CE IVD

U-TOP™ COVID-19 Detection Kit

For in vitro diagnostic use only

For Prescription Use only

For use under Emergency Use Authorization (EUA) only

A diagnostic kit for detection of SARS-CoV-2 (COVID-19)
in clinical samples using Real-time PCR

Instructions for Use | V2.2

Store at –20°C

Date of Revision: January 11, 2022

Certificate of KGMP

ISO 13485 LL-C (Certification) Certificate of ISO13485

SEASUN BIOMATERIALS

Indications of Medical Devices Act

1. Product Category:	IVD Reagent for Infectious Agents
2. Product Name:	U-TOP™ COVID-19 Detection Kit
3. Product Catalogue Number:	SS-9930
4. Purpose of use:	See 1. in this User Guide

Warnings and Precautions

Contact us for detailed information for the safe use of the U-TOP™ COVID-19 Detection Kit. Please check storage temperature and attention points for accurate diagnosis of the product. Sample and Assay waste must be disposed of in a legally designated manner.

Warranty and Responsibility

All products of SEASUN BIOMATERIALS Inc. are tested under rigorous quality management processes. SEASUN BIOMATERIALS Inc. guarantees to ensure the quality of the product during warranty period. If any problems relating to the quality of the product are found, please contact the headquarters immediately.

Quality Control System

All aspects of the quality management system, product creation, quality assurance, and supplier qualifications are certified to ISO 13485, ISO 9001, KGMP.

Inquiries and customer service (A/S)

Send us an e-mail (as@seasunbio.com) to inquire about the product.

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1. Intended Use

The U-TOP™ COVID-19 Detection Kit is a one-step real-time RT-PCR test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in oropharyngeal and nasopharyngeal swab specimens, anterior nasal and mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, as well as bronchoalveolar lavage and sputum specimens 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 U.S.C. §263a, that meet requirements to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA which is generally detectable in upper and lower respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinically 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.

Testing with the U-TOP™ COVID-19 Detection 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 U-TOP™ COVID-19 Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

2. Product Description

The U-TOP™ COVID-19 Detection Kit is a qualitative test based on real-time reverse transcription polymerase chain reaction (RT-PCR) for detection of the SARS-CoV-2 RNA extracted from clinical specimens.

The U-TOP™ COVID-19 Detection Kit uses dual-labeled Peptide Nucleic Acid (PNA) probes that target two distinct regions in ORF1ab and one region in N gene of SARS-CoV-2 genome. Detection probes for two amplicons of Orf1ab are labeled with FAM and the probe for N is labeled with HEX reporter dye. The kit evaluates the presence of three individual amplicons of the SARS-CoV-2 genome. A sample is determined to be SARS-CoV-2 positive if the Ct value of one or both fluorescence channels are ≤ 38.

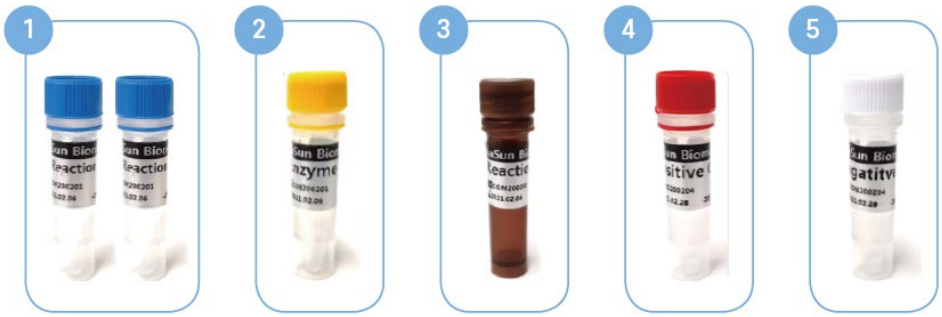
Since the PNA probes are labeled with the fluorescent dye reporter and quencher, the fluorescent signals of the intact probes are suppressed by the quencher dye. During the PCR amplification step, hybridization of the probes to the specific single-stranded DNA template results in separation of the reporter and quencher dyes and the generation of a fluorescent signal. With each PCR cycle, increasing amounts of target DNA amplicon are generated and the signal of the reporter dye increases concomitantly. Each reporter dye is measured at defined wavelengths, which enables simultaneous detection and discrimination of the amplified coronavirus target and the internal control.

The kit includes a primer/probe set to detect human RNase P gene (RP) as an internal control (IC) intended to examine RNA extraction efficiency, the enzyme activity of the kit as well as the assay performance. The IC probe is labeled with Texas Red fluorescent dye which uses an independent fluorescence detection channel from SARS-CoV-2 targets. The RP primer/probe set is included in the Reaction Mix tube.

In addition, the kit utilizes external Positive (PC) and Negative (NC) controls. The PC contains templates for SARS-CoV-2 Orf1ab and N targets as well as the human RNase P gene. The NC contains RNase/DNase free distilled water.

3. Kit Components and Packaging Specifications

The kit composed of 2X Reaction buffer, Enzyme Mix, Reaction Mix, Positive control and Negative Control.



	Reagent label	Part #	Descriptions/Contents	Volume / Quantity	Store at
1	2X Reaction buffer	SS-9930COP	PCR buffer	750㎕ / 2 tubes	-20°C
2	Enzyme Mix	SS-9930COE	Reverse transcriptase Taq polymerase	100㎕ / 1 tube	
3	Reaction Mix	SS-9930COM	Primer, probe mixture	400㎕ / 1 tube	
4	Positive Control	SS-9930COS	Template for SARS-CoV-2 and RNase P	200㎕ / 1 tube	
5	Negative Control	SS-9930CON	Nuclease free DW	200㎕ / 1 tube	

Control Materials to be Used with U-TOP™ COVID-19 Detection Kit

Negative Control (NC): contains nuclease-free water intended to evaluate cross contamination of the kit, supplements, reagents and PCR instrument used in the test. Detection accuracy as well as non-specific signals may be caused by primer dimer, primer-probe non-specific binding also can be evaluated with this control. Negative control should be run using 10 µL in one well per test.

Positive Control (PC): contains in vitro transcribed RNA of SARS-CoV-2 Orf1ab and N genes and a plasmid containing human RNase P that is intended to evaluate RNA extraction, enzyme activity, and analytical and clinical performance of the assay. The positive control should be run using 10 µL in one well per test.

Internal Control (IC): The Reaction Mix tube of the kit consists of a primer set and a probe that detects human RNase P. The internal control is intended to evaluate the RNA extraction process, test accuracy as well as the real-time PCR instrument performance.
Both PC and NC should be used directly with the test without prior dilution.

4. Storage and Handling Requirements

Store all reagents at –20°C (both un-opened and in-use product).
Use the reagents within 3 months once opened.
Do not use reagents past their expiration date.
Completely thaw the reagents except the Enzyme mix at room temperature before each use.
Place all reagents on ice once thawed during whole test procedure.
Place Enzyme Mix on ice during whole test procedure.
Avoid excessive freeze/thaw cycles.
Vortex and spin down briefly the reagents before each use.

