ioneBio

iLAMP Novel-CoV19 Detection Kit

Instructions For Use

REF **CoV19-iLAMP**

IVD **For In Vitro Diagnostic Use**

For use under Emergency Use Authorization (EUA) only

For in vitro diagnostic use only

RX only

CE IOB-IFU-D01 REV.1.2020-12 Dec, 2020

Table of Contents

1	Warnings and Precautions……	3
2	Symbols……....	3
3	Intended Use……...	4
4	Summary and Explanation of the Test……	4
5	Kit Components…….....	5
6	Storage and Handling of Kit Components…….....	5
7	Equipment & Materials required (but not provided with the kit)……..	5
8	Specimens Collection, Handling and Storage……..	7
9	RNA(Nucleic Acid) Extraction……..	7
10	Quality Control……	8
11	Reagent Preparation and Test Procedure…….	8
12	Instrument Setup……	10
13	Interpretation of Results…….....	11
14	Trouble Shooting……	12
15	Limitations……..	13
16	Performance Characteristics……	13
17	Contact Information and Technical Support……	19

1. Warnings and Precautions

The instruction for use must be read carefully prior to use and followed accordingly. Reliability of results cannot be guaranteed if there are any modifications from these instructions.

▪ For in vitro diagnostic use (IVD).

▪ Samples and controls should always be treated as if infectious and/or biohazardous in accordance with safe laboratory procedures.

▪ Follow necessary precautions when handling specimens. Use personal protective equipment(PPE) consistent with current guidelines for the handling of potentially infectious samples.

▪ Use of non-recommended reagent volumes may result in a loss of performance and may also decrease the reliability of the test results.

▪ Use of non-recommended consumables with instruments may adversely affect test results.

▪ Reagents must be stored and handled as specified in these instructions for use. Do not use the kit after the indicated expiry date.

▪ All reagents must be thawed and maintained on a cold block at all times during preparation and use.

▪ Do not mix reagents from different lots.

▪ Do not eat, drink, smoke, or apply cosmetic products in the work areas.

▪ Dispose of waste in compliance with local, state, and federal regulations.

2. Symbols

IVD	In vitro diagnostic use	Σ	Sufficient for <n> tests
	Consult instructions for use	LOT	Batch code
	Use by (MMDDYYYY)	REF	Catalog number
	Manufacturer		Temperature limitation
	Caution, consult accompanying documents		

3. Intended Use

The **iLAMP Novel-CoV19 Detection Kit** is intended for the qualitative detection of nucleic acid from SARS-CoV-2 in upper respiratory specimens such as nasopharyngeal swabs and oropharyngeal swabs from individuals suspected of COVID-19 by their healthcare provider.

Results are for the identification of SARS-CoV-2 RNA. The RNA of the SARS-CoV-2 RNA is generally detectable in upper 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 infective status. Positive results do not rule out bacterial co-infection with other viruses.

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 **iLAMP Novel-CoV19 Detection kit** is intended for use by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time nucleic acid amplification and in vitro diagnostic procedures. The **iLAMP Novel-CoV19 Detection Kit** is only for use under the Food and Drug Administration's Emergency Use Authorization.

4. Summary and Explanation of the Test

The **iLAMP Novel-CoV19 Detection Kit** is a real-time reverse transcription LAMP PCR test and is designed to detect nucleic acid sequences from the nucleocapsid(N) gene using LAMP (Loop-mediated isothermal amplification) method.

The Key differentiator of LAMP assays from current rRT PCR assays is its ability to amplify under one temperature condition without heat-induced denaturation by using a strand-displacing Bst polymerase, not Taq polymerase commonly used in current rRT PCR.

For this assay, the entire amplification process from the beginning to the end of the DNA can be identified in real time through fluorescence measurements. The fluorescence detection method used in this assay is SYBR green. SYBR green specifically binds to the DNA amplicons produced by six primers after they bind to the target gene. SYBR green acting as a DNA binding dye (Intercalating) and synthesized in the DNA extension phase becomes fluorescent when combined with DNA amplicons. At this time, the more amplification occurs, the higher the intensity of fluorescence.

The SARS-CoV-2 primer sets are designed to detect RNA from the SARS-CoV-2 in upper respiratory specimens from patients suspected of COVID-19 by their healthcare provider.

