Rapid COVID-19 PoaCheck™ Coronavirus (2019-nCoV) Detection Kit

INSTRUCTIONS FOR USE (IFU)

For in vitro diagnostic use Only

For Prescription (Rx) use Only

BIOPOA

Revision No.: LAMP-100-01(Rev.00)

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1. Intended use

Rapid COVID-19 PoaCheck™ is a real-time RT-PCR test intended for the qualitative detection of RNA from the SARS-CoV-2 in nasopharyngeal, nasal, and oropharyngeal swab specimens from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories - certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests, or by similarly qualified non-U.S. laboratories.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper respiratory 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 positive results to the appropriate public health authorities.

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

The Rapid COVID-19 PoaCheck™ is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. Validation of this test has not been reviewed by FDA - Review under the EUA program is pending.

2. Summary and Explanation of the Test

Rapid COVID-19 PoaCheck™ (Orf1a gene) kit was designed to amplify the Orf1a target gene of COVID-19 (2019-nCoV) in an isothermal reaction condition.

LAMP (Loop-Mediated Isothermal Amplification) consists of *Bst* polymerase which has the strand displacement DNA synthesis and 3 primer sets. First, the inner primer binds to the DNA and elongates, causing strand displacement, and the first strand is released. The loop structure is formed from the 5'-end of the separated single strand, and the same process is repeated at the 3'-end and the loop structure is extended. To perform LAMP, four different primers designed specifically to recognize the six distinct positions of the gene to be amplified are used. An additional pair of "loop primers" can further accelerate the reaction. Compared to the general PCR recognition of two positions, LAMP has a very high specificity for the target DNA. The primers used in this product are designed to amplify the Orf1a gene of COVID-19.

Rapid COVID-19 PoaCheck™ (Orf1a gene) kit was designed to amplify the Orf1a target gene of COVID-19 (2019-nCoV) in an isothermal reaction condition. LAMP (Loop-Mediated Isothermal Amplification) consists of Bst polymerase which has the strand displacement DNA synthesis and 3 primer sets. First, the inner primer binds to the DNA and elongates, causing strand displacement, and the first strand is released. The loop structure is formed from the 5'-end of the separated single strand, and the same process is repeated at the 3'-end and the loop structure is extended. To perform LAMP, four different primers designed specifically to recognize the six distinct positions of the gene to be amplified are used. An additional pair of "loop primers" can further accelerate the reaction. Compared to the general PCR recognition of two positions, LAMP has a very high specificity for the target DNA. The primers used in this product are designed to amplify the Orf1a gene of COVID-19.

Compared to the general PCR method which recognizes two positions, LAMP has higher specificity because the primers are designed to recognize six gene positions.

Due to the characteristics of isothermal amplification, there is no loss or damage of RNA/DNA from temperature change. The amplification efficiency is very high and since temperature control is unnecessary, it makes the response time becomes short (65℃, 30 min).

3. Principles of the Procedure

The Rapid COVID-19 PoaCheck™ is a real-time reverse transcription polymerase chain reaction (rRT-PCR) test. The SARS-CoV-2 primer and probe set(s) are designed to detect RNA from the SARS-CoV-2 in nasopharyngeal, nasal, or oropharyngeal swabs from patients suspected of COVID-19 by their healthcare provider.

Test Principle

Rapid COVID-19 PoaCheck™ is a diagnostic kit with a method of amplifying a specific part of the Orf1a gene of the new coronavirus via LAMP method for 30 minutes at 65°C. Results are confirmed through the monitor of a real time PCR machine, and positive test results are measurable within 5 to 20 minutes while 10 more minutes are required to confirm the negative.

Loop Mediated Isothermal Amplification (LAMP)

The Loop-mediated Isothermal Identification (LAMP) diagnostic method is an isothermal gene amplification method first developed by Notomi and others in 2000, but unlike the Taq DNA polymerase used in conventional PCR-based diagnostics, using Bst DNA polymerase can amplify the target gene in large quantities through the strand displacement method. Therefore, the LAMP diagnostic method is much more efficient. In addition, unlike conventional PCR-based diagnostics, genetic amplification is performed in isothermal conditions with no temperature change, which not only reduces test time, but also allows low-cost equipment such as water bath tanks to react. Also, the specificity is higher than existing PCR-based diagnostic methods because amplification is done using 4-6 specific primer sets that are unique in 6-8 areas of the target gene, and it has high sensitivity with an amplification efficiency of 109 copies or higher given an hour of reaction time.

