CE IVD

AQ-TOP™ COVID-19 Rapid Detection Kit PLUS

For In Vitro Diagnostic Use Only

For Prescription Use Only

For use under Emergency Use Authorization (EUA) Only

A molecular rapid diagnostic kit for detection of SARS-CoV-2 (COVID-19)

Instructions for Use | V2.0

Store at –20°C

Date of Revision: Sep-2020

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:	AQ-TOP™ COVID-19 Rapid Detection Kit PLUS
3. Product Catalogue Number:	SS-9940
4. Purpose of use:	See 1. in this User Guide

Warnings and Precautions

Contact us for detailed information for the safe use of the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS. 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 ISO13485, ISO9001, KGMP.

Inquiries and customer service (A/S)

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

CONTENTS

1. Intended Use

The AQ-TOP™ COVID-19 Rapid Detection Kit PLUS is a real-time reverse transcription loop mediated isothermal amplification (RT-LAMP) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens including oropharyngeal and nasopharyngeal swabs, anterior nasal and mid-turbinate nasal swabs, nasopharyngeal washes/aspirates or nasal aspirates as well as bronchoalveolar lavage (BAL) 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, that meet requirements 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 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 AQ-TOP™ COVID-19 Rapid Detection Kit PLUS is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR/ LAMP and *in vitro* diagnostic procedures. The AQ-TOP™ COVID-19 Rapid Detection Kit PLUS is intended for use only under the Food and Drug Administration’s Emergency Use Authorization.

2. Product Description

The AQ-TOP™ COVID-19 Rapid Detection Kit PLUS uses dual-labeled Peptide Nucleic Acid (PNA) probes that target ORF1ab and N gene for detection of SARS-CoV-2 RNA with FAM in two separate wells, and human RNase P for the internal control (IC) with HEX

fluorescence channel in both two wells. Both reverse transcription and LAMP reactions take place at 60°C using the enzyme mixture of reverse transcriptase and Bst DNA polymerase. During the amplification, fluorescence resonance energy transfer (FRET) probes are incorporated in the amplification products. Upon incorporation, fluorescence is generated and can be monitored by the fluorescence reader on the CFX 96 and ABI 7500 real-time PCR platforms in a real time fashion.

In addition, the kit utilizes external Positive (PC) and Negative (NC) controls. The PC contains template specific for the SARS-CoV-2 ORF1ab and N genes as well as the human RNase P gene. The NC contains RNase/DNase free distilled water.

3. Kit Components and Packaging Specifications

The AQ-TOP™ COVID-19 Rapid Detection Kit PLUS is composed of 2X Reaction Buffer, Enzyme Mix, Reaction Mix 1 and 2, Positive Control and Negative Control.

Table 1. Kit components and the characteristics

	Reagent label	Part #	Descriptions/Contents	Volume / Quantity	Store at
1	2X Reaction Buffer	SS-9940CVPB	PCR buffer	1500㎕/2 tubes	-20°C
2	Enzyme Mix	SS-9940CVPE	Reverse transcriptase, Bst DNA polymerase	200㎕/1 tube	
3	Reaction Mix 1	SS-9940CVM1	Primer, probe mixture for ORF1ab and RNase P	400㎕/1 tube	
4	Reaction Mix 2	SS-9940CVM2	Primer, probe mixture for N gene and RNase P	400㎕/1 tube	
5	Positive Control	SS-9940CVPP	Templates for SARS-CoV-2 and RNase P	200㎕/1 tube	
6	Negative Control	SS-9940CVPN	Nuclease free DW	200㎕/1 tube	

Quality Controls

Negative Control (NC): contains nuclease-free water intended to evaluate cross contamination of the kit reagents and PCR instrument used in the test. The NC must be run using 10 µL for each Reaction mix 1 and 2 in a total of two wells per test directly without dilution or rehydration.

Positive Control (PC): contains DNA plasmids with inserts of the SARS-CoV-2 specific ORF1ab and N templates as well as the human RNase P that are intended to evaluate thermocycler malfunction, enzyme activity, as well as analytical and clinical performance of the kit. The PC must be run using 10 µL for each Reaction mix 1 and 2 in a total of two wells per test directly without dilution or rehydration.

Internal Control (IC): Both Reaction Mix 1 and 2 tubes consist 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 performance of the thermocycler.

***Negative Extraction Control (NEC)**: It is also highly recommended to run a Negative Control through the nucleic acid extraction procedure to monitor for potential contamination that could occur during processing. Any molecular grade nuclease-free water (e.g., Thermo Fisher Scientific, Cat # 10977015) can be purchased and function as the negative extraction control.

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 the whole test procedure.
Place Enzyme Mix on ice during the whole test procedure.
Avoid excessive freeze/thaw cycles.
Vortex and spin down the reagents briefly before each use.