5. Test kit Components

Table 1: Components Included in the Kit

No.	Cap Color	Image	Component	Part Number	Pack Size (2000 tests per kit)	Volume per Vial
1	Clear		RT LAMP Premix	PM0420	20 vials	1.28mL (100 tests per vial)
2	Amber		Primer Set	PS0420	20 vials	720uL (100 tests per vial)
3	Red		Positive Control	PC0420	20 vials	100uL

6. Storage and Handling of Kit Components

▪ The iLAMP Novel-CoV19 Detection Kit is shipped on dry ice. The components of the kit should arrive frozen.

▪ All components should be **stored at -25°C ~ -15°C freezer** to prevent degradation of reagents.

▪ Thaw **in a refrigerator at 4°C** before use.

▪ Once the reagents(components) are thawed, always work with them **on ice or cold rack/block to keep them at around 4°C**

▪ Do not repeat thawing and freezing **more than twice.**

▪ Master Mix(After two reagents are combined) **must be used in a PCR instrument within 30 minutes.**

▪ Do not open the tubes during or after PCR amplification and discard them immediately after use.

7. Equipment & Materials required (but not provided with the kit)

1) Control Material

Table 2: Negative Control Material

Control	Description	Recommended Supplier	Catalog Number
Negative Control	DNase/RNase-Free Distilled Water	Thermo fisher	10977015

2) RNA Extraction Equipment/Kit

Table 3: RNA Extraction Equipment

	Name	Description	Supplier	Catalog Number	Image
1	QIAcube*	RNA Extraction Instrument	Qiagen	9001293	
2	QIAamp DSP* Virus Kit	RNA Extraction Kit	Qiagen	60704	

*QIAamp DSP Virus Kit is used on QIAcube automatic instrument.

3) PCR Instrument

Table 4: PCR Instrument

Option	Equipment	Software	Supplier	Catalog Number	Image
1	Bio-Rad CFX96 Real-time PCR System	CFX Manager 3.1 software	Bio-Rad	184509	

4) Additional Equipment and Consumables

Table 5: Additional Equipment and Consumables

No.	Description	Recommended Supplier	Catalog Number
1	Vortex mixer	Scientific industries	SI-0236
2	Centrifuge for 1.5ml tubes	Eppendorf	5424R
3	Mini centrifuge for 0.2mL 8strip tubes	USA Scientific	#2621-0016
4	Adjustable sterile pipettes with corresponding filter-plugged pipette tips*	Eppendorf	4920000938
5	Disposable latex gloves	Uni Gloves	UN.PSXXS2

5	Cold block	Thermo Scientific	1225W62
6	0.2 ml Flat qPCR Tube 8-Cap Strips	Bio-Rad	#TCS0803EDU
7	1.5 microcentrifuge tube	Axygen	MCT-150-A

8. Specimen Collection, Handling and Storage

1) Collecting the Specimen

Refer to Interim Guidelines for Collecting, Handling and Testing Clinical Specimens from Patients Under Investigations (PUIs) for 2019 Novel Coronavirus(2019-nCoV)
https://www.cdc.gov/coronavirus/2019-nCoV/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 table2 below) Store the samples at 2-8 ℃ up to 72 hours if necessary. If a delay in shipping or extraction is expected to exceed 72 hours, store samples at -70℃

Recommended Product Name	Recommended supplier	Cat#	FDA
Teknova Viral Transport Medium	Teknova	#4V2020	FDA Listed

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℃ and ship to the lab on ice packs. If a specimen is frozen at -70℃, 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℃ over 72hours. 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.

9. RNA(Nucleic Acid) Extraction

The iLAMP Novel CoV-19 Detection Kit does not include viral RNA extraction reagents. Th e following combination of extraction kit and automatic instrument have been validated with

the **iLAMP Novel-CoV19 Detection Kit**:

- QIAamp DSP Virus Kit (Qiagen, Cat. #60704)
- QIAcube Automatic Instrument (Qiagen, Cat. #9001293)

Proceed with the extraction as indicated in the Instructions For Use for the equipment and kit. The extraction combination requires 250uL of clinical sample input and yields 60uL of purified nucleic acid eluent. Following the extraction, RNA should be used immediately or stored at 70℃ (for up to 1 month) for use later.