5. Additional Materials and Equipment

The kit does not include sample collection and preservation instruments/buffers, RNA extraction reagents and Real-time PCR detection systems. Components required for detection of SARS-CoV-2 but not included with the kit are:

1. Sample collection / Storage / Shipping consumables
   A. TOP™ Virus Collection Kit (Seasun Biomaterials, Cat.No SS-1200), Viral sample collection kit (Noble Bio, Cat. No UTNFS-3B-1) or BD™ Universal Viral Transport System (BD, UVT Cat No. 220531) for collection and transport of upper respiratory tract specimens.
   B. Sputum collection container: sterile container with screw cap (BD, Cat # 90004-118).
2. RNA extraction kit for extracting RNA from clinical specimen.
   A. QIAamp DSP virus kit (Qiagen, Cat. No 60704)
   B. PANAMAX™48 Nucleic acid extraction system (Software version: panaMAX 01.00.03) with PANAMAX™ Viral DNA/RNA Extraction Kit (Panagene, Cat.No PNAK-1001, PNAK-1002)]
   C. TOP™ Viral DNA/RNA Extraction Kit (Seasun Biomaterials, Cat. No SS-1300)
3. Real-time PCR system and the consumables

A. Real-time PCR detection systems can be used with the U-TOP™ COVID-19 assay are
-CFX 96 real-time PCR detection system (Bio-Rad) with software CFX manager V3.1
-Applied Biosystems real-time PCR system 7500 with Software 2.0.6

B. PCR Consumables
- 96 well white PCR plate (Bio-rad MLL9651, Applied Biosystems AB0900W or equivalent)
- Sealing Film or 8-12 well PCR plate cap (Bio-rad MSB 1001 or equivalent)
- Vortex and Micro centrifuge
- Sterilized pipette tips with filter (10 µL, 200 µL and 1000 µL)
- 1.5 mL DNase/RNase free microcentrifuge tubes and racks
- Disposable powder-free gloves and laboratory gowns

6. Warnings and Precautions

- For Emergency Use Authorization Only.
- For *in vitro* diagnostic use only.
- For Prescription Use Only (Rx).
- This product 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, that meet requirements to perform high complexity tests.
- This test has been authorized only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.
- 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 Act, 21 U.S.C. § 360bbb-3(b)(1), unless the authorization is terminated or revoked sooner.
- Use under the guidance of physicians and specialists.
- Sensitivity of reagents may be lowered with prolonged exposure to room temperature or light.
- Store all assay contents at -20℃ away from UV/sunlight.
- Avoid use of the kit if contaminated with test sample.

- Keep clear the external environment, always use in a clean place.
- Only use sterilized single-use micro filter tips.
- Strong external impact may damage screw tubes filled with reagents or control materials.
- If any abnormality is observed, stop the experiment, contact the manufacturer.

7. Specimen Collection, Handling and Storage

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality.

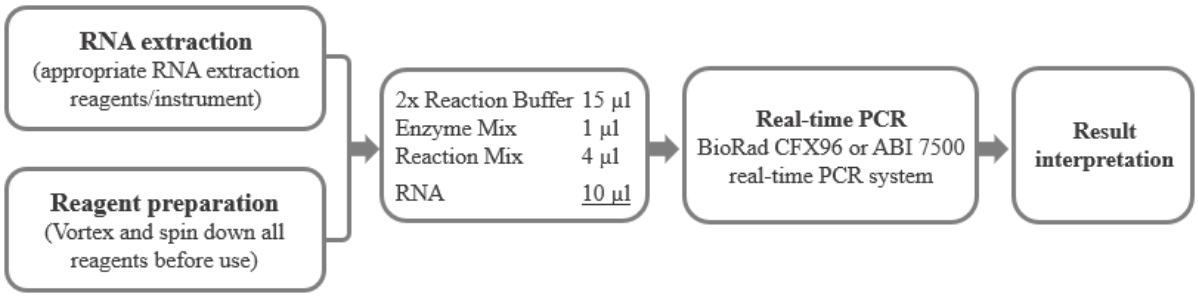
Collecting specimen: Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (SARS-CoV-2). The U-TOP™ COVID-19 detection kit has been validated with TOP™ Virus Collection Kit (Seasun Biomaterials, Cat.No SS-1200), Viral sample collection kit (Noble Bio, Cat.No UTNFS-3B-1) and BD™ Universal Viral Transport System (BD, UVT Cat#220531) for collection and transport of upper respiratory tract specimens. Sputum, BALs, and washes/aspirates can be collected and transported in a sterile sputum collection container (BD, Cat # 90004-118). Follow specimen collection device manufacturer instructions for proper collection methods. Store the samples at 2-8°C up to 72 hours. If a delay in shipping or extraction is expected, store samples at –70°C.

Shipping: Specimens must be packaged and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Store specimens at 2-8°C and ship to the lab on ice pack. If a specimen is frozen at -70°C, ship to the lab on dry ice. Additional useful and detailed information on packing, shipping, and transporting specimens can be found at Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19).

Rejection criteria: Specimens will be rejected prior to the test 1) if the specimens were stored at 2-8°C over 72 hours. 2) Specimens without sufficient volume for the test (less than 1 mL). 3) Label is damaged (cannot be read or recognized) or without labeling/identifying documents.

8. Test procedure/Protocol

8.1 Summary of Preparation and Testing Process



Work flow of U-TOP™ COVID-19 Detection Kit

8.2 RNA extraction from clinical specimens

The U-TOP™ COVID-19 Detection Kit does not include viral RNA extraction reagents. A silica dioxide-coated magnetic bead based automated nucleic acid extraction system PANAMAX™48 with PANAMAX™ Viral DNA/RNA extraction kit (Panagene, Cat# PNAK-1001, PNAK-1002), spin column based nucleic acid extraction reagents QIAamp DSP virus kit (Qiagen, Cat # 60704) and TOP™ Viral DNA/RNA extraction kit (Seasun Biomaterials, Cat#SS-1300) have been validated with the U-TOP™ COVID-19 Detection Kit. All three extraction kits require 300 µL sample input (both upper and lower respiratory tract specimens) and yields 60 µL of purified nucleic acid eluent. Following the extraction, RNA should be used immediately or stored at -70°C (for up to 1 month) for use later.

8.3 Reaction master mix and Assay set up

Note: Plate set-up configuration can vary with the number of specimens. Negative and Positive control must be included in each run. Prepare reaction master mix in separate area (Assay preparation area) from nucleic acid handling.

1. Clean and decontaminate all work surfaces, equipment as well as small supplements e.g. pipette, vortex, micro centrifuge with 70% ethanol prior to use to minimize the risk of nucleic acid cross-contamination.

2. Place enzyme mix on ice until thawed. Other reagents can be thawed at room temperature. Keep all reagents on ice once thawed during the whole test procedure.

3. Vortex for 5 sec and spin down all reagents before use.

4. Determine the number of reactions (N) to set up the assay. It is necessary to make excess reaction mix for PC, NC and for possible pipetting error.

5. Prepare the reaction master mix in 1.5 mL microcentrifuge tube according to the following table. It is recommended to prepare 110% of the calculated amount of PCR mix to account for pipetting carryovers.

Master mix for one reaction

Reagents	Volume (µL)
2X Reaction Buffer	15
Enzyme Mix	1
Reaction Mix	4
Total (w/o RNA sample)	**20**

6. Vortex the prepared master mix for 5 sec and centrifuge briefly to collect contents at the bottom of the tube and place the tube in a cold rack (ice or cold block).

