specimen at 2-8°C and ship overnight to the testing laboratory on ice pack.
  Note: Nasopharyngeal swab: Insert a swab into the nostril parallel to the palate. Leave the swab in place for a few seconds to absorb secretions. Swab both nasopharyngeal areas with the same swab. Oropharyngeal swab (e.g., throat swab): Swab the posterior pharynx, avoiding the tongue.

5.2.2 Sample Storage: Samples are best kept refrigerated at 2-8°C and tested immediately. If there is a delay on testing, serum should be separated from whole blood and stored frozen. Stored samples can be aliquoted in 0.5 mL aliquots. The WHO indicates that all types of specimens may be kept at -20°C for up to 7 days. For storage longer than 7 days, specimens should be frozen at -70°C.

5.2.3 Sample Handling: real-time RT-PCR analysis on clinical samples from patients who are suspected or confirmed to be infected with COVID-19 coronavirus (SARS-CoV-2) should be conducted in a biosafety cabinet Class 2 in a Biosafety Level 2 (BSL-2) containment facility as described in the WHO Laboratory Biosafety Manual, 3rd ed. Any testing for the presence of viruses should be performed in appropriately equipped laboratories by staff trained in the relevant technical and safety procedures. National guidelines on laboratory biosafety should be followed in all circumstances (WHO, 2020).

6 Product description

GeneOne COVID-19 Nucleic Acid Diagnostic Kit is a TaqMan probe real-time qRT-PCR reagent for detecting the RNA of upper respiratory tract samples from suspected patients of SARS-CoV-2 viral infection. The kit includes premix of components required for reverse transcription and PCR, as well as primers and TaqMan probe specific to the RNA of SARS-CoV-2 allowing sensitive detection in a one-step RT-PCR format. In a nucleic acid test, reverse transcriptase converts RNA into cDNA, which is subsequently amplified by PCR enzymes and primers. TaqMan probe binds specifically to PCR products during thermal cycles and the detected fluorescence is proportional to the amounts of products. The kit enables rapid, easy, and highly sensitive detection of the RNA of SARS-CoV-2, and is suitable for clinical diagnostic use.

The GeneOne COVID-19 test includes an internal control to identify possible inhibition of reverse transcriptase enzyme and PCR to confirm the integrity of the reagents, and to verify the quality of sample extraction. The GeneOne COVID-19 test also includes a positive control which contains synthetic DNA molecules carrying sequences identical to the part of RNA sequence of SARS-CoV-2 targeted by this assay. The positive control represents a source of SARS-CoV-2 specific sequence a source of COVID-19 specific sequence, identifying problems related to COVID-19 specific primers and the TaqMan probe. Precautions should be taken to prevent and to minimize the risk.

G1 Primer Mix included in the GeneOne COVID-19 test are:

- G1C Primer Mix targets SARS-CoV-2 with fluorophore FAM™ labeled probe.
- G1I Primer Mix targets the RNase P RNA with fluorophore HEX™ labeled probe.

G1C Primer Mix was designed by referring the current sequence alignment between SARS-CoV-2 and other coronavirus strains. This design allows the detection and differentiation the RNA of SARS-CoV-2. GeneOne Diagnostics Corporation will perform routine in silico analysis and post-market surveillance to ensure when new mutations arise in the virus genome during the outbreak, a proper update of the test will be performed, and customers will be properly notified.

The test is a one-step reverse transcription qPCR test utilizing PCR amplification of the targets, and simultaneous detection of PCR amplicons by fluorophore labelled G1 Primer Mix. The test contains internal controls that monitor the performance of the test. All kit components are manufactured ready to use immediately upon arrival.

7 Materials and devices (required but not provided)

- Appropriate 2~4 channel real-time PCR instrument, compatible with the fluorophores used in this test.
- Three real-time PCR instruments have been used and tested with the product, the Roche LightCycler® 96 Instrument、Applied Biosystems QuantStudio® 3 or Qiagen Rotor-Gene® Q. Other validation exercises will include testing more thermocyclers.
- Appropriate nucleic acid extraction system or kit
- Vortex mixer and Ice
- Centrifuge with a rotor for 2 mL reaction tubes
- Pipettes (adjustable) and Pipette tips with filters (disposable)
- Powder-free gloves (disposable)
- Biosafety cabinet, ideally BSL-2 facility

8 Procedure

The World Health Organization recommends recording the full name, date of birth, contact information and the time and the date of collection of the patient sample. Additionally, the following information could be collected:

- Symptoms, date of onset, duration of symptoms, contact with known COVID-19 cases (e.g. family member, recent travel history);
- Comprehensive travel history (dates, place, duration of visit); and
- Vaccination history, especially any vaccinations for flaviviruses including yellow fever virus, Japanese encephalitis virus, and dengue virus.

