Genetron SARS-CoV-2 RNA Test

Instructions for Use

For Rx Use Only

For IVD Use Only

For Emergency Use Authorization Only

[Product Name]

Genetron SARS-CoV-2 RNA Test

[Packing Specifications]

Table 1. Packing Specifications

Product Name	**Art. No**	**Specification**
Detection Kit for Novel Coronavirus (SARS-CoV-2) RNA (PCR-Fluorescence Probing)	RPQ021	50 Tests/Kit
Detection Kit for Novel Coronavirus (SARS-CoV-2) RNA (PCR-Fluorescence Probing)	RPQ022	100 Tests/Kit

[Intended Use]

The Genetron SARS-CoV-2 RNA Test is a real-time RT-PCR test intended for the qualitative detection of SARS-CoV-2 nucleic acid in upper respiratory specimens (such as oropharyngeal, nasopharyngeal, anterior nasal and mid-turbinate nasal swab specimens) from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests.

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

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

The Genetron SARS-CoV-2 RNA Test is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. The Genetron SARS-CoV-2 RNA Test is only for use under the Food and Drug Administration's Emergency Use Authorization.

[Testing Principle]

The kit is based on a real-time fluorescent PCR platform, using RNA reverse transcription, polymerase chain reaction and TaqMan probe technology to target the highly conserved regions of the ORF1ab gene and N gene of SARS-CoV-2. The primers and probes are specifically designed to target these sites; the specific primers amplify the target sequence by PCR. The TaqMan probe, bound to the template, is cleaved by Taq enzyme (5 '→ 3' exonuclease activity). The fluorophore is separated from the quenching group to generate and accumulate fluorescence signals. Based on the changes in the fluorescence signal as a function of the number of amplification cycles, a real-time amplification curve can be obtained, thereby realizing the detection of SARS-CoV-2 nucleic acid. This kit contains dUTP and UDG enzymes to prevent contamination.

[Precautions]

1. Please read the instructions carefully before use. This product is intended for in vitro diagnostic use under Emergency Use Authorization only.
2. The Genetron SARS-CoV-2 RNA Test has not been FDA cleared or approved; the test has been authorized by FDA under an Emergency Use Authorization (EUA) for use by laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, to perform high complexity tests.
3. The Genetron SARS-CoV-2 RNA Test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
4. The Genetron SARS-CoV-2 RNA Test is only authorized for the duration of the declaration that circumstances exist justifying the authorization of emergency use of in vitro diagnostic tests for detection and/or diagnosis of COVID-19 under Section 564(b)(1) of the Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
5. Due to the source of the sample itself, the sample collection process, sample quality control, sample transportation, sample pretreatment, etc., and due to the restrictions of the quality of RNA extraction, the model of the fluorescence-detection PCR instrument, the operating environment, and the limitations of current molecular biology technology, false positive or false negative results may occur. The user must understand the potential errors and limitations of accuracy of the test.
6. Nasopharyngeal, anterior nasal and mid-turbinate nasal swab specimens are additional acceptable upper respiratory specimens that can be tested with the Genetron SARS-CoV-2 RNA Test; however, performance with these specimen types have not been determined.
7. The kit should be transported and stored at -20°C. Before use, the components in the kit should be fully thawed, mixed and centrifuged. Avoid repeatedly freezing and thawing the reagents in the kit.
8. All test samples, calibrators and controls should be considered as infectious substances. Wear appropriate personal protective equipment such as gowns, masks

and disposable gloves and frequently change them during the experiment to avoid cross-contamination between samples. Sample handling and waste disposal must comply with requirements of relevant regulations.

9. The use of positive controls and reaction reagents should be strictly distinguished to prevent cross-contamination and cause false positives.
10. During the experiment, care should be taken to prevent contamination of reagents with exogenous RNA. No template control substances should be added first, then test samples, and finally the positive control. Separate and dedicated pipettes and filter tips should be used when preparing reaction reagents and adding RNA templates. The reagent preparation area, sample/control preparation area and nucleic acid amplification area should be strictly distinguished.
11. Only operators with professional training who wear proper personal protective equipment can operate in a laboratory with proper safety protections. After an experiment, handle the workbench and pipette according to the specifications of the standard PCR operation room.
12. 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.