Due to the nature of LAMP, which reacts at isothermal conditions, it can only be inspected with equipment that maintains a constant temperature, so it can be easily used in front-line clinical diagnosis rooms or clinical sites that do not have expensive special equipment or professional personnel. With such advantages, LAMP has recently been used worldwide to diagnose various pathogens in both humans and animals.

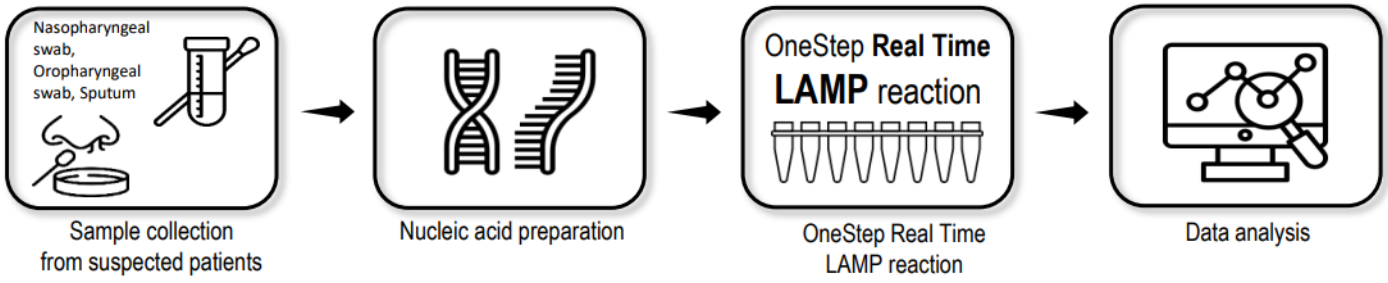
In a LAMP PCR, four primers, forward outer primer, forward inner primer, backward outer primer, and backward inner primer, are used. Amplification initiates from strand invasion by forward inner primer. DNA polymerase extends the primer and separates the target DNA duplex. The first product is then displayed by the synthesis initiated by forward outer primer, which anneals to the upstream target region. As the first product is displaced, the 5’ end of the product forms a self-hybridizing loop structure due to inclusion of a reverse primer sequence in the forward inner primer sequence. This annealing and displacement cycle repeats on the opposite end of the target sequence. And the resulting product is LAMP dumbbell (both ends flanked by loops) structure that serves as a seed for amplification. This LAMP dumbbell structure has multiple sites for initiation of synthesis. From the 3’ ends of open loops, and both forward and backward inner primers, as amplification proceeds from these sites, the product grow and form long concatemers, each with more sites of initiation. The result is rapid accumulation of products and amplification byproducts that can be detected by a variety of methods.

A detailed video explanation of LAMP principle can be found at:
https://www.youtube.com/watch?v=L5zi2P4lggw