8.1 Patient Sample Collection

CDC recommends collecting both nasopharyngeal AND oropharyngeal swab (NP/OP swab) when collecting upper respiratory swabs for investigation of COVID-19. If the user would like additional information on when and how to collect the sample, refer to section 5.2.1 above.

- COVID-19: Collect lower respiratory specimen (e.g. bronchoalveolar lavage, sputum, tracheal aspirate), upper respiratory tract (e.g. nasopharyngeal fluids, nasal swab), and serum.

8.2 Sample Preparation

The quality of the extraction of the RNA from the samples is essential for the performance of GeneOne COVID-19 Nucleic Acid Diagnostic kit. The extraction protocol to be followed should be performed following manufacturer's instructions or an internally validated protocol. However, due to the mucoid and mucopurulent, therefore, viscous nature of sputum specimen a pre-processing of the samples is recommended before extraction. A protocol provided by the CDC and evaluated for COVID-19 for the processing of sputum samples is available by the CDC in the following link: https://www.cdc.gov/coronavirus/2019-ncov/downloads/processing-sputum-specimens.pdf (CDC, 2020). The extraction method validated with GeneOne COVID-19 and recommended is the example: QIAamp® Viral RNA Mini Kit (Cat No.:52904/52906).

Alternative nucleic acid extraction systems and kits might also be appropriate. The suitability of the other nucleic acid extraction procedure for use with GeneOne COVID-19 Nucleic Acid Diagnostic Kit must be validated by the user.

Extraction of RNA using the QIAamp® Viral RNA Mini Kit must be performed following the manufacturer's instructions using 140 µL of sample, and a modified elution using 60 µL of buffer AVE. It is highly recommended prior to the elution of nucleic acids to ensure the removal of all ethanol. For column-based kits that include washing with buffers containing ethanol, an additional centrifugation step (see extraction procedure) using a new collection tube is recommended.

8.3 GeneOne COVID-19 Nucleic Acid Diagnostic Kit Reagent Setup

- When preparing reagents, clean all working surfaces with a fresh 10% bleach solution followed by molecular grade alcohol or another equivalent method of cleaning, that disinfects and degrades nucleic acids.
- All GeneOne COVID-19 Nucleic Acid Diagnostic Kit, Positive Control (PC), No Template Control nuclease free water (NTC), and sample tubes should be vortexed for 3 seconds, and briefly spun down before using to ensure properly mixed reagents, and to remove any condensation or residue from the lids.
- Thaw all reagents and samples on ice, or on a cold block, before starting setup

8.4 Reaction Setup

8.4.1 Every reaction setup should include enough reaction wells for the number of patient samples, along with at least one positive control and one NTC (**# patient samples + 2 = total reaction wells needed**). Example: 5 patient samples to be tested + 1 PC well + 1 NTC well = 7 total reaction wells.

Reaction Mixture (for Positive control and Specimen)	
Component	**Single reaction**
Deoxy+ Fast One-Step qRT-PCR Premix	12.5 µl
G1C Primer Mix	2 µl
Sterilized ddH2O	5.5 µl
Volume of reaction mixture	**20 µl**

Reaction Mixture (for Internal control)	
Component	**Single reaction**
Deoxy+ Fast One-Step qRT-PCR Premix	12.5 µl
G1I Primer Mix	2 µl
Sterilized ddH2O	5.5 µl
Volume of reaction mixture	**20 µl**

8.4.2 All pipetting should be done on ice, whenever possible. Pipetting of PC and sample elution is recommended to be performed in a designated area, spatially or temporally separated from the Master Mix and NTC. Change pipette tips between different patient sample elutions and discard pipette tips after pipetting each component. Pipet PC after all other pipetting is finished if possible, to avoid accidental contamination

8.4.3 Pipet 20 µL of Master Mix into each well being used in an appropriate optical plate or optical reaction tube (example: Roche LC96 real-time PCR instrument accepts 8-tube strips).

8.4.4 Pipet 5 µL of patient sample (elution from nucleic acid extraction) or 5 µL of a control (NTC and PC) to the appropriate well(s). At least one positive control and one NTC must be included in each run.