5. Additional Materials and Equipment

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. # SS-1200) for collection and transport of swab specimens
   B. Sterile Collection Container (BD, Cat. # 9004-118) for collection and transport of

washes/aspirates/BALs

2. RNA extraction kit for extracting RNA from clinical specimens
   A. TOP Viral DNA/RNA Extraction Kit (Seasun Biomaterials, Cat. # SS-1300)
   B. PANAMAX 48 Nucleic acid extraction system (Software version: panaMAX 01.00.03) with PANAMAX Viral DNA/RNA Extraction Kit (Panagene, Cat. # PNAK-1001, PNAK-1002)
   C. QIAamp DSP Virus Kit (Qiagen, Cat. # 60704)

3. Real-time PCR system and the consumables
   A. CFX 96 real-time PCR detection system with software CFX manager V3.1
   B. Applied Biosystems real-time PCR system 7500 with Software 2.0.6

- 96 well white PCR plate (Bio-Rad, Cat. # MLL9651) or 96-well 0.2 mL PCR reaction plate (Applied Biosystems, Cat. # AB0900W or equivalent)
- Sealing Film (Bio-Rad, Cat. # MSB 1001), 8 or 12 well PCR plate cap (Seasun Biomaterials, Cat. # SS-1103 and SS-1104)
- 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

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 Federal Food, Drug and Cosmetic 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 the external environment clean; always use in a clean workplace/bench.
- 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 clinical specimens for testing: Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (SARS-CoV-2) (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html). Follow specimen collection device manufacturer instructions for proper collection methods. Specimens should be collected using only the products validated with this kit (See Section 5. Additional Materials and Reagents). Store the samples at 2-8°C up to 72 hours if necessary. If a delay in shipping or extraction is expected to exceed 72 hours, 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 packs. 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) If the specimens have insufficient volume for the test (less than 1 mL); 3) If the label is damaged (cannot be read or recognized) or if the specimens are received without labeling/identifying documents.

8. Test Procedure

8.1 Test workflow

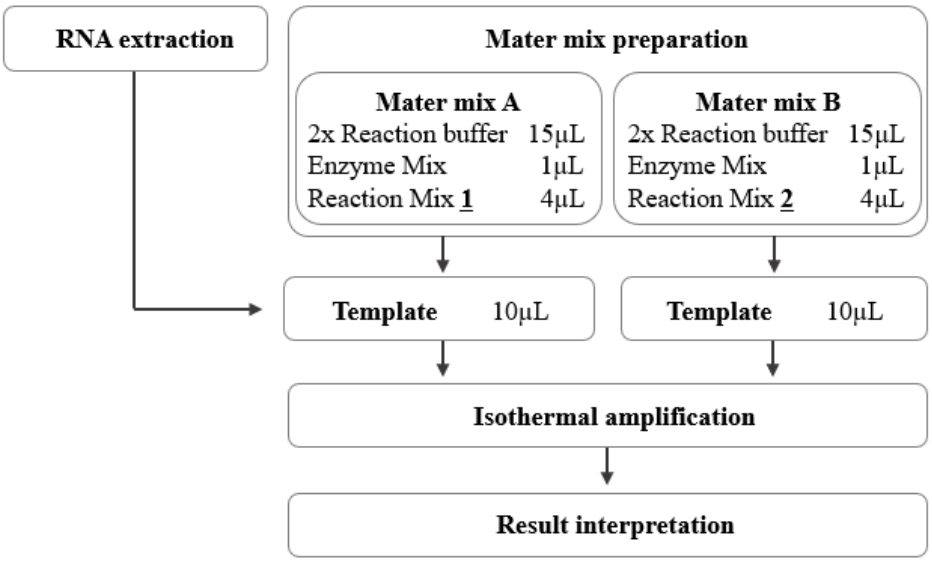


Figure 1. Workflow of AQ-TOP™ COVID-19 Rapid Detection Kit PLUS

8.2 RNA extraction from clinical specimens

The AQ-TOP™ COVID-19 Rapid Detection Kit PLUS does not include viral RNA extraction reagents. The following extraction kits have been validated with the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS:

- A silica magnetic bead based automated nucleic acid extraction system, PANAMAX48 with the PANAMAX Viral DNA/RNA extraction kit (Panagene, Cat. # PNAK-1001, PNAK-1002)
- Spin column based nucleic acid extraction reagent, QIAamp DSP Virus Kit (Qiagen, Cat. # 60704)
- Spin column based nucleic acid extraction reagent, TOP Viral DNA/RNA extraction kit (Seasun Biomaterials, Cat. # SS-1300)

All three extraction kits require 300 µL of clinical sample input 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 Master mix preparation and Assay set up

Note: *Two separate master mixes, A and B, must be prepared using the Reaction mix 1 and 2, respectively for testing each clinical sample. Negative and Positive controls must be tested for each master mix, A and B.*

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 the enzyme mix on ice during the whole test procedure. 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 briefly before each use.
4. Determine the number of reactions to set up for the assay. Be sure to make excess reaction mix for the positive and negative controls and for possible pipetting error.
5. Prepare the two individual master mixes: A and B in 1.5 mL microcentrifuge tubes according to Table 2. It is recommended to prepare 110% of the calculated amount of each master mix to account for pipetting carryover.