7. Set up 96 well PCR plate.

8. Dispense 20 µL of master mix into the wells of 96-well PCR plate.

9. Pipette **10 µL** of NC into NC sample well (dispensing sample and control in 96 well plate are irrelevant, no fixed position is required)

8.4 Nucleic acid template addition

Note: Always change pipette tips in-between patient sample handling and after pipetting each component. Add the Positive Control in PCR plate last, to avoid the contamination. Positive control contains high concentration of viral template.

1. Gently vortex nucleic acid sample tubes for approximately 5 sec and spin down the tubes to collect contents at the bottom of the tubes. Always keep the sample tubes on ice or in a cold block.

2. Dispense nucleic acid samples of **10 µL** into the 96 well PCR plate containing the aliquoted

reaction master mix.

3. Carefully pipette **10 µL** of PC into a PCR plate well last.

4. Seal the PCR plate with cap strip or sealing film. Ensure the sealing film is completely absorbed to the plate by using a roller.

5. Spin down briefly using a micro plate centrifuge to downward the contents and remove extra air bubbles. It is recommended to centrifuge for 30 sec at 500 x g, 4°C.

8.5 Set up Real-time PCR run

Note: U-TOP™ COVID-19 detection kit running protocol and PCR conditions are the same for both the CFX 96 and ABI 7500 real-time PCR detection systems; however, the method for the run set up (program set up) of the two PCR platforms are different. The run protocol and fluorescence channels for the targets are shown in Tables 1 and 2.

Table 1. RT-PCR Thermocycling Conditions

Step	Temp	Time	Repeat
cDNA synthesis	50°C	30 min	1
Amplification	95°C	15 min	1
	95°C	30 sec	41
	60°C	30 sec*	
	72°C	30 sec	

* Collect fluorescence signal after reaction for 60°C / 30 sec

Table 2. Fluorescence Channel for Probes

Fluorescence	Target
FAM*	<Orf1ab> SARS-CoV-2
HEX/VIC**	<N> SARS-CoV-2
Texas Red	<RNase P> human

*Two individual PNA probes for two distinct amplicons of Orf1ab own the same fluorescence signal FAM

** HEX for Bio-rad CFX 96, VIC for ABI 7500 platform

CFX96 and Software Operation - 1 (New experiment)

① Turn on a computer and CFX 96 > Display the 96-well thermal block > Place the 96 well plate prepared in previous step.

② Run the CFX Manager software on the computer connected to the CFX96. Go to File > New > Protocol > input the run information as shown in table 1. > Set the sample volume to 30 μL.

③ Go to Plate > Edit Selected > Set Fluorophores > Select fluorescence channel FAM, HEX and Texas Red.

④ Specify the positive control well, select "Positive Control" from sample type, and input 3 detection fluorescence in the load (Select FAM, HEX, Texas Red).

⑤ Specify the negative control well, select "Negative Control" from sample type, and input 3 detection fluorescence in the load (Select FAM, HEX, Texas Red).

⑥ Wells with clinical specimens should be specified as Unknown, input 3 detection fluorescence in the load (Select FAM, HEX, Texas Red).

⑦ Go to Setting > plate type > Select BR white.

⑧ Go to Start Run > Select Block Name (PCR instrument) to use > Close Lid and Start Run.

CFX96 and Software Operation - 2 (Pre-Programmed Run Settings)

① If you have a previous run file, you can re-use for further. Double click on a previous run file and select sequentially File > Repeat Run.

② Go to Plate tab > set Control and Sample information > Start Run. The fluorescence channel, plate type, and volume are already selected with previous run.

ABI 7500 and Software Operation – 1 (New experiment)

① Turn on a computer and ABI 7500 > display the 96 well thermal block > Place the 96 well plate prepared in previous step.

③ Run 7500 Software on the computer connected to the ABI 7500. Select sequentially 7500 (96well) > Quantitation-Standard curve > TaqMan@ Reagents > Standard (2 hours to complete a run).

④ Go to Plate Setup > Define Targets and Samples > Define targets > Add New Target > set

Target Name and Reporter as shown below:

Target 1. ORF1ab: Reporter FAM; Quencher NFQ-MGB

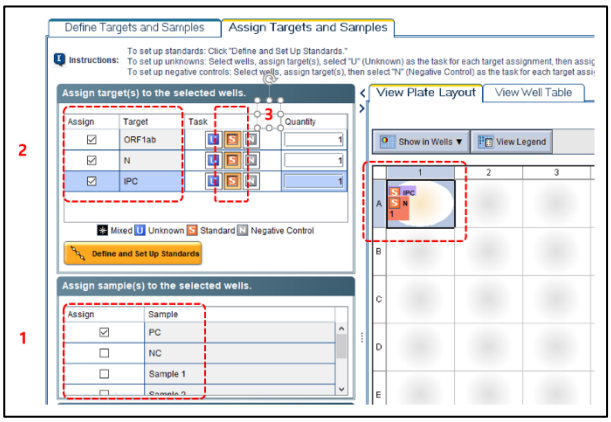
Target 2. N gene: Reporter VIC; Quencher NFQ-MGB

Target 3. IC: Reporter TEXAS RED; Quencher NFQ-MGB

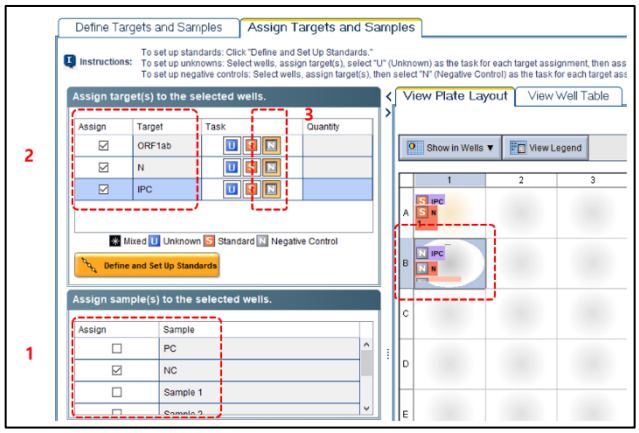
⑤ Go to Define Samples > Add New Sample > Input PC, NC and Sample (Test Specimen).

⑥ Go to "Assign Target and Samples" to set targets and well positions for PC, NC and Samples to be analyzed.

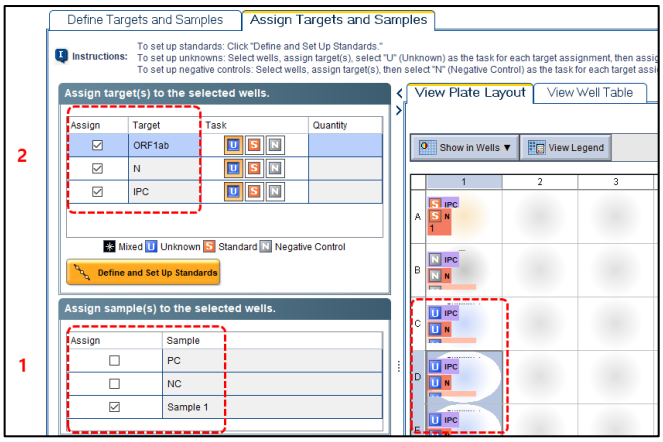
1. Positive Control: Click Positive Control Well from "View Plate Layout". Select "PC" from ① (shown in figure below) and activate all targets from ② (shown in figure below) then activate "**S**" for all 3 targets from ③ (shown in figure below).