Detection Target

Target virus	Target genes	Channel
SARS-CoV-2	ORF1a	FAM

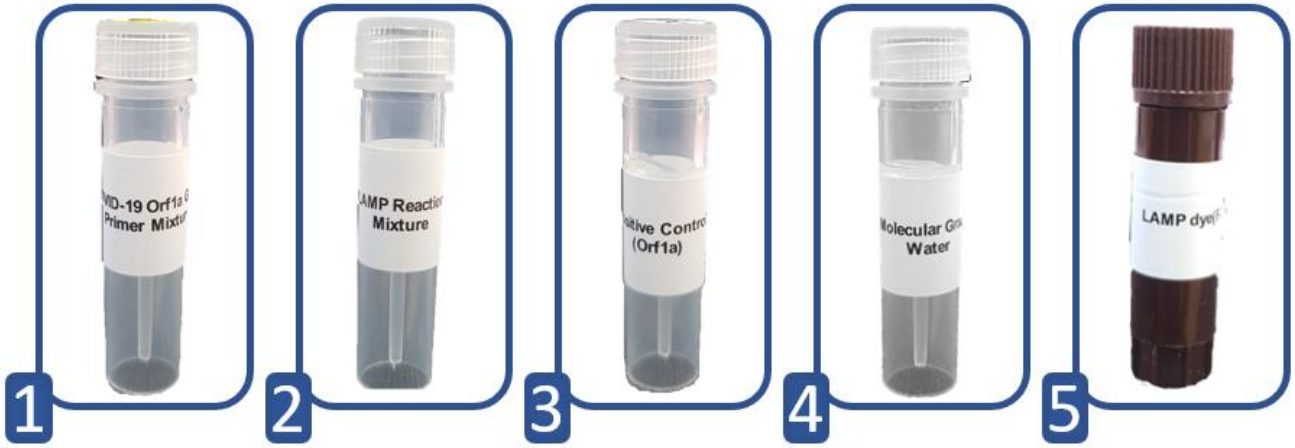
Testing Process: Summary



4. Assay Materials

Materials Provided

Number	Components	Cap label	Volume	Storage
1	COVID-19 Orf1a gene primer mixture	Orf	300 ㎕, 1 ea	–20 to –15℃
2	LAMP reaction mixture	Mix	1 mL, 1 ea	–20 to –15℃
3	Positive control (Orf1a)	OC	30 ㎕, 1 ea	–20 to –15℃
4	Molecular grade water	DW	1 mL, 1 ea	–20 to –15℃
5	LAMP dye (FAM)	Dye	40 ㎕, 1 ea	–20 to –15℃



The Rapid COVID-19 PoaCheck™ can be shipped and stored at –20 to –15℃ until the expiration date printed on the label. All components of the Rapid COVID-19 PoaCheck™ are must be stored at –20 to –15℃, before and after opening. Do not repeat the freeze/thaw procedure more than 3 times after opening. Exposure to light, heat, or humidity may affect the shelf life of some of the kit components and should be avoided.

Materials required but not provided

- Real-time PCR instrument and equipment
- Laboratory freezers -20~-15℃
- Centrifuge with a rotor for 2 mL reaction tubes
- Centrifuge with a rotor for microtiter plates, if using 96 well reaction plates
- Laboratory mixer, Vortex or equivalent
- Pipettes (adjustable 1.00 µL to 1,000.00 µL)
- Cold block or ice
- Appropriate nucleic acid extraction system or kit:
- Nuclease-free water (not DEPC-treated)

- Disposable polypropylene micro-tubes or 96 well reaction plates with corresponding (optical) closing materials

5. Warnings and Precautions

- For in vitro diagnostic use (IVD) only.
- For prescription use only.
- Laboratories should include a statement such as 'the test has been validated but FDA's independent review of this validation is pending' in test reports to healthcare providers.
- Specimens should always be considered potentially infectious and handled in accordance with safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus disease 2019 (COVID-19). https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html.
- Specimen processing should be performed in accordance with national biological safety regulations.
- If infection with 2019-nCoV is suspected based on current clinical screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where reagents and human specimens are handled.
- Perform all manipulations of live virus samples within a class II (or higher) biological safety cabinet.
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents while performing this assay and handling materials including samples, reagents, pipettes, and other equipment and reagents.
- Wash hands thoroughly after handling specimens and reagents.
- The Rapid COVID-19 PoaCheck™ is a single-use device.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- Do not use a kit after its expiration date. The product will maintain performance through the control date printed on the label.
- Material Safety Data Sheets (MSDS) can be requested through BioPOA website (www.biopoa.co.kr) or e-mail (biopoa@biopoa.co.kr).
- The laboratory process must be one-directional, it should begin in the Extraction Area move to the Amplification and Detection Area. Do not return samples, equipment, and reagents in the area where you performed the previous step.
- Please be careful not to contaminate the Primer/Reaction mixture and LAMP dye (FAM) with PCR products or Positive control (Orf1a) through pipetting. To prevent contamination, use of filter tips is recommended.
- Store extracted positive materials (samples, controls, and other amplicons) away from all other reagents and add it to the reaction mix in a separate area.
- Thaw all components thoroughly on ice before starting, experiment. When thawed, mix the components and centrifuge briefly.
- Dispose of all samples that have come into contact with specimens and reagents in accordance with applicable national, international, and regional regulations.

- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectants.
- Avoid contact of specimens and reagents with the skin, eyes, and mucosa. If contact with skin, eyes, and mucosa, immediately flush with water and seek medical attention.

6. Storage and Handling Conditions

1) Carefully read this instruction before starting the procedure.

2) Clinical samples should be regarded as potentially infectious materials and prepared the LAMP reaction mixture is to be prepared in a laminar flow hood.

3) Do not use the kit after its expiration date written on box.

4) Avoid repeatedly thawing and freezing of the reagents. This may cause an inaccurate test result.

5) Once the reagents have been thawed, they are to be vortexed, and spun down briefly the tubes before use.