Table 2. Master Mix Preparation for Testing of Each Clinical Specimen

Mater mixture A	
Reagents	**Volume (µL)**
2X Reaction Buffer	15
Enzyme Mix	1
Reaction Mix **1**	4
Total (w/o template)	**20**

Master mixture B	
Reagents	**Volume (µL)**
2X Reaction Buffer	15
Enzyme Mix	1
Reaction Mix **2**	4
Total (w/o template)	**20**

6. Vortex each 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, and dispense 20 µL of master mixture into the wells of 96-well plate according to the Figure 2.
8. Pipette **10 µL** of NC into the 2 designated NC wells (10 µL for master mix A well and 10 µL for master mix B well). The NC should be loaded first and side-by-side for master mix A and B as displayed in Figure 2.
9. If incorporating the NEC, pipette **10 µL** into the 2 designated NEC wells. One NEC from each extraction batch should be run for each Reaction mix.

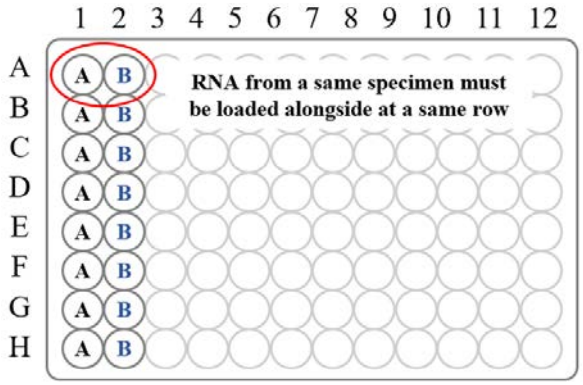


Figure 2. Master mix A should be loaded at wells in odd columns (1,3,5,7,9,11), and Master mix B should be loaded at wells in even columns (2,4,6,8,10,12)

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 to the PCR plate last, to avoid possible contamination. The Positive Control contains a high concentration of viral template (5X assay LoD). Change gloves often to avoid cross contamination between samples and control reagents*

1. Gently vortex clinical RNA extraction tubes for approximately 5 sec and spin down 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 A and B (10 µL for master mix A well and 10 µL for master mix B well). **RNA extracted from one clinical specimen must be loaded side-by-side for master mix A and B as shown in Figure 2.**

3. After the loading all clinical specimens, carefully pipette **10 µL** of PC into designated PC wells (10 µL for master mix A well and 10 µL for master mix B well). PC must be loaded last and side-by-side for master mix A and B.
4. Seal the PCR plate with a 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 collect 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 LAMP run

AQ-TOP™ COVID-19 Rapid Detection Kit PLUS run protocol is slightly different for the CFX 96 and ABI 7500 real-time PCR detection systems. The run protocol and fluorescence channels for the targets are shown in Tables 3 and 4.

Table 3. RT-LAMP Conditions

Instrument	Temp	Time	Repeat
CFX 96	60℃	50 sec	30*
ABI 7500		60 sec	

* Collect fluorescence signal in each repeat

Table 4. Fluorescence Channel for Probes

Fluorescence	Target
FAM	ORF1ab and N
HEX/VIC or JOE*	Internal control RNase P

* HEX for CFX 96; VIC or JOE for ABI 7500 system

CFX 96 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 CFX 96. 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 and HEX.

④ Specify the positive control wells (2 wells: one for master mix A and one for master mix B), select “Positive Control” from “Sample type”, and load the fluorophores.

⑤ Specify the negative control wells (2 wells: one for master mix A and one for master mix B), select “Negative Control” from “Sample type”, and load the fluorophores.

⑥ Wells with clinical specimens should be specified as Unknown, and load the fluorophores.

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

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

CFX 96 and Software Operation - 2 (Pre-Programmed Run Settings)

① If you have a previous run file, you can re-use the programmed conditions for additional runs. 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 (96 well) > 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 reporters as shown below:

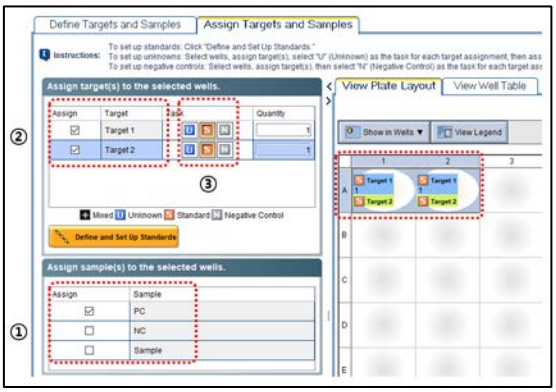
Target 1; Reporter FAM; Quencher NFQ-MGB

Target 2; Reporter VIC (or JOE); Quencher NFQ-MGB

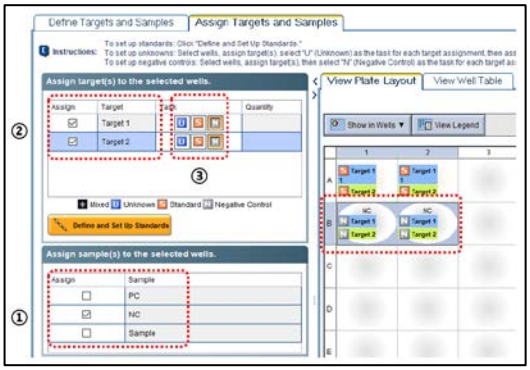
⑤ Go to Define Samples > Add New Sample > Input PC, NC and Sample (Test Specimen).