10. Quality Control

▪ Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures.

▪ Quality control procedures are intended to monitor reagent and assay performance.

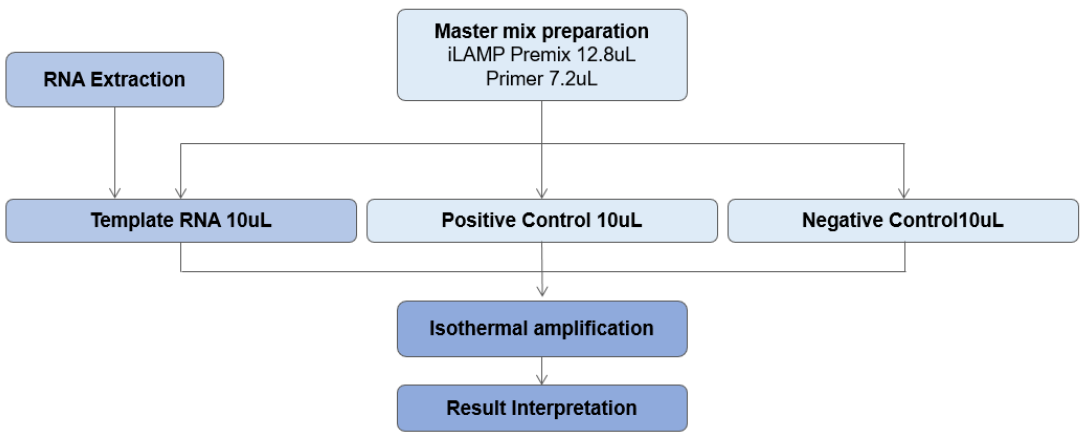
▪ Always include a negative control(NC) and a positive control(PC) in each amplification and detection run.

▪ PC – Positive Control contains N gene plasmid DNA and is designed to evaluate not only analytical and clinical performance, but also PCR operation and reagent activity. Positive Control is used once in each instrument run. 10ul of the positive control is used for each run.

▪ NC – Negative Control – RNase-free water. [Recommended supplier is Thermo fisher, and product name is UltraPure™ DNase/RNase-Free Distilled Water, Cat.# is SM-W01-100.] This control is needed to verify analyte contamination and is used once in each instrument run. 10uL of the negative control is used for each run.

11. Reagent Preparation and Test Procedure

*Test Workflow



1) Preparation before test

Thaw all reagents at 4℃ 5 minutes before use and keep on ice or cold rack. The testing personnel must wear latex gloves to avoid contamination.

Table 6: Preparation of all reagent, Template RNA and Controls

No.	Item	Description	Provided/Not Provided	Volume per test
1	Real-time LAMP Premix	Clear cap	Provided	12.8 µℓ
2	Primer Set	Amber cap	Provided	7.2 µℓ
3	Template RNA (or cDNA)	Extracted RNA using RNA extraction kit	Not Provided	10 µℓ
4	Positive control	Red cap	Provided	10 µℓ
5	Negative control	RNase-free water	Not Provided	10 µℓ

2) Master mix preparation

1.Vortex and spin down 'Real-time LAMP Premix' and 'Primer' Set each.
2.Refer to the table below and prepare a master mix by adding (12.8 uL x (N+3)) RT LAMP Premix(clear cap) and (7.2uL x (N+3)) Primer Set(amber cap) in a 1.5ml e-tube
3.Vortex and spin down the master mix.

Table 7: Master Mix Preparation

Item	Volume (µℓ) per 1 test	Volume (µℓ) per 10 specimen samples
RT LAMP Premix	12.8	12.8 µℓ x (10+3)* = 140.8 µℓ
Primer Set	7.2	7.2 µℓ x (10+3)* = 79.2 µℓ
Total volume	20	128 µℓ + 72 µℓ = 220 µℓ

*Add extra 1 test to each component due to the expected loss while dispensing and extra 2 tests for positive control and negative control respectively.

