GeneFinder™ COVID-19 Plus RealAmp Kit

FOR USE UNDER EMERGENCY USE AUTHORIZATION (EUA) ONLY

Instructions for Use

REF IFMR-45

Rx USE ONLY

IVD

REF **IFMR-45**

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In Vitro Diagnostic

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1. Intended Use

The GeneFinder™ COVID-19 Plus Real*Amp* Kit is a real-time reverse transcription-PCR assay for the qualitative detection of SARS-CoV-2 nucleic acids in nasopharyngeal, oropharyngeal, anterior nasal, and mid-turbinate nasal swab specimens, bronchoalveolar lavage fluid (BAL), and sputum from individuals who are suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 USC §263a, to perform high complexity tests.

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

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

The GeneFinder™ COVID-19 Plus Real*Amp* Kit is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The GeneFinder™ COVID-19 Plus Real*Amp* Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Summary and Explanation

An outbreak of pneumonia of unknown etiology in Wuhan City, Hubei Province, China was initially reported to WHO on December 31, 2019. Chinese authorities identified a novel coronavirus (SARS-2019-nCoV), which has resulted in thousands of confirmed human infections in many countries including the United States. Cases of asymptomatic infection, mild illness, severe illness, and some deaths have been reported.

The GeneFinder™ COVID-19 Plus RealAmp Kit is a molecular in vitro diagnostic test that aids in the detection and diagnosis SARS-2019-nCoV and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers, labeled oligonucleotide probes, and control material used in real-time RT-PCR for the in vitro qualitative detection of SARS-2019-nCoV RNA in respiratory specimens.

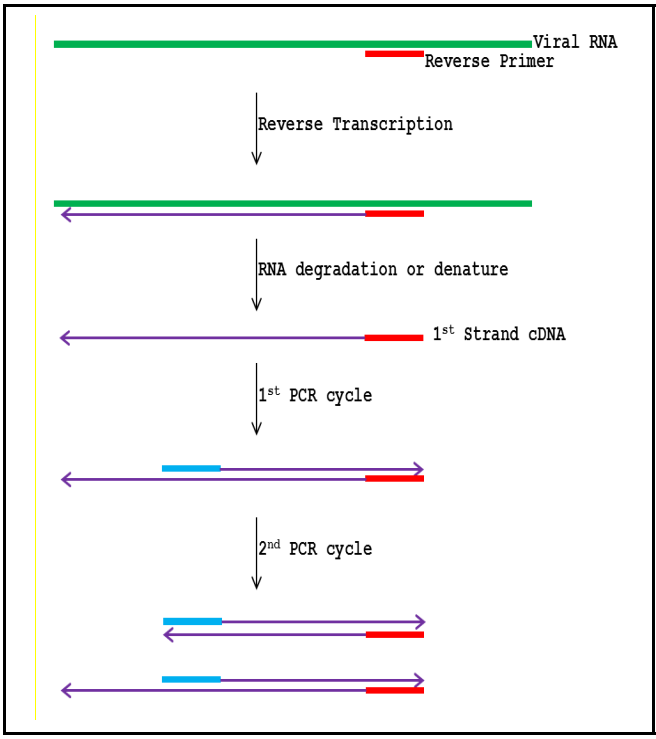
2. Principle of the Assay

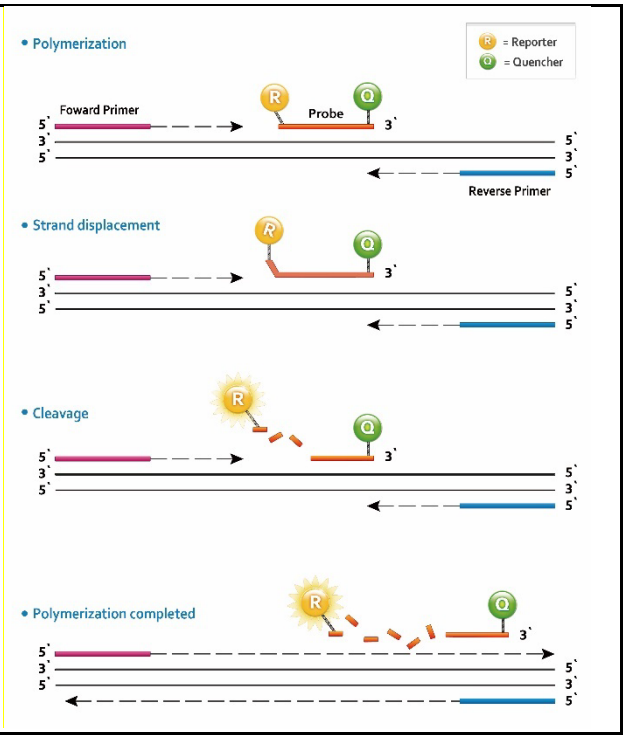
The GeneFinder™ COVID-19 Plus Real*Amp* Kit is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe set(s) is designed to detect RNA from the SARS-CoV-2 in sputum, bronchoalveolar lavage fluid (BAL), nasopharyngeal, oropharyngeal, anterior nasal, and nasal mid-turbinate swabs from individuals who are suspected of COVID-19 by their healthcare provider.

To develop the Real-Time Fluorescent RT-PCR Kit for Detecting SARS-2019-nCoV, the whole genome of SARS-CoV-2 was sequenced and compared to other known Coronavirus genes to deliberately select a specific target region in the ORF1ab region of SARS-CoV-2 genome. Further, human housekeeping gene RNAse P was developed as the target gene for the internal control.

