Allplex™ 2019-nCoV Assay

(Cat no. RP10243X)

Instructions for Use

CFX96™ Real-time PCR Detection System-IVD/CFX96™ Touch Real-Time PCR Detection System ; CFX Manager™ Software V1.6 & V3.1/CFX Maestro™ Software V1

For in vitro diagnostic use

Prescription Use only

Kit for 100 tests

Seegene
V1.4, 12/2021

I. Intended Use

The Allplex™ 2019-nCoV Assay is an in vitro diagnostic (IVD) real-time RTPCR test intended for the presumptive qualitative detection of nucleic acid from the 2019-nCoV in Nasopharyngeal, Oropharyngeal swab, and Sputum from individuals with signs and symptoms of infection who are suspected of COVID-19.

Results are for the identification of 2019-nCoV RNA. The 2019-nCoV RNA is generally detectable in Nasopharyngeal, Oropharyngeal swab, and Sputum during the acute phase of infection. Positive results are indicative of active infection. Laboratories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude 2019-nCoV 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 Allplex™ 2019-nCoV Assay is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

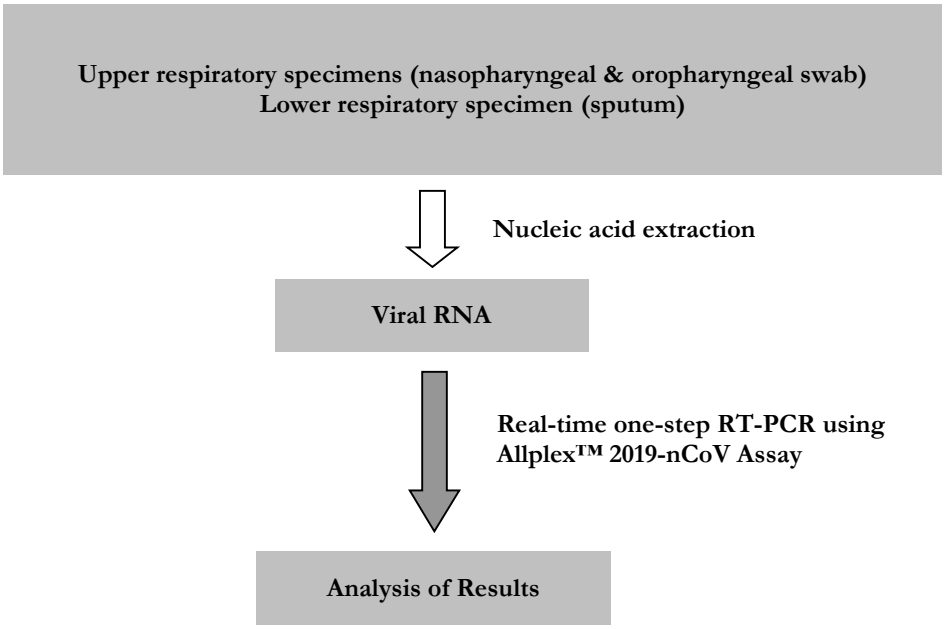
II. Summary and Explanation of the Test

The technology of the Allplex™ 2019-nCoV Assay is a real-time reverse transcription polymerase chain reaction (RT-PCR) test. The 2019-nCoV primer and probe set (s) is designed to detect RNA from the 2019-nCoV in upper respiratory specimens (nasopharyngeal and oropharyngeal swab) and lower respiratory specimen (sputum) from patients who meet CDC 2019-nCoV clinical criteria (e.g. signs and symptoms associated with 2019-nCoV infection) in conjunction with CDC 2019-nCoV epidemiological criteria (e.g., history of residence in or travel to a geographic region with active 2019-nCoV transmission at the time of travel), or other epidemiologic criteria for which 2019-nCoV testing may be indicated.

III. Description of Test Steps

Nucleic acids are isolated and purified from upper respiratory specimens (nasopharyngeal and oropharyngeal swab) and lower respiratory specimen (sputum) using an automated nucleic acid extraction system*. Nucleic acids are isolated from 300 uL of specimens. 10 uL of Internal Control (RP-V IC) must be added before the extraction. Detailed extraction procedures are followed by manufacturer's instruction. 8 uL of purified nucleic acid is reverse transcribed using 5X Real-time One-step Buffer/Real-time One-step Enzyme into cDNA which is then subsequently amplified in a CFX96™ and CFX96™ Touch Real-Time PCR Detection System. During the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5' nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by the CFX96™ and CFX96™ Touch Real-Time PCR Detection System. The result of amplification is reported through 'Seegene

viewer' analysis. The 'Seegene viewer' shows whether the exported data is 2019-nCoV Detected, Presumptive positive, or Negative for easy retrieval of result by the user.



** Automated nucleic acid extraction system:*

System	Applicable extraction kit
Microlab NIMBUS IVD (Hamilton Co.) Microlab STARlet IVD (Hamilton Co.) Seegene NIMBUS (Seegene Inc.) Seegene STARlet (Seegene Inc.)	STARMag 96 X 4 Universal Cartridge Kit (Seegene Inc.)