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

[Main Composition]

Table 2. Main Composition

Name	Specifications and Quantity		Main Ingredients
	50 Tests/Kit	100 Tests/Kit	
PCR Detection Mix	175 μL×1 tube	350 μL×1 tube	Primers and probes
Reaction Buffer	750 μL×1 tube	750 μL×2 tube	Buffer, dNTP, dUTP, etc.
Enzyme Mix	75 μL×1 tube	150 μL×1 tube	Taq enzyme, reverse transcriptase, UDG enzyme
Positive Control	100 μL×1 tube	200 μL×1 tube	Synthetic viral RNA and human RNA
No Template Control	200 μL×1 tube	400 μL×1 tube	RNase-free ddH_2O

Note: The components in the kits of different batches cannot be interchanged.

Materials required but not provided:

- DNA/RNA Shield Collection Tube w/Swab (Beijing Tianmo Technology Development Co., Ltd (Catalog No. TS003-2); other flocked swabs can be

collected and transported in media such as VTM and UTM and processed with the Genetron workflow

- QIAamp Viral RNA Mini Kit (Qiagen, Item No. 52904) manufactured by QIAGEN
- Microcentrifuge
- 1.5mL tube (Axygen, Cat# 22718392)
- Vortex mixer
- Pipettes
- Pipette tips with filters
- MicroAmp™ Fast 8-Tube Strip, 0.1 mL (Applied Biosystems; Catalog #:4358293)
- MicroAmp™ Optical 8-Cap Strips (Applied Biosystems; Catalog #:4323032)
- MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL (Applied Biosystems; Catalog #:4346906 or 4366932)
- MicroAmp™ Optical Adhesive Film (Applied Biosystems; Catalog #:4311971)

[Storage Conditions and Shelf Life]

This kit should be stored away from light at -18°C or below. The shelf life is empirically set for 6 months.
After the reagent vial is opened, it should be used within 2 months. The allowable number of repeated freeze-thaw cycles of the components in the kit is no more than 5 times.
See the package label for the production date and expiration date.
Do not use reagents past their expiration date.

[Applicable Instruments]

Applied Biosystems 7500 Real-Time PCR System.(7500 software version 2.3)

[Sample Requirements]

1. Sample types: oropharyngeal swabs, nasopharyngeal swabs, anterior nasal swabs and mid-turbinate nasal swabs

2. Sample collection, transportation and storage:

1) Sample collection

Collection of swab samples: Use swabs to remove nasal and pharyngeal secretions, place them in a spiral plastic tube with sampling solution (DNA/RNA Shield Collection Tube w/Swab), discard the tail, screw the tube cap tightly, and send it for detection.

2) Sample transportation

Samples must be packaged, shipped and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when

sending potential SARS-CoV-2 samples. Seal and transport the samples at a room temperature.

3) Sample storage

Test the sample as soon as possible after collection. If storage is required, samples can be stored for no more than 24 hours at 2-8°C; for no more than 1 week at -18°C or below; for longer periods at -70°C or below. Repeated freezing and thawing should be avoided.

[Control Materials]

The following assay controls are to be used with the Genetron SARS-CoV-2 RNA Test:

- A No Template Control (NTC) contains RNase-free ddH_2O and is intended to monitor false positives caused by extraneous nucleic acid contamination from the assay reagents or the environment. One NTC (10 µL) should be run per plate. The NTC is included as a component of the kit (50 tests/kit; RPQ021 or 100 tests/kit; RPQ022).
- A Positive Control (PC) is intended to evaluate enzyme activity, analytical and clinical performance of the assay. One positive control (10 µL) should be run per plate. The positive control is composed of SARS-CoV-2 synthetic RNA containing the ORF1ab and N sequences as well as the internal human RNase P control sequence at a weak positive concentration (2X LoD). This control is provided with both the 50 tests and 100 tests per kit.
- An internal extraction control is needed to verify adequate sample lysis and efficient nucleic acid extraction. Human RNase P serves as the extraction control to monitor specimen quality, extraction efficiency, and successful completion of reverse transcription. This control ensures that samples with a negative result contain nucleic acid for testing. The RNase P primer/probe set is included in the master mix setup and will detect and amplify this gene if present in a clinical sample. No additional RNase P is spiked into the clinical samples prior to processing.

[Testing Method]

After unpacking, place the positive control from the kit into the sample preparation area.