For the detection of SARS-CoV-2 RNA, nucleic acids are isolated and purified from sputum, bronchoalveolar lavage fluid (BAL), nasopharyngeal, oropharyngeal, anterior nasal, and nasal mid-turbinate swabs. The purified nucleic acid is reverse transcribed, using the GeneFinder™ COVID- 19 Plus Real*Amp* RT-PCR master mix into cDNA (Fig. 1), which is then subsequently amplified in the rRT-PCR instrument. During the amplification process, the probe anneals to a specific target sequence located between the forward and reverse primers. In the extension phase of the PCR cycle, the 5' nuclease activity of Taq DNA polymerase degrades the bound probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal (Fig. 2). Fluorescence intensity is monitored at each PCR cycle by the rRT-PCR instrument.

Figure 1. Reverse Transcription reaction diagram	Figure 2. Real time PCR application principle





3. Kit Contents

(100 tests / Kit)

Reagents / Materials	Cap color	Specifications	Quantity	Storage	Catalogue No.
COVID-19 Plus Reaction Mixture	Purple	1,050 µL/vial	1 vial	-20℃	IFMR-45
COVID-19 Plus Probe Mixture	Brown	550 µL/vial	1 vial	-20℃	IFMR-45
COVID-19 Plus Positive Control	Red	50 µL/vial	1 vial	-20℃	IFMR-45
COVID-19 Plus Negative Control	Green	50 µL/vial	1 vial	-20℃	IFMR-45

4. Reagent Storage, Handling, and Stability

- GeneFinder™ COVID-19 Real*Amp* kit is shipped at -20 ℃.
- All components of the kit arrive in solution and frozen.
- All components of the kit must be stored at -20°C upon arrival.
- The COVID-19 Plus Probe Mixture must be stored at -20°C and in the dark.
- Do not use kit components after expiration date printed on the tube label.
- If there is damage to the packaging, do not use.
- Dispose of unused reagents and waste in accordance with country, federal, state, and local regulations.
- Repeated freezing and thawing may lead to inaccurate results.

Note: Inaccurate results may be obtained if the kit is not handled according to the instructions provided.

5. Product Description

a. COVID-19 Plus Reaction Mixture
Reaction mixture containing reagents and enzymes for reverse transcription and amplification.

b. COVID-19 Plus Probe Mixture
Buffer solution containing specific primers and probes that hybridize with the nucleic acid sequences of SARS-CoV-2 targets and human RNAse P target.

c. COVID-19 Plus Positive Control
The Positive Control verifies that the assay run is performing as intended and is used with every batch, starting at the master mixture.
Caution: Care should also be taken to avoid cross-contamination of other specimens when adding positive control.

d. COVID-19 Plus Negative Control
The Negative Control is used to monitor for contamination on the assay run and is used with every batch, starting at the master mixture.

6. Required Materials

6.1 Provided with the kit
Please see Kit Contents, Section 3.

6.2 Required but not provided in the product
- Applied Biosystems® 7500 Real-Time PCR Instrument (ABI 7500), Applied Biosystems® 7500 Fast Real-Time PCR Instrument (ABI 7500 Fast), or CFX96 Real-Time PCR Instrument (CFX96).
- The following extraction kits were validated for use with the GeneFinder™ COVID-19 Plus Real*Amp* Kit:

Instrument/Manufacturer	Extraction Kit	Catalog No.
QIAGEN	QIAamp viral RNA Mini Kit	50 preps (52904) 250 preps (52906)
Roche MagNA Pure 96	DNA and Viral NA Small Volume Kit	576 Extraction (06543588001) External Lysis buffer (06374913001)

- Molecular Grade Water, Nuclease-free
- Racks for 1.5 mL microcentrifuge tubes
- Pipettes (1- 20 µL, 20-200 µL, 200-1,000 µL)
- Pipettes tips with aerosol barrier (RNase, DNase-free)
- Powder-free gloves (disposable)
- Vortex mixer or equivalent
- 1.5 ml tube
- PCR tube or 96 well plate
- Bench microcentrifuge
- DNAZap™ solutions (ThermoFisher Scientific, Cat.no: AM9890), or equivalent

7. Warnings and Precautions

For prescription use only.
For *in vitro* Diagnostics only (IVD).
For Emergency Use only (Rx)

This product has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories that are certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. 263a, that meet requirements to perform high complexity tests

This product has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
The emergency use of this product is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.

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. Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

Please read the package insert carefully prior to operation. Each step of operation, from specimen collection, storage and transportation, and laboratory testing, should be strictly conducted in line with relevant biosafety regulations and molecular laboratory management.

False positive and false negative results can be caused by poor specimen quality, improper specimen collection, improper transportation, improper laboratory processing, or a limitation of the testing technology. The operator should understand the principles of the procedures, including its performance limitations, in advance of operation to avoid potential mistakes.

Separate laboratory areas, dedicated to performing predefined procedures of the assay, are required. a) 1st Area: Preparation Area—Prepare testing reagent: b) 2nd Area: specimen processing—Process the specimen and controls: c) 3rd: Amplification Area—PCR conducted.

All materials used in one area should remain in that area and should not be moved or used in other areas. After the assay procedures, the workbench and lab supplies should be cleaned and disinfected immediately.

All contents in this package are prepared and validated for the intended testing purpose. Replacement of any of the package contents will affect the testing performance of the kit. Components contained within a kit are intended to be used together. Do not mix components from different kit lots.

Prior to beginning each assay, each component must be thoroughly thawed and briefly centrifuged. Avoid repeated freeze-thaw cycles.