2. Negative Control: Click Negative Control Well from "View Plate Layout". Select "NC" from ① (shown in figure below) and activate all targets from ② (shown in figure below) then activate "**N**" for all 3 targets from ③ (shown in figure below)



3. Sample: Click Wells with test samples from “View Plate Layout”. Select “Sample “from ① (shown in figure below) and activate all targets from ② (shown in figure below).



⑦ Select “None” from “Select the dye to use as the passive reference.”

⑧ Go to Run Method > Input the PCR condition as shown in table 1. Setting with Tabular View is easier than with Graphical View. Set “Reaction volume Per Well” to 30 µL.

⑨ Save the protocol from File > Save As, then Go to Run and click “START RUN“ to start amplification.

ABI7500 and Software Operation - 2 (Pre-Programmed Run Settings)

① A previous run file can be used as a template. Go to File > Open > Select the file.

② Input the sample information in the “Plate setup” and proceed in the same order as above.

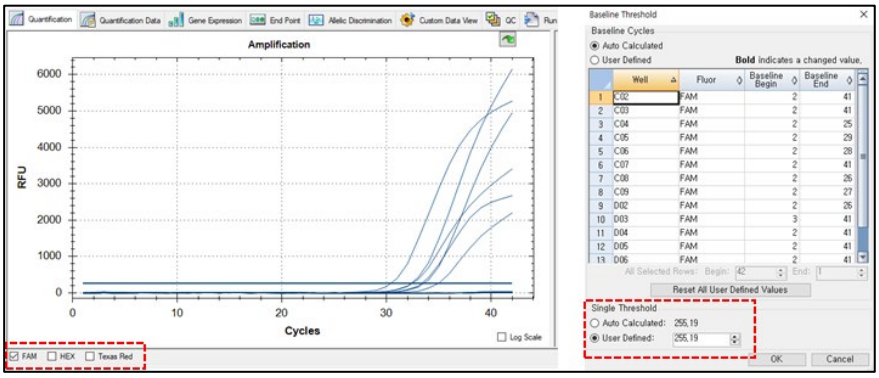
9. Result Interpretation

Base line and threshold setting

The U-TOP™ COVID-19 Detection Kit has been validated using the baseline setting which is automatically adjusted by the CFX 96 and ABI 7500 Real-Time instruments. However, the baseline setting can be adjusted manually in case of production of background noise signal in PCR initiation phase. For adjusting manually, be sure the Thresholds fall within the exponential phase of the fluorescence curves and above any background signal. The threshold value for different instruments varies due to different signal intensities. If adjusting the threshold baseline manually follow the instructions below.

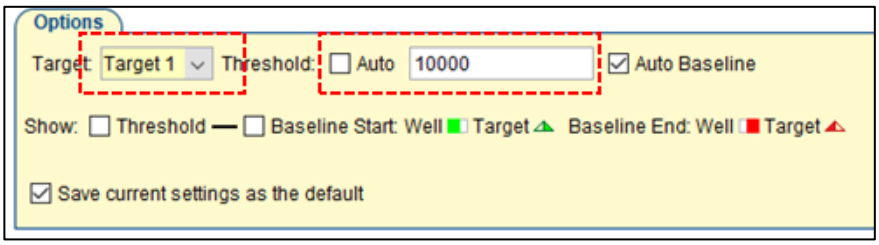
1. CFX 96 real time PCR detection system

Check the box in front of the reporter (FAM, HEX, Texas Red) that needs to be adjusted. Go to Setting > Baseline Threshold > Activate the User defined > Adjust the Threshold value (shown in the figure below).



2. ABI 7500 real time PCR detection system

Go to Amplification Plot > Select target (Reporter) to be adjusted > Adjust the Threshold value (shown in the figure below).



Result Interpretation

All controls should be examined prior to interpretation of patient results (Table 3). If the controls are not valid, the patient results cannot be interpreted.

Negative Control: The NC reaction for fluorescence channels should not exhibit any fluorescence growth curves (Ct) that cross the threshold line (≤ 38). If NC reaction exhibits a growth curve that crosses the threshold at or before Ct 38, sample contamination may have occurred. Invalidate the run and repeat the assay using the same NC. If the NC continues to show a Ct of ≤ 38, discard the NC tube and use a new NC or DNase/RNase free distilled water (Thermo Fisher Scientific Cat.10977015).

Positive Control: The PC will yield a positive result with the FAM, HEX, and Texas Red fluorescence channels (FAM for SARS-CoV-2 Orf1ab amplicon 1 and 2, HEX for SARS-CoV-2 N gene, and Texas Red for human RNase P).

If negative and positive control results are not as described above, the test results of the entire batch are invalid. The controls should meet the requirements listed in table 3 to ensure valid results.

Table 3. Interpretation of Results for Quality Control

Control	Ct value		
	ORF1ab (FAM)	N (HEX)	IC (Texas Red)
Negative	ND	ND	ND
Positive	≤ 38	≤ 38	≤ 38

ND= Not detectable

If the values of the controls are conclusive, refer to the table 4 below to determine the infection status.

Table 4. Clinical Sample Results Interpretation

Ct value			Interpretation	Action
ORF1ab (FAM)	N (HEX/VIC)	IC (Texas Red)		
> 38 or ND	> 38 or ND	≤ 38	Negative (Absence of SARS-CoV-2 RNA)	Report results to healthcare provider. Consider testing for other viruses that may cause similar symptoms.
≤ 38	/	/	Positive (Presence of SARS-CoV-2 RNA)*	Report results to healthcare provider and appropriate public health authorities.
/	≤ 38	/	Positive (Presence of SARS-CoV-2 RNA)*	
≤ 38	≤ 38	/	Positive (Presence of SARS-CoV-2 RNA)	
> 38 or ND	> 38 or ND	> 38 or ND	Invalid**	Repeat test with same RNA extract if available. If result remains invalid, repeat the extraction procedure with the remaining clinical specimen and repeat the test. If all markers remain negative after re-test, report the results as invalid and re-collect patient sample.

*Result is suggestive of: 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in one of the target regions, or 3) other factors.

**Re test after confirmation of RNA extraction step, PCR mixture preparation step, PCR protocol and Kit storage condition and Validity.

/ = No requirement of Ct value. If one or both SARS-CoV-2 target have Ct value of ≤ 38, Ct value of IC and remained SARS-CoV-2 are not required to be considered.

ND= not determined (No detectable Ct value)

Internal Control (IC): All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line at or before Ct 38, thus indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimens may indicate:

- Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.
- Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.
- Improper assay set up and execution. Reagent or equipment malfunction.

If the IC assay does not produce a positive result for human clinical specimens, interpret as follows:

- If the SARS-CoV-2 is positive even in the absence of a positive Ct value of IC (≤ 38.00 Ct), the result should be considered valid. It is possible, that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative IC signal does not preclude the presence of SARS-CoV-2 virus RNA in a clinical specimen.
- If all SARS-CoV-2 markers and RNase P are negative for the specimen, the result should be considered invalid for the specimen. If residual RNA extract remains, repeat the test with this material. If the result remains invalid, repeat the extraction procedure with residual specimen and repeat the test. If all markers remain negative after re-test, report the results as invalid and re-collect patient sample.