1. Sample preparation (sample preparation area)

Sample processing: Use freshly collected samples for RNA extraction.

RNA should be extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Item No. 52904) manufactured by QIAGEN following the manufacturer's instructions for use as follows. Sample input volume is 140 µL and the final elution volume is 60 µL.
The extracted RNA samples should be tested immediately or stored at -70°C or below for later use. Viral RNA is stable for up to one year when stored at -70°C or below.

2. Reagent preparation (reagent preparation area)

Remove the kit from freezer. Remove the Enzyme Mix from the box, centrifuge briefly and immediately place it on ice for later use. Remove the PCR Detection Mix and Reaction Buffer from the kit, thaw at room temperature, mix well, centrifuge briefly and keep on ice for later use.

Using Table 3 below, prepare the PCR reaction system based on the number of samples to be tested with up to 5% allowance to make sure that there is sufficient volume for pipetting. A no template control and a positive control should be included in each test run (i.e., 96-well plate).

Table 3. Preparation of PCR Reaction System

Components of reaction solution	Added volume (μL)/test
Reaction Buffer	15
Enzyme Mix	1.5
PCR Detection Mix	3.5
Total volume	20

After mixing the prepared PCR reaction system, pipette 20 μL into the corresponding reaction wells of a 96-well plate (or 8-tube strip), and then transfer to the sample processing area.

3. Sample loading (sample processing area)

Add 10 μL of the sample RNA to be tested, the positive control, and the no template control to the corresponding reaction wells. After sealing the wells with the MicroAmp™ Optical Adhesive Film (Applied Biosystems; Catalog #:4311971), shake and mix thoroughly and centrifuge immediately.

Note: For quality control, a positive control and a no template control should be included in each test.

4. PCR amplification (nucleic acid amplification area)

Put the 96-well PCR plate (or 8-tube strip) into the sample slot of the qPCR instrument and record the placement order. Set the instrument amplification program and instrument parameters according to Table 4. The combination of fluorescence channels is: VIC, FAM, and ROX.

Table 4. Amplification Program

System	Total volume 30 μL			
Collect	Fluorescence signals from the novel coronavirus (SARS-CoV-2) -VIC and FAM channels and collect fluorescence signals from the internal reference gene-ROX channel. Collect these three types of signals simultaneously.			
Steps	**Cycles**	**Temperature**	**Time**	**Collection of fluorescence signals**
RT incubation	1 cycle	55	15 min	No

Enzyme activation	1 cycle	95°C	30 sec	No
Amplification	45 cycles	95°C	15 sec	No
		60°C	45 sec	Yes

5. Result Analysis

Threshold setting: It is necessary to respectively set the threshold values for the FAM, VIC, and ROX channels, and then read the Ct value. After the PCR run is complete, the instrument automatically saves the result. Adjust the Start value, End value and Threshold value of Baseline according to the image after analysis (the user may make self-adjustment according to the actual situation; the Start value may be set at 3 ~ 15; the End value may be set at 5 ~ 20; set the value of Threshold in the "Log graph" window, so that the threshold line is located in the exponential phase of amplification curve; the amplification curve of No template control is straight or lower than the threshold line); click "analysis" to automatically obtain the analysis result, and read the detection result in the "Report" window.

5.1. Verification of the Run Validity

1) Positive Control: The amplification curves of VIC (detects the N gene target), FAM (detects the ORF1ab target), and ROX (detects the RNase P internal control (IC) target) channels were all S- shaped, and the Ct value was ≤ 35.
2) No Template Control: There is no significant amplification of VIC, FAM and ROX channels or no display of Ct value or Ct value > 42.

The positive and no template controls must perform as expected before the run can be considered valid. Otherwise, this run is invalid and should be retested with residual extracted nucleic acid (Table 5).

5.2. Interpretation of Test Results

All test controls should be within the expected Ct values to consider the run valid. A valid run allows patient results to be interpreted (Table 6).

- For samples with positive internal control signal (i.e., The ROX channel should have an obvious S-shaped amplification curve, and Ct value ≤ 35):

  - **Positive:**
    If both the N gene and the ORF1ab gene are positive (Ct $\leq$38) and RNase P IC is +/-, then the sample test result is positive, given the positive and negative controls perform as expected.
    If only the N gene or the ORF1ab gene is positive (Ct $\leq$38) and RNase P IC is +/-, then the sample test result is positive, given the positive and negative controls perform as expected.

