Genematrix

REF NR05A

NeoPlex™ COVID-19 Detection Kit

Multiplex RT-Real-time PCR Reagents for SARS-CoV-2 Detection

For use under the US Emergency Use Authorization (EUA) only

For *in vitro* diagnostic use only

Rx only

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GENERAL INFORMATION

Product Name

NeoPlex™ COVID-19 Detection Kit

Kit Contents

96 Tests

INTENDED USE

NeoPlex™ COVID-19 Detection Kit is a multiplex *in vitro* real-time PCR assay intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper respiratory specimens (such as nasopharyngeal, oropharyngeal, mid-turbinate, and anterior nasal swabs) and lower respiratory specimens (such as sputum, tracheal aspirates, and bronchoalveolar lavage) from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 is generally detectable in 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 NeoPlex™ COVID-19 Detection Kit is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time RT-PCR and *in vitro* diagnostic procedures. The NeoPlex™ COVID-19 Detection Kit is only for use under the US Food and Drug Administration's Emergency Use Authorization (EUA).

Summary and Explanation of the Test

SARS-CoV-2 is a novel coronavirus belonging to the family of Coronaviruses and causes coronavirus disease 2019 (also called COVID-19). Beginning in December 2019 in Wuhan City, China, SARS-CoV-2 has been spreading globally and the World Health Organization (WHO) declared the spate of infections caused by SARS-CoV-2 a pandemic on March 2020. More than 350,000 people were confirmed as SARS-CoV-2 infected and 15,000 people were dead currently. The most common symptoms are: fever, cough, fatigue, and shortness of breath. However, individuals can develop severe symptoms including pneumonia or respiratory failure.

The NeoPlex™ COVID-19 Detection Kit Assay is a qualitative *in vitro* test for the simultaneous detection and confirmation of RdRp and N genes in SARS-CoV-2 virus causing COVID-19 from upper respiratory specimens (such as nasopharyngeal, oropharyngeal, mid-turbinate and anterior nasal swab) and lower respiratory specimens (such as sputum, BAL, and tracheal aspirate) from individuals suspected of COVID-19 by their healthcare provider. The NeoPlex™ COVID-19 Detection Kit Assay is real-time reverse transcription polymerase chain reaction (rRT-PCR) assay. Testing requires a small sample volume and short hands-on time with results available in approximately 3 hours. This test kit is intended for professional use.

Principles of the Procedure

The test is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper and lower respiratory specimens. NeoPlex™ COVID-19 Detection Kit is based on two major processes;

1) Isolation of nucleic acid from specimens, 2) Multiplex real-time amplification.

Virus Target	Gene Targets
SARS-CoV-2	RdRp gene[1]
	N gene[2]

The primer & probe system is based on the standard TaqMan® Technology. The SARS-CoV-2 specific probes are labelled with the FAM fluorophore and JOE fluorophore to target COVID-19 RdRp and N genes, respectively. The internal control is labelled with the Cy5 fluorophore.

1) Isolation of nucleic acid from specimens: Nucleic acids are extracted from specimens using the QIAamp DSP Viral RNA Mini Kit (manual) (Qiagen, 61904).

2) Multiplex real-time PCR: Nucleic acid isolated from specimens is reverse transcribed to cDNA and subsequently amplified using the Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument with SDS software version 1.4

Assay controls

Controls that will be provided with the test kit include:

a) A “no template” (negative) control, consisting of RNase-free water, is needed monitor for contamination during the extraction and RT-PCR process and is used through the entire sample processing procedure. At least one negative control should be included with each run.

b) A positive template control is needed to monitor if the instrument and device work properly and is used through the entire sample processing procedure. The COVID-19 PC includes RdRp and N target genes as in vitro transcript (IVT) RNA at approximately 2x LoD (i.e., 20 copies/µl) respectively. At least one positive control should be included in each run.

c) A negative extraction control (EC) is a previously characterized negative patient sample. It serves both as a negative extraction control to monitor for any cross-contamination that occurs during the extraction process, as well as an extraction control to validate extraction reagents and successful RNA extraction.

d) An internal control targeting human RNaseP mRNA is needed to monitor if any potential PCR inhibitor exists in the specimen and is used though the entire sample processing procedure.

COVID-19 PPM in NeoPlex™ COVID-19 Detection Kit contains primers and probes for targeting human RNaseP mRNA as an internal control. A positive internal control signal is required when interpreting any negative test results. The COVID-19 PPM also serves as the extraction control to ensure that samples resulting as negative contain nucleic acid for testing.