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

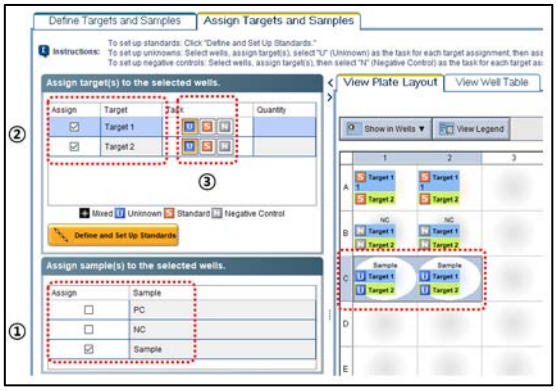
1. Positive Controls (2 wells for one run): Click Positive Control Wells from “View Plate Layout”. Select “PC“ from ① (shown in figure below) and activate both targets from ② (shown in figure below) then activate ”**S**“ for both targets from ③ (shown in figure below).



2. Negative Controls (2 wells for one run): Click Negative Control Well from “View Plate Layout”. Select “NC“ from ① (shown in figure below) and activate both targets from ② (shown in figure below) then activate ”**N**“ for both targets from ③ (shown in figure below)



3. Sample (2 wells for each specimen): Click Wells with test samples from “View Plate Layout”. Select “Sample “from ① (shown in figure below) and activate both 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 conditions 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.

ABI 7500 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.
Input the sample information in the “Plate setup” and proceed in the same order as above.

9. Result Interpretation

9.1 Base line and threshold setting

The AQ-TOP™ COVID-19 Rapid Detection Kit PLUS has been validated using the baseline threshold setting which is automatically adjusted by both the CFX 96 and ABI 7500 Real-Time PCR instruments.

9.2 Interpretation of Quality control

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

Negative Control (NC): Both NC reactions should not exhibit any fluorescence growth curves (Ct) that cross the threshold line ≤ 30. If any NC shows amplification of ORF1ab/N/RNase P, invalidate the run and repeat testing using the same NC and residual nucleic acid from clinical samples. If the NC continues to show a Ct of ≤ 30 for any assay target, discard the kit’s NC tube and use a new negative control or DNase/RNase free distilled water (Thermo Fisher Scientific, Cat. # 10977015). If the new NC continues to show amplification, discard the entire kit and contact the local distributor or the manufacturer.

Positive Control (PC): Both PC reactions must yield positive results with all FAM and HEX or VIC/JOE fluorescence channels. If the PC reactions do not exhibit positive amplification for the ORF1ab/N/RNase P targets, PCR inhibition or thermocycler/kit malfunction may have

occurred. Invalidate the run and repeat the assay using the same PC and residual nucleic acid from clinical samples. If the PC continues to not exhibit ORF1ab/N/RNase P detection, discard the kit's PC tube and contact local distributor or the manufacturer.

Negative Extraction Control (NEC): Both NEC reactions should not exhibit any fluorescence growth curves (Ct) that cross the threshold line ≤ 30. If the NEC shows amplification of ORF1ab/N/RNase P, contamination may have occurred during the nucleic acid extraction process or with the reagents of the extraction kit. Invalidate the run and re-extract RNA from residual clinical specimens using the same NEC. If the NEC continues to show a Ct of ≤ 30 for any assay target, discard the NEC and use a new DNase/RNase free distilled water (e.g., Thermo Fisher Scientific, Cat # 10977015).

Internal Control (IC): Failure to detect RNase P in any clinical specimen 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.

The controls must meet the requirements listed in Table 5 to ensure valid results.

Table 5. Interpretation of Results for Quality Controls

Control	Ct value (Master mix A)		Ct value (Master mix B)	
	ORF1ab (FAM)	IC (HEX or VIC/JOE)	N (FAM)	IC (HEX or VIC/JOE)
Negative	ND	ND	ND	ND
Positive	≤ 30	≤ 30	≤ 30	≤ 30
NEC*	ND	ND	ND	ND

ND= Not detectable (no Ct value)

*Negative Extraction Control - Optional but highly recommended

Interpretation of Clinical Samples

If the control results are valid, refer to Table 6 to interpret the results of clinical samples.

Table 6. Clinical Sample Results Interpretation

Ct values (Master mix A)		Ct values (Master mix B)		Interpretation	Action
ORF1ab (FAM)	IC (HEX or VIC/JOE)	N (FAM)	IC (HEX or VIC/JOE)		
ND	≤ 30	ND	≤ 30	Negative	Report results. Consider testing for other viruses that may cause similar symptoms.
≤ 30	/	/	/	Positive*	Report results.
/	/	≤ 30	/		
≤ 30	/	≤ 30	/	Positive	
ND	ND	ND	ND	Invalid**	- Repeat test with left-over RNA extract. - If result of the left-over RNA extract remains invalid, repeat the extraction procedure with left-over clinical specimen and repeat the test. - If result of the new RNA extract remains invalid after re-test, report the results as invalid and re-collect patient sample.
ND	≤ 30	ND	ND		
ND	ND	ND	≤ 30		

ND = Not detectable (no Ct value)

/ = No requirement of Ct value. If at least one SARS-CoV-2 target has Ct value of ≤30, Ct values for the IC and the remaining SARS-CoV-2 target are not required to be considered positive.