- **Negative:** If both the N gene and the ORF1ab gene are negative (Ct > 38) and RNase P IC is positive (Ct ≤ 35), then the sample test result is negative.

- If none of the VIC, FAM and ROX channels show positive results, the sample may not have been loaded sufficiently, and it should be tested again.

- However, if the RNase P IC does not produce a positive result for a clinical specimen and the N and/or ORF1ab targets are positive, the sample should be considered a valid positive result because it is possible that some samples may fail to exhibit RNase P detection due to low cell numbers in the original clinical sample. A negative RNase P signal does not preclude the presence of SARS-CoV-2 virus RNA in a clinical specimen.

Table 5. Interpretation of Control Results

Positive Control	No Template Control	RNase P Gene	Result interpretation	Action
+	-	+	Both run and sample are valid	Report sample result
+	-	-	Run valid, but sample may be invalid	Repeat testing with residual extracts unless the specimen is considered positive when ORF1ab and/or N gene is positive.
+	+	+/-	Run invalid	Repeat the run with residual extracts
-	-	+/-	Run invalid	Repeat the run with residual extracts
-	+	+/-	Run invalid	Repeat the run with residual extracts

Table 6. Interpretation of Test Result

ORF1ab gene Ct	N gene Ct	RNase P	Result interpretation	Action
≤38	≤38	+/-	SARS-CoV-2 Detected	Report positive result
≤38	>38	+/-	SARS-CoV-2 Detected	Report positive result**

>38	≤38	+/-	SARS-CoV-2 Detected	Report positive result**
>38	>38	+	SARS-CoV-2 Not Detected	Report negative result
>38	>38	-	Invalid	Repeat the run using residual extracted material***.

Note: ** If only one of the two targets is "positive" and RNase P is +/-, this would still be considered a valid positive result

***** If run controls are valid, but the test was invalid: first, conduct the repeat test by re-extracting RNA from the same specimen. If the test fails again, collect a new specimen from the patient and repeat the test.**

[Limitations of Testing Methods]

1. The test results of this kit are for clinical reference only and should not be used as the sole basis for clinical diagnosis and treatment. The clinical management of patients should be considered in combination with signs/symptoms, medical history, other laboratory tests, and treatment response. The test results cannot be used as a basis for clinical diagnosis or exclusion of cases, and are for reference only by clinicians.
2. Possible reasons for inaccurate results include: inadequate sample collection, transportation, storage and processing in that low RNA content in the sample can cause detection failure or inaccurate results; variation in the target sequence region detected or sequence changes caused by other reasons may lead to inaccurate results; other unproven interference factors or nucleic acid response inhibitors may also cause inaccurate results.

Conditions of Authorization for the Laboratory

The Genetron SARS-CoV-2 RNA Test Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations#covid19ivd.

However, to assist clinical laboratories using the Genetron SARS-CoV-2 RNA Test the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using your product will include with test result reports, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may

include mass media.

B. Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

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

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

E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and Genetron Health (via email: support_USA@genetronhealth.com) any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

F. All laboratory personnel using your product must be appropriately trained in PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit, and use your product in accordance with the authorized labeling.

G. You, authorized distributors, and authorized laboratories using your product 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, "Laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

[Product Performance Index]

1. Appearance: The appearance of the package is clean and intact without leakage or damage; the components are complete; the labels on reagent vials are clear, the reagent vials are clean and intact without leakage or damage; each liquid component is clear and transparent without obvious turbidity, sediment or

flocculation.

[Performance Characteristics]

2. Limit of Detection (LoD):

The detection limit of this kit is 1000 copies/mL. Viral RNA isolated from clinically confirmed COVID-19 patients was used as the testing material to determine the LoD. The concentration of the RNA copy number was determined by Quantstudio 3D Digital PCR System. One SARS-CoV-2 positive oropharyngeal swab sample was detected by a commercial SARS-CoV-2 (Shanghai BerGem) approved by the Chinese National Medical Product Authority (NMPA), was serially diluted to 4000, 2000, 1000 and 500 copies/mL with confirmed, pooled negative oropharyngeal swab sample matrix. Four replicates per dilution were tested to estimate the LoD. The LoD was determined as the lowest titer with 95% detection rate. The estimated LoD was 1000 copies/mL (Table 7) and was confirmed using 20 individual extraction replicates (Table 8).