3) Reaction assembly

1. Carefully pipette out (20 x (N+3))µℓ of master mix into (0.2ml+(N+2)) qPCR strip tubes.
2. Add 10 µℓ of each extracted template RNA into each qPCR strip tube containing the master mix.
3. Carefully pipette out 10 µℓ positive control(red cap, provided with the test kit) into a separate qPCR strip tube containing the master mix. One strip tube of 10 µℓ positive control is used once in each instrument run.
4. Carefully pipette out 10 µℓ negative control(RNase-free water, not provided with the test kit) into

a separate qPCR strip tube containing the master mix. One strip tube of 10 μl negative control is used once in each instrument run.
5. Spin down qPCR strip tubes containing the master mix added with the extracted template RNA, positive control and negative control to ensure the liquid is in the bottom of the strip tubes.

12. Instrument Setup

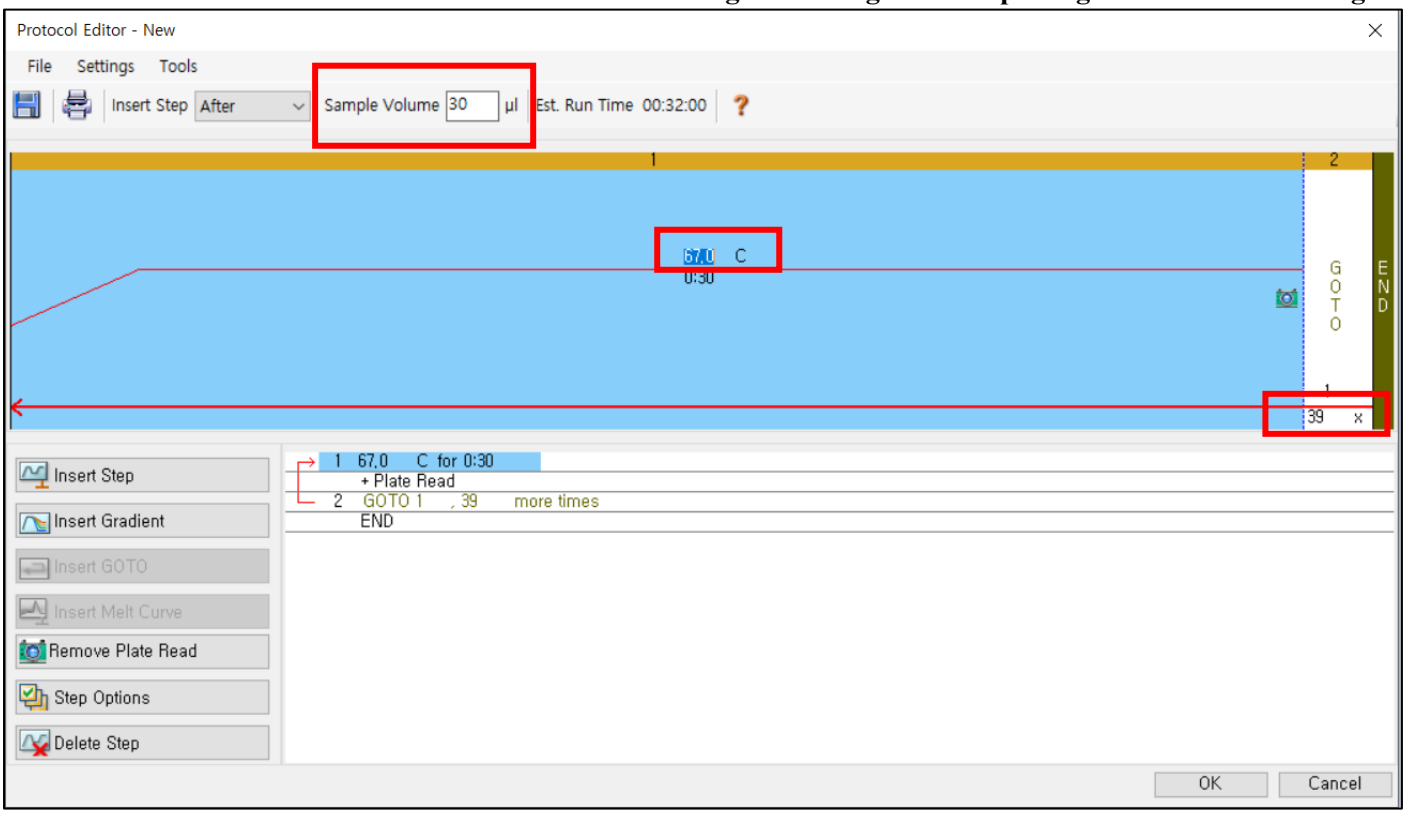
1. Please refer to the instruction manual provided with the instrument. Run setup using Bio-Rad CFX96 Manager Software(3.0 or higher) connected to the Bio-Rad CFX96 Real-Time PCR System:

2. Refer to the table below and correctly set the temperature and fluorescence of the instrument. Otherwise, the results may not be accurate.