*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

** IC signals for master mix A and B must cross the threshold line together at or before cycle 30 to validate the run and to indicate that proper RNA extraction has occurred in the case of negative samples.

10. Limitations

- This assay is for in vitro diagnostic use under FDA Emergency Use Authorization only. 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 the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS has only been established with nasopharyngeal swab samples. Oropharyngeal, mid-turbinate and anterior nares swabs, nasopharyngeal washes/aspirates or nasal aspirates as well as bronchoalveolar lavage specimens are also considered acceptable specimen types for use but performance has not been established.
- 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 has only been validated with the CFX 96 Real-Time PCR Detection system and Applied Biosystems 7500 (ABI 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.

11. Conditions of Authorization for the Laboratory

The AQ-TOP™ COVID-19 Rapid Detection Kit PLUS assay's Letter of Authorization, User Manual, and Labeling are available on FDA website:

https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas

To assist clinical laboratories using the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS, the

relevant Conditions of Authorization are listed below.

a) Authorized laboratories[1] using the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS 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 the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS will use your product as outlined in the authorized labeling. 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 the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

d) Authorized laboratories using the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS 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 the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS 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 the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS must be appropriately trained in molecular 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 the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS 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."

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 yielded at least a 95% positivity rate with the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS. A preliminary LoD was determined using whole viral genomic RNA (NCCP No. 43326. National Culture Collection for Pathogens) spiked into pooled clinical negative nasopharyngeal swab (NP) matrix. In the first part of the study, 10-fold dilutions of known concentrations of whole viral genomic SARS-CoV-2 RNA were prepared and processed using the Qiagen QIAamp DSP Virus Kit and run on the CFX 96 Real-Time PCR detection system. Three PCR replicates per concentration were tested, and results are summarized in Table 7.

Table 7. Summary of Preliminary LoD Testing Data Using the QIAamp DSP Virus Kit

LoD for ORF1ab target

	10,000 copies/µL		1,000 copies/µL		100 copies/µL		10 copies/µL		**1 copy/µL**		0.1 copies/µL	
	Orf1ab	RNase P	Orf1ab	RNase P	Orf1ab	RNase P	Orf1ab	RNase P	**Orf1ab**	**RNase P**	Orf1ab	RNase P
Detection rate	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	**3/3**	**3/3**	1/3	3/3
Mean Ct	13.23	23.23	16.32	23.69	20.35	25.00	23.46	22.93	**27.32**	**24.07**	-	23.40
SD	0.16	0.49	0.13	0.97	0.16	1.07	0.13	0.43	**0.15**	**0.37**	-	0.51

LoD for N gene target

	10,000 copies/µL		1,000 copies/µL		100 copies/µL		10 copies/µL		**1 copy/µL**		0.1 copies/µL	
	N	RNase P	N	RNase P	N	RNase P	N	RNase P	**N**	**RNase P**	N	RNase P
Detection rate	3/3	3/3	3/3	3/3	3/3	3/3	3/3	3/3	**3/3**	**3/3**	1/3	3/3
Mean Ct	14.29	23.57	17.22	24.07	20.27	25.34	24.43	23.74	**27.20**	**24.22**	-	24.04
SD	0.19	0.69	0.21	0.36	0.21	1.51	0.12	0.40	**0.08**	**0.17**	-	0.62

Based on these results, additional 3-fold dilutions of SARS-CoV-2 genomic RNA were prepared in negative NP swab matrix, and processed using the PANAMAX Viral DNA/RNA Extraction Kit, TOP™ Viral DNA/RNA Extraction Kit and QIAamp DSP Virus Kit with 20 independent extraction replicates. RNA was tested on both the CFX 96 and ABI 7500 Real-Time PCR systems. The lowest target level at which at least 95% of 20 replicates produced

positive results was 1 copy/µL for all 3 validated extraction methods on both PCR platforms. Result are summarized in Table 8.