Immediately after the addition of the Nucleic Acid reaction Mix, the 96 well plate for real-time PCR should be covered and transferred to a separate specimen processing area.

To prevent contamination from exogenous RNA, samples should be prepared in the following sequence: 1) no template (negative) control, 2) specimen RNA, and 3) positive control. In addition, filtered pipette tips are required and should be replaced after the addition of each reagent or sample.

Be sure to deposit samples with the pipette directly into the reaction mix in PCR tubes. Do not deposit samples with the pipette to the inside plate well wall. The plates should be sealed immediately after the addition of sample.
Following the amplification protocol, PCR plates should be placed into a sealable plastic bag for autoclaving and decontamination.

Be sure not to introduce any foam or bubbles into the tubes when aliquoting Nucleic Acid reaction Mix. All PCR plates should be sealed prior to being loaded into the thermocycler to avoid any possible leakage and contamination.

All lab workbench and supplies should be cleaned and disinfected regularly using 75% Ethanol or UV light.

All pipette tips and centrifuge tubes in the assay should be DNase/RNase-free. The used centrifuge tubes and pipette tips should be discarded in waste bin with bleach and discarded after decontamination.

Avoid exposure to light of the COVID-19 Plus Probe Mixture.

8. Control Material for GeneFinder™ COVID-19 Plus Real*Amp* kit.

8.1 COVID-19 Plus Positive Control (PC):
The PC verifies that the assay run is performing as intended and is used with every batch. starting at the master mixture. The PC is comprised of four individual non-infectious DNA plasmids coding for the RdRp gene, the E gene, the N gene, and the RNAse P gene. The PC should yield a positive result for each target in the GeneFinder™ COVID-19 Plus Real*Amp* Kit.
※ Note. Always use a new aliquot of PC

8.2 COVID-19 Plus Negative Control (NC):
The NC is used to monitor contamination on the assay run and is used with every batch, starting at the master mixture. The NC is comprised of DEPC-treated water. The NC should yield a negative result for each target in the GeneFinder™ COVID-19 Plus Real*Amp* Kit.

8.3 An internal control (IC), targeting the endogenous human RNase P gene is used to verify that nucleic acid is present in every sample and to ensure that samples resulting as negative for SARS-CoV-2 RNA contain nucleic acid for testing.

9. Procedure

9.1 Equipment Preparation
Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used including 10% bleach, 70% ethanol, and DNAZapTM solutions. (ThermoFisher Scientific, Cat.no: AM9890) to minimize the risk of nucleic acid contamination.

9.2 Specimen Collection
The GeneFinder™ COVID-19 Plus Real*Amp* Kit is intended for use with RNA extracted from bronchoalveolar lavage fluid, nasopharyngeal swabs, oropharyngeal swabs, nasal swabs, mid-turbinate nasal swabs or sputum specimens

Collection should avoid possible contamination in collection, storage, and transportation. The specimen should be presumed contagious and collection, storage, and transportation should operate according to relevant regulations.

Specimen Storage: The specimen may be tested immediately after collection, or it may be stored at 2-8°C for up to 72 hours after collection. If a delay in extraction is expected, the specimen may be stored at -70°C or lower. Avoid repeated freeze-thaw cycles. Refer to the 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) or the FDA FAQs on Diagnostic Testing for SARS-CoV-2 (https://www.fda.gov/medical-devices/emergency-situations-medical-devices/faqs-testing-sars-cov-2).

9.3 Transporting Specimens
Specimens must be packaged and transported in accordance with the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens. Store specimens at 2-8ºC and ship overnight. If a specimen is frozen at -70ºC or lower, ship overnight on dry ice

9.4 RNA Extraction
RNA should be extracted from a fresh specimen to ensure suitable RNA quality and quantity. Use only RNA extraction kits validated for use with the GeneFinder™ COVID-19 Plus Real*Amp* kit. RNA extraction should be performed according to the RNA Extraction kit manufacturer's instructions. Use extracted RNA samples immediately or store at -70°C. The input volume and elution volume of the specimen for each extraction system are listed in Table 1.

Table 1. Sample preparation

Instrument / Extraction kit	Manufacturer	Patient specimen	Lysis buffer	Elution volume
QIAamp viral RNA mini kit	QIAGEN	140 uL	560 uL	50 uL

MagNA pure 96 /DNA and Viral RNA Small Volume kit	Roche	250 uL	-	50 uL

9.5 Preparation of Reagents

Thaw all components at 15 to 25℃. Mix gently and centrifuge the contents at low speed for 5 seconds.

a) Prepare the GeneFinder™ COVID-19 Plus Real*Amp* master mixture by combining the components as listed in Table 2.

Table 2. Master Mixture preparation

Component \ Calculation	Volumes for 1 reaction (µL)	Volumes for N patient samples (µL)
COVID-19 Plus Reaction Mixture	10 µL	10*(N+3)
COVID-19 Plus Probe Mixture	5 µL	5*(N+3)
Total (COVID-19 Plus Master Mixture)	15 µL	15*(N+3)