IV. Allplex™ 2019-nCoV Assay Control Material(s) :

Controls that will be provided with the test kit include:

1) **"No template" or Negative Control (NC)** is used to confirm test validity, and the absence of any contaminants during testing. The "No template" control is prepared using RNase-free Water added to the Master Mix prior to PCR. NC must be included in each test run. No signal should be detected with the NC.
2) **Positive Control (PC)** is used to confirm test validity, and functions as the validation control for PCR amplification and the test detection steps. The PC is constructed from plasmids encoding 2019-nCoV sequences and must be included in each test run. The PC must generate a detectable signal.
3) **Internal Control (IC)** or "**Extraction control**" is used to confirm test validity. The IC is composed of MS2 phage genome. Prior to sample preparation, a defined quantity of the IC is dispensed into the specimen before extraction. Following the extraction process, the IC will be amplified by the PCR reagents and measured by the Allplex™ 2019-nCoV Assay to demonstrate proper specimen processing and test validity.

NOTE : We recommend AccuPlex™ SARS-COV-2 reference material (Seracare life Sciences, Inc., Cat no. 0505-0126)as an extraction control (for all targets).

V. Interpretation of Results

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

1) *Allplex™ 2019-nCoV Assay Controls – Positive, Negative and Internal Control*

The validity or acceptance criteria of control results are indicated in Table below.

The validity of the experiment is confirmed when the PCR run results for the PC (Positive Control) and NC (Negative Control) are considered acceptable by meeting the criteria listed below.

Table : Allplex™ 2019-nCoV Assay; Control Acceptance Criteria

Control	Seegene Viewer Result (Ct value)				
	IC (HEX)	E gene (FAM)	RdRP gene (CalRed 610)	N gene (Quasar 670)	Interpretation
2019-nCoV Positive Control	≤ 40	≤ 40	≤ 40	≤ 40	Positive Control (+)
	>40 or N/A	>40 or N/A	>40 or N/A	>40 or N/A	Positive Control (invalid)
Negative Control	>40 or N/A	>40 or N/A	>40 or N/A	>40 or N/A	Negative Control (-)
	≤ 40	≤ 40	≤ 40	≤ 40	Negative Control (invalid)
Internal Control	≤ 40	-	-	-	Valid
	>40 or N/A	-	-	-	Invalid

In cases of a validity failure, the sample results should not be interpreted or reported, and the run must be repeated.

2) *Examination and Interpretation of Results :*

The results are analyzed by Seegene Viewer software. Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable as described above.

- Interpretation of Results:

Ct value	Result
≤ 40	Detected
> 40 or N/A	Not detected

Inter-pretation	IC (HEX)	E gene (FAM)	RdRP gene (CalRed 610)	N gene (Quasar 670)	Auto-Interpretation	Results
Case 1	+/-	+	+	+	**2019-nCoV Detected**	**All Target Results are valid. Result for SARS-CoV-2 RNA is Detected.**
Case 2	+/-	+	-	+	**2019-nCoV Detected**	**All Target Results are valid. Result for SARS-CoV-2 RNA is Detected. Negative target result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the corresponding target region, or 3) other factors.**
Case 3	+/-	+	+	-		
Case 4	+/-	-	+	+		
Case 5	+/-	-	-	+		
Case 6	+/-	-	+	-		
Case 7	+/-	+	-	-	**Presumptive positive**	**All Target Results are valid. Result for Sarbecovirus RNA is detected. Result for SARS-CoV RNA is Presumptive Positive. Negative target result is suggestive of 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the corresponding target region, or 3) other factors. Repeat test with more nucleic acids (up to 13 uL) instead of RNase-free water. For sample with the same result on a repeated test, additional confirmatory testing may be conducted, if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management.**
Case 8	+	-	-	-	**Negative**	**All Target Results are valid. Result for SARS-CoV-2 RNA is Not Detected.**
Case 9	-	-	-	-	**Invalid**	**Results are invalid. Repeat test. If the result is still invalid, a new specimen should be obtained.**

VI. Reagents

Allplex™ 2019-nCoV Assay		
Contents	**Volume**	**Description**
2019-nCoV MOM	500 µL	MuDT Oligo Mix (MOM): - Amplification and detection reagent
Real-time One-step Enzyme	200 µL	Enzyme mix for one-step RT-PCR
5X Real-time One-step Buffer	500 µL	Buffer for one-step RT-PCR - Buffer containing dNTPs
2019-nCoV PC	80 µL	Positive Control (PC): - Mixture of pathogen and IC clones
RP-V IC	1,000 µL	Exogenous Internal Control (IC) of Allplex™ 2019-nCoV Assay
RNase-free Water	1,000 µL	Ultrapure quality, PCR-grade

Store at -20°C or below

VII. Assay Materials

1) Materials provided

The reagents contained in one kit are sufficient for 100 reactions.

- 2019-nCoV MOM
- Real-time One-step Enzyme
- 5X Real-time One-step Buffer
- 2019-nCoV PC
- RP-V IC
- RNase-free Water

2) Materials required but not provided

Additional materials and equipment required

- STARMag 96 X 4 Universal Cartridge kit (Cat. No. 744300.4.UC384, Seegene Inc.)
- Disposable powder free gloves (latex or nitrile)
- Pipettes (adjustable) and sterile pipette tips
- 1.5 mL microcentrifuge tubes
- Clean bench
- Ice Maker
- Desktop centrifuge
- Vortex mixer
- Hard-Shell® PCR plates 96-well WHT/WHT (Cat. No. HSP9655, Bio-Rad)
- Optical Flat 8-Cap Strips (Cat. No. TCS0803, Bio-Rad)
- Permanent Clear Heat Seal (Cat. No. 1814035, Bio-Rad)*
- PX1 PCR plate sealer (auto-sealer, Cat. No. 181-4000, Bio-Rad)*
- CFX96™ and CFX96™ Touch Real-Time PCR Detection System (BIO-RAD) ; CFX Manager™ Software V1.6 & V3.1/CFX Maestro™ Software V1
- Seegene Viewer Software for analysis and interpretation of result (Seegene Inc.)