Table 8. Summary of LoD Confirmation Studies Using All 3 Extraction Methods

		# Detected / # Tested							
		CFX 96				ABI 7500			
		Orf1ab	RNase P	N	RNase P	Orf1ab	RNase P	N	RNase P
TOP Virus kit	1 copy/µL	20/20	20/20	20/20	20/20	20/20	20/20	20/20	20/20
	0.3 copies/µL	14/20	20/20	11/20	20/20	16/20	20/20	11/20	20/20
	0.1 copies/µL	8/20	20/20	7/20	20/20	7/20	20/20	6/20	20/20
PANAMAX	1 copy/µL	20/20	20/20	20/20	20/20	20/20	20/20	20/20	20/20
	0.3 copies/µL	15/20	20/20	12/20	20/20	16/20	20/20	15/20	20/20
	0.1 copies/µL	7/20	20/20	6/20	20/20	9/20	20/20	6/20	20/20
QIAamp DSP	1 copy/µL	20/20	20/20	20/20	20/20	20/20	20/20	20/20	20/20
	0.3 copies/µL	11/20	20/20	10/20	20/20	15/20	20/20	13/20	20/20
	0.1 copies/µL	6/20	20/20	6/20	20/20	8/20	20/20	5/20	20/20

12.2 Inclusivity (Analytical Reactivity)

Analytical reactivity (inclusivity) of the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS was evaluated in September 2020 using a total of 7313 publicly available SARS-CoV-2 whole genome sequences that were downloaded from the following databases

- 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/)
- NMDC (https://microbiomedata.org/)

Analysis was performed using the assay's primer and probe sequences against the downloaded SARS-CoV-2 sequences on <Find binding sites and create fragment> tool of CLC main workbench 20.0.3 software. All alignments of the primer and probe sets against the SARS-CoV-2 sequences showed 100% identity (absence of mismatch base against the SARS-CoV-2 target). In summary, *in silico* analysis predicted that the assay can detect all SARS-CoV-2 strains analyzed in this study.

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 AQ-TOP™ COVID-19 Rapid Detection Kit PLUS primers and probes 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.

All primer and probe sequences targeting the N gene of SARS-CoV-2 showed less than 40% sequence similarity to the target sequences. However, some of the ORF1ab primers showed high homologies to specific microorganisms: SARS-coronavirus, *Haemophilus influenzae, Legionella pneumophila, Streptococcus pneumoniae, Streptococcus pyogenes, Mycoplasma pneumoniae, Staphylococcus epidermidis, Streptococcus salivarius* and *Staphylococcus aureus.* Since the amplification and detection of RT-LAMP requires simultaneous binding of 6 primers and a detection probe to the target nucleic acid, it is not expected that these microorganisms will be amplified or produce cross-reactive signal because simultaneous homologies were only exhibited to 4 primers at most. Results of the *in silico* analysis are summarized in Table 9 and 10.

Table 9. *In silico* Cross-Reactivity Analysis of ORF1ab Oligonucleotides

Microorganism	Reference No	Primer AB						Probe
		AB3-F3	AB3-B3	AB3-FIP	AB3-BIP	AB3-LPF	AB3-LPB	AB
Human coronavirus 229E	NC_002645.1	/	/	/	/	/	/	/
Human coronavirus OC43	NC_006213.1	/	/	/	/	/	/	/
Human coronavirus HKU1	NC_006577	/	/	/	/	/	/	/
Human coronavirus NL63	NC_005831.2	/	/	/	/	/	/	/
SARS-coronavirus	NC_004718.3	100%	/	/	86%	90%	100%	/
MERS-coronavirus	KJ556336.1	/	/	/	/	/	/	/
Adenovirus type 1	MH183293.1	/	/	/	/	/	/	/
Adenovirus type 2	J01917.1	/	/	/	/	/	/	/
Adenovirus type 3	AY599836.1	/	/	/	/	/	/	/
Human Metapneumovirus	KJ627437.1	/	/	/	/	/	/	/
Parainfluenza virus 1	KX639498.1	/	/	/	/	/	/	/
Parainfluenza virus 2	KM190939.1	/	/	/	/	/	/	/
Parainfluenza virus 3	NC_001796.2	/	/	/	/	/	/	/
Parainfluenza virus 4	JQ241176.1	/	/	/	/	/	/	/

Microorganism	Reference No	Primer AB						Probe
		AB3-F3	AB3-B3	AB3-FIP	AB3-BIP	AB3-LPF	AB3-LPB	AB
Influenza A	GCF_000865085	/	/	/	/	/	/	/
Influenza B	BLee1940	/	/	/	/	/	/	/
Enterovirus	NC_001472.1	/	/	/	/	/	/	/
Respiratory syncytial virus	NC_001803.1	/	/	/	/	/	/	/
Rhinovirus	NC_009996.1	/	/	/	/	/	/	/
Chlamydia pneumoniae	NC_005043.1	/	/	/	/	/	/	/
Haemophilus influenzae	NZ_LN831035.1	/	/	68%	/	/	/	/
Legionella pneumophila	NZ_LR134380.1	78%	/	/	/	/	/	/
Mycobacterium tuberculosis	NC_000962.3	/	/	/	/	/	64%	/
Streptococcus pneumoniae	NZ_LN831051.1	61%	/	/	/	/	/	/
Streptococcus pyogenes	NZ_LN831034.1	/	/	/	/	94%	/	80%
Bordetella pertussis	NC_018518.1	/	/	/	/	/	/	/
Mycoplasma pneumoniae	NZ_CP010546.1	/	/	/	/	/	76%	/
Pneumocystis jirovecii	CAKM01000281	/	/	/	/	/	/	/
Candida albicans	GCA_003454745	/	/	/	/	/	/	/
Pseudomonas aeruginosa	NC_002516.2	/	/	/	/	/	/	/
Staphylococcus epidermidis	NZ_CP035288.1	/	78%	73%	/	70%	82%	/
Streptococcus salivarius	GCF_900636435	/	/	63%	/	/	64%	/
Staphylococcus aureus	BX571856.1	/	89%	/	/	/	76%	/