Table 7. Estimation of LoD

Copies per mL	ORF1ab Target			N Target			RNase P		
	Positive Replicates	Average Ct	Standard Deviation	Positive Replicates	Average Ct	Standard Deviation	Positive Replicates	Average Ct	Standard Deviation
4000	4/4	33.25	0.27	4/4	33.42	0.28	4/4	26.96	0.13
2000	4/4	34.15	0.37	4/4	34.25	0.25	4/4	27.91	0.06
1000	4/4	35.16	0.83	4/4	35.42	0.86	4/4	28.75	0.04
500	2/4	36.28	1.35	2/4	35.05	0.42	4/4	28.88	0.06
NTC	0/3	N\A	N\A	0/3	N\A	N\A	0/3	N\A	N\A
PC	3/3	32.95	0.16	3/3	33.24	0.14	3/3	31.20	0.09

Table 8. LoD Confirmatory Study

Copies per mL	ORF1ab Target			N Target			RNase P		
	Positive Replicates	Average Ct	Standard Deviation	Positive Replicates	Average Ct	Standard Deviation	Positive Replicates	Average Ct	Standard Deviation
1000	20/20	35.44	0.40	20/20	35.06	0.37	20/20	33.15	0.20

3. Inclusivity：

The inclusivity study was conducted in silico by mapping the assays to all analyzed

SARS-CoV-2 sequences in the NCBI database as of May 20, 2020. A total of 1497 full genome sequences of SARS-CoV-2 and 484 partial SAR-CoV-2 sequences from 27 countries / regions (Australia, Brazil, China, Colombia, Czech Republic, France, Greece, Hong Kong, India, Iran, Israel, Italy, Malaysia, Nepal, Netherlands, Pakistan, Peru, South Africa, South Korea, Spain, Sri Lanka, Sweden, Taiwan, Turkey, USA, Vietnam and Netherlands) were analyzed against the primers and probes used in the Genetron SARS-CoV-2 RNA Test. Both the ORF1ab and N primer and probe sets showed 100% homology to 1433/1497 (95.7%) of the evaluated sequences; the oligonucleotides showed mismatches to 64 of the SARS-CoV-2 sequences that were analyzed. However, no mutations/mismatches were in the 3′ end of the primer or probe sequences (defined as the last 5 nucleotides) which can potentially affect assay sensitivity. The mismatches of the ORF1ab and/or N oligonucleotides compared to the 64 SARS-CoV-2 sequences (64/1497) are not predicted to adversely affect primer binding or reduce assay efficiency. The mapping results showed that the Genetron SARS-CoV-2 RNA Test can detect all the strains in the published database.

4. Cross-reactivity:

4.1 *In silico* analysis

To assess the potential cross-reactivity of the Genetron SARS-CoV-2 RNA Test, both *in silico* analysis (Table 9) and wet testing (Table 10) against normal and pathogenic organisms found in the respiratory tract was completed. The study evaluated whether there is any significant amplification of non-target sequences that could either result in cross-reactivity or potentially interfere with the detectability of the assay's analyte. The result shows that the Genetron SARS-CoV-2 RNA Test does not react with related pathogens or other high prevalence disease agents.

The *in silico* analysis was conducted by comparing the available sequences of potential cross-reactive organisms in https://www.ncbi.nlm.nih.gov/nucleotide/. The potential cross-reactive organisms included six types of 229E and HKU1, 53 types H1N1 and H7N9, as well as 20 types of bacteria and yeast. Representative bacteria, yeast, and viruses from the in silico cross-reactivity analysis is shown in Table 9. The analysis results showed that some microorganism sequences have >80% homology to one of the primers or probes (see table below) and none of them will be expected to be amplified by the test due to one of the following reasons (as the circled numbers represent):

① Only one primer showed >80% homology to a sequence, while the other primer showed significantly less homology to the same sequence.

② While the probe showed >80% homology to a sequence, neither of the primers showed significant homology to the same sequence.

③ While one or both primers showed >80% homology to a sequence, there

are >3 mismatches at the 3' end of the primers.