8.4.5 Seal the reaction plate with an optical adhesive film or the reaction tubes with appropriate lids.

8.4.6 Place plate or tubes into real-time PCR instrument in the correct orientation and start a run.

8.5 PCR Instrument Setup

8.5.1 If using Roche LightCycler® 96 Instrument, visit website at: https://lifescience.roche.com/documents/LightCycler96_Manual_Version2016.pdf for downloading the template file. The template file comes pre-programmed with the PCR instrument setup described in this section. When not using a template, or when using another device, use the settings outlined below to program the PCR instrument.

8.5.1.1 In order to achieve optimal performance from the test, it is important to make sure that the instrument is compatible with the conditions outlined below.

8.5.2 Program PCR instrument with the cycling conditions below:

Parameter		Temp. (℃)	Hold 【hh:mm:ss】	Cycles	Acquisition
Reverse Transcription		45℃	00:05:00	1	
Enzyme Activation		95℃	00:01:00		
Cycling	Denaturation	95℃	00:00:03	40	
	Annealing and Extension	60℃	00:00:20		Signal 【FAM/HEX】

8.5.3 Ensure that the PCR instrument being used is compatible with fluorophores below. Some devices may not have options for the quencher. If needing help or having questions, contact GeneOne Diagnostics Corporation technical support at +886-2-2377-6331 or Mail: sales@geneonedx.com

8.5.4 Define the fluorescence detectors (dyes):

Target	Detector Name	Reporter	Quencher
G1C Positive control	COVID-19	FAM	BHQ1
G1 Internal Control	RNase P	HEX	BHQ1

- When the run is finished, ensure that the run file is saved.

9 Data analysis

For basic information regarding data analysis on specific real-time PCR instruments please refer to the user manual of the respective instrument.

Verification and validation studies performed for GeneOne COVID-19 Nucleic Acid Diagnostic Kit (GFYV103-001) were conducted following Good Laboratory Practices for Molecular Biology assays (Viana & Wallis, 2011). If these conditions are not met, the performance will show higher variability due to user errors during experiment.

9.1 Validity of Diagnostic Test Runs

9.1.1 Valid Diagnostic Test Run

- Check to see if both the positive and no template control pass.

9.1.1.1 Interpretation of test results

Control Type	Control Name	Purpose of Control	COVID-19 FAM channel	Internal Control (RNaseP) HEX channel
COVID-19 Positive Control	COVID-19 (FAM channel)	Verifies the performance of the master mix	+	-
COVID-19 Internal Control	RNase P (HEX channel)		-	+
No Template Control	Master Mix + Water	Verifies the reagents are free of contamination	-	-

- If controls pass, interpret the sample results.

9.1.2 Invalid Diagnostic Test Run

9.1.2.1 If any of the controls fail, the run is invalid. Document the run and initiate troubleshooting.

9.2 Interpretation of Results

Once the controls have passed, the unknown samples can be interpreted based on three possible outcomes:

- Positive
- Internal
- Negative

		Type of sample	Expected Cq values
Controls	Positive	G1C Positive Control	23≤ Cq ≤27
	Internal	G1 Internal Control	25≤ Cq ≤32
	Negative	No template control (NTC)	Cq ≥38 or None detected

An Inconclusive result follows if any of the controls fails. See troubleshooting.

The interpretation of results can be translated to the following table:

	Sample Result		Internal Control (RNaseP) HEX channel	No Template Control (NTC) (Master Mix + Water)	Interpretation of Results
	COVID-19 (SARS-CoV-2)	COVID-19 Positive Control			
Instrument Reading	+	+	+	-	COVID-19 +
	-	+	+	-	COVID-19 -
	Any Result (+/-)	-	+	-	Inconclusive: See Troubleshooting
		+	-	-	
		+	+	+	
Any Cq value less than 36 cycles is considered a positive reading (+). Any Cq value larger than 38 cycles is considered a negative reading (-). The Cq value between 36~38 is considered an inconclusive result, and please test again or check other clinical data for further evaluation. When possible, always check that the medical history and/or symptoms match the final result prior to treatment.					