Kit Components and Packaging Specification

The NeoPlex™ COVID-19 Detection Kit (catalog number: NR05A-US-IR0) contains sufficient reagents for 96 reactions and the components are as follow:

Components		Volume	Storage condition	Lid color
COVID-19 PPM	RdRp (Primer/Probe) N (Primer/Probe) IC (Primer/Probe) Tris EDTA Buffer Solution	500ul/vial (1ea)	≤ -20℃	Gray
One-step Master Mix		500ul/vial (1ea)	≤ -20℃	Yellow
COVID-19 Positive Control(PC)	RdRp IVT RNA (100 copies/rxn) N IVT RNA (100 copies/rxn) IC IVT RNA (100 copies/rxn)	100ul/vial (1ea)	≤ -20℃	Red
DW(RNase-free water) (No template control)		1ml/vial (1ea)	≤ -20℃	Blue

Materials Required but Not Provided

- MicroAmp™ Optical 8-Tube Strip, 0.2-mL (Cat No. 4316567)
- MicroAmp™ Optical 8-Cap Strip (Cat No. 4323032)
- QIAamp DSP Viral RNA Mini Kit (QIAGEN, Cat No.61904)
- Pipettes set, P2/P10, P20, P200, and P1000
- Aerosol barrier pipette tips
- Real-Time PCR instrument
  - Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument with SDS software version 1.4 (Thermo Fisher Scientific, Cat No. 4406985 / 510(K) : K141220)
- Micro Centrifuge
- Vortexing mixer
- Disposable powder-free gloves

⚠ Use PCR plate strip caps only. Do not use PCR plate sealing film.

KIT STORAGE AND STABILITY

- Store the kit at ≤ -20℃.
- Kit materials are stable until the expiration date printed on the label under un-opened condition.
- Kit's shelf life is twelve **(12)** months.
- Use the reagents within four **(4)** weeks of opening.
- Limit freeze/thaw cycles for kit reagents.

WARNINGS AND PRECAUTIONS

1. **For *In Vitro* Diagnostic use under the FDA Emergency Use Authorization (EUA) Only.**
2. This test has not been FDA cleared or approved, but has been authorized for emergency use by FDA under an EUA for use by authorized laboratories;
3. This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens; and
4. The emergency use of this test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostics for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Federal Food, Drug, and Cosmetic Act, 21 U.S.C. § 360bbb-3(b)(1), unless the declaration is terminated or authorization is revoked sooner.
5. For prescription use only.
6. Do not smoke, drink or eat or apply cosmetic products in the work areas.
7. Laboratories within the United States and its territories are required to report all results to the appropriate public health authorities.
8. Positive results are indicative of SARS-CoV-2 RNA.
9. Care should be taken when handling, storing and disposing of potentially infectious materials. Suitable barrier protection against potential pathogens is recommended during all stages of use. Gloves and laboratory coats should be worn at all times. Adherence to appropriate local biosafety and biohazard guidelines or regulation is recommended when working with any human-derived blood. Body fluid, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. Handle waste disposal in accordance with accepted medical practice and applicable regulations.
10. Always use pipette tips with aerosol barriers. Tips that are used must be sterile and free of DNases and RNases. Use only supplied or pre-specified required consumables to ensure optimal test performance.
11. All human-sourced materials should be considered potentially infectious and should be handled with universal pre-cautions. If spillage occurs, immediately disinfect with a freshly prepared solution of 0.5% sodium hypochlorite in distilled or deionized water (dilute household bleach 1:10) or follow appropriate site procedures.
12. Fresh clean gloves must be worn in each area and must be changed before leaving that area.
13. Do not pipette by mouth.
14. Do not use reagents from different lots or from different tubes of the same lot.
15. Keep a kit in a refrigerator as listed in shelf life recommendations.
16. Do not freeze/thaw more than four times. Repeated frozen/thawed product may result in false negative and false positive results.