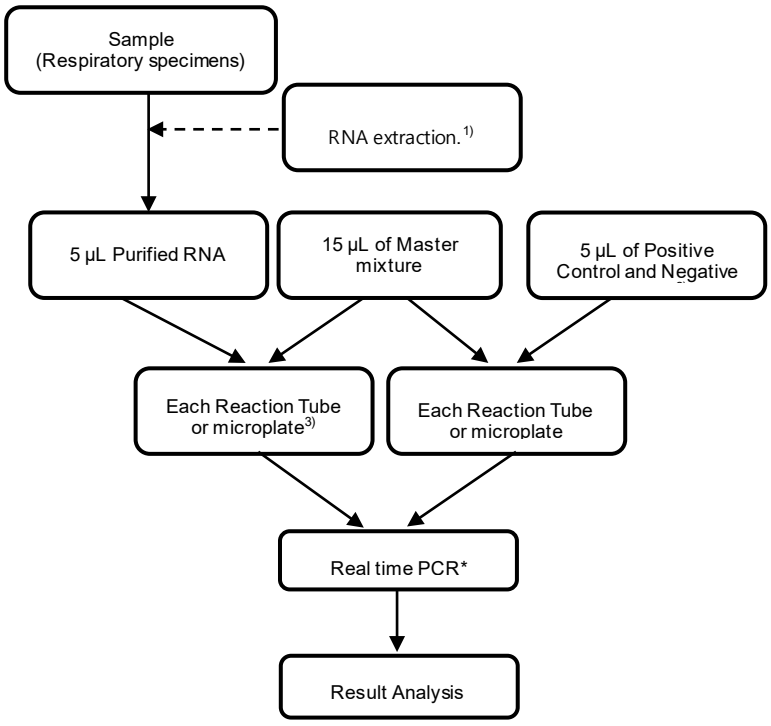
Important: Controls should be processed in each batch to ensure reliable results.

b) Prepare 96-well plates for real-time RT-PCR based on the estimated number of reactions (N). Pipette 15 µL of master mixture into each well. Cover and transfer the plate into sample processing area.

c) Add 5 µL of the extracted RNA and controls to the well pre-filled with reagent mix in the following order: NC, patient specimen(s), and PC. Seal the plate and centrifuge at 2000 rpm for 10 seconds. Place the plate into the real-time RT-PCR instrument and record the location of controls and each specimen.

9.6 Real-Time PCR

Figure 3 Schematic Workflow for Test



1) Use only the RNA extraction kits validated with the GeneFinder COVID-19 Plus RealAmp kit.
2) The PC and NC should be run with each batch.
3) The PCR tubes and plates listed in Table 3 are recommended for use with the GeneFinder COVID-19 Plus RealAmp kit

Table 3. PCR tubes and plates recommended for use with the GeneFinder™ COVID-19 Plus Real*Amp* kit.

Instrument	Manufacturer	Plate / Tube	Cat. #	Package
ABI 7500	Thermo-Fisher scientific	MicroAmp Optical 8-tube strip	4316567	125strips/pack
		MicroAmp Optical 8-cap strip	4323032	300strips/pack
		MicroAmp Optical Adhesive Film	4311971	100 sheets/pack
		MicroAmp® Optical 96-Well Reaction Plate	N8010560	10 plates/pack
ABI 7500 Fast		MicroAmp Fast 8-tube strip	4358293	125 Strips/pack
		MicroAmp Optical 8-cap strip	4323032	300strips/pack
		MicroAmp® Fast Optical 96-Well Reaction Plate	4346907	10 plates/pack
		MicroAmp Optical Adhesive Film	4311971	100 sheets/pack
CFX 96	Bio-Rad	0.2 ml 8-Tube PCR Strips without Caps, low profile, white	TLS-0851	120strips/pack
		0.2 ml 8-Tube PCR Strips without Caps, low profile, clear	TLS-0801	120 strip/pack
		Optical Flat 8-cap strips for 0.2ml tube	TCS-0803	120strips/pack
		Multiplate PCR plates 96 well, white, low-profile	MLL9651	25 plates/pack
		Microseal 'B' Seal ('B' Clear Adhesive Seal)	MSB-1001	100 sheets/pack

※ Real-Time PCR Instruments

The GeneFinder™ COVID-19 Plus Real*Amp* kit is to be used with the following Real-time PCR instruments:
- Applied Biosystems® 7500 Real-Time PCR System (software version 2.3, catalogue no: 4351104)
- Bio-Rad CFX Real-Time PCR Detection System (software version 1.6, catalogue no: BR181-51-95)
- Please ensure that all instruments used have been installed, calibrated, and maintained according to the manufacturer's instruction and recommendations.

※ Real-Time PCR cycling conditions

Step		Temperature	Time	Cycles
1	Reverse Transcription	50℃	20 min	1 cycle
2	Pre-denaturation	95℃	5 min	1 cycle
3	Denaturation	95℃	15 sec	45 cycles
	Annealing*	58℃	60 sec	

*Collection of data

※ Fluorescence channel settings

Target	Fluorescence (Reporter dye)	Quencher dye
RdRp gene	FAM	None
E gene	Texas Red	None
N gene	JOE (ABI 7500/ABI 7500 Fast) / VIC (CFX96)	None
Internal Control	Cy5	None

9.7 Data Analysis

1. Select Amplification Plot in Analysis Mode.
2. Select Analysis Settings.
3. Set Threshold Values and Baseline start and end values.

Target	Threshold		Baseline	
	ABI 7500/ ABI 7500 Fast	CFX 96	Begin	End
RdRp gene (FAM)	30,000	300	3	15
E gene (Texas Red)	30,000	300	3	15
N gene (JOE/VIC)	30,000	300	3	15
Internal Control (Cy5)	10,000	100	3	15

10. 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. 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. Always include a NC and PC in each batch.

The NC should show no amplification curve in the FAM, Texas Red, JOE/VIC, and Cy5 channels.