- distribution by Seegene Canada (Canada)
- Microlab STARlet IVD & Microlab NIMBUS IVD (Hamilton Co.)
- Seegene STARlet & Seegene NIMBUS (Seegene Inc.)

* The above mentioned heat seal and plate sealer must be used in combination.

VIII. Warnings and Precautions

The Allplex™ 2019-nCoV Assay should be performed by qualified and trained personnel.

- ***For in vitro diagnostic use only.***
- **The performance of this device has not been assessed in a population vaccinated against COVID-19.**
- **Based on in silico analysis and wet testing of in vitro transcription RNA containing mutations S235F (VUI202012/01) and T205I (501Y.V2). This assay can detect the sequence for "VUI202012/01" and "501Y.V2" variants. (As of Jan 5th, the sequence collected from the GISAID database for "VUI202012/01" and "501Y.V2".)**
- **Based on in silico analysis and wet testing of in vitro transcription RNA containing virial strains of new B.1.1.529 lineage. This assay can detect the sequence for B.1.1.529 variants. (As of Nov 26th, sequences were collected from the GISAID data base for B.1.1.529.)**
- ***Reliability of the results depends on adequate specimen collection, storage, transport, and processing procedure.***
- ***This test has been validated for the following specimen types: upper respiratory specimens (nasopharyngeal and oropharyngeal swab) and lower respiratory specimen (sputum).***
- ***This test has not been validated for any other types of specimens.***
- ***Store RNA samples at ≤ -20℃ until use and keep on ice during use.***
- ***Sensitivity of the assay may decrease if samples are repeatedly frozen/thawed or stored for a longer period of time.***
- ***Workflow in the laboratory should proceed in an unidirectional manner.***
- ***Wear disposable gloves and change them before entering different areas. Change gloves immediately if contaminated or treat them with DNA decontaminating reagent.***
- ***Supplies and equipments must be dedicated to working areas and should not be moved from one area to another.***
- ***Do not pipette by mouth.***
- ***Do not eat, drink or smoke in laboratory work areas. Wear disposable powder-free gloves, laboratory coats and eye protections when handling specimens and reagents. Wash hands thoroughly after handling specimens and test reagents.***
- ***Avoid contamination of reagents when removing aliquots from reagent tubes. Use of sterilized aerosol resistant disposable pipette tips is recommended.***
- ***Do not pool reagents from different lots or from different tubes of the same lot.***
- ***Do not use the product after its expiry date.***
- ***Do not reuse all disposable items.***
- ***Use screw-capped tubes and prevent any potential splashing or cross-contamination of specimens during preparation.***
- ***Please be careful not to contaminate reagents with extracted nucleic acids, PCR products, and positive control. To prevent contamination of reagents, use of filter-tips is recommended.***

- ***Use separated and segregated working areas for each experiment.***
- ***To avoid contamination of working areas with amplified products, open PCR reaction tubes or strips only at designated working areas after amplification.***
- ***Store positive materials separated from the kit's reagents.***
- ***Laboratory safety procedures (refer to Biosafety in Microbiological and Biomedical Laboratories & CLSI Documents) must be taken when handling specimens. Thoroughly clean and disinfect all work surfaces with 0.5% sodium hypochlorite (in de-ionized or distilled water). Product components (product residuals, packaging) can be considered as laboratory waste. Dispose of unused reagents and waste in accordance with applicable federal, state, and local regulations.***
- ***Expiry date is 8 months from the date of manufacture at ≤ -20℃. Please refer to label for expiry date.***
- ***Seegene NIMBUS and Seegene STARlet are the same equipment as Microlab NIMBUS IVD and Microlab STARlet IVD, although the legal manufacturer is different. Since there are no hardware changes on the device, the test results are identical.***
- ***This kit is a qualitative in vitro test for the single or multiple detection of 3 target genes (E gene, RdRP gene, and N gene)***

IX. Storage and Handling Conditions

1) *Reagent storage and handling*

- All reagents of the Allplex™ 2019-nCoV Assay kit should be stored at -20 °C or below.
- Completely thaw all reagents on ice.
- Do not store any reagents in a frost-free freezer.
- Do not use kits or reagents beyond indicated expiry date.
- Always check the expiry date on the reagent tubes prior to use.

NOTE: The performance of kit components is unaffected for up to 7 freezing and thawing cycles. If the reagents are used only intermittently, they should be stored in aliquots.

2) *Specimen Storage and Transport*

- Specimen types: upper respiratory specimens (nasopharyngeal and oropharyngeal swab) and lower respiratory specimen (sputum).
- Specimen collection media/device : UTM(Universal Transport Medium) and VTM(Universal Viral Transport Medium).
- Temperature : 2~8 °C
- Duration : 3 days

NOTE: (1) Performance may be affected by prolonged storage of specimens.
(2) Specimens should also adhere to local and national instructions for transport of pathogenic material.