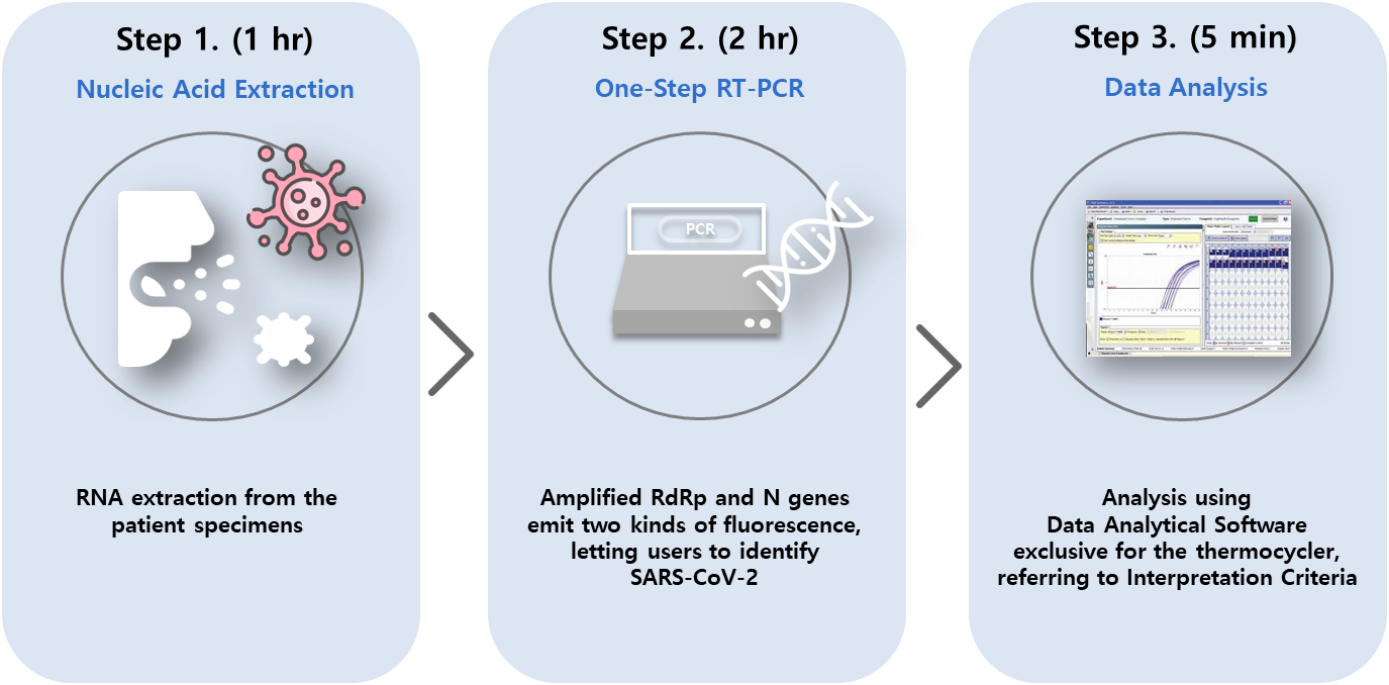
17. Take caution to not contaminate the product when extracting nucleic acid, amplifying PCR product, using positive control (PC, Positive Control). To prevent contamination, store patient specimens separately from the positive control (PC, Positive Control).
18. Use sterilized consumable laboratory supplies. Do not reuse them.
19. Perform the procedure given in this package insert as described. Any deviation from the outlined protocols may result in assay failure or cause erroneous results. Modification to assay reagents, assay protocol or instrumentation is not permitted, and is in violation of the product Emergency Use Authorization.
20. Add the extracted nucleic acid sample and positive control (PC, Positive Control) into the reaction solution in a space separate from the PCR reaction solution preparation space.
21. Use calibrated measuring tools. (e.g. pipette)
22. Check the expiration date before using the kit reagents.
23. Keep Positive Control separated from Test Kit reagents when using, to avoid contamination.
24. Before starting the PCR, make sure the lid is closed properly.

ASSAY PROCEDURES

Compatible Real-time PCR Instruments

- Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument with SDS software version 1.4

Schematic View of Assay Procedure



Specimen Collection and Handling

The recommended sample type for NeoPlex™ COVID-19 Detection Kit is an upper respiratory specimen (such as nasopharyngeal, oropharyngeal, mid-turbinate, or nasal swab) or lower respiratory specimen (such as sputum, BAL, or tracheal aspirate) specimen. Swabs should be a Universal Transport Media (UTM™) or equivalent.

1. Store specimens at 2-8 °C for no longer than seventy-two (72) hours. For pro-longed storage, freeze at ≤ -70°C.
2. Extracted nucleic acids should be stored at ≤ -70°C.
3. Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents.

Upper Respiratory Specimen (Naso/Oropharyngeal swab, Mid-turbinate or nasal swab)	Sputum
Place the specimen at room temperature for 10 minutes before running assay.	Place the specimen at room temperature for 10 minutes before running assay.
Prepare the sample by vortexing for 20 seconds before use.	Add saline or PBS to the specimen (1 part specimen to 2 parts of saline or PBS) and vortex it for 1 minute. Leave it at room temperature for 20 minutes. Vortex for 30 seconds

For more information, refer to:

1. Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Persons for Coronavirus Disease 2019 (COVID-19)
2. Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19)

⚠

- Handle all specimens as if they are capable of transmitting infectious agents.
- Use only the specimen type listed in the instruction manual.
- The specimen volume should be above 0.5ml.
- Wear eye protection, laboratory coats and disposable gloves when handling specimens.
- Specimens should be stored under the storage conditions listed above to ensure accurate results.
- Sample information should be recorded to avoid confusion.