/ = no alignment found

Table 10. *In-silico* Cross-Reactivity Analysis of N Gene Oligonucleotides

Microorganism	Reference No	Primer N						Probe
		N4-F3	N4-B3	N4-FIP	N4-BIP	N4-LPF	N4-LPB	N
Human coronavirus 229E	NC_002645.1	/	/	/	/	/	20%	/
Human coronavirus OC43	NC_006213.1	/	/	/	20%	/	/	21%
Human coronavirus HKU1	NC_006577	/	/	/	21%	/	22%	/
Human coronavirus NL63	NC_005831.2	/	/	/	/	21%	/	/
SARS-coronavirus	NC_004718.3	32%	28%	/	37%	21%	34%	/
MERS-coronavirus	KJ556336.1	/	/	/	26%	21%	21%	/
Adenovirus type 1	MH183293.1	/	21%	/	/	/	/	/
Adenovirus type 2	J01917.1	/	/	/	/	/	/	/
Adenovirus type 3	AY599836.1	/	/	/	/	/	/	/
Human Metapneumovirus	KJ627437.1	/	/	/	/	/	/	/
Parainfluenza virus 1	KX639498.1	/	/	/	/	/	/	/
Parainfluenza virus 2	KM190939.1	/	/	/	/	/	/	/
Parainfluenza virus 3	NC_001796.2	/	/	/	/	/	/	/
Parainfluenza virus 4	JQ241176.1	21%	/	/	/	/	/	/
Influenza A	GCF_000865085.1	/	/	/	/	/	/	/
Influenza B	BLee1940	/	/	/	/	/	/	/
Enterovirus	NC_001472.1	/	/	/	/	/	/	/
Respiratory syncytial virus	NC_001803.1	/	/	/	/	/	/	/
Rhinovirus	NC_009996.1	/	/	/	/	/	/	/
Chlamydia pneumoniae	NC_005043.1	23%	23%	/	/	/	/	/
Haemophilus influenzae	NZ_LN831035.1	27%	23%	/	/	/	/	23%
Legionella pneumophila	NZ_LR134380.1	25%	23%	/	/	/	/	20%
Mycobacterium tuberculosis	NC_000962.3	23%	/	/	/	/	/	23%
Streptococcus pneumoniae	NZ_LN831051.1	25%	/	/	/	/	/	26%
Streptococcus pyogenes	NZ_LN831034.1	23%	/	/	/	/	/	26%
Bordetella pertussis	NC_018518.1	25%	/	/	/	/	/	/
Mycoplasma pneumoniae	NZ_CP010546.1	23%	/	/	/	/	/	/
Pneumocystis jirovecii	CAKM01000281.1	/	/	/	/	/	/	/
Candida albicans	GCA_003454745.1	/	/	/	/	/	/	/
Pseudomonas aeruginosa	NC_002516.2	/	/	/	/	/	/	21%
Staphylococcus epidermidis	NZ_CP035288.1	/	/	/	/	/	/	21%
Streptococcus salivarius	GCF_900636435.1	/	/	/	/	/	/	/
Staphylococcus aureus	BX571856.1	/	/	/	/	/	/	/

/ = no alignment found

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 (spiking of cultured isolates or inactivated strains into negative clinical nasopharyngeal specimens) shown in Table 11 was tested using three extraction replicates 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 in the FAM detection channel for SARS-CoV-2 (ORF1ab and N genes) when using the CFX 96 platform. As expected, the internal control in the HEX (VIC or JOE) detection channel did show 100% detection for all three tested replicates for all organisms evaluated for potential cross-reactivity.

Table 11. Cross-Reactivity Wet Testing Analysis

Microorganism	Source	% Detection (#detected / #tested)			
		Reaction mixture A		Reaction mixture B	
		ORF1ab	IC	N	IC
Human coronavirus 229E	KBPV[a] VR-9	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Human coronavirus OC43	KBPV VR-8	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Human coronavirus HKU1	ATCCVR-3262SD[b]	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Human coronavirus NL63	NCCP 43214	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
SARS-coronavirus	Clinical isolate[c]	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
MERS-coronavirus	Clinical isolate	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Adenovirus type 1	KBPV VR-1	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Adenovirus type 2	KBPV VR-58	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Adenovirus type 3	KBPV VR-2	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Human Metapneumovirus	KBPV VR-86	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Parainfluenza virus 1	KBPV VR-44	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Parainfluenza virus 2	KBPV VR-45	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Parainfluenza virus 3	KBPV VR-46	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Parainfluenza virus 4	KBPV VR-69	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Influenza A (H3N2)	KBPV VR-32	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Influenza A (H1N1)	KBPV VR-33	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Influenza B	KBPV VR-34	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Enterovirus	KBPV VR-12	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Respiratory syncytial virus	KBPV VR-48	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Rhinovirus 1	KBPV VR-1	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Rhinovirus 14	KBPV VR-39	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Rhinovirus 7	KBPV VR-82	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Chlamydia pneumoniae	ATCC 53592	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Haemophilus influenzae	CCARM 9257	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Legionella pneumophila	CCARM 19001	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Mycobacterium tuberculosis	NCCP15972	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Streptococcus pneumoniae	CCARM 4157	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Streptococcus pyogenes	CCARM 4528	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)