The PC should show a sigmoidal amplification curve in the FAM, Texas Red, JOE/VIC, and Cy5 channels with Ct values as indicated in Table 4.

Table 4. Acceptance Criteria for Controls

Acceptance Criteria for Controls (Target Ct range for Controls)				
Control	RdRp gene (FAM)	E gene (Texas Red/ROX)	N gene (JOE/VIC)	IC (RNase P) (Cy5)
COVID-19 Plus Positive control	Detected (Ct ≤22)	Detected (Ct ≤22)	Detected (Ct ≤22)	Detected (Ct ≤21)
COVID-19 Plus Negative control	Not detected (no Ct or Ct ≥ 40)	Not detected (no Ct or Ct ≥ 40)	Not detected (no Ct or Ct ≥ 40)	Not detected (no Ct or Ct ≥ 40)

The amplification curves for the IC of the test specimens should appear to be sigmoidal in shape with a Ct value not higher than 40 in the Cy5 channel. Table 4 provides further details for interpretation of results for quality control.

11. Results Interpretation

All controls must be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. If any control does not perform as described in Section 10, the run is considered invalid and all specimens should be repeated from extraction step. Please note, all clinical samples should yield positive results for the IC, with Ct values not higher than 40. However, in the absence of IC amplification or IC Ct ≥ 40, the result is still valid if a patient specimen detects a SARS-CoV-2 target.

A specimen is positive for a SARS-CoV-2 target if there is a sigmoidal amplification curve in the FAM, Texas Red, and/or JOE/VIC channel, with Ct values not higher than 40. See Table 5 for interpretation of results.

Table 5. Interpretation of results

SARS-CoV-2 RdRp	SARS-CoV-2 N	SARS-CoV-2 E	RNase P	Result Interpretation	Report	Actions
+	+	+	+/-	SARS-CoV-2 Detected	POSITIVE	Report results to sender and appropriate health authorities.
If only one or both targets are positive		+/-	+/-	SARS-CoV-2 Detected	POSITIVE	Report results to sender and appropriate health authorities.
-	-	+	+/-	SARS-CoV-2 is Presumptive Positive	PRESUMPTIVE POSITIVE	Sample is repeated once from RT-PCR. If the repeated result remains "PRESUMPTIVE POSITIVE", report results to sender and appropriate health authorities.
-	-	-	+	SARS-CoV-2 Not Detected	NEGATIVE	Report results to sender and appropriate health authorities.
-	-	-	-	Invalid Result	INVALID	Repeat extraction and RT-PCR. If additional clinical sample is unavailable, report Invalid Results, which will request a new specimen be collected, if clinically indicated.

12. Limitations

The use of this assay as an in vitro diagnostic under the 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.

This assay is for in vitro diagnostic use under FDA Emergency Use Authorization only.

Laboratories are required to report all results to the appropriate public health authorities.

False-negative results may arise from degradation of the viral RNA during storage and transport of the specimens.

Negative results do not preclude infection with the SARS-CoV-2 virus and should not be the sole basis of a patient treatment/management or public health decision. Follow up testing should be performed according to the current CDC recommendations.

Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.

The performance of the GeneFinder™ COVID-19 Plus Real*Amp* Kit was established using upper respiratory specimens[1] and sputum specimens. Nasal swabs and mid-turbinate nasal swabs are also considered acceptable specimen types for use with the GeneFinder™ COVID-19 Plus RealAmp Kit but performance has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected 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.

The clinical performance has not been established in all circulating variants but is anticipated to be reflective of the prevalent variants in circulation at the time and location of the clinical evaluation. Performance at the time of testing may

[1] Upper respiratory specimen was a mix of nasopharyngeal and oropharyngeal specimens, presumed negative for SARS-CoV-2, for the purpose of establishing performance characteristics of the GeneFinder™ COVID-19 Plus RealAmp Kit.

vary depending on the variants circulating, including newly emerging strains of SARSCoV-2 an their prevalence, which change over time.

* Procedure Limitations

Extraction and amplification of nucleic acid from clinical specimens must be performed according to the instruments and kits listed in this procedure. Other extraction approaches and processing systems have not been evaluated.

False-negative results may arise from:
- o Improper specimen collection
- o Degradation of the viral RNA during shipping/storage
- o Using unauthorized extraction or assay reagents
- o The presence of RT-PCR inhibitors
- o Mutation in the SARS-CoV-2 virus
- o Failure to follow instructions for use

False-positive results may arise from:
- o Cross contamination during specimen handling or preparation
- o Cross contamination between patient samples
- o Specimen mix-up
- o RNA contamination during product handling

The effect of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated.

Negative results do not preclude infection with SARS-CoV-2 virus and should not be the sole basis of a patient management decision.

A positive result indicates the detection of nucleic acid from SARS-CoV-2.

Nucleic acid may persist even after the virus is no longer viable.