10 Troubleshooting

GeneOne Diagnostics Corporation values customer feedback and wants to be informed of any issues with the GeneOne COVID-19 Nucleic Acid Diagnostic Kit, even if the recommended steps for troubleshooting solves the issue. To give feedback please fill out the Customer Feedback Form by visiting Mail: sales@geneonedx.com

10.1 Stability

Real-time and accelerated shelf-life and in-use stability studies are currently under testing. Presently, the shelf-life of this product has been established as 12 months. Using expired kit reagents is not recommended, as doing so may lead to inaccurate results.
Always use the most recent version of this document for updates as more stability information will be added when studies are completed.

10.2 User Errors

Good Laboratory Practices for Molecular Biology Diagnostics (Viana & Wallis, 2011) are necessary for the use of this product. This product is not intended to be used by untrained personnel.
It is essential for the user to have some molecular biology experience and be familiar with proper pipetting technique to prevent errors, such as splashes, crossover contamination, and errors on volume selection. Pipette tips must be replaced after every pipetting. Gloves must be replaced often.
Equipment, such as pipettes and real-time PCR instruments, should be calibrated when applicable.
Ninety 90 minutes of online training for Good Laboratory Practices for Molecular Genetics Testing (CDC, 2020) is available at the CDC website at the following link
https://www.cdc.gov/labtraining/trainingcourses/good-lab-practices-molecular-genetics-testing.html

10.3 Invalid Results/Inconclusive Results

10.3.1 Positive Control (PC) not amplifying

No amplification from the PC could be the result of one or multiple reasons, such as:

- Pipetting errors (pipetting controls into the wrong well, missing a well, pipetting inadequate amount of reagent),
- Incorrect placement of plates or tubes into the real-time PCR instrument,

- Degradation of GeneOne COVID-19 Nucleic Acid Diagnostic Kit Master Mix or GeneOne COVID-19 Nucleic Acid Diagnostic Kit Positive Control (result of reagents being at temperatures above -20°C for an extended period),
- Use of expired reagents,
- or the wrong reagents being used.

Without further evidence, it is best to disregard the results from the patient samples and re-test by re-amplification. If the positive control fails again, then an investigation should be conducted to identify possible causes for error, depending on the results of the investigation and risks identified in the process, the test may be reprocessed from extraction. If failure of the positive control happens a third time after re-extraction and re-amplification, open a new GeneOne COVID-19 Nucleic Acid Diagnostic Kit, a new Positive Control, or a new Master Mix, and then re-test and retest. If failure remains, please contact GeneOne Diagnostics Corporation technical support by calling Tel: +886-2-2377-6331 or contacting us at: Mail: sales@geneonedx.com.

10.3.2 Internal Control (IC) not amplifying

No amplification from the Internal Control channel could be the result of one or multiple reasons, such as:

- Degradation of the Internal Control (IC) cell line,
- Loss of activity of the Reverse Transcriptase (RT) Enzyme,
- PCR inhibitors such as: ethanol and heparin,
- The extraction was performed incorrectly,
- The extraction kit used is not compatible with GeneOne COVID-19 Nucleic Acid Diagnostic Kit or does not preserve RNaseP RNA.

Note:

If the IC fails again, then samples should be re-extracted and re-amplified. If it fails a third time an investigation should be conducted to identify possible causes for error. If the cause for the error is clear, A re-test can be performed after all known causes, such as PCR inhibitors or extraction failure, have been eliminated. If the cause for error is unclear, please contact GeneOne Diagnostics Corporation technical support by calling Tel: +886-2-2377-6331 or contacting us at: Mail: sales@geneonedx.com.

10.3.3 No Template Control (NTC) showing amplification

Amplification of COVID-19 in the No Template Control indicates contamination in one or more of the

reagents, incorrect placement of plate or tube into the real-time PCR instrument, or pipetting errors. None of the results can be trusted and re-testing by re-amplification should be performed. If the NTC fails again, then an investigation should be conducted to identify possible causes for error, and the test must be reprocessed from extraction or not, depending on the investigation results and risks identified in the process. If failure of the NTC, after re-extraction and re-amplification, happens a third time, open a new nuclease-free water and retest. If it still failing, please contact GeneOne Diagnostics Corporation technical support by calling Tel: +886-2-2377-6331 or contacting us at: Mail: sales@geneonedx.com.