X. Nucleic Acid Extraction

NOTE: (1) 10 uL of RP-V IC must be added to each specimen before nucleic acid extraction.

(2) Vortex specimen before use. If the specimen is still viscous, let it cool down or add saline solution.

(3) Store RNA samples at ≤ -20℃ until use and keep on ice during use.

(4) For Microlab STARlet IVD/NIMBUS IVD and Seegene STARlet/NIMBUS, use 300 uL of specimen and 10 uL of RP-V IC. For further information, please refer to the operation manual.

XI. Amplification and Detection

1) Preparation for Real-time PCR

NOTE: (1) Centrifuge all reagents stored at ≤ -20℃ after thawing them completely.

(2) Positive control amplification and clinical samples require special caution in handling to avoid carry-over contamination.

(3) PCR setup can be performed on NIMBUS and STARlet. Please contact Seegene Technologies (US) for method and protocol file.

① Prepare following reagents in a labeled sterile 1.5 mL tube. Set up all reagents on ice.

One-step RT-PCR Mastermix for different number of reactions (unit: uL)

No. of Reactions	1	2	3	4	5
2019-nCoV MOM	5	10	15	20	25
RNase-free Water	5	10	15	20	25
5X Real-time One-step Buffer	5	10	15	20	25
Real-time One-step Enzyme	2	4	6	8	10

② Mix by inverting the tube 5 times or quick vortex, and briefly centrifuge.

③ Aliquot 17 uL of the One-step RT-PCR Mastermix into PCR tubes*.
NOTE: The PCR tubes must be centrifuged before running PCR reaction. It needs to force the liquid to the bottom and to eliminate air bubbles.

④ Add 8 uL of each sample's nucleic acids, 2019-nCoV PC and NC (RNase-free water) into the tube containing aliquot of the One-step RT-PCR Mastermix.

⑤ Cover with Permanent Clear Heat seal for 96-Well Skirted PCR Plates on PX1™ PCR Plate sealer, and briefly centrifuge the PCR tubes.

⑥ Verify that the liquid containing all PCR components is at the bottom of each PCR tube. If not, centrifuge again at a higher rpm and for a longer time.

⑦ Immediately initiate the PCR.

2) Real-time PCR Instrument Set Up

① Protocol Setup

- In the main menu, select File → New → Protocol to open Protocol Editor.

- In Protocol Editor, define the thermal profile as table below.
- Click the box next to Sample Volume to directly input 25 uL.
- Click OK and save the protocol to open the Experiment Setup window.

Step	No. of cycles	Temperature	Duration
1	1	50℃	20 min
2	1	95℃	15 min
3	45	94℃	15 sec
4*		58℃	30 sec
5	GOTO Step 3, 44 more times		

*** Plate Read at Step 4. Fluorescence is detected at 58°C**

② **Plate Setup**

- From Plate tab in Experiment Setup, click Create New to open Plate Editor window.
- Click Select Fluorophores to indicate the fluorophores (FAM, HEX, Cal Red 610 and Quasar 670) that will be used and click OK.
- Select the desired well(s) and then its sample type from the Sample Type drop-down menu.
- Unknown: Clinical samples
- Negative Control
- Positive Control
- Click on the appropriate checkboxes (FAM, HEX, Cal Red 610 and Quasar 670) to specify the fluorophores to be detected in the selected wells.
- Type in Sample Name and press enter key.
- In Settings of the Plate Editor main menu, choose Plate Size (96 wells) and Plate Type (BR White).
- Click OK to save the new plate.
- You will be returned to the Experiment Setup window.

③ **Start Run**

- From Start Run tab in Experiment Setup, click Close Lid to close the instrument lid.
- Click Start Run.
- Store the run file either in My Documents or in a designated folder. Input the file name, click SAVE, and the run will start.

[Analytes]

Fluorophore	Analyte
FAM	E gene
HEX	Internal Control (IC)
Cal Red 610	RdRP gene

Quasar 670	**N gene**

XII. Performance Evaluation

1) Limit of Detection (LoD) - Analytical Sensitivity:

Part 1: *in vitro* transcription RNA

a) Samples for limit of detection (LoD) measurement were prepared by spiking *in vitro* transcription RNA diluent into each negative sample matrix (upper or lower respiratory sample). *In vitro* transcription was performed by MEGAscript® T7 Kit (Ambion®) with 3 targets: E gene, RdRP gene and N gene. Real-time PCR for Allplex™ 2019-nCoV Assay was performed on CFX96™, and CFX96 Touch ™ Real-time PCR Detection System, and the data was analyzed with the Seegene viewer. Since no quantified virus isolates of the 2019-nCoV were available, assays designed for detection of the 2019-nCoV RNA were tested with characterized stocks of *in vitro* transcribed RNA for 3 targets (E gene, RdRP gene and N gene). The sequences are based on the GenBank accession number NC_045512.2.

b) *In vitro* transcription RNA of known titer (Unit: Copies/mL) was spiked into a diluent of upper (nasopharyngeal & oropharyngeal) or lower (sputum) respiratory negative sample matrix to mimic clinical specimen.

c) Tentative LoD (5 replicates in 10-fold dilution in Table 1. LoD of each target gene was determined on the CFX96™ Real-time PCR Detection System followed by a confirmatory LoD test (20 replicates spiked at tentative LoD) for each clinical negative matrix. If 20/20 replicates were detected in the confirmatory LoD test, the next lower concentration, using a 3-fold dilution, was tested until achieving a positive rate of < 20/20.

d) Using the CFX96 Touch ™ Real-time PCR Detection System, a tentative LoD (5 replicates at 2X, 1X and 0.5X LoD) was determined followed by the confirmatory LoD test (20 replicates spiked at tentative LoD) for each clinical negative matrix.