Preparation before testing

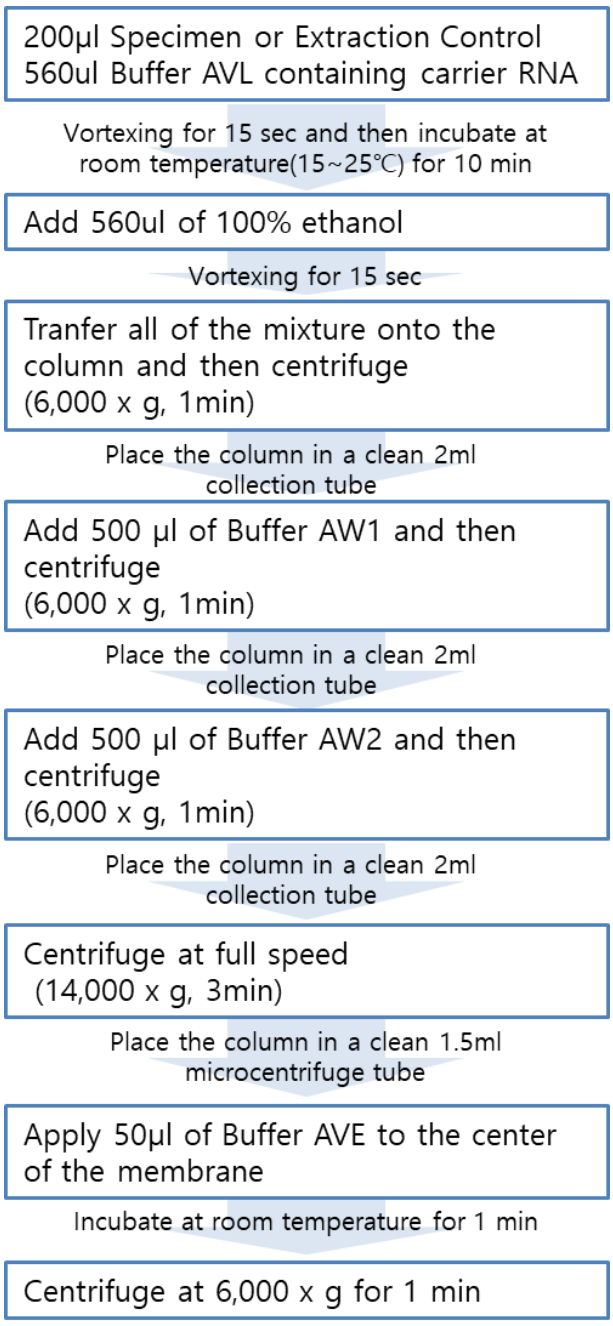
1. Prepare all the devices and reagents before use.
2. Place the kit at room temperature for at least 10 minutes to equilibrate, before PCR Master Mix.
3. After preparing PCR Master Mix, place them on ice.

⚠ **Limit freeze/thawing more than four times.**

[Step 1] Nucleic Acid Extraction

Nucleic acid should be collected from a fresh specimen to ensure suitable Nucleic acid quality and quantity.

Nucleic acid extraction is performed using the manual (QIAamp DSP Viral RNA Mini Kit(Qiagen, 61904). Follow the manufacturer's protocol as linked below:
(https://www.qiagen.com/us/resources/resourcedetail?id=46638e95-df58-4874-9015-732e75587524&lang=en)



[Step 2] Prepare PCR Master Mix and sample

1. Prepare the PCR Master Mix as described in the table below:

Contents	Volume per test
COVID-19 PPM	5ul
One-step Master Mix	5ul
DW(RNase-free Water)	5ul
Total Volume	**15ul**

Note : Calculate the required amount of each reagent based on the number of reactions (patient samples + controls).

2. Vortex and briefly centrifuge the PCR Master Mix using a microfuge.
3. Place 15μL aliquots of the PCR Master mix into 0.2ml PCR tubes and close the lids. This step should be performed on ice.
4. To prepare the patient samples, add 5μl of each extracted, patient nucleic acid sample to its respective tube as described in the table below.

Contents	1 test (Volume)
PCR Master Mix	15ul
Nucleic acid (either extracted patient specimen or control)	5ul
Total Reaction Volume	**20ul**

⚠

- It is recommended that the PCR mixture be prepared just before use.
- Aerosol-resistant filter tips and tight gloves should be used when preparing samples. Take great care to avoid cross contamination.
- Defrost the reagents completely
- Centrifuge the reagent tubes briefly to remove the drops from the inside of the lids.

5. To prepare the controls, add 5μl of COVID-19 PC or DW (RNase-free water) to its respective tube as described above.

⚠

- Use a new pipette tip with each different sample.
- Avoid cross-contamination of PCR Master mix and samples with Positive Control.
- Do not label on the cap of the reaction tubes as fluorescence is detected through the cap.
- Centrifuge the PCR tube thoroughly for 30 seconds

[Step 3] PCR Setup and Amplification

1. Set up and run the Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument. Follow the instrument Reference Guides for detailed instructions.

2. Select fluorescence channels according to the following:

Instruments	RdRp gene	N gene	IC
ABI 7500(Fast)	FAM	JOE	Cy5

3. Program the PCR protocol as following:

Segment	Temperature (°C)	Time	Cycles
1	50	30 min	1
2	95	15 min	1
3	95	15 sec	40
4*	60	60 sec	

* Segment 4: Fluorescence data should be collected during the 60°C incubation step

4. For the analysis of the test result after PCR amplification, take the Ct result and interpret them according to the interpretation criteria for result analysis.