11 Limitations

- Strict compliance with this document is required for optimal results. Please always use the most recent version of this document. This can be downloaded for free at GeneOne website: http://www.gene1dx.com/.
- Use of this product is to be limited to trained and instructed personnel in real-time PCR techniques and IVD procedures.
- Good laboratory practices are essential for the proper performance of this assay. It is also recommended that upon receipt of reagents a test run be performed to check the purity, integrity, and performance of the reagents prior to testing on patient samples.
- Appropriate specimen collection, transport, storage, and processing procedures are required for optimal results.
- Do not use the GeneOne COVID-19 Nucleic Acid Diagnostic Kit components directly on the specimens collected. Perform an appropriate nucleic acid extraction prior to using this assay.
- The presence of PCR inhibitors may cause false negatives or invalid results.
- Potential mutations of the target regions of the COVID-19 genome covered by this test kit may result in failure to detect the presence of the pathogens.
- As with any diagnostic test, results of the GeneOne COVID-19 Nucleic Acid Diagnostic Kit are to be interpreted with consideration of all clinical and laboratory findings.

12 Performance evaluation

Diagnostic Evaluation is based on contrived samples with upper respiratory tract (e.g. oropharyngeal swab).

Table: Performance for GeneOne COVID-19 Nucleic Acid Diagnostic Kit

Application	Specimen	Strain	Estimated LOD
Limit of Detection (copies/mL)	Oropharyngeal swab	SARS-CoV-2 RNA	*4 x 10^3 copies/mL

Concentration Copies/mL	#GE/Reaction	Mean Cq	Cq SD	Detection rate	Positive rate
4E+06	2E+04	25.13	0.45	20/20	20/20
4E+05	2E+03	28.67	0.55	20/20	20/20
4E+04	2E+02	31.74	1.61	20/20	20/20
4E+03	2E+01	35.01	0.78	20/20	20/20
4E+02	2E+00	39.54	-	1/20	1/20
NTC	-	-	-	0/20	0/20

Data is based upon 200 measurements on contrived samples (Spiked samples were created by serial dilution of whole viral RNA spiked into QIAamp extracted pooled clinical oropharyngeal matrix).

#genome equivalent per reaction (GE/reaction) was determined from calibration curve established using synthetic SARS-CoV-2 RNA (ATCC® VR3276SD™)

Characteristics	
Intended Use	Qualitative detection of Coronavirus disease 2019 (COVID-19) strain SARSCoV-2, in patients that meet the clinical criteria for COVID-19 (e.g. fever, cough, shortness of breath) in upper respiratory tract (oropharyngeal swab)
User	Technicians trained in molecular diagnostics procedures
Detection	Gene Nucleocapsid Protein of strain SARS-CoV-2 responsible for Coronavirus disease

<table>
<tr><td>details</td><td>2019(COVID-19)</td></tr>
<tr><td>Time to detection</td><td>50-70 minutes, depending on the thermocycler used</td></tr>
<tr><td>Extraction System</td><td>QIAamp Viral RNA Mini kit (QIAGEN)</td></tr>
<tr><td>Thermocycler</td><td>Roche LightCycler® 96 Instrument</td></tr>
<tr><td>Inclusivity (analytical sensitivity)</td><td>BLASTn analysis queries alignments were performed with the SARS-CoV-2 Nucleocapsid Protein primer and probe sequences with all publicly available nucleic acid sequences for 2019-nCoV in GenBank to demonstrate the predicted inclusivity of the GeneOne COVID-19 Nucleic Acid Diagnostic Kit.
All the alignments show 100% identity to the available 2019-nCoV sequences.