Table 8: Instrument Setup

Step	Sample Volume	Temperature	Cycle	Time
Isothermal amplification	30	67 ℃	39	30 sec

Figure 1: Program set-up using Bio-Rad CFX Manager



2. Put the reaction tubes into the sample holder in the real-time PCR machine, and make sure that the tubes are placed according to the Plate Editor setup. Then close the lid, and start the reaction run.

3. After the run, take out the sample tubes/plate and discard them immediately. To avoid contamination. Do not open the reaction tube after the reaction and do not bring tubes in the sample processing area.

13. Interpretation of Results

1) Interpretation of controls: all test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted.

Table 9: Ct value for controls

Control	Ct	Interpretation
Positive Control	≤ 25	It should show exponential curve. If amplification is not detected, the test is invalid, and the results cannot be used for diagnosis. Also, The Ct value should always be consistent(±3) for each run.
Negative Control	No detection	No exponential amplification curve should be shown, otherwise the test is invalid, and the results cannot be used for diagnosis.

2) Set threshold as shown in the table below.

Table 10: Threshold

Target	Threshold
SARS-CoV-2	5000

3) Interpretation of test results: Check the Ct value in the table below for identification of SARS-CoV-2. An exponential amplification curve showing up and crossing the background threshold indicates the presence of corresponding analyte as indicated below:

Table 11: Interpretation of Results and Actions

No.	SARS-CoV-2 (Ct value)	Positive control (Ct value)	Negative control	Interpretation	Action
1	- **(> 35)**	+ **(≤ 25)**	-	SARS-CoV-2 not detected	Report results to healthcare provider. Consider test for other viruses that cause similar symptoms
2	+ **(≤ 35)**	+ **(≤ 25)**	-	SARS-CoV-2 positive	Report results to healthcare provider and appropriate public health authorities
3	**+ (≤ 35)**	- **(> 25)**	+	Invalid	Repeat test. If result remains invalid, see trouble shooting
4	**-**	-	+	Invalid	Repeat test. If result remains

	(> 35)	**(> 25)**			invalid, see trouble shooting

14. Trouble Shooting

Table 12: Possible causes for trouble

Problem	Possible Causes	Suggestion
No signal detection in Positive Control	□ Pipetting errors(control in wrong well, missing a well, inadequate amount of a reagent) □Degraded reagents due to incorrect storage temperature □Use of incorrect reagent	▪ If the PC is invalid, the run should be considered invalid and the user should re-test the samples by re-extraction and use a fresh aliquot of the PC
Signal detection in Negative Control	□ Contamination of one or more reagents during set-up □ Contamination of the test room or the test tools □ Pipetting errors(control in wrong well, missing a well, inadequate amount of a reagent) □ Use of incorrect reagent	▪ If the NC is invalid, the run should be considered invalid and the user should re-test the samples by re-extraction. ▪ If the NC fails again, than an investigation should be conducted to identify possible causes for error. Check if the site or tools are contaminated. ▪ Repeat the experiment with new aliquots of all reagents.
SARS-CoV-2 not being detected	□ The extraction was performed incorrectly. □ Pipetting errors(control in wrong well, missing a well, inadequate amount of a reagent) □ Use of incorrect reagent □ Degraded reagents due to incorrect storage temperature or exposure to light □Degraded reagents due to repeated thawing and freezing	▪ Repeat the experiment with new aliquots of all reagents.

15. Limitations

▪ All users should be qualified by training or experience to perform molecular diagnostic test procedures.

▪ The test was validated for use only with upper respiratory specimens. Sputum is considered acceptable specimen type for use with **iLAMP Novel-CoV19 Detection Kit** but performance with sputum type has not been established

▪ Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions.

▪ Improper collection, transport, or storage of specimens may impact the ability of the assay to perform as indicated.