INTERPRETATION OF RESULTS

Control Testing Result Interpretation

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. Control results should be interpreted according to the criteria outlined below.

Acceptance criteria for a Valid Test

Case	Positive Control	Negative Control	Internal Control	Interpretation
1	+	-	+	Acceptable
2	+	-	- *	
3	+	+	+	Invalid/Re-test
4	+	+	-	
5	-	+	+	
6	-	+	-	
7	-	-	+	
8	-	-	-	

* If the IC is negative, but the positive and negative controls yield expected results, and the patient specimen has at least one target detected. A positive result may be assigned.

Patient Specimen Result Interpretation

For the analysis of the test result after PCR amplification, take the amplification curve (or amplification plot) result. Interpret the results according to the two below interpretation tables, first which describes the individual target gene Ct thresholds, and the second which outlines the patient specimen result interpretation algorithm.

1. Individual target gene Ct threshold

Target	fluorescence	Ct threshold	Interpretation
COVID-19 RdRp gene	Refer to the fluorescence channels table of **[Step 3] PCR Setup and Amplification.**	≤ 40	Positive (+)
		N/A	Negative (-)
COVID-19 N gene		≤ 40	Positive (+)
		N/A	Negative (-)
IC*		≤ 40	Positive (+)
		N/A	Negative (-)

* The Internal Control (IC) gene is to monitor the nucleic acid isolation procedure and the possibility of PCR inhibition. Extraction Control (EC, not provided) signal can be confirmed in this channel.

2. Patient Specimen Result Interpretation Algorithm

	FAM	JOE	Cy5	Interpretation
Case	RdRp gene	N gene	IC	
1	+	+	+	SARS-CoV-2 Positive
2	+	-	+	
3	-	+	+	
4	+	+	-	
5	+	-	-	
6	-	+	-	
7	-	-	+	Negative
8	-	-	-	Invalid/Re-test

Troubleshooting

If the Internal control signal is not observed

Potential causes	Solution
Error in specimen collection	If both the target and IC signal were not observed, recollect the specimen
Nucleic acid extraction failure	Read carefully the instruction for use of nucleic acid extraction kit and extract the nucleic acid from specimen again. To validate nucleic acid extraction step, you must use negative extraction control (Previously characterized negative patient sample).
Incorrect PCR setting	Repeat the detection procedure with a correct setting
Incorrect PCR cycle or machine temperature	Check the PCR conditions and repeat the PCR under the correct setting if necessary.
The fluorescence for data analysis do not comply with the protocol	Select the correct fluorescence for each target listed in this Instruction guide for data analysis
Leaving reagents at room temperature for a long time or incorrect storage condition	Check the storage conditions and the expiration date of the reagents and use a new kit
Presence of inhibitor	Dilute the template nucleic acid in distilled water (10-100x) and repeat the PCR with the diluted nucleic acid (If specimen is still present, restart from nucleic acid extraction procedure)
High load of pathogen's nucleic acid	Dilute the template nucleic acid in distilled water (10-100x) and repeat the PCR with the diluted nucleic acid

If signals are observed at the negative control or extraction control (i.e., a false positive result)

Potential causes	Solution
Presence of cross contamination	Decontaminate all surfaces and instruments with sodium hypochlorite or ethanol. Use filter tips during the extraction procedure. Change tips among tubes. Repeat the nucleic acid extraction with the new set of reagents
The fluorescence for data analysis do not comply with the protocol	Select the correct fluorescence for each target listed in this Instruction guide for data analysis

If no signal is observed at the positive control (i.e., a false negative result)

Potential causes	Solution
Error in specimen collection	Recollect the specimen
Incorrect storage of the specimen	Recollect the specimen and repeat the whole process. Make sure the product is stored in recommended conditions
Error in nucleic acid extraction	Re-extract the nucleic acid
Incorrect PCR setting	Repeat the PCR with corrected setting
The fluorescence for data analysis do not comply with the protocol	Select the correct fluorescence for each target listed in this Instruction guide for data analysis
Error in adding nucleic acid to corresponding PCR tubes	Check the sample numbers for nucleic acid containing tubes and make sure to add nucleic acid into correct PCR tubes during detection process
Incorrect PCR mixture	Check whether all components are added or not (If you use to pre-composed premix, should be reduce sensitivity) Each reagent should be used after homogenization and spin down before put the real-time PCR

Quality Control

NeoPlex™ COVID-19 Detection Kit includes COVID-19 PC as Positive Control and DW(RNase-free water) as Negative control to monitor the reliability of the results from nucleic acid extraction to PCR amplification. For all runs, valid test results must be obtained for both Positive and Negative control. Positive Control result must be Positive (Valid). Negative Control result must be Negative (Valid). A negative extraction control (EC) is as previously characterized negative patient sample which is not provided in this kit. If the controls are not valid, the results cannot be interpreted. If the positive and negative control results are consistently invalid, contact us for technical assistance.