Part 2: Reference Material

a) We purchased AccuPlex™ SARS-COV-2 as reference material (Seracare life Sciences, Inc. Cat no. 0505-0126), performed spiking on lower (sputum) respiratory negative sample matrix, diluted the sample, then performed additional LoD tests. As the titer RNA sold at Seracare was 5,000 copies/mL, we tested 1.2X LoD, 1X LoD, 0.1X LoD, and 0.01X LoD based on existing *in vitro* transcription RNA test results. After that, we performed 20 repetitive tests on a concentration level where all targets were detected, then decreased the concentration by 3-fold to establish the LoD.

b) The LoD test on each pathogen was performed using the CFX96™, and CFX96 Touch ™ Real-time PCR Detection System.

c) As a result, we could confirm the LoD of each target as shown on Table 1. LoD of each target gene. As all targets were detected on concentration level of 4,167

Copies/mL (Table 2. LoD of pathogen types), we confirm the final LoD of the assay as the concentration stated in Table 2. This result was identical to the results from the *in vitro* transcription RNA test.

Table 1. LoD of each target gene

PCR instrument	Target type		Limit of Detection	Unit
CFX96 ™	***in vitro* transcription RNA**	E gene	4,167	Copies/mL
		RdRP gene	4,167	Copies/mL
		N gene	4,167	Copies/mL
	Reference Material	E gene	4,167	Copies/mL
		RdRP gene	1,250	Copies/mL
		N gene	1,250	Copies/mL
CFX96 Touch™	***in vitro* transcription RNA**	E gene	4,167	Copies/mL
		RdRP gene	4,167	Copies/mL
		N gene	4,167	Copies/mL
	Reference Material	E gene	4,167	Copies/mL
		RdRP gene	4,167	Copies/mL
		N gene	1,250	Copies/mL

Table 2. LoD of pathogen types

PCR instrument	Target type	Limit of Detection	Unit
CFX96™	***in vitro* transcription RNA**	4,167	Copies/mL
CFX96 Touch™	**Reference Material**	4,167	Copies/mL

2) Inclusivity (Analytical Sensitivity):

The *in silico* analysis for all sequences of SARS-CoV-2, available from NCBI and GISAID databases, was conducted by mapping the primers and probes of the Allplex™ 2019-nCoV Assay. If the *in silico* analysis revealed < 100% homology between the SARS-CoV-2 sequences and primers/probes, its results were confirmed in a wet test. As of March 13, 2020, *in silico* analysis through GISAID (n = 533) and NCBI (n = 141) sequences, generated data as shown in Table 6 below. Of these, 3 cases with homology of '< 100%' in the primer / probe region were identified.

Table 3: *In silico* analysis for detection of SARS-CoV-2 sequences (as of March 13, 2020)

DB (Data Base)	Target gene	Homology		
		F' Primer	Probe	R' Primer
GISAID (n=533)	RdRP	100 %	100 %	100 %
	E	100 %	99.8 % (n=1 case1))	99.8 % (n=1 case 2))
	N	89.8 % (n=54 case 3))	100 %	100 %
NCBI (n=141)	RdRP	100 %	100 %	100 %
	E	100 %	99.3 % (n=1 case1))	100 %
	N	99.3 % (n=1 case 3))	100 %	100 %

Since no quantified virus isolates of the 2019-nCoV variant are currently available, assays designed for detection of the 2019-nCoV variant RNA were tested with characterized stocks of *in vitro* transcribed RNA (Case 1): NCBI accession no. MT039890, Case 2): GISAID accession no. EPI_ISL_412459, Case 3): NCBI accession no. MT163714). *In vitro* transcription RNA of known titer (Unit: Copies/mL, Concentration: 3X LoD = 12,500 Copies/mL) were spiked into a negative sample matrix (lower respiratory specimen; sputum) to mimic clinical specimen. The Allplex™ 2019-nCoV Assay was tested for 3 cases of mismatch types. It was performed three times under the same condition, and all cases were detected (Table 4).

Table 4: Allplex™ 2019-nCoV Assay testing of 3 cases of mismatch types

No.	Type	Rep.	E gene	IC	RdRP gene	N gene
1	Case 1; E gene probe region 1mer mismatch.	1	31.23	29.61	33.15	35.45
		2	31.27	29.47	32.37	35.3
		3	31.09	29.38	32.32	34.6
2	Case 2; E gene R' primer region 1mer mismatch.	1	30.6	29.35	32.26	35.91
		2	31.39	29.41	32.73	34.91
		3	31.23	29.22	32.8	35.39
3	Case 3; N gene F' primer region 3mer mismatch.	1	30.93	29.45	32.72	35.5
		2	31.13	29.55	32.71	35.15
		3	30.7	29.51	32.25	35.1

Although mismatch was confirmed in 3 cases, it was concluded that the concentration of 3X LoD was detected unequivocally. In summary, the major base mismatch did not affect assay performance.