④ While both primers showed >80% homology to a sequence, they are mapped to the same strand in the same direction.

Table 9. *In Silico* Analysis Results

Organism	GenBank ID	ORF1ab %Homology			N gene %Homology			RNase P gene %Homology		
		F	R	Probe	F	R	Probe	F	R	Probe
coronavirus_229E	NC_002645.1	54.55%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
coronavirus_NL63	NC_005831.2	50.00%	0.00%	0.00%	90.48% ①	68.42%	0.00%	47.37%	50.00%	0.00%
coronavirus_OC43	NC_006213.1	0.00%	72.73%	0.00%	76.19%	0.00%	78.57%	36.84%	50.00%	56.52%
coronavirus_HKU1	NC_006577.2	81.82% ①	0.00%	85.00% ②	0.00%	0.00%	0.00%	0.00%	45.00%	0.00%
coronavirus_MERS	NC_019843.3	0.00%	0.00%	85.00% ②	66.67%	68.42%	0.00%	78.95%	70.00%	0.00%
coronavirus_SARS	NC_004718.3	100.00 %④	100.00% ④	60.00%	90.48% ①	47.37%	100.00 %②	0.00%	90.00% ①	73.91%
Human adenovirus	AC_000017.1	0.00%	54.55%	55.00%	71.43%	63.16%	0.00%	0.00%	0.00%	69.57%
Human metapneumovirus	NC_039199.1	50.00%	0.00%	0.00%	0.00%	84.21% ①	39.29%	0.00%	0.00%	0.00%
Human parainfluenza virus	NC_003461.1	0.00%	68.18%	0.00%	0.00%	68.42%	0.00%	73.68%	55.00%	47.83%
Influenza A virus	NC_026437.1	0.00%	59.09%	0.00%	0.00%	68.42%	0.00%	0.00%	50.00%	0.00%
Influenza B virus	NC_002204.1	0.00%	0.00%	0.00%	14.29%	78.95%	0.00%	0.00%	0.00%	65.22%
Human enterovirus 68 strain	NC_038308.1	0.00%	72.73%	70.00%	71.43%	52.63%	0.00%	68.42%	0.00%	0.00%
Respiratory syncytial virus	NC_001803.1	0.00%	90.91% ①	0.00%	0.00%	0.00%	0.00%	89.47% ①	0.00%	69.57%
Human rhinovirus 1 strain	NC_038311.1	0.00%	68.18%	90.00% ②	0.00%	0.00%	46.43%	78.95%	75.00%	0.00%
Chlamydia pneumoniae	NC_005043.1	90.91% ①	68.18%	0.00%	71.43%	68.42%	71.43%	89.47% ①	75.00%	0.00%
Haemophilus influenzae	NZ_LN831035.1	81.82% ①	72.73%	0.00%	85.71% ①	0.00%	92.86% ②	0.00%	75.00%	0.00%
Legionella pneumophila	NZ_LR134380.1	90.91% ①	72.73%	0.00%	76.19%	68.42%	0.00%	78.95%	85.00% ①	0.00%
Mycobacterium tuberculosis	NC_000962.3	63.64%	0.00%	0.00%	66.67%	57.89%	67.86%	78.95%	90.00% ①	0.00%

Organism	GenBank ID	ORF1ab %Homology			N gene %Homology			RNase P gene %Homology		
		F	R	Probe	F	R	Probe	F	R	Probe
Streptococcus pneumoniae	NZ_LN831051.1	63.64%	63.64%	0.00%	76.19%	78.95%	0.00%	68.42%	90.00% ①	0.00%
Streptococcus pyogenes	NZ_LN831034.1	0.00%	72.73%	90.00% ②	85.71% ①	0.00%	0.00%	68.42%	75.00%	47.83%
Bordetella phage BPP-1	NC_005357.1	0.00%	0.00%	70.00%	71.43%	78.95%	0.00%	68.42%	0.00%	0.00%
Mycoplasma pneumoniae	NZ_CP010546.1	59.09%	63.64%	0.00%	66.67%	73.68%	0.00%	78.95%	0.00%	0.00%
Pneumocystis jirovecii RU7	NW_017264775.1	36.36%	54.55%	80.00% ②	23.81%	68.42%	0.00%	68.42%	80.00% ③	91.30%②
Candida albicans	NC_032089.1	72.73%	72.73%	80.00% ②	71.43%	68.42%	0.00%	89.47% ①	70.00%	21.74%
Pseudomonas aeruginosa	NC_002516.2	72.73%	0.00%	80.00% ②	80.95% ①	0.00%	71.43%	36.84%	80.00% ①	91.30%②
Staphylococcus epidermidis	NC_004461.1	45.45%	0.00%	0.00%	61.90%	89.47% ①	78.57%	57.89%	0.00%	39.13%
Streptococcus salivarius	GCF_000286295.1	45.45%	54.55%	90.00% ②	42.86%	0.00%	0.00%	0.00%	0.00%	0.00%