10. Limitations

- This test is for *in vitro* diagnostic use under FDA Emergency Use Authorization. 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.
- The performance of this test was established using nasopharyngeal swab and sputum specimens. Oropharyngeal swab specimens, anterior nasal and mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates, as well as bronchoalveolar (BALs) specimens are also considered acceptable specimen types for use but performance has not been established with the U-TOP™ COVID-19 Detection Kit.
- This test is a qualitative test and does not provide the quantitative value of viral load in the original specimens.
- The specimens to be tested shall be collected, processed, stored and transported in accordance with the conditions specified in the instructions. Inappropriate specimen preparation and operation may lead to inaccurate results.
- Extraction and amplification of nucleic acid from clinical samples must be performed according to the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- Amplification and detection of SARS-CoV-2 with this kit Detection Kit has only been validated with the CFX-96 Real-time PCR Detection system and Applied Biosystems® 7500 Real-Time PCR instrument. Use of other instrument systems may cause inaccurate results.

- False-negative results may occur if the viruses are present at a level that is below the analytical sensitivity of the assay or if the virus has genomic mutations, insertions, deletions, or rearrangements or if performed very early in the course of illness.
- 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.

11. Conditions of Authorization for the Laboratory

The U-TOP™ COVID-19 Detection Kit assay's Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on 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 U-TOP™ COVID-19 Detection Kit, 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 only. Deviation from the authorized procedures, such as 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.

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

SEASUN BIOMATERIALS (via email: info@seasunbio.com) if they become aware of any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product.

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

g) SEASUN BIOMATERIALS, 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, that meet requirements to perform high complexity tests" as "authorized laboratories."

12. Assay Performance

12.1 Limit of Detection (LoD)

The LoD study established the lowest SARS-CoV-2 viral RNA concentration (genomic copies/ µL) that consistently yields a 95% positivity rate with the U-TOP™ COVID-19 Detection Kit.

A preliminary LoD for the SARS-CoV-2 specific targets (ORF1ab and N genes) was determined using whole viral genomic RNA (NCCP No. 43326. National Culture Collection for Pathogens) with a starting concentration of 120 ng/µL spiked into pooled negative clinical nasopharyngeal swab and sputum matrices. In the first part of this study, a total of three 10-fold dilutions of known concentrations of genomic RNA were prepared in negative clinical matrix (both nasopharyngeal swab and sputum) and processed using the Qiagen QIAamp DSP virus kit and run on the CFX 96-real time PCR detection system. Five PCR replicates per concentration and 3 lots of the kit were tested. See Table 5 for a summary of the LoD range finding study:

Table 5. Summary of Preliminary LoD Testing

Sputum									
	100 copies/µL			10 copies/µL			1 copy/µL		
	Orf1ab	N	RNase P	Orf1ab	N	RNase P	Orf1ab	N	RNase P
Detection rate	15/15	15/15	15/15	15/15	15/15	15/15	15/15	15/15	15/15
Mean Ct	30.12	30.03	30.64	34.12	34.35	30.92	37.13	36.92	30.77

SD	0.51	0.46	0.83	0.54	0.52	0.88	0.58	0.50	0.79
Nasopharyngeal swab									
	100 copies/µL			10 copies/µL			1 copy/µL		
	Orf1ab	N	RNase P	Orf1ab	N	RNase P	Orf1ab	N	RNase P
Detection rate	15/15	15/15	15/15	15/15	15/15	15/15	15/15	15/15	15/15
Mean Ct	30.27	30.37	30.68	34.17	34.00	30.59	36.90	36.95	30.93
SD	0.67	0.56	0.66	0.52	0.44	0.84	0.48	0.43	0.81

Based on these results, additional 3-fold dilutions of known concentrations of genomic RNA were prepared in negative clinical matrix (nasopharyngeal swab and sputum) and processed using the QIAamp DSP virus kit, PANAMAX48 and TOP™ Virus kit, individually. Twenty individual extraction replicates per dilutions were tested on both the CFX 96 and ABI 7500 real-time PCR systems. The lowest target level at which more than 95% of 20 replicates produced positive results was 1 copy/µL for all three nucleic acid extraction methods for both upper and lower tract specimens on both PCR platforms CFX 96 and ABI 7500 system. Results of the confirmatory LoD studies are summarized in Table 6 and 7.

Table 6. Summary of Confirmatory LoD Test on Bio-Rad CFX 96 System

Sputum		1 copy/µL			0.3 copies/µL			0.1 copies/µL		
		ORF1ab	N	RNaseP	ORF1ab	N	RNaseP	ORF1ab	N	RNaseP
QIAamp DSP	Detection rate	**20/20**	**20/20**	**20/20**	**18/20**	**17/20**	**20/20**	**13/20**	**14/20**	**20/20**
	Mean Ct	36.93	37.05	30.51	-	-	30.93	-	-	30.53
	SD	0.45	0.56	0.84	-	-	0.83	-	-	0.77
PANAMAX™48	Detection rate	**20/20**	**20/20**	**20/20**	**11/20**	**11/20**	**20/20**	**6/20**	**5/20**	**20/20**
	Mean Ct	36.56	35.97	30.20	-	-	30.28	-	-	29.64
	SD	0.52	0.80	0.93	-	-	0.84	-	-	1.11
TOP™ Virus kit	Detection rate	**20/20**	**20/20**	**20/20**	**8/20**	**9/20**	**20/20**	**4/20**	**6/20**	**20/20**
	Mean Ct	36.09	36.22	29.59	-	-	30.05	-	-	29.16
	SD	0.71	0.63	1.13	-	-	1.11	-	-	1.11

Nasopharyngeal swab		1 copy/µL			0.3 copies/µL			0.1 copies/µL		
		ORF1ab	N	RNaseP	ORF1ab	N	RNaseP	ORF1ab	N	RNaseP
QIAamp DSP	Detection rate	**20/20**	**20/20**	**20/20**	**17/20**	**16/20**	**20/20**	**12/20**	**11/20**	**20/20**
	Mean Ct	37.08	37.23	30.62	-	-	30.81	-	-	30.69
	SD	0.47	0.48	0.83	-	-	0.74	-	-	0.91
PANAMAX™48	Detection rate	**20/20**	**20/20**	**20/20**	**6/20**	**13/20**	**20/20**	**3/20**	**4/20**	**20/20**
	Mean Ct	37.03	36.27	29.16	-	-	29.63	-	-	28.98
	SD	0.58	0.74	1.10	-	-	1.07	-	-	1.26
TOP™ Virus kit	Detection rate	**20/20**	**20/20**	**20/20**	**9/20**	**12/20**	**20/20**	**6/20**	**6/20**	**20/20**
	Mean Ct	36.30	36.27	30.03	-	-	29.26	-	-	29.83
	SD	0.46	0.87	1.47	-	-	1.19	-	-	0.97