Control Type	Channel	Use
Negative Control	N/A	Monitors for environmental contamination.
Positive Control	FAM JOE Cy5	Monitors the NeoPlex™ COVID-19 Detection Kit and assay protocols to ensure proper function.
Extraction Control (not provided)	Cy5	Verifies proper nucleic acid extraction (both extraction kit and procedure), assay reagents and procedure.

LIMITATIONS

1. Performance of the NeoPlex™ COVID-19 Detection Kit has only been established with nasopharyngeal swab specimens, oropharyngeal swab specimens and sputum.
2. Analyte targets (viral sequences) may persist *in vivo,* independent of virus viability. Detection of analyte target(s) does not imply that the corresponding virus(es) are infectious. or are the causative agents for clinical symptoms.
3. All results from this and other tests must be considered in conjunction with the clinical history. epidemiological data and other data available to the clinician evaluating the patient.
4. The detection of pathogen nucleic acids is dependent upon proper specimen collection, handling, transportation, storage and preparation (including extraction). Failure to observe proper procedures in any one of these steps can lead to incorrect results. There is a risk of false negative values resulting from improperly collected, transported, or handled specimens.
5. The performance of NeoPlex™ COVID-19 Detection Kit was established using nasopharyngeal swabs, oropharyngeal swabs and sputum. Nasal swabs, mid-turbinate nasal swabs, BAL and tracheal aspirates are also considered acceptable specimen types for use with the NeoPlex™ COVID-19 Detection Kit, but performance has not been established.
   Testing of nasal and mid-turbinate nasal swabs (self-collected or collected by a healthcare provider) and BAL and tracheal aspirates (collected by a healthcare provider) is limited to patients with symptoms of COVID-19.
6. A specimen yielding a negative result may contain respiratory pathogens not probed by the assay.
7. The performance of this assay was not established in immunocompromised patients,
8. The performance for some viruses and subtypes may vary depending on the prevalence and population tested.
9. The performance of this test has not been established for screening of blood or blood product.
10. This test cannot rule out infections caused by other viral or bacterial pathogens not present on this test.
11. The results of this test should not be used as the sole basis for diagnosis, treatment, or other patient management decisions.
12. This device has been evaluated for use with human specimen material only.
13. The performance of this device has not been evaluated for patients without signs and symptoms of infection.
14. The performance of this device has not been evaluated for monitoring treatment of infection.
15. This assay may cross-react with SARS-coronavirus.
16. This test is a qualitative test and does not provide the quantitative value of detected organisms present.
17. False-negative results may occur by:
    - Error in specimen collection
    - Incorrect storage of the specimen
    - Error in nucleic acid extraction
    - Incorrect PCR setting
    - Error in adding nucleic acid to corresponding PCR tubes
    - Incorrect PCR mixture
18. False-positive results may occur by:
    - Presence of cross contamination by target organisms. Their nucleic acids or amplified product, or from non-specific signals in the assay.
19. This device may not be able to differentiate newly emerging SARS-CoV-2 subtypes.
20. The performance of this test was established based on the evaluation of a limited number of clinical specimens. Clinical performance has not been established with 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 vary depending on the variants circulating, including newly emerging strains of SARS-CoV-2 and their prevalence, which change over time.

Conditions of Authorization for the Laboratory

The NeoPlex™ COVID-19 Detection Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/in-vitro-diagnostics-euas

However, to assist clinical laboratories using the NeoPlex™ COVID-19 Detection Kit ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using NeoPlex™ COVID-19 Detection Kit will include with result reports of NeoPlex™ COVID-19 Detection Kit, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using NeoPlex™ COVID-19 Detection Kit will use NeoPlex™ COVID-19 Detection 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 NeoPlex™ COVID-19 Detection Kit are not permitted.

C. Authorized laboratories that receive NeoPlex™ COVID-19 Detection Kit will notify the relevant public health authorities of their intent to run NeoPlex™ COVID-19 Detection Kit prior to initiating testing.

D. Authorized laboratories using NeoPlex™ COVID-19 Detection 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 NeoPlex™ COVID-19 Detection Kit and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and GeneMatrix Inc. (support@genematrix.net) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of NeoPlex™ COVID-19 Detection Kit of which they become aware.

F. All laboratory personnel using NeoPlex™ COVID-19 Detection Kit must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use NeoPlex™ COVID-19 Detection Kit in accordance with the authorized labeling.

G. GeneMatrix, Inc., authorized distributors, and authorized laboratories using NeoPlex™ COVID-19 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."