3) Cross-reactivity (Analytical Specificity):

a) Evaluation of Cross-reactivity with high priority pathogens

Cross-reactivity studies were performed to demonstrate that the test does not react with pathogens listed on Table 5. List of Pathogens analyzed *in silico*. that are reasonably likely to be encountered in the clinical specimen. In addition, the pathogens listed in Table 6, were also wet tested.

Table 5. List of Pathogens analyzed *in silico*

Other high priority pathogens from the same genetic family	High priority pathogens likely in the circulating area
Human coronavirus 229E	Adenovirus (e.g. C1 Ad. 71)
Human coronavirus OC43	Human Metapneumovirus (hMPV)
Human coronavirus HKU1	Parainfluenza virus 1-4
Human coronavirus NL63	Influenza A & B
SARS-coronavirus	Enterovirus (e.g. EV68)
MERS-coronavirus	Respiratory syncytial virus
	Rhinovirus
	Chlamydia pneumoniae
	Haemophilus influenzae
	Legionella pneumophila
	Mycobacterium tuberculosis
	Streptococcus pneumoniae
	Streptococcus pyogenes
	Bordetella pertussis
	Mycoplasma pneumoniae
	Pneumocystis jirovecii (PJP)
	Candida albicans
	Pseudomonas aeruginosa
	Staphylococcus epidermis
	Streptococcus salivarius

In silico analysis test results:

In silico cross-reactivity is defined as greater than 80% homology between 'oligo set' and any sequence present in the targeted microorganism as table above. Cross-reaction is likely to occur when first, the amplicon size is below 500 bp, and second, when the homology of the binding site between the oligo set (forward primer, reverse primer, and probe) and the microorganism is greater or equal to 80% (Table 6. *In silico* analysis results of targeted pathogens).

Table 6. *In silico* analysis results of targeted pathogens

Pathogen	RdRP gene	E gene	N gene	Complex*
Human coronavirus 229E	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Human coronavirus OC43	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Human coronavirus HKU1	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Human coronavirus NL63	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
SARS-coronavirus	Amp. Mis. #	100% Match	Amp. Mis. #	Amp. Mis. #
MERS-coronavirus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Adenovirus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Human Metapneumovirus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Parainfluenza virus 1	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Parainfluenza virus 2	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Parainfluenza virus 3	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Parainfluenza virus 4	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Influenza A virus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Influenza B virus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Enterovirus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Respiratory syncytial virus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Rhinovirus	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Chlamydia pneumoniae	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Hemophilus influenzae	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Legionella pneumophila	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Mycobacterium tuberculosis	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Streptococcus pneumoniae	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Streptococcus pyogenes	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Bordetella pertussis	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Mycoplasma pneumoniae	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Pneumocystis gynoecia (PJP)	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Candida albicans	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Pseudomonas aeruginosa	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Staphylococcus epidermis	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #
Streptococcus salivary	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #	Amp. Mis. #

Note: (*) Complex: Amplicon formation for any combination of oligo primers and probe

(#) Amp. Mis: Amplicon mismatch. Amplicon is not formed. It indicates that the combination of oligos with each microorganism did not achieve above 80% homology and was greater than 500 bp.

As a result of analysis, there were no cases of amplicon formation with potential non-specific or cross-reactive organisms except with E gene sequences that showed a 100% match with SARS-coronavirus. E gene is a target gene for Sarbecovirus, so the results of the *in silico* analysis is valid (see IFU interpretation Case 7 on page 5).
Thus, through *in silico* analysis for cross-reactivity, no sequence homology to generate amplicons was observed, but the corresponding organism was further identified during wet test. In conclusion, no detection in targets other than SARS-Coronavirus was observed in the wet test, which matches the *in silico* analysis results.

Table 7 below lists 60 pathogens, and 1 pooled nasal swab wash evaluated by wet testing. The bacterial species were tested at ≥ 1 x 10^6 CFU/mL, and viral species at ≥ 1 x 10^5 PFU/mL or 1 x 10^6 genome copies/rxn.

Table 7. List of Pathogens evaluated by Wet Testing

No.	Usage	Pathogen	Source	Isolate No.
1	*Exclusivity*	human coronavirus HKU1	*Korean isolate*	
2	*Exclusivity*	human coronavirus OC43	ATCC	VR-1558
3	*Exclusivity*	human coronavirus NL63	*Korean isolate*	
4	*Exclusivity*	human coronavirus 229E	ATCC	VR-740
5	*Exclusivity*	human Severe Acute Respiratory Syndrome, SARS	*Korean isolate*	
6	*Exclusivity*	human Middle East Respiratory Syndrome Coronavirus: MERS-CoV	*Korean isolate*	
7	*Exclusivity*	influenza A virus (H1N1)	ATCC	VR-95
8	*Exclusivity*	Influenza A virus (H3N2)	ATCC	VR-547
9	*Exclusivity*	influenza B virus	ATCC	VR-523
10	*Exclusivity*	Human Rhinovirus 1	KBPV	VR-81
11	*Exclusivity*	Rhinovirus 21	KBPV	VR-40
12	*Exclusivity*	Human rhinovirus type 90	ATCC	VR-1291
13	*Exclusivity*	Human rhinovirus type 16	ATCC	VR-283
14	*Exclusivity*	Human rhinovirus type 42	ATCC	VR-338
15	*Exclusivity*	Human rhinovirus type 8	ATCC	VR-488
16	*Exclusivity*	Human rhinovirus type 14	ATCC	VR-284
17	*Exclusivity*	Human enterovirus type 68	ATCC	VR-1826
18	*Exclusivity*	Human enterovirus type 70	ATCC	VR-836
19	*Exclusivity*	Human enterovirus type 71	ATCC	VR-784