Table 7. Summary of Confirmatory LoD Test on ABI7500 System

Sputum		1 copy/μL			0.3 copies/μL			0.1 copies/μL		
		ORF1ab	N	RNaseP	ORF1ab	N	RNaseP	ORF1ab	N	RNaseP
QIAamp DSP	Detection rate	**20/20**	**20/20**	**20/20**	**16/20**	**16/20**	**20/20**	**10/20**	**14/20**	**20/20**
	Mean Ct	37.38	37.21	30.93	-	-	30.50	-	-	30.75
	SD	0.48	0.51	0.79	-	-	0.79	-	-	0.88
PANAMAX™48	Detection rate	**20/20**	**20/20**	**20/20**	**8/20**	**10/20**	**20/20**	**5/20**	**4/20**	**20/20**
	Mean Ct	37.14	35.60	28.76	-	-	29.99	-	-	30.36
	SD	0.54	0.69	1.02	-	-	1.15	-	-	0.98
TOP™ Virus kit	Detection rate	**20/20**	**20/20**	**20/20**	**9/20**	**13/20**	**20/20**	**9/20**	**7/20**	**20/20**
	Mean Ct	35.77	35.71	29.48	-	-	29.37	-	-	30.59
	SD	0.40	0.82	0.79	-	-	0.88	-	-	1.12

Nasopharyngeal swab		1 copy/μL			0.3 copies/μL			0.1 copies/μL		
		ORF1ab	N	RNaseP	ORF1ab	N	RNaseP	ORF1ab	N	RNaseP
QIAamp DSP	Detection rate	**20/20**	**20/20**	**20/20**	**16/20**	**16/20**	**20/20**	**10/20**	**14/20**	**20/20**
	Mean Ct	37.38	37.21	30.93	-	-	30.50	-	-	30.75
	SD	0.48	0.51	0.79	-	-	0.79	-	-	0.88
PANAMAX™48	Detection rate	**20/20**	**20/20**	**20/20**	**8/20**	**10/20**	**20/20**	**5/20**	**4/20**	**20/20**
	Mean Ct	37.14	35.60	28.76	-	-	29.99	-	-	30.36
	SD	0.54	0.69	1.02	-	-	1.15	-	-	0.98
TOP™ Virus kit	Detection rate	**20/20**	**20/20**	**20/20**	**8/20**	**11/20**	**20/20**	**7/20**	**5/20**	**20/20**
	Mean Ct	35.67	35.84	30.11	-	-	30.01	-	-	30.27
	SD	0.45	0.75	1.02	-	-	0.67	-	-	0.97

12.2 Inclusivity (analytical reactivity)

Analytical reactivity (inclusivity) of the U-TOP™ COVID-19 Detection Kit was evaluated using publicly available full and partial SARS-CoV-2 genome sequences. 5282 sequences were downloaded from the following databases including National Genomics Data Center China (https://bigd.big.ac.cn/), GenBank (https://www.ncbi.nlm.nih.gov/genbank/), GISAID (https://www.gisaid.org/), GWH (https://bigd.big.ac.cn/gwh/) and NMDC (https://microbiomedata.org/).

Analysis was performed using the <Find binding sites and create fragment> tool in CLC main workbench 20.0.3 software. 496 sequences which comprise whole genome information of SARS-CoV-2 were analyzed against primer and probes contained in the kit. All the alignments

of the kit's primer and probe sets against the available 496 SARS-CoV-2 sequences showed 100% identity (absence of mismatch base against the SARS-CoV-2 target).

12.3 Specificity (Cross-Reactivity)

Evaluation of analytical specificity of the kit was conducted using both *in silico* analysis and wet testing against pathogenic organisms mainly found in the human respiratory tract.

***In-silico* Analysis:**

BLASTn analysis queries of the U-TOP™ COVID-19 Detection Kit primers and probes (2 ORF1ab primer/probe sets and 1 N primer/probe set) were performed against public domain nucleotide sequences with the following database search parameters:

- Mask low complexity regions = Yes
- Expectation value = 10
- Match/Mismatch = Match 2 Mismatch -3
- Gap Costs = Existence 5 Extension 2
- Max number of hit sequence = 250
- Mask lower case = No
- Mask low complexity regions = Yes
- Number of threads = 16
- Filter out redundant results = No.

Table 8. *In-silico* Cross-Reactivity Analysis

	Microorganism	Ref.Seq	ORF1ab (4 primer, 2 probe)	N (2 primer, 1 probe)
1	Human coronavirus 229E	NC_002645.1	No alignment found[a]	No alignment found
2	Human coronavirus OC43	NC_006213.1	No alignment found	No alignment found
3	Human coronavirus HKU1	NC_006577	No alignment found	No alignment found
4	Human coronavirus NL63	NC_005831.2	No alignment found	No alignment found
5	SARS-coronavirus**	NC_004718.3	Amplicon-1 primers: Forward 38%, Reverse 38% Amplicon-2 primers: Forward 42%, Reverse 40% Probes (both amplicons): No alignment found	Forward primer: 21% Reverse primer: 40% Probe: No alignment found
6	MERS-coronavirus	KJ556336.1	No alignment found	No alignment found
7	Adenovirus type 1	MH183293.1	No alignment found	No alignment found
8	Adenovirus type 2	J01917.1	No alignment found	No alignment found
9	Adenovirus type 3	AY599836.1	No alignment found	No alignment found
10	Human Metapneumovirus	KJ627437.1	No alignment found	No alignment found

	Microorganism	Ref.Seq	ORF1ab (4 primer, 2 probe)	N (2 primer, 1 probe)
11	Parainfluenza virus 1	KX639498.1	No alignment found	No alignment found
12	Parainfluenza virus 2	KM190939.1	No alignment found	No alignment found
13	Parainfluenza virus 3	NC_001796.2	No alignment found	No alignment found
14	Parainfluenza virus 4	JQ241176.1	No alignment found	No alignment found
15	Influenza A	GCF_000865085.1	No alignment found	No alignment found
16	Influenza B	BLee1940	No alignment found	No alignment found
17	Enterovirus	NC_001472.1	No alignment found	No alignment found
18	Respiratory syncytial virus	NC_001803.1	No alignment found	No alignment found
19	Rhinovirus	NC_009996.1	No alignment found	No alignment found
20	*Chlamydia pneumoniae*	NC_005043.1	No alignment found	No alignment found
21	*Haemophilus influenzae*	NZ_LN831035.1	No alignment found	No alignment found
22	*Legionella pneumophila*	NZ_LR134380.1	No alignment found	No alignment found
23	*Mycobacterium tuberculosis*	NC_000962.3	No alignment found	No alignment found
24	*Streptococcus pneumoniae*	NZ_LN831051.1	No alignment found	No alignment found
25	*Streptococcus pyogenes*	NZ_LN831034.1	No alignment found	No alignment found
26	*Bordetella pertussis*	NC_018518.1	No alignment found	No alignment found
27	*Mycoplasma pneumoniae*	NZ_CP010546.1	No alignment found	No alignment found
28	*Pneumocystis jirovecii* (PJP)	CAKM01000281.1	No alignment found	No alignment found
29	*Candida albicans*	Database*	No alignment found	No alignment found
30	*Pseudomonas aeruginosa*	NC_002516.2	No alignment found	No alignment found
31	*Staphylococcus epidermis*	NZ_CP035288.1	No alignment found	No alignment found
32	*Streptococcus salivarius*	GCF_900636435.1	No alignment found	No alignment found
33	*Staphylococcus aureus*	BX571856.1	No alignment found	No alignment found

[a]; No alignment found: Greatest bit score of all the primer/probe against reference sequence is lower than 50 (lower than 50 % of the sequence similarity).
*BLAST against online database www.candidagenome.org
**Partial sequence homologies under 50% were found between primers and SARS-Coronavirus, whereas no homology was found in detection probes. Expected melting temperatures of primer with 50% of homology were 30-40°C however the annealing temperature of this assay is 60°C. Thus, nonspecific amplification of SARS-Coronavirus cannot be occurred. Even if SARS-coronavirus is amplified in non-specific manner the PCR product is not detected with the probes with no sequence similarity.