PERFORMANCE CHARACTERISTICS

Limit of Detection (LoD)

1. The Limit of Detection (LoD) study established the lowest SARS-CoV-2 viral concentration (copies per reaction) that can be detected by the NeoPlex™ COVID-19 Detection Kit at least 95% of the time using synthetic RNA (from Twist Bioscience). Negative nasopharyngeal specimens (NPS), and negative sputum from a few patients were used in this study. Synthetic RNA was diluted into extraction buffer and then spiked into negative NPS or negative sputum to generate 24 individual samples at concentrations ranging from 100 - 12.5 copies/rxn. All samples were extracted using the manual QIAamp DSP Viral RNA Mini Kit (Qiagen, 61904), and run on the Applied BioSystems 7500 Fast Dx Real Time PCR Instrument. The lowest concentration that achieved greater than 95% positivity was confirmed to be 50 copies/rxn for both nasopharyngeal swabs and sputum.

Analytical Specificity

(Inclusivity, Cross reactivity)

1. Inclusivity

An *In silico* Inclusivity study was performed to assess the ability of the NeoPlex™ COVID-19 Detection Kit to detect SARS-CoV-2 sequences in the NCBI database. *In silico* inclusivity analyses of the primer and probe sequences for the SARS-CoV-2 RdRp and N sets were performed against all SARS-CoV-2 U.S. sequences available in the NCBI database as of Jan 16, 2021. The analysis included 33,595 sequences in the RdRp gene region and 33,595 sequences in the N gene region. All primer/probe sets targeting the RdRp and N gene in the NeoPlex™ COVID-19 Detection Kit exhibited 100% homology with all sequences in the NCBI database.

2. Cross Reactivity

An *In silico* cross-reactivity analysis was conducted with all primer and probe sequence in NeoPlex™ COVID-19 Detection Kit against Genbank sequences from NCBI nt database available on April 16th, 2020 for organisms listed on the below table.

Results from the in silico cross reactivity analysis showed the only organisms in the below table with oligo-hit sequence homology >80% are SARS-coronavirus, Human coronavirus HKU1, and MERS-coronavirus. However, it is not anticipated that Human coronavirus HKU1 or MERS-coronavirus will cross-react with the NeoPlex™ COVID-19 Detection Kit, as only a single primer or probe sequence exhibited >80% homolog, and therefore amplification is unlikely to occur. This assay may cross-react with SARS-coronavirus.

No.	Pathogens	No.	Pathogens
1	Human coronavrius 229E	20	Human bocavirus

2	Human coronavirus OC43	21	Parechovirus
3	Human coronavirus HKU1	22	*Chlamydia pneumoniae*
4	Human coronavirus NL63	23	*haemophilus influenzae*
5	SARS-Coronavirus	24	*Legionella pneumophila*
6	MERS-Coronavirus	25	*Mycobacterium tuberculosis*
7	Human adenovirus sp.	26	*Streptococcus pneumoniae*
8	Human metapneumovirus	27	*Streptococcus pyogenes*
9	Human parainfluenza virus 1	28	*Bordetella pertussis*
10	Human parainfluenza virus 2	29	*Bordetella parapertussis*
11	Human parainfluenza virus 3	30	*Mycoplasma pneumoniae*
12	Human parainfluenza virus 4	31	*Pneumocystis jirovecii(PJP)*
13	Influenza A virus	32	*Candida albicans*
14	Influenza B virus	33	*Pseudomonas aeruginosa*
15	Human Enterovirus	34	*Staphylococcus epidermidis*
16	Human Enterovirus D68	35	*Staphylococcus salivarius*
17	Human respiratory syncytial virus A	36	*corynebacterium diphtheriae*
18	Human respiratory syncytial virus B	37	*Moraxella catarrhalis*
19	Human rhinovirus	38	*Coxiella burnetii* (Q-Fever)

* GenBank IDs of 6 SARS coronavirus which showed high identity to our assay is as follows
: MK062183.1, MK062184.1 MK062182.1, MK062181.1, MK062180.1, MK062179.1

Clinical Performance

Clinical study using natural clinical specimens

A clinical study was performed to compare the performance of the NeoPlex™ COVID-19 Detection Kit in detecting SARS-CoV-2 from individual upper respiratory clinical specimens (i.e.,OP or NP swabs) and lower respiratory clinical specimens (i.e., sputum) with an FDA-authorized real-time RT-PCR assay. For the NeoPlex™ COVID-19 Detection Kit, nucleic acid extraction and PCR amplification were performed using QIAamp DSP Viral RNA Mini Kit (Qiagen) and Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument (ThermoFisher Scientific), respectively. In total, 50 positive clinical specimens, including 25 upper respiratory and 25 lower respiratory specimens were tested, which included 20 low positive specimens (10 upper respiratory and 10 lower respiratory specimens) as defined as samples with Ct values within 3 Ct of the mean LoD of the comparator assay (ranging from 30 to 36). In total, 30 negative clinical specimens (15 upper respiratory and 15 lower respiratory specimens) were tested. Performance of the NeoPlex™ COVID-19 Detection Kit demonstrated 100% PPA and 100% NPA vs the comparator assay. Results of testing natural clinical upper and lower respiratory specimens are illustrated in the following tables, respectively.