<table>
<tr><th>Strain</th><th>GenBank</th><th>in silico analysis for % identity to *target 1</th><th>in silico analysis for % identity to *target 2</th><th>in silico analysis for % identity to *target 3</th><th>The likelihood of being detected</th></tr>
<tr><td>SARS-coronavirus 2 isolate Wuhan-Hu-1</td><td>MN908947.3</td><td>100</td><td>100</td><td>100</td><td>Can be detected</td></tr>
<tr><td>SARS-coronavirus 2 isolate USA-WA1</td><td>MN985325.1</td><td>100</td><td>100</td><td>100</td><td>Can be detected</td></tr>
<tr><td>SARS-coronavirus 2 isolate Australia/VIC01</td><td>MT007544.1</td><td>100</td><td>100</td><td>100</td><td>Can be detected</td></tr>
<tr><td>SARS-coronavirus 2 isolate BRA/SP02</td><td>MT126808.1</td><td>100</td><td>100</td><td>100</td><td>Can be detected</td></tr>
<tr><td>SARS-coronavirus 2 isolate PER/Peru-10</td><td>MT263074.1</td><td>100</td><td>100</td><td>100</td><td>Can be detected</td></tr>
<tr><td>SARS-coronavirus 2 isolate HKG/90</td><td>MT215195.1</td><td>100</td><td>100</td><td></td><td>Can be detected</td></tr>
<tr><td>SARS-coronavirus 2 isolate TKYE6968</td><td>LC542976.1</td><td>100</td><td>100</td><td></td><td>Can be detected</td></tr>
<tr><td>SARS-coronavirus 2 isolate HKU-SZ-005b</td><td>MN975262.1</td><td>100</td><td>100</td><td></td><td>Can be detected</td></tr>
<tr><td>SARS-coronavirus 2 isolate SNU01</td><td>MT039890.1</td><td>100</td><td>100</td><td></td><td>Can be detected</td></tr>
<tr><td>SARS-coronavirus 2 isolate HZ-1</td><td>MT039873.1</td><td>100</td><td>100</td><td></td><td>Can be detected</td></tr>
</table>
* target 1/2/3 are used in the G1C Primer Mix (GHYV103-A01)</td></tr>
<tr><td>Analytical</td><td>In silico analysis was performed for the gene regions targeted by the GeneOne COVID-</td></tr>
</table>

Specificity (wet-test or in silico analysis)	19 test to evaluate cross-reactivity. GeneOne conducted a primer BLAST search of the NCBI database against Human coronavirus 229E, Human coronavirus OC43, Human coronavirus HKU1, Human coronavirus NL63, SARS-coronavirus, MERS-coronavirus. The BLAST searches did not identify any cross-reactivity with the exception of SARS coronavirus, which is with one mismatch in the same subgenus (Sarbecovirus) as SARS-CoV-2 and therefore show limited possibility of being detected with the GeneOne COVID-19 Nucleic Acid Diagnostic Kit.

Strain	GenBank	*in silico* analysis for % identity to *target 1	*in silico* analysis for % identity to *target 2	*in silico* analysis for % identity to *target 3	The likelihood of being detected
CoV 229E	NC_002645.1	52.63	45		Will not be detected
CoV OC43	AY391777.1	52.63	60		Will not be detected
CoV HKU1	NC_006577.2	47.37	45		Will not be detected
CoV NL63	NC_005831.2	47.37	55		Will not be detected
SARS-coronavirus	NC_004718.3	100	95	100	May be detected
MERS	KT006149.2	57.89	45		Will not be detected
Human parainfluenza virus 1	NC_003461.1	47.37	50		Will not be detected
Human parainfluenza virus 2	AB176531.1	63.16	60		Will not be detected
Human parainfluenza virus 3	NC_001796.2	52.63	57.89	48	Will not be detected
Human parainfluenza virus 4a	KF878965.2	42.11	50		Will not be detected
Human parainfluenza virus 4b	EU627591.1	47.37	45	52	Will not be detected
Influenza A virus	NC_002019.1	52.63	45	56	Will not be detected
Influenza B virus	NC_002208.1	52.63	60		Will not be detected
Influenza C virus	MK050100.1	47.37	45		Will not be detected
Human adenovirus type 1	AC_000017.1	68.42	70		Will not be detected
Human adenovirus type 7	AC_000018.1	57.89	75		Will not be detected
Respiratory syncytical virus type B	JN032120.1	52.63	40		Will not be detected
Human rhinovirus A	DQ473509.1	47.37	50		Will not be detected
Human metapneumovirus isolate 00-1	AF371337.2	57.89	45		Will not be detected
Enterovirus E type 1	MG571548.1	52.63	60		Will not be detected
Measles virus	NC_001498.1	84.21	50		Will not be detected
Human cytomegalovirus	X17403.1	73.68	55		Will not be detected
Epstein Barr Virus (Human herpesvirus 4)	NC_009334.1	63.16	55		Will not be detected
Mumps virus	NC_002200.1	57.89	65		Will not be detected