13. Troubleshooting

Problems	Possible Causes	Action
No fluorescent signal is detected in any samples, including positive control	Error in the preparation of the master mixture	Ensure the volumes of reagent dispensed during preparation of the master mixture are correct.
	Probe degradation	Use a new probe aliquot.
	Omitted components	Verify each component and repeat the PCR mixture preparation.
	Instrument settings error	Verify the rRT-PCR instrument settings are correct.
If the fluorescent signal is detected in a negative control reaction.	Carry-over contamination	Change tips between samples; clean pipettes; use filter tips.
	Tube cap not properly sealed	Ensure plates are sealed correctly.
	Contamination of the master mixture	Prepare a new master mix and retest samples from RT-PCR.
	Contamination of the extraction/preparation area	Clean surfaces and instruments with aqueous detergents, wash lab coats, and replace test tubes and tips in use.
If the fluorescent signal does not exhibit sigmoidal	Poor quality of RNA samples	Extract RNA from samples using only the kits validated with the GeneFinder™ COVID-19 Plus Real*Amp* Kit and store the extracted RNA at -70℃.

characteristic in the patient samples.	Not enough volume of RNA samples added	Repeat samples from RT-PCR.
If the fluorescent signal does not exhibit sigmoidal characteristic in the positive control	Probe degradation	Use a new probe aliquot. Repeat samples from RT-PCR
If inconsistent Intensity of fluorescent signals appear	Pipetting error	Repeat samples from RT-PCR
	Contamination in the outer surface of PCR tubes and plate	Repeat samples from RT-PCR
	Bubbles in wells	Repeat samples from RT-PCR

14. Conditions of Authorization

The GeneFinder™ COVID-19 Plus Real*Amp* kit assay's Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patient and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas.

To assist clinical laboratories using the GeneFinder™ COVID-19 Plus Real*Amp* kit ("your product" in the conditions below), the relevant Conditions of Authorization are listed below.

a) Authorized laboratories[2] using the GeneFinder™ COVID-19 Plus Real*Amp* kit will include with test result reports, all authorized Fact Sheets. . Under exigent circumstances, other appropriate methods for disseminating Fact Sheets may be used, which may include mass media.
b) Authorized laboratories using the GeneFinder™ COVID-19 Plus Real*Amp* kit will use the GeneFinder™ COVID-19 Plus Real*Amp* kit 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 the GeneFinder™ COVID-19 Plus Real*Amp* kit are not permitted.
c) Authorized laboratories that receive the GeneFinderTM COVID-19 Plus RealAmp kit will notify the relevant public health authorities of their intent to run the GeneFinder™ COVID-19 Plus Real*Amp* kit prior to initiating testing.
d) Authorized laboratories using the GeneFinder™ COVID-19 Plus Real*Amp* kit 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 the GeneFinder™ COVID-19 Plus Real*Amp* kit and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Osang Healthcare (sales@osanghc.com)any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of the GeneFinder™ COVID-19 Plus Real*Amp* kit of which they become aware.
f) All laboratory personnel using the GeneFinder™ COVID-19 Plus Real*Amp* kit must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use the GeneFinder™ COVID-19 Plus Real*Amp* kit in accordance with the authorized labeling.
g) Osang Healthcare, authorized distributors, and authorized laboratories using the GeneFinder™ COVID-19 Plus Real*Amp* 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.

[2] 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, that meet requirements to perform high complexity tests" as "authorized laboratories."

15. Performance Characteristics

Analytical Sensitivity: LoD

The LoD study established the lowest concentration of SARS-CoV-2 (genome copies(cp)/µL) that can be detected by the GeneFinder COVID-19 Plus RealAmp Kit at least 95% of the time. The LoD was determined using both upper respiratory specimen and sputum, extracted using both the QIAamp Viral RNA mini Kit and Roche MagNA Pure 96 kit and measured on the ABI 7500, ABI 7500 Fast, and CFX96 instruments.

A preliminary LoD of 0.5 cp/µL was established by testing five different dilutions of SARS-CoV-2 viral genomic RNA (1.5 cp/µL, 1 cp/µL, 0.5 cp/µL, 0.25 cp/µL, and 0.05 cp/µL). The preliminary LoD was then confirmed by testing twenty replicates of three different dilutions of SARS-CoV-2 viral genomic RNA (0.5 cp/µL, 0.25 cp/µL, and 0.05 cp/µL). The study results, summarized in table 6, establish an LoD for the GeneFinder™ COVID-19 Plus Real*Amp* Kit of 0.5 cp/µL for both upper respiratory and sputum specimen types using the QIAamp Viral RNA mini Kit and Roche MagNA Pure 96 kit and measured on the ABI 7500, ABI 7500 Fast, and CFX96 instruments.

Table 6. Summary of LoD confirmation at 0.5 cp/µL

Target	QIAamp Viral RNA mini kit						Roche MagNA Pure 96 kit					
	ABI 7500		ABI 7500fast		CFX 96		ABI 7500		ABI 7500fast		CFX 96	
	Lower Respiratory Tract specimen (Sputum)	Upper respiratory Tract specimen	Lower Respiratory Tract specimen (Sputum)	Upper respiratory Tract specimen	Lower Respiratory Tract specimen (Sputum)	Upper respiratory Tract specimen	Lower Respiratory Tract specimen (Sputum)	Upper respiratory Tract specimen	Lower Respiratory Tract specimen (Sputum)	Upper respiratory Tract specimen	Lower Respiratory Tract specimen (Sputum)	Upper respiratory Tract specimen
RdRp (n/20), mean Ct	20/20 37.3	20/20 37.1	20/20 37.7	20/20 37.1	20/20 37.4	20/20 37.4	20/20 37.4	20/20 37.6	20/20 36.4	20/20 36.7	20/20 36.1	20/20 36.4
E gene (n/20), mean Ct	20/20 37.3	20/20 37.0	20/20 37.2	20/20 37.1	20/20 37.0	20/20 37.4	20/20 37.3	20/20 37.3	20/20 36.4	20/20 36.5	20/20 36.4	20/20 36.5
N gene (n/20), mean Ct	20/20 36.2	20/20 36.1	20/20 36.1	20/20 35.9	20/20 36.1	20/20 36.0	20/20 37.2	20/20 36.4	20/20 36.2	20/20 36.7	20/20 36.4	20/20 36.4

FDA SARS-CoV-2 Reference Panel

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to corroborate the LoD. of the GeneFinder™ COVID-19 Plus Real*Amp* kit. The extraction method and instrument used were QIAamp Viral RNA mini Kit and CFX 96 Real-Time PCR Detection System. The results are summarized in Table 7.