▪ False-negative results may arise from: Improper sample collection, storage and transport and resulting in degradation of the SARS-CoV-2 RNA, the presence of RT-PCR inhibitors, mutation in the SARS-CoV-2 virus, and/or failure to follow instructions for use.

▪ This test cannot rule out diseases caused by other bacterial or viral pathogens

▪ Laboratories are required to report results consistent with local, state and federal public health authorities.

16. Performance Characteristics

1) Limit of Detection (LoD) – Analytical Sensitivity

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 iLAMP Novel-CoV19 Detection Kit. A tentative LoD was determined using whole viral genomic RNA (NCCP No. 43326. National Culture Collection for Pathogens) spiked into 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 QIAamp DSP Virus Kit(automated on QIAcube) and run on the CFX 96 Real-Time PCR detection system. Sample input and elution volumes were 250µL and 60 µL, respectively. Three individual extraction replicates per concentration were tested, and results are summarized in Table 13. The lowest target level which all 3 extraction replicates produced positive results was 1 copy/ µL for N gene of SARS-CoV-2

Table 13: Summary of Tentative LoD Study

Category	10,000 copies/uL	1,000 copies/uL	100 copies/uL	10 copies/uL	1 copies/uL	0.1 copies/uL
	N	N	N	N	N	N
Positive/Total	3/3	3/3	3/3	3/3	3/3	3/3
Mean Ct	20.27	21.34	22.77	24.72	27.21	-
SD	0.27	0.32	0.37	0.31	0.76	-

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 QIAamp DSP Virus Kit(automated on QIAcube) with 26 independent extraction replicates. Sample input and elution volumes were 250µL and 60 µL, respectively. RNA was tested on the CFX 96 Real-Time PCR systems. The lowest target level at which at least 95% of 26 replicates produced positive results was 1 copy/µL. Results are shown in Table 14.

Table 14: Summary of Tentative LoD Study

PCR Instrument: BioRad CFX96	
Targe: N	
10 copies/uL	26/26
1 copies/uL	26/26
0.1 copies/uL	20/26

2) Inclusivity (Analytical Sensitivity)

Analytical reactivity (inclusivity) of the iLAMP Novel-CoV19 Detection Kit was evaluated in December 2020 using a total of 7,313 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/)

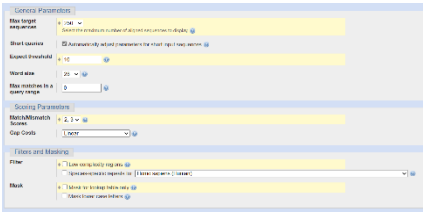
Analysis was performed using the assay's primer sequences against the downloaded SARS-CoV-2 sequences on tool of CLC main workbench 20.0.3 software. All alignments of the primer sets against the SARSCoV-2 sequences showed 100% identity (absence of mismatch base against the SARS-CoV-2 target). In summary,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

3) Cross-Reactivity (Analytical Specificity)

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 **iLAMP Novel-CoV19 Detection Kit** primers 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 sequences targeting the N gene of SARS-CoV-2 showed less than 40% sequence similarity to the target sequences. However, some of the primers showed high homologies to specific microorganism, SARS-coronavirus.
Since the amplification and detection of RT-LAMP requires simultaneous binding of six (6) primers 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 15

Table 15: In silico Cross-Reactivity Analysis of 6 Primers

No.	Organism	Reference No	N Gene					
			F3	B3	FIP	BIP	LF	LB
1	Human coronavirus 229E	NC_002645.1	22.3%	26.4%	15%	27.5%	26.4%	26.4%
2	Human coronavirus OC43	NC_006213.1	11.2%	15.8%	37.5%	20%	15.8%	10.6%
3	Human coronavirus HKU1	NC_006577	22.2%	26.3%	20%	45%	31.5%	10.5%
4	Human coronavirus NL63	NC_005831.2	/	/	/	/	/	/