No.	Usage	Pathogen	Source	Isolate No.
20	*Exclusivity*	human respiratory syncytial virus A	ATCC	VR-26
21	*Exclusivity*	human respiratory syncytial virus B	ATCC	VR-955
22	*Exclusivity*	Parainfluenza 1 virus	ATCC	VR-1380
23	*Exclusivity*	Human parainfluenza virus 2	ATCC	VR-92
24	*Exclusivity*	Human parainfluenza virus 3	ATCC	VR-93
25	*Exclusivity*	human parainfluenza 4 virus 4a	ATCC	VR-1378
26	*Exclusivity*	Human parainfluenza virus 4b	ATCC	VR-1377
27	*Exclusivity*	Human Metapneumovirus (MPV)	KBPV	VR-87
28	*Exclusivity*	Human adenovirus 1	ATCC	VR-1
29	*Exclusivity*	Human adenovirus 11	KBPV	VR-63
30	*Exclusivity*	Human adenovirus 18	ATCC	VR-1095
31	*Exclusivity*	Human adenovirus 23	ATCC	VR-1101
32	*Exclusivity*	Human adenovirus 3	ATCC	VR-3
33	*Exclusivity*	Human adenovirus 4	ATCC	VR-1572
34	*Exclusivity*	Human adenovirus 8	ATCC	VR-1368
35	*Exclusivity*	Human adenovirus type 31	ATCC	VR-1109
36	*Exclusivity*	Human adenovirus type 40	ATCC	VR-931
37	*Exclusivity*	Human adenovirus type 5	KBPV	VR-61
38	*Exclusivity*	Human adenovirus type 35	ATCC	VR-718
39	*Exclusivity*	*Legionella pneumophila Serotype 2*	ATCC	33154
40	*Exclusivity*	*Legionella pneumophila subsp. fraseri Serotype 4*	ATCC	33156
41	*Exclusivity*	*Legionella pneumophila Serotype 7*	ATCC	33823
42	*Exclusivity*	*Legionella pneumophila Serotype 10*	ATCC	43283
43	*Exclusivity*	*Legionella pneumophila Serotype 11*	ATCC	43130
44	*Exclusivity*	*Legionella pneumophila Serotype 12*	ATCC	43290
45	*Exclusivity*	*Legionella pneumophila Serotype 13*	ATCC	43736
46	*Exclusivity*	*Legionella pneumophila Serotype 14*	ATCC	43703
47	*Exclusivity*	*Legionella pneumophila subsp. fraseri Serotype 15*	ATCC	35251
48	*Exclusivity*	*Mycoplasma pneumoniae*	ATCC	15293
49	*Exclusivity*	*Mycoplasma pneumoniae M129-B7*	ATCC	29342
50	*Exclusivity*	*Chlamydophila pneumoniae*	ATCC	53592
51	*Exclusivity*	*Bordetella pertussis*	ATCC	BAA-589
52	*Exclusivity*	*Pseudomonas aeruginosa (Z139; VIM-1)*	Zeptometrix	801908
53	*Exclusivity*	*Mycobacterium tuberculosis*	ATCC	25177
54	*Exclusivity*	*Haemophilus influenzae*	ATCC	51907
55	*Exclusivity*	*Streptococcus pneumoniae*	KCCM	40410
56	*Exclusivity*	*Streptococcus pyogenes*	ATCC	19615
57	*Exclusivity*	*Staphylococcus epidermidis*	KCCM	40416
58	*Exclusivity*	*Candida albicans*	KCCM	11282

No.	Usage	Pathogen	Source	Isolate No.
59	*Exclusivity*	*Pneumocystis pneumonia jirovecii (PJP)*	*Korean isolate*	
60	*Exclusivity*	*Staphylococcus salivarius*	*Korean isolate*	
61	*Exclusivity*	Pooled human nasal wash	*Clinical sample*	

Allplex™ 2019-nCoV Assay was tested for cross-reactivity to 61 different pathogens. It was performed three times under the same conditions. As a result, none of the 61 pathogens generated detectable signals.

b) Endogenous Interference Substances Studies:

Allplex™ 2019-nCoV Assay uses well-established bead type auto-extraction methods and based on our experience with other similar assays from Seegene (e. g. syndromic respiratory panels with CE-IVD marking), we do not anticipate inference from common endogenous substances.

4) Clinical Evaluation:

A clinical evaluation study was performed to evaluate the performance of Allplex™ 2019-nCoV Assay using upper respiratory specimens (nasopharyngeal and oropharyngeal swab) and lower respiratory specimens (sputum).