Table 7. Summary of LoD Confirmation Result Using the FDA SARS-CoV-2 Reference Panel

Reference Materials Provided by FDA	Specimen Type	Product LoD	Cross-Reactivity
SARS-CoV-2	Nasopharyngeal swab	$1.8x10^5$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL:RNA NAAT detectable units/mL
N/A: Not Applicable
ND: Not Detected

Inclusivity

The inclusivity of the GeneFinder™ COVID-19 Plus Real*Amp* Kit was evaluated in silico by comparison of the primer and probe sequences with 100 SARS-CoV-2 sequences published in the NCBI database and 2,980 SARS-CoV-2 sequences published in the GISAID database as of 31 March 2020. The primers and probes for SARS-CoV-2 targets exhibited 100% homology with the all 3,080 target sequences with one exception. MT039890 (Severe acute respiratory syndrome coronavirus 2 isolate SNU01, complete genome) had 96.2% homology (25/26 nt) with the E gene probe, showing a mismatch at the 4th position of the 3' end of the probe. However, since the E target is a presumptive marker of SARS-CoV-2, it does not affect the diagnosis of SARS-CoV-2.

Analytical Specificity: Cross Reactivity

The analytical specificity of GeneFinder COVID-19 Plus RealAmp Kit was evaluated both *in silico* and by wet testing of other organisms and viruses that may be present in respiratory specimens.

In silico analysis of the primers and probes was performed against the organisms and viruses listed in Table 8. *In silico* analysis suggests cross-reactivity of the GeneFinder COVID-19 Plus RealAmp Kit primers/probe sets for the RdRp and E targets with only SARS-coronavirus (Figure 2).

Table 8. Organisms and viruses, evaluated for cross-reactivity by *in silico* analysis, against the primers and probes for SARS-CoV-2 from the GeneFinder™ COVID-19 Plus Real*Amp* Kit.

Human coronavirus OC43
Human coronavirus HKU1
Human coronavirus 229E
Human coronavirus NL63
MERS-coronavirus
SARS-coronavirus
Human Metapneumovirus (hMPV)
Enterovirus (e.g. EV68)
Pseudomonas aeruginosa
Chlamydia pneumoniae
Haemophilus influenzae
Legionella pneumophila
Mycobacterium tuberculosis
Streptococcus pneumonia
Streptococcus pyrogenes
Bordetella pertussis
Candida albicans
Pneumocystis jirovecii (PJP)

The 20 organisms and viruses, listed in Table 9, were wet-tested for cross-reactivity with the GeneFinder COVID-19 Plus RealAmp Kit. All organisms and viruses were tested as nucleic acid and were spiked directly into the GeneFinder COVID-19 Plus RealAmp RT-PCR mix at the concentrations listed in Table 9. Each organism/virus was tested for cross-reactivity, in triplicate, on the ABI 7500, ABI Fast, and CFX96. No cross-reactivity was observed for the organisms and viruses listed Table 9 except with the primer/probe set for the E target, against SARS-coronavirus. A mean Ct value of 15.6 ± 0.1[3] for this target, at a concentration of 1.2×10^9 cp/Rx of SARS-Coronavirus RNA, was determined. Based on the *in silico* analysis, cross-reactivity with the primer/probe set for the E target against SARS-coronavirus is expected.

[3] Mean Ct value of nine replicates across the ABI 7500, ABI fast, and CFX 96.

Table 9. Organisms and viruses used in wet-testing cross-reactivity of the GeneFinder™ COVID-19 Plus Real*Amp* Kit.

Name	Source	Concentration
Influenza A (H1N1/09)	NCCP (42004)	6.9×10^7 cp/Rx
Influenza A (H3N2)	NCCP (42471)	6.9×10^7 cp/Rx
Influenza A (H5N1)	KVCC (R1300047)	6.9×10^7 cp/Rx
influenza B	NCCP (42469)	6.5×10^7 cp/Rx
Rhinovirus	NCCP (42601)	1.1×10^8 cp/Rx
Respiratory syncytial virus (A/B)	NCCP (43179)	6.2×10^7 cp/Rx
Parainfluenza 1	Vircell (MBC105)	6.2×10^7 cp/Rx
Parainfluenza 2	Vircell (MBC038)	6.2×10^7 cp/Rx
Parainfluenza 3	Vircell (MBC039)	6.2×10^7 cp/Rx
Parainfluenza 4	Vircell (MBC050)	6.2×10^7 cp/Rx
Adenovirus	NCCP (43193)	2.0×10^7 cp/Rx
Human Bocavirus	KCDC	1.7×10^8 cp/Rx
Measles virus	NCCP (40204)	1.7×10^8 cp/Rx
Mycoplams pneumoniae	Vircell (MBC035)	6.0×10^6 cp/Rx
Human coronavirus OC43	plasmid*	1.2×10^9 cp/Rx
Human coronavirus HKU1	plasmid*	1.2×10^9 cp/Rx
Human coronavirus 229E	plasmid*	1.2×10^9 cp/Rx
Human coronavirus NL63	plasmid*	1.2×10^9 cp/Rx
MERS-coronavirus	plasmid*	1.2×10^9 cp/Rx
SARS-coronavirus	plasmid*	1.2×10^9 cp/Rx

*The target sequences for the primers/probe sets against the RdRp gene, N gene, and E gene of each Coronavirus strain were cloned into the pTOP Blunt V2 vector. Plasmids were spiked directly into the GeneFinder COVID-19 Plus RealAmp master mixture at the concentrations listed in Table 9.