Cross-Reactivity Wet Testing:

Wet testing against normal and pathogenic organisms of the respiratory tract was performed to confirm the results of the *in silico* analysis. Each organism (cultured isolates or inactivated strains) identified in the Table 9 was tested using three extraction replicates with the U-TOP™ COVID-19 Detection Kit at concentrations of 10^6 CFU/mL or higher for bacteria and 10^5 pfu/mL or higher for viruses. No detectable amplification curve (Ct) was observed for the ORF1ab and N targets. As expected, the internal control did show 100% detection for all three tested replicates for all organisms evaluated for potential cross-reactivity.

Table 9. Cross-Reactivity Wet Testing Analysis

Microorganism	Source	% Detection (#detected / #tested)		
		ORF1ab	N	IC
Human coronavirus 229E	KBPV[a] VR-9	0% (0/3)	0% (0/3)	100% (3/3)
Human coronavirus OC43	KBPV VR-8	0% (0/3)	0% (0/3)	100% (3/3)
Human coronavirus HKU1	ATCCVR-3262SD*	0% (0/3)	0% (0/3)	100% (3/3)
Human coronavirus NL63	NCCP 43214	0% (0/3)	0% (0/3)	100% (3/3)
SARS-coronavirus	Clinical isolate[b]	0% (0/3)	0% (0/3)	100% (3/3)
MERS-coronavirus	Clinical isolate[b]	0% (0/3)	0% (0/3)	100% (3/3)
Adenovirus type 1	KBPV VR-1	0% (0/3)	0% (0/3)	100% (3/3)
Adenovirus type 2	KBPV VR-58	0% (0/3)	0% (0/3)	100% (3/3)
Adenovirus type 3	KBPV VR-2	0% (0/3)	0% (0/3)	100% (3/3)
Human Metapneumovirus	KBPV VR-86	0% (0/3)	0% (0/3)	100% (3/3)
Parainfluenza virus 1	KBPV VR-44	0% (0/3)	0% (0/3)	100% (3/3)
Parainfluenza virus 2	KBPV VR-45	0% (0/3)	0% (0/3)	100% (3/3)
Parainfluenza virus 3	KBPV VR-46	0% (0/3)	0% (0/3)	100% (3/3)
Parainfluenza virus 4	KBPV VR-69	0% (0/3)	0% (0/3)	100% (3/3)
Influenza A (H3N2)	KBPV VR-32	0% (0/3)	0% (0/3)	100% (3/3)
Influenza A (H1N1)	KBPV VR-33	0% (0/3)	0% (0/3)	100% (3/3)
Influenza B	KBPV VR-34	0% (0/3)	0% (0/3)	100% (3/3)
Enterovirus	KBPV VR-12	0% (0/3)	0% (0/3)	100% (3/3)
Respiratory syncytial virus	KBPV VR-48	0% (0/3)	0% (0/3)	100% (3/3)
Rhinovirus 1	KBPV VR-1	0% (0/3)	0% (0/3)	100% (3/3)
Rhinovirus 14	KBPV VR-39	0% (0/3)	0% (0/3)	100% (3/3)
Rhinovirus 7	KBPV VR-82	0% (0/3)	0% (0/3)	100% (3/3)
Chlamydia pneumoniae	ATCC 53592	0% (0/3)	0% (0/3)	100% (3/3)
Haemophilus influenzae	CCARM 9257	0% (0/3)	0% (0/3)	100% (3/3)
Legionella pneumophila	CCARM 19001	0% (0/3)	0% (0/3)	100% (3/3)
Mycobacterium tuberculosis	NCCP15972	0% (0/3)	0% (0/3)	100% (3/3)
Streptococcus pneumoniae	CCARM 4157	0% (0/3)	0% (0/3)	100% (3/3)
Streptococcus pyogenes	CCARM 4528	0% (0/3)	0% (0/3)	100% (3/3)
Bordetella pertussis	NCCP 13671	0% (0/3)	0% (0/3)	100% (3/3)
Mycoplasma pneumoniae	ATCC 29342	0% (0/3)	0% (0/3)	100% (3/3)
Pneumocystis jirovecii (PJP)	Lab culture	0% (0/3)	0% (0/3)	100% (3/3)
Candida albicans	CCARM 14004	0% (0/3)	0% (0/3)	100% (3/3)
Pseudomonas aeruginosa	CCARM 0220	0% (0/3)	0% (0/3)	100% (3/3)
Staphylococcus epidermis	CCARM 3711	0% (0/3)	0% (0/3)	100% (3/3)
Streptococcus salivarius	NCCP 14735	0% (0/3)	0% (0/3)	100% (3/3)
Staphylococcus aureus	NCCP 15920	0% (0/3)	0% (0/3)	100% (3/3)
Nasal wash	-	0% (0/3)	0% (0/3)	100% (3/3)

[a]; KBPV: Korean bank of pathogenic virus (https://www.kbpv.re.kr/index.php)
[b]; Clinical isolate, Culture: Clinical isolates in department of diagnostics, Hospital of Chungnam University, Korea.
*human coronavirus HKU1 was tested using the spike with isolated nucleic acid at concentration of 5×10^5 copies/ml.

12.3 Interfering substances study

Interfering substances studies were performed using nasopharyngeal swab specimens collected from healthy individuals spiked with or without SARS-CoV-2 genomic RNA at the concentration of 5xLoD. The samples were previously confirmed to be negative using the U-Top™ COVID-19 test. The interfering substances indicated in table 10 were added to the positive or negative contrived samples at the indicated concentrations, and the samples were processed using three extraction methods shown in table 10 following IFU supplied with the products. Each substance was tested at the highest medically relevant concentration in three replicates for both positive and negative contrived samples. Results indicate that all three extraction methods and the U-TOP COVID-19 test can well tolerate all the substances at the concentration equal or lower than the indicated values without significant interference. The study results are summarized in table 10.