Microorganism	Source	% Detection (#detected / #tested)			
		Reaction mixture A		Reaction mixture B	
		ORF1ab	IC	N	IC
Bordetella pertussis	NCCP 13671	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Mycoplasma pneumoniae	ATCC 29342	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Pneumocystis jirovecii (PJP)	Lab culture[c]	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Candida albicans	CCARM 14004	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Pseudomonas aeruginosa	CCARM 0220	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Staphylococcus epidermidis	CCARM 3711	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Streptococcus salivarius	NCCP 14735	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Staphylococcus aureus	NCCP 15920	0% (0/3)	100% (3/3)	0% (0/3)	100% (3/3)
Nasal wash	-	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)

[a]; KBPV: Korean bank of pathogenic virus (https://www.kbpv.re.kr/index.php)

[b]; human coronavirus HKU1 was tested using spiked isolated nucleic acid at a concentration of $5x10^5$ copies/mL

[c]; Clinical isolate, Culture: Clinical isolates in Department of Diagnostics, Hospital of Chungnam University, Korea.

12.4 Interfering Substances Study

Interfering substances studies were performed using nasopharyngeal swab specimens collected from healthy (previously confirmed to be negative) individuals spiked with and without SARS-CoV-2 genomic RNA at a concentration of 5X LoD. The interfering substances were added to the positive or negative contrived samples at the indicated concentrations, and the samples were processed using the three validated extraction methods. Each substance was tested at the highest medically relevant concentration in three replicates for both positive and negative contrived samples. Results indicated that all three extraction methods were able to remove these potentially interfering substances and performance of the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS was not impacted. The results are summarized in Table 12.

Table 12. Summary of Interference Studies

			Tobramycin	Mucin	Whole blood	Fluticasone	Mupirocin	Nasal gel (Centella asiatica, Neomycin, Hydrocortisone)	Nasal drop (Oxymetazoline)	Oseltamir (Tamiflu)	Cepacol Sore Throat (Benzocaine/Menthol lozenges)	Zanamir
			5ug/mL	2.5mg/mL	2.5%(v/v)	5%(v/v)	5mg/mL	5mg/mL	10`%(v/v)	2.5ug/mL	5mg/mL	3mg/mL
PANAMAX	Positive	ORF1ab	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
		RNaseP	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
		N	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
		RNaseP	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
	Negative	ORF1ab	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
		RNaseP	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
		N	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
		RNaseP	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
TOP Virus Kit	Positive	ORF1ab	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
		RNaseP	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
		N	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
		RNaseP	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
	Negative	ORF1ab	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
		RNaseP	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
		N	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
		RNaseP	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

QIAamp DSP kit	Positive	ORF1ab	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
		RNaseP	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
		N	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
		RNaseP	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
	Negative	ORF1ab	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
		RNaseP	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)
		N	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)	0% (0/3)
		RNaseP	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)	100% (3/3)

12.5 Clinical Evaluation

A clinical study of the AQ-TOP™ COVID-19 Rapid Detection Kit PLUS was performed that evaluated a total of 85 (35 positive and 50 negative) individual, leftover, de-identified nasopharyngeal swab specimens collected from the hospital of Chungnam National University, Rep. of Korea. Specimens were previously tested using another EUA authorized molecular test. RNA was extracted from 300 µL of each clinical specimen using the PANAMAX48 extraction system according to the instructions supplied by manufacturer and run on the CFX 96 Real-Time PCR system in blinded manner.

Both the positive percent agreement (PPA) and negative percent agreement (NPA) between AQ-TOP™ COVID-19 Rapid Detection Kit PLUS and the EUA authorized molecular comparator assay was 100%. The results are summarized in Table 13.

Table 13. Summary of Clinical Evaluation Results

		EUA Authorized Molecular Comparator Assay		
		Positive	**Negative**	**Total**
AQ-TOP™ COVID-19 Rapid Detection Kit PLUS	**Positive**	35	0	35
	Negative	0	50	50
	Total	35	50	85
Positive Percent Agreement		100% (35/35); 95% CI: 91.00%-100.00%		
Negative Percent Agreement		100% (50/50); 95% CI: 93.89%-100.00%		

13. Troubleshooting

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. References

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)		

SEASUN BIOMATERIALS Inc.

Address N317, 11-3, Techno 1-ro, Yuseong-gu, Daejeon, 34015, Korea
Tel +82-42-716-0301
Fax +82-42-716-0302
US Technical Support **1-800-660-1952**
E-mail as@seasunbio.com / info@seasunbio.com
Web www.seasunbio.com