A total of three hundred (300) clinical specimens were tested:

- 50 positive upper respiratory specimens
- 50 positive lower respiratory specimens
- 100 negative upper respiratory specimens
- 100 negative lower respiratory specimens

The results from testing upper respiratory specimens including nasopharyngeal + oropharyngeal swabs shown in Table 7A generated a Positive Percent Agreement (PPA): 100.00% (49/49) [95% CI: 92.75% ~ 100.00%], and a Negative Percent Agreement (NPA): 94.00% (94/100) [95% CI: 87.40% ~ 97.77%].

The results from testing lower respiratory specimens (sputum) shown in Table 7B, generated Positive Percent Agreement (PPA): 100.00% (49/49) [95% CI: 92.75% ~ 100.00%], and a Negative Percent Agreement (NPA): 97.87% (92/94) [95% CI: 92.52% ~ 99.74%]

Table 8A: Upper respiratory samples (nasopharyngeal + oropharyngeal swab)
n=150

Final results		2019-Novel Coronavirus(2019-nCoV) Real-time RT-PCR Panel			
		2019-nCoV Detected	Inconclusive	2019-nCoV Not Detected	Total
Allplex™ 2019-nCoV Assay	2019-nCoV Detected	49	1[1)]	6[2)]	56
	Presumptive Positive	0	0	0	0
	Negative	0	0	94	94
	Total	49	1	100	150

1) Sequencing result was positive.

2) Sequencing results were positive for 5 cases, and negative for 1 remaining case.

A. Positive Percent Agreement (PPA): 100.00% (49/49) [95% CI: 92.75% ~ 100.00%]

B. Negative Percent Agreement (NPA): 94.00% (94/100) [95% CI: 87.40% ~ 97.77%]

Table 8B: Lower Respiratory samples (Sputum) n=150

Final results		2019-Novel Coronavirus(2019-nCoV) Real-time RT-PCR Panel			
		2019-nCoV Detected	Inconclusive	2019-nCoV Not Detected	Total
Allplex™ 2019-nCoV Assay	2019-nCoV Detected	49	1[1)]	2[2)]	52
	Presumptive Positive	0	0	0	0
	Negative	0	0	92	92
	Total	49	1	94	144

*** Due to criteria for failure, invalid results (6 Sputum samples) were excluded from statistical processing.**

1) Sequencing result was positive.

2) Sequencing results were all positive for 2 cases.

A. Positive Percent Agreement (PPA): 100.00% (49/49) [95% CI: 92.75% ~ 100.00%]

B. Negative Percent Agreement (NPA): 97.87% (92/94) [95% CI: 92.52% ~ 99.74%]

XIII. Conclusion

In the clinical evaluation study, left-over samples from COVID-19 PUIs that were stored at the Seegene Medical Foundation were tested. A total of 300 samples (150 upper respiratory samples, 150 lower respiratory samples); 100 positive samples (50 upper respiratory samples, 50 lower respiratory samples) and 200 negative samples (100 upper respiratory samples, 100 lower respiratory samples) were tested. The purpose of this clinical study was to assess the clinical equivalence of Seegene's Allplex™ 2019-nCoV assay to US FDA EUA approved '2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Panel' (CDC). By comparing the clinical efficacy with the predicate, 100.00% (49/49) ([95% CI: 92.75% ~ 100.00%] Positive Percent Agreement (PPA) and 94.00% (94/100) [95% CI: 87.40% ~ 97.77%] Negative Percent Agreement (NPA) was obtained for upper respiratory samples. In case of lower respiratory samples, 100.00% (49/49) ([95% CI: 92.75% ~ 100.00%] Positive Percent Agreement (PPA) and 97.87% (92/94) [95% CI: 92.52% ~ 99.74%] Negative Percent Agreement (NPA) was obtained when compared with the predicate.

In conclusion, we confirm a high clinical concordance between Seegene's Allplex™ 2019-nCoV assay and CDC's FDA EUA cleared 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Panel.

XIV. Assay limitations

- The use of this assay as an *in vitro* diagnostic.
- Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.
- Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
- False-negative results may arise from:
  - Improper sample collection
  - Degradation of the viral RNA during shipping/storage
  - Specimen collection after nucleic acid can no longer be found in the specimen matrix
  - The presence of RT-PCR inhibitors
  - Mutation in the SARS-CoV-2 virus
  - Failure to follow instructions for use
- Negative results do not preclude infection with SARS-CoV-2 virus, and should not be the sole basis of a patient management decision.
- Laboratories are required to report all positive results to the appropriate public health authorities.

XV. Key to Symbols

Symbol	Explanation
IVD	In vitro diagnostic medical device
LOT	Batch code
REF	Catalog number
	Use-by date
	Upper limit of temperature
PRIMER	Oligonucleotide mix for amplification and detection
ENZYME	Enzyme Mix
BUFFER	Buffer
WATER	RNase-free Water
CONTROL +	Positive Control (PC)
CONTROL IC	Internal Control (IC)
	Consult instructions for use
	Manufacturer
	Date of manufacture
EC REP	Authorized representative in the European Community
	Caution
	Contains sufficient for <n> tests
$\mathrm{R_x}$ Only	Prescription Use only
EUA	Emergency Use Authorization

XVI. ORDERING INFORMATION

The product will be distributed by Seegene Inc., located at Taewon Bldg., 91. Ogeum-ro, Songpa-gu, Seoul, Republic of Korea, 05547 and Seegene CANADA Inc. located at 240 Richmond Street West Toronto ON M5V1V6 Canada.