Table 10. Interference studies

Interfering substances	Conc.	Detection % (#Detected / #tested)																	
		QIAamp DSP virus kit						TOP virus kit						PANAMAX					
		Positive			Negative			Positive			Negative			Positive			Negative		
		ORF1ab	N	IC	ORF1ab	N	IC	ORF1ab	N	IC	ORF1ab	N	IC	ORF1ab	N	IC	ORF1ab	N	IC
Tobramycin	5 ug/ml	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)
Mucin	2.5 mg/ml	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)
Whole blood	2.5% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)
Fluticasone	5% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)
Mupirocin	5 mg/ml	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)
Nasal gel (Centella asiatica, Neomycin, Hydrocortisone)	5 mg/ml	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)
Nasal drop (Oxymetazoline)	10% (v/v)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)
Oseltamir (Tamiflu)	2.5 ug/ml	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)
Cepacol Sore Throat (Benzocaine/Menthol lozenges)	5 mg/ml	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)
Zanamir	3 mg/ml	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	0% (0/3)	0% (0/3)	100% (3/3)

12.4 Clinical Evaluation

Performance of the U-TOP™ COVID-19 Detection Kit was evaluated using contrived clinical nasopharyngeal swab and sputum specimens. A total of 60 contrived positive specimens (30 contrived positive nasopharyngeal swab specimens and 30 contrived positive sputum specimens) and 60 negative specimens were tested (30 negative nasopharyngeal swab and 30 negative sputum specimens). Leftover individual unique clinical nasopharyngeal swab and sputum matrices were determined to be negative using FDA-authorized comparator real-time RT-PCR assay and the U-TOP™ COVID-19 Detection Kit prior to spiking in the RNA.
SARS-CoV-2 viral genomic RNA (NCCP No. 43326. National Culture Collection for Pathogens) was spiked into 30 negative nasopharyngeal swab matrices and 30 negative sputum matrices at various concentrations relative to the assay's LoD. Of the 30 contrived nasopharyngeal swab positive samples, 20 were spiked at concentrations equivalent to 2X the LoD (2 copies/µL), and 10 were spiked with concentrations equivalent to 3X the LoD (3 copies/µL). Of the 30 contrived sputum positive samples, 20 were spiked at 2X LoD (2 copies/µL) and 10 were spiked with concentrations of 3X LoD (3 copies/µL). The remaining 30 nasopharyngeal swabs and 30 sputum samples were tested as negative clinical samples.

Prepared samples were randomized and blinded, and RNA was extracted using the QIAamp DSP Virus Kit. Testing was performed in a total of two RT-PCR runs with one positive and one negative control included per run. All negative samples were non-reactive and positive spiked samples at 2X and 3X LoD for both nasopharyngeal swabs and sputum showed 100% detection. Results of the study are summarized in Table 11.

Table 11. Clinical Evaluation with Contrived Nasopharyngeal Swab and Sputum Specimens

Specimen Type	SARS-CoV-2 concentration	# of samples	Detection rate		Mean Ct		
			ORF1ab	N	ORF1ab	N	IC (RNaseP)
Nasopharyngeal swab	2xLoD	20	20/20	20/20	36.1	36.2	30.6
	3xLoD	10	10/10	10/10	35.8	35.7	31.0
	Negative	30	0/30	0/30	-	-	30.7
Sputum	2xLoD	20	20/20	20/20	36.2	36.0	30.6
	3xLoD	10	10/10	10/10	35.6	36.2	30.7
	Negative	30	0/30	0/30	-	-	30.6

An additional study was performed to evaluate the performance of the U-TOP™ COVID-19 Detection Kit testing individual, leftover, de-identified nasopharyngeal swab and sputum clinical specimens. RNA was extracted from randomized and blinded clinical specimens using the QIAmp DSP virus kit according to the instructions supplied with the kit. A total of 35 clinical positive specimens including 20 nasopharyngeal swab, 15 sputum samples, and 40 clinical negative specimens including 25 nasopharyngeal swab, 15 sputum samples were analyzed on CFX 96 Real time PCR system by U-TOP™ COVID-19 Detection Kit. Specimens were previously tested using an FDA-authorized comparator real-time RT-PCR assay.

Both positive percent agreement (PPA) and negative percent agreement (NPA) between the 2 assays for both specimen types were 100%. The results are summarized in Table 12 and 13.

Table 12. Clinical performance of nasopharyngeal swab specimens.

Nasopharyngeal Swabs		Comparator Assay		
		Positive	Negative	Total
U-TOP™ COVID-19 Detection Kit	Positive	20	0	20
	Negative	0	25	25
	Total	20	25	45
Positive Agreement		100.0% (20/20); 95% CI: 83.89% - 100.00%		
Negative Agreement		100.0% (25/25); 95% CI: 86.68% - 100.00%		

Table 13. Clinical performance of sputum specimens.

Sputum specimens		Comparator Assay		
		Positive	Negative	Total
U-TOP™ COVID-19 Detection Kit	Positive	15	0	15
	Negative	0	15	15
	Total	15	15	30
Positive Agreement		100.0% (15/15); 95% CI: 79.62% - 100.00%		
Negative Agreement		100.0% (15/15); 95% CI: 79.62% - 100.00%		

Appendix A. 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 corroborate the LoD. The extraction method and instrument used were QIAamp DSP virus kit (Qiagen, Cat No.60704) and CFX 96 Real-Time PCR Detection System. The results are summarized in Table 14.

Table 14. 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	NP Swab	$0.6x10^3$ NDU/mL	N/A
MERS-CoV		N/A	ND

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

13. Trouble shooting

Problem	Cause	Solution
Fluorescence signal is not detected in all samples	Error of the PCR reaction	Review if any reagent was missed during the preparation process
	If the storage conditions of the kit are not appropriate, or the expiration date has expired	Repeat the test after checking the storage conditions and expiration date
Fluorescent signal is low in all samples	If the PCR reagents were not mixed correctly	Proceed with the test after review of PCR mix
	Long storage at room temperature or light exposure	Dispose the kit.
	If the expiration date has passed	Check the expiration date of the kit
Signal detection in Negative Control	If the PCR mixture or Negative control are contaminated	Discard and use new
	If the experiment place or the tool is contaminated	Check whether the test site or tool is contaminated. Repeat the experiment with new aliquots of all reagents
If there are different results in the same sample	Pipetting error	Check the pipette
	Cross contamination	Be careful with DNA splitting and repeat the test
	Contaminated 96-well plate	Test with a new 96-well plate
- SEASUN BIOMATERIALS Inc. guarantees all its products before the expiration date - Contact our A/S team if a problem not mentioned in this table has occurred		

14. Reference

1. Victor M Corman et al., Diagnostic detection of SARS-CoV-2 by real-time RT-PCR. Euro Surveill 2020. 25(3): 2000045
2. Leo Poon et al., Detection of 2019 novel coronavirus (SARS-CoV-2) in suspected human cases by RT-PCR. www.who.int/docs/default-source/coronaviruse/peiris-protocol-16-1-20.pdf.
3. Mary Johnson. Wuhan 2019 Novel Coronavirus – 2019–nCoV. MATER METHODS. 2020. 10:2867
4. Zheng-Li Shi et al., Discovery of a novel coronavirus associated with the recent pneumonia outbreak in 2 humans and its potential bat origin. Nature. 2020 579(7798): 270-273
5. Naganori Nao et alk., Detection of second case of SARS-CoV-2 infection in Japan. NIID. 2020.
6. 2019-Novel coronavirus (SARS-CoV-2) real-time rRT-PCR panel primers and probes. 2020. US Centers for Disease Control and Prevention.
7. SARS-CoV-2 detection real-time RT-PCR protocol. 2020. KCDC v1.5

15. Symbols

REF	Catalogue Number		Expiration Date
	Temperature limitation (Storage temperature)		Manufacturer
IVD	In vitro Diagnostic Medical Device	LOT	Lot number
	Do Not Reuse (For single use only)		

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