Interference

Four interfering substances (Table 10) were evaluated for potential inhibition of the GeneFinder™ COVID-19 Plus Real*Amp* Kit.

Substances were spiked into negative upper respiratory matrix containing viral genomic RNA at 2X LoD, in quadruplicate, at the concentrations listed in Table 10. Specimens were then extracted using QIAamp viral RNA mini Kit and measured for SARS-CoV-2 on the ABI 7500. No inhibition was observed for any target in the presence of any of the interfering substances.

Table 10. Summary of Interfering Substances tested.

	Panel Member	Concentration
Panel Reagents	Mucin	10%, 1%, 0.1%
	Blood	20%, 5%, 1%
	Respiratory syncytial virus A	2000 cp/µL, 200 cp/µL, 20 cp/µL
	PBS	1X

Clinical Evaluation

The performance of the GeneFinder™ COVID-19 Plus Real*Amp* Kit was evaluated in a contrived clinical study using 60 individual upper respiratory specimens and 60 sputum specimens collected from patients with signs and symptoms of a respiratory infection. Positive specimens were prepared by spiking viral genomic RNA into 30 upper respiratory specimens and 30 sputum specimens at 1X, 2X, 3X, and 4X LoD. All specimens were extracted using the MagNA Pure 96 DNA and Viral NA Small volume kit (Roche) and were measured on the ABI 7500. All samples were tested in randomized and blinded fashion. The positive and negative percent agreements between the GeneFinder COVID-19 Plus RealAmp Kit and the expected results from the upper respiratory and sputum specimens are shown below.

Table 11. Clinical performance of the GeneFinder™ COVID-19 Plus Real*Amp* Kit with contrived upper respiratory specimens.

SARS-CoV-2 concentration	Number of NP/OP swabs	ORF1a/b target		E target		N target	
		Positive (95% CIs)	Mean Ct Value	Positive (95% CIs)	Mean Ct Value	Positive (95% CIs)	Mean Ct Value
1X LoD	10	10/10 (72.3% - 100%)	35.9 ± 1.9	10/10 (72.3% - 100%)	35.6 ± 1.8	10/10 (72.3% - 100%)	36.3 ± 1.4
2X LoD	10	10/10 (72.3% - 100%)	33.2 ± 3.0	10/10 (72.3% - 100%)	36.3 ± 1.5	10/10 (72.3% - 100%)	34.4 ± 1.5
3X LoD	5	5/5 (56.6% - 100%)	32.4 ± 2.7	5/5 (56.6% - 100%)	33.7 ± 1.7	5/5 (56.6% - 100%)	33.0 ± 3.1
4X LoD	5	5/5 (56.6% - 100%)	32.6 ± 1.9	5/5 (56.6% - 100%)	34.2 ± 1.9	5/5 (56.6% - 100%)	32.7 ± 1.0
Negative	30	0/30	NA	0/30	NA	0/30	NA

NA = Not available

Performance of the GeneFinder™ COVID-19 Plus Real*Amp* Kit with contrived upper respiratory specimens against the expected results are:

Positive Percent Agreement 30/30 = 100% (95% CI: 88.6% - 100%)
Negative Percent Agreement 30/30 = 100% (95% CI: 88.6% - 100)

Table 12. Clinical performance of the GeneFinder™ COVID-19 Plus Real*Amp* Kit with contrived sputum specimens.

SARS-CoV-2 concentration	Number of NP/OP swabs	ORF1a/b target		E target		N target	
		Positive (95% CIs)	Mean Ct Value	Positive (95% CIs)	Mean Ct Value	Positive (95% CIs)	Mean Ct Value
1X LoD	10	10/10 (72.3% - 100%)	37.3 ± 0.6	10/10 (72.3% - 100%)	36.7 ± 1.2	10/10 (72.3% - 100%)	36.2 ± 1.1
2X LoD	10	10/10 (72.3% - 100%)	37.4 ± 1.2	10/10 (72.3% - 100%)	36.8 ± 0.9	10/10 (72.3% - 100%)	36.8 ± 0.5
3X LoD	5	5/5 (56.6% - 100%)	36.8 ± 0.9	5/5 (56.6% - 100%)	36.2 ± 0.8	5/5 (56.6% - 100%)	35.8 ± 1.1
4X LoD	5	5/5 (56.6% - 100%)	36.4 ± 0.8	5/5 (56.6% - 100%)	36.6 ± 1.4	5/5 (56.6% - 100%)	36.4 ± 1.2
Negative	30	0/30	NA	0/30	NA	0/30	NA

NA = Not available

Performance of the GeneFinder COVID-19 Plus RealAmp Kit with contrived sputum specimens against the expected results are:

Positive Percent Agreement 30/30 = 100% (95% CI: 88.6% - 100%)
Negative Percent Agreement 30/30 = 100% (95% CI: 88.6% - 100%)

16. Product Label Symbols

LOT	Lot or batch number		Caution
REF	Catalogue number		Store below temperature shown
IVD	In Vitro Diagnostic Medical Device		Expiry date
	Consult Instruction for Use		Manufacturer

17. Reference

1. WHO COVID-19 report 2020

18. Contact Information

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