6) Quickly prepare the reaction mixture on ice.

7) Always use sterile pipette tips with filters.

8) Wear separate coats and gloves in each area.

9) Collect test samples in sterile tubes.

10) Test samples should be extracted immediately or frozen at -20℃ to -80℃.

7. Assay Control Material

Controls that will be provided with the test kit include:

a) A “no template” (negative) control is needed to monitor contamination on the assay run and is used with every batch. The molecular grade water included in the kit is used as a negative control. A negative control tube is prepared as below and run alongside the samples on each LAMP RT-PCR run.

Component	Volume (µL)
Negative control (molecular grade water)	1.0
Primer mixture	3.0
LAMP RT-PCR Reaction mixture (dNTP, DNA polymerase, and reverse transcriptase)	10.0
LAMP dye	0.4
Molecular grade water	4.6 – 1.6
Total	20

b) A positive template control is needed to verify that the assay is working as intended and is used with every batch.

Positive control material is composed of 2019 nCoV-19 Orf1a plasmid DNA at a concentration of 40 pg/µL. Each test kit contains 30 µL in one vial. A tube containing the positive control should be prepared as follow, alongside with tubes containing the RNA samples, and run together with the samples. The limit of detection (LOD) of the kit is 1 copy of the Orf1a gene/µL.

Component	Volume (µL)
Positive control	1.0
Primer mixture	3.0
LAMP RT-PCR Reaction mixture (dNTP, DNA polymerase, and reverse transcriptase)	10.0
LAMP dye	0.4
Molecular grade water	4.6 – 1.6
Total	20

c) An internal control is not needed because, thorough analytical specificity experiment for interference, we have validated that materials potentially arising from or during the sampling do not cause inhibition of LAMP-PCR methods employed in our test. When in doubt, we recommend the negative samples be re-tested with increased amount of the sample in question.

8. PCR Procedures

Sample collection, transport, and storage

Collect Nasopharyngeal swab (NP), oropharyngeal swab (OP) or nasal swab according to CDC guidelines and/or manufacturer's protocol for sample collection, storage and handling.

Nucleic acid extraction

The assay was validated with the extraction options listed below. Perform the RNA extraction on samples according to the manufacturer's instructions for use.

Instrument / Extraction kit	Manufacturer	Specimen input volume	Lysis buffer	Elution volume
QIAamp viral RNA mini kit	QIAGEN	140 uL	560 uL	50 uL
MagNA pure 96 /DNA and Viral RNA Small Volume kit	Roche	250 uL	-	50 uL

Amplification and Detection

* Use the reagents which are stored at –20℃. Once they are melted, do a brief spin down before use.

* Be careful of contamination when you use the positive control for amplification.

1) Please make the reaction mixture on the ice. Mix the reagents well according to the table below.

No. of Reactions (Unit: $\mu\ell$)	1	8	16	32
COVID-19 Orf1a gene primer mixture	**3.0**	**24**	**48**	**96**
LAMP reaction mixture	**10**	**80**	**160**	**320**
Molecular grade water	**4.6-1.6**	**36.8-12.8**	**73.6-25.6**	**147.2-51.2**
LAMP dye	**0.4**	**3.2**	**6.4**	**12.8**
RNA (*positive control: 1$\mu\ell$)	**2.0-5.0**	**2.0-5.0**	**2.0-5.0**	**2.0-5.0**
Total	**20**	**160**	**320**	**640**

2) Mix well by vortexing thoroughly and then spinning down.

3) Add aliquot 15-18 $\mu\ell$ of one-step RT-LAMP master mix to each PCR tube.

4) Add positive and negative control (DW) to each PCR tube.

* It is highly recommended that the mixture for negative control should be made separately to avoid cross contamination.

5) Close the lid of PCR tube and then spin down briefly to remove any bubbles.

6) Real time RT-LAMP reaction should be done within 30 minutes.

7) Set the program as below table.

Step	No. of Cycle	Temperature	Duration	Threshold setting	Fluorophore
1	40	65℃	45 sec	14,000	FAM

8) Plate setup

- Type the sample names in the each tube.

* Unknown: Clinical sample

* Negative control

* Positive control

9) Click Start Run

Multiple reactions can be setup with the following tube/strips.