Strain	GenBank	*in silico* analysis for % identity to *target 1	*in silico* analysis for % identity to *target 2	The likelihood of being detected
Mycobacterium tuberculosis	AL123456.3	No significant similarity found	No significant similarity found	Will not be detected
Bordetella pertussis	CP011448.1	No significant similarity found	No significant similarity found	Will not be detected
Chlamydia pneumoniae	NC_002491.1	No significant similarity found	No significant similarity found	Will not be detected
Escherichia coli	AE005174.2	No significant similarity found	No significant similarity found	Will not be detected
Haemophilus influenzae	CP000672.1	No significant similarity found	No significant similarity found	Will not be detected
Streptococcus salivarius	CP015282.1	No significant similarity found	No significant similarity found	Will not be detected
Streptococcus pyogenes	AE009949.1	No significant similarity found	No significant similarity found	Will not be detected
Streptococcus pneumoniae	CP019299.1	No significant similarity found	No significant similarity found	Will not be detected
Staphylococcus epidermidis	CP043845.1	No significant similarity found	No significant similarity found	Will not be detected
Staphylococcus aureus	AP017922.1	No significant similarity found	No significant similarity found	Will not be detected
Pseudomonas aeruginosa	CP000438.1	No significant similarity found	No significant similarity found	Will not be detected
Neisseria sp.	CP022527.1	No significant similarity found	No significant similarity found	Will not be detected
Neisseria meningitidis	CP023814.1	No significant similarity found	No significant similarity found	Will not be detected
Mycoplasma pneumoniae	CP014267.1	No significant similarity found	No significant similarity found	Will not be detected
Moraxella catarrhalis	CP018059.1	No significant similarity found	No significant similarity found	Will not be detected
Lactobacillus sp.	CP027190.1	No significant similarity found	No significant similarity found	Will not be detected
Corynebacterium sp.	NZ_CP008913.1	No significant similarity found	No significant similarity found	Will not be detected
Legionella longbeachae	CP020894.3	No significant similarity found	No significant similarity found	Will not be detected
Legionella sainthelensi	CP025491.2	No significant similarity found	No significant similarity found	Will not be detected
Legionella pneumophila	CP015928.1	No significant similarity found	No significant similarity found	Will not be detected
Legionella israelensis	CP038254.1	No significant similarity found	No significant similarity found	Will not be detected
Legionella geestiana	CP038271.1	No significant similarity found	No significant similarity found	Will not be detected

* target 1/2/3 are used in the G1C Primer Mix (GHYV103-A01)

For in vitro testing, 4 panels were sourced:

- Enterovirus (Type 71)
- Human immunodeficiency virus
- Influenza virus (H5N1)
- Dengue virus (Type I)

The samples from these panels are representative of true clinical human specimens and evaluated by the GeneOne COVID-19 Nucleic Acid Diagnostic Kit in quadruplicates,

each concentration with triplicates. The results of the in vitro cross-reactivity testing are presented below:

Microorganism	Strain	Concentration	Detection/replicates	Final result
Enterovirus	Type 71	1E+05 (PFU/ml)	0/4	Negative
Human immunodeficiency virus		1E+05 (PFU/ml)	0/4	Negative
Influenza	H5N1	1E+05 (PFU/ml)	0/4	Negative
Dengue virus	Type I	1E+05 (PFU/ml)	0/4	Negative
NTC		-	-	
G1C Positive control		1E+04 (copies/μl)	24.68	

Clinical performance evaluation

Clinical evaluation of the GeneOne COVID-19 Nucleic Acid Diagnostic Kit was conducted with contrived oropharyngeal swabs (30 positive and 30 negative) in PBS-Buffered solution. 60 swabs were contrived with Quantitative Synthetic SARS-CoV-2 RNA (ATCC® VR3276SD™) and tested blindly to generate the Positive Percentage Agreement (PPA) and Negative Percentage Agreement (NPA).The 60 oropharyngeal samples were tested in a blinded fashion (samples were prepared and capped, then all the tubes were mixed in a box and extracted using QIAamp® Viral RNA Mini Kit (Cat No.:52904/52906) in a random order). Testing was performed in a total of three RT-PCR runs with one positive and one negative control included per run. Results of the study are summarized below.

SARS-CoV-2 concentration	Results (detected/ tested)	GeneOne COVID-19 Kit % positive (95% CI)
1x LoD	10 / 10	100%
2x LoD	10/ 10	100%
4x LoD	10/ 10	100%
Negative	30 / 30	100%

13 Quality control

In accordance with the GeneOne Diagnostics Corporation ISO 13485 and GMP - certified Quality Management System, each lot of GeneOne COVID-19 Nucleic Acid Diagnostic Kit is tested against predetermined specifications to ensure consistent product quality.

14 Technical assistance

For technical assistance, please contact our Technical Support:

- Website: http://www.gene1dx.com/
- Email: sales@geneonedx.com
- Phone: 886-2-2377-6331

15 References

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