5	SARS-coronavirus	NC_004718.3	20%	83.3%	40%	28.9%	28.5%	27.2%
6	MERS-coronavirus	KJ556336.1	/	/	/	/	/	/
7	Adenovirus type 1	MH183293.1	/	/	/	/	/	/
8	Adenovirus type 2	J01917.1	/	/	/	/	/	/
9	Adenovirus type 3	AY599836.1	/	/	/	/	/	/
10	Human Metapnemovirus	KJ627437.1	/	/	/	/	/	/
11	Parainfluenza virus 1	KX639498.1	/	/	/	/	/	/
12	Parainfluenza virus 2	KM190939.1	/	/	/	/	/	/
13	Parainfluenza virus 3	NC_001796.2	/	/	/	/	/	/
14	Parainfluenza virus 4	JQ241176.1	/	/	/	/	/	/
15	Influenza A	GCF_000865085.1	/	/	/	/	/	/
16	Influenza B	BLee1940	/	/	/	/	/	/
17	Enterovirus	NC_001472.1	/	/	/	/	/	/
18	Respiratory syncytial virus	NC_001803.1	/	/	/	/	/	/
19	Rhinovirus	NC_009996.1	/	/	/	/	/	/
20	Chlamydia pneumoniae	NC_005043.1	/	/	/	/	/	/
21	Haemophilus Influenzae	NZ_LN831035.1	/	/	/	/	/	/
22	Legionella pneumophila	NZ_LR134380.1	/	/	/	/	/	/
23	Mycobacterium tuberculosis	NC_000962.3	/	/	/	/	/	/
24	Streptococcus pneumoniae	NZ_LN831051.1	/	/	/	/	/	/
25	Streptococcus pyogenes	NZ_LN831034.1	/	/	/	/	/	/
26	Bordetella pertussis	NC_018518.1	/	/	/	/	/	/
27	Mycoplasma pneumoniae	NZ_CP010546.1	/	/	/	/	/	/
28	Pneumocystis jirovecii	CAKM01000281.1	/	/	/	/	/	/
28	Candidua albicans	GCA_003454745.1	/	/	/	/	/	/
29	Pseudomonas aeruginosa	NC_002516.2	/	/	/	/	/	/
30	Staphylococcus epidermidis	NZ_CP035288.1	/	/	/	/	/	/
31	Streptococcus salivarius	GCF_900636435.1	/	/	/	/	/	/
32	Streptococcus aureus	BX571856.1	/	/	/	/	/	/

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 16 as tested using three extraction replicates at concentrations of 105 FU/mL or higher for bacteria and 105 pfu/mL or higher for viruses. No detectable amplification curve (Ct) was observed in the SYBR Green detection channel for the SARS-CoV-2 when using the CFX 96 platform. However, some of the primers showed high homologies to specific microorganism, SARS-coronavirus.

Since the amplification and detection of RT-LAMP requires simultaneous binding of six (6) primers 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, which ensures specificity for SARS-CoV-2. Also, SARS-CoV, is not currently prevalent and poses minimal detection risk. Furthermore, the reaction mix includes primers specific for SARS-CoV-2.

Table 16: Wet Testing Cross-Reactivity Analysis of 6 Primers

No.	Organism	Source	% Detection
			N
1	Human coronavirus 229E	KBPV[a]-VR-9	0% (0/3)
2	Human coronavirus OC43	KBPV-VR-8D	0% (0/3)
3	Human coronavirus HKU1	Clinical specimen[b]	0% (0/3)
4	Human coronavirus NL63	KBPV-VR-88D	0% (0/3)
5	SARS-coronavirus	Clinical specimen	100% (3/3)
6	MERS-coronavirus	Clinical specimen	0% (0/3)
7	Adenovirus type 1	Clinical specimen	0% (0/3)
8	Adenovirus type 2	Clinical specimen	0% (0/3)
9	Adenovirus type 3	Clinical specimen	0% (0/3)
10	Human Metapneumovirus	KBPV-VR-86	0% (0/3)
11	Parainfluenza virus 1	Clinical specimen	0% (0/3)
12	Parainfluenza virus 2	Clinical specimen	0% (0/3)
13	Parainfluenza virus 3	Clinical specimen	0% (0/3)
14	Parainfluenza virus 4	Clinical specimen	0% (0/3)
15	Influenza A	KBPV-VR-32D	0% (0/3)
16	Influenza B	KBPV-VR-34D	0% (0/3)
17	Enterovirus	KBPV-VR-55D	0% (0/3)
18	Respiratory syncytial virus	Clinical specimen	0% (0/3)
19	Rhinovirus	Clinical specimen	0% (0/3)
20	Chlamydia pneumoniae	Clinical specimen	0% (0/3)
21	Haemophilus Influenzae	CCARM[c] 9257	0% (0/3)
22	Legionella pneumophila	KCCM 41777	0% (0/3)
23	Mycobacterium tuberculosis	Clinical specimen	0% (0/3)
24	Streptococcus pneumoniae	Clinical specimen	0% (0/3)
25	Streptococcus pyogenes	KCCM 11817	0% (0/3)
26	Bordetella pertussis	NCCP 13671	0% (0/3)
27	Mycoplasma pneumoniae	Clinical specimen	0% (0/3)
28	Pneumocystis jirovecii	Clinical specimen	0% (0/3)
29	Candidua albicans	KCCM 11282	0% (0/3)
30	Pseudomonas aeruginosa	CCARM 0220	0% (0/3)
31	Staphylococcus epidermidis	KCCM 35494	0% (0/3)