Instrument	Manufacturer	Plate / Tube	Cat. #	Package
ABI 7500	Thermo Fisher scientific	MicroAmp Optical 8-tube strip	4316567	125strips/pack
		MicroAmp Optical 8-cap strip	4323032	300strips/pack
		MicroAmp Optical Adhesive Film	4311971	100sheets/pack
		MicroAmp® Optical 96-Well Reaction Plate	N8010560	10plates/pack
ABI 7500 Fast		MicroAmp Fast 8-tube strip	4358293	125Strips/pack
		MicroAmp Optical 8-cap strip	4323032	300strips/pack
		MicroAmp® Fast Optical 96-Well Reaction Plate	4346907	10plates/pack
		MicroAmp Optical Adhesive Film	4311971	100sheets/pack
CFX 96	Bio-Rad	0.2 ml 8-Tube PCR Strips without Caps, low profile, white	TLS-0851	120strips/pack
		0.2 ml 8-Tube PCR Strips without Caps, low profile, clear	TLS-0801	120strip/pack
		Optical Flat 8-cap strips for 0.2ml tube	TCS-0803	120strips/pack
		Multiplate PCR plates 96 well, white, low-profile	MLL9651	25plates/pack
		Microseal 'B' Seal ('B' Clear Adhesive Seal)	MSB-1001	100sheets/pack

9. Reading the Result

Interpretation of Results

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

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. If Ct value of the sample is equal to or less than 30 cycles, the sample is positive for SARS-CoV-2. If the Ct value is greater than 30 cycles, conduct a retest. Although it is not necessary to review fluorescent curves, we recommend reviewing the curves to determine a retest.

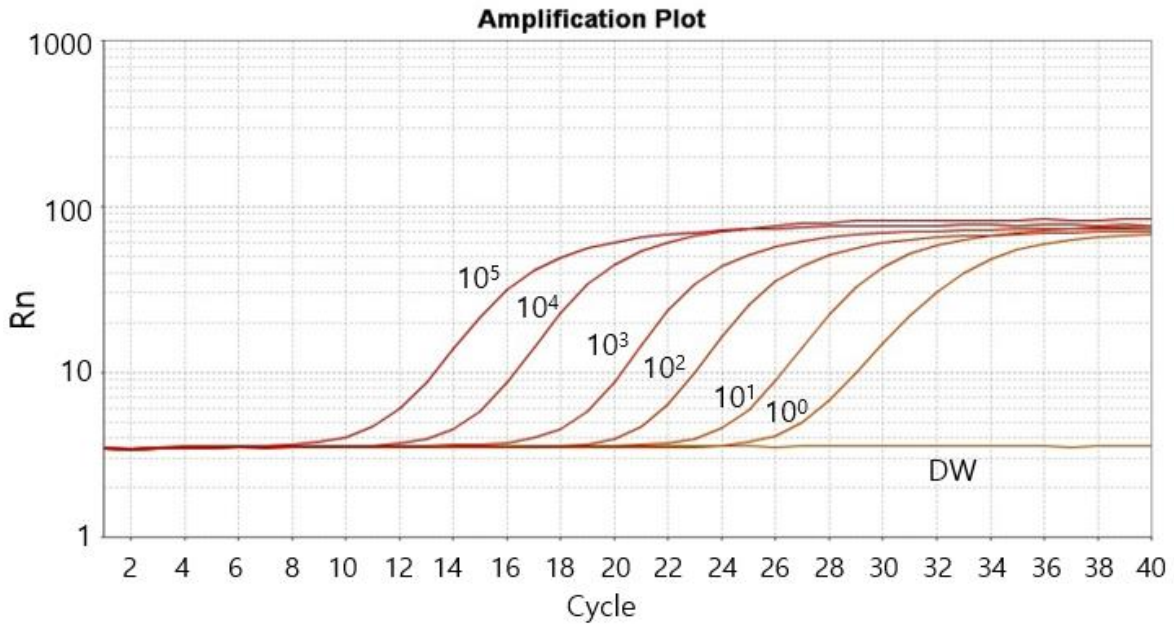
Positive control: the amplification curve for the positive control should appear as sigmoidal in shape, and its Ct value should be 20±2 cycles.

Negative control: negative control should not be detectable

Invalid control results: repeat extraction, and RT-PCR. If additional clinical sample is unavailable, report Invalid or inconclusive results, which will request a new specimen be collected, if clinically indicated.

Case	Positive control	Negative control	Orf1a gene	Interpretation (Ct Value)
1	+	-	+	2019-nCoV detected (≤30)
2	+	-	-	2019-nCoV not detected (>30)
3	+	-	+	retest (>30)
4	