Specimen Type: Upper Respiratory (i.e., NP or OP)		**FDA Authorized Comparator Assay**	
		Positive	**Negative**
NeoPlex™ COVID-19 Detection Kit	**Positive**	25	0
	Negative	0	15
Positive Percent Agreement (PPA)		100% (25/25), 95% CI: (86.28, 100%)	

Negative Percent Agreement (NPA)	100% (15/15), 95% CI: (78.20, 100%)

Specimen Type: Lower Respiratory (i.e., Sputum)		**FDA Authorized Comparator Assay**	
		Positive	**Negative**
NeoPlex™ COVID-19 Detection Kit	**Positive**	25	0
	Negative	0	15
Positive Percent Agreement (PPA)		100% (25/25), 95% CI: (86.28, 100%)	
Negative Percent Agreement (NPA)		100% (15/15), 95% CI: (78.20, 100%)	

Clinical study using contrived samples

The clinical performance of the NeoPlex™ COVID-19 Detection Kit assay was also established using residual nasopharyngeal swab, oropharyngeal swab and sputum specimens collected from individual patients with signs and symptoms of COVID-19 from a South Korean site. Prior to testing with the NeoPlex™ COVID-19 Detection Kit, the specimens were confirmed negative for SARS-CoV-2 by a sequencing panel established by the Ministry of Health of Japan[3].

For each specimen type (i.e., nasopharyngeal swabs, oropharyngeal swabs and sputum), 40 contrived positives were generated for testing, of which twenty (20) were prepared at 2x LoD and twenty (20) at 10x LoD. Contrived positive samples were generated using extracted RNA from contrived SARS-CoV-2 positive patient specimens.

Forty (40) clinical negative nasopharyngeal swabs, oropharyngeal swabs and sputum from individual patients were also included in this study. Contrived specimens were tested along with 40 distinct negative clinical specimens in a randomized, blinded fashion. The results of the NeoPlex™ COVID-19 Detection Kit testing contrived specimens are described below and demonstrated a Positive Percent Agreement (PPA) and Negative Percent Agreement (NPA) of 100% for each specimen type.

Specimen Type	**SARS-CoV-2 concentration**	**Clinical Evaluation of the NeoPlex™ COVID-19 Detection Kit**		
		Number tested	**Number Detected**	**% Detection**
Nasopharyngeal swab	2x LoD	20	20	100% (N=20/20)
	10x LoD	20	20	100% (N=20/20)
	Negative	40	0	0 (N=0/40)
	PPA: 40/40, 100% (95% CI: 91.19-100%)			
	NPA: 40/40, 100% (95% CI: 91.19-100%)			
Oropharyngeal swab	2x LoD	20	20	100% (N=20/20)
	10x LoD	20	20	100% (N=20/20)
	Negative	40	0	0 (N=0/40)
	PPA: 40/40, 100% (95% CI: 91.19-100%)			
	NPA: 40/40, 100% (95% CI: 91.19-100%)			

Sputum	2x LoD	20	20	100% (N=20/20)
	10x LoD	20	20	100% (N=20/20)
	Negative	40	0	0 (N=0/40)
	PPA: 40/40, 100% (95% CI: 91.19-100%)			
	NPA: 40/40, 100% (95% CI: 91.19-100%)			

FDA SARS-CoV-2 Reference Panel Testing

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to confirm the LoD. The extraction method and instrument used were as below.

1) Isolation of nucleic acid from specimens: Nucleic acids were extracted from specimens using the QIAamp DSP Viral RNA Mini Kit (manual) (Qiagen, Cat No. 61904).
2) Multiplex real-time PCR: Nucleic acid isolated from specimens is reverse transcribed to cDNA and subsequently amplified using the Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument with SDS software version (Thermo Fisher Scientific, Cat No. 4406985 / 510(K): K141220).

The results are summarized in the below table.

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	$5.4x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

REFERENCES

1. Chen, Huijun, et al., Clinical characteristics and intrauterine vertical transmission potential of COVID-19 infection in nine pregnant women: a retrospective review of medical records. The Lancet 395.10226 (2020): 809-815.

2. Centers for Disease Control and Prevention, Respiratory Viruses Branch, Division of Viral Diseases, 2019-Novel Coronavirus (2019-nCoV) Real-time rRT-PCR Panel Primers and Probes. 24 Jan 2020.

3. Shirato, Kazuya, et al. Development of genetic diagnostic methods for novel coronavirus 2019 (nCoV-2019) in Japan. Japanese Journal of Infectious Diseases (2020): JJID-2020.

SYMBOLS

REF	LOT		
Catalogue number	Batch code	Date of manufacture	Use-by date
IVD	-20℃ (-4℉)		
In vitro diagnostic medical device	Upper limit of temperature	Caution	Consult instruction for use
	Σ <n>	Rx ONLY	
Manufacturer	Contains sufficient for <n> tests	Prescription Use Only	

IVD

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