The result of silico analysis shows that the Genetron SARS-CoV-2 RNA Test does not react with related pathogens or other high prevalence disease agents.

4.2 Cross-Reactivity Wet Testing

Coronavirus MERS, OC43 NL63 HKU1 and 229e are high priority pathogens from the same genetic family, of which these viruses showed higher similarity to the assay's probes and primers in the *in silico* study. For in vitro testing, the panel was sourced from the 2020 "2019-nCoV Coronavirus EQA (External Quality Assessment) programme" obtained from the NCCL; National Center for Clinical Laboratories. The organisms on the panel consist of armored RNA specimens and were evaluated by the Genetron SAS-CoV-2 RNA Test in triplicate. The results of the in vitro cross-reactivity testing are presented below in Table 10:

Table 10. Cross-Reactivity Wet Testing

Sample number	Virus type	Maximum similarity of probes and primers	Concentration □ copies/mL□	Results from 3 replicates			Positive detection Rate
2020001	SARS	100%	3.0×10^6	Negative	Negative	Negative	0/3

Sample number	Virus type	Maximum similarity of probes and primers	Concentration □ copies/mL□	Results from 3 replicates			Positive detection Rate
2020006	NL63	90.48%	1.6×10^6	Negative	Negative	Negative	0/3
2020007	HKU1	85%	9.4×10^5	Negative	Negative	Negative	0/3
	229E	54.55%	2.7×10^6				
2020012	MERS	85%	1.0×10^4	Negative	Negative	Negative	0/3
	OC43	72.73%	1.0×10^6				
NTC	RNase-free ddH2O	-	/	Negative	-	-	-
PC	SARS-CoV-2	-	2×10^3	Positive	-	-	3/3

5. Clinical Evaluation:

For the clinical validation of the Genetron SARS-CoV-2 RNA Test, a method comparison study was conducted to assess performance compared to the Maccura Biotechnology Co., Ltd., EUA authorized assay, SARS-CoV-2 Fluorescent PCR Kit (received US FDA EUA authorization on April 15, 2020).

A total of 85 oropharyngeal swab clinical specimens were included in the method comparison analysis. Of the 85 clinical specimens, 50 were SARS-CoV-2 positive and 35 were SARS-CoV-2 negative based on the Maccura EUA assay.

The Genetron SARS-CoV-2 RNA Test detected 50 positives and 34 negatives. The positive and negative percent agreements between the Genetron SARS-CoV-2 RNA Test and the comparator method are shown below:

Table 11. Clinical Study Results

		Comparator - Maccura		Total
		Positive	Negative	
Genetron Health	Positive	50	1	51
	Negative	0	34	34
Total		50	35	85

Positive percent agreement (PPA) = 50/50; 100%, (95% CI: 95.7% - 100%)
Negative percent agreement(NPA) = 34/35; 97.1%, (95% CI: 91.1% - 99.1%)
Kappa value = 0.95

6. 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 QIAamp Viral RNA Mini Kit (Qiagen, Item No. 52904) and ABI7500. The results are summarized in Table12.

Table 12: 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	Oropharyngeal swabs	1.8×10^3 NDU/mL	N/A
MERS-CoV		N/A	ND

NDU/mL: RNA NAAT detectable units/mL

N/A: Not Applicable

ND: Not Detected

[Interpretation of Identifiers]

Identifier	Meaning	Identifier	Meaning
	Indicates that the shipping package should be upright		Indicates that fragile items are contained in the shipping package and should be handled with care.
	Temperature Limitation		Indicates that the shipping package should be protected from rain/moisture and should be kept
IVD	In Vitro Diagnostic Medical Device.		

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[Basic Information]

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