32	Streptococcus salivarius	KCCM 11926	0% (0/3)
33	Streptococcus aureus	NCCP 15920	0% (0/3)

[a]KBPV: Korean bank of pathogenic virus

[b]Clinical isolate: Clinical isolates in department of laboratory medicine, Hospital of Danguk University, Korea.

[c]Culture Collection of Antimicrobial Resistance Microbes, Korea

3) Endogenous Interference Substances Studies

Interfering substances studies were performed using nasopharyngeal swab specimens collected from healthy (previously confirmed to be negative) individuals spiked with and without SARSCoV-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 validated extraction methods. Each substance was tested at the highest medically relevant concentration in three replicates for both positive and negative contrived samples. Results indicate that iLAMP Novel-CoV19 Detection Kit can tolerate the substances at the concentrations tested without significant interference. The results are summarized in Table 17.

Table 17: Summary of Interference Studies

Category		Whole blood	mucin(Bovine submaxillary galnd, type 1-S)	Afrin Original Nasal spray	Whituben Nasal Spray	Otrivin Nasal Spray	GoodSense All Day Allergy, Ceririzine HCL tablets 10mg	Cepacol Sore Throat(Benxocaine/ Menthol)	Zanamivir	Tamiflu	Mupirocin Nasal Ointment	Tobra-mycin
		2.5% v/v	2.5mg/ml	15%	0.1% v/v	0.05% v/v	1mg/ml	5mg/ml	3.3mg/ml	2.2ug/ml	5.4mg/ml	3ug/ml
Positive	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)	100%(3/3)
Negative	N	0%(3/3)	0%(3/3)	0%(3/3)	0%(3/3)	0%(3/3)	0%(3/3)	0%(3/3)	0%(3/3)	0%(3/3)	0%(3/3)	0%(3/3)

4) Clinical Evaluation

A clinical study was performed to evaluate the clinical performance of the **iLAMP Novel-CoV19 Detection Kit** using leftover nasopharyngeal swab specimens collected from the Department of Laboratory medicine, Hospital of Danguk University, Republic of Korea. RNAs were extracted from blinded 250 ul of clinical specimens using the QIAamp DSP Virus Kit(automated on QIAcube) according to the instructions supplied with the kit and eluted with 60 ul of elution buffer. A total 80 clinical specimens including 40 positive(12 low-positives), and 40 negative nasopharyngeal swabs were analyzed on CFX 96 Real time PCR system. Specimens were previously tested using an EUA authorized molecular test STANDARD MnCoV Real-Time Detection Kit targeting ORF1ab and E genes of the SARSCoV-2. The positive and negative controls were included per run for the validation. Both positive percent agreement (PPA) and negative percent agreement (NPA) between the 2 assays were 100% (PPA 40/40, NPA 40/40). The results are summarized in Table 18.

Table 18: Summary of Interference Studies

Target		STANDARD MnCoV Real-Time Detection Kit (SDbiosensor)		
		Positive	Negative	Total
iLAMP Novel-CoV19 Detection Kit	Positive	40	0	40
	Negative	0	40	40
	Total	40	40	80
Positive Agreement		100% (92.00-100)		
Negative Agreement		100% (92.00-100)		

17. Contact Information and Technical Support

Information and technical support can be obtained from:

Contact: iONEBIO Customer Support
Email: iONEBIO@iONEBIO.com
Phone: +82-031-360-1210
Website: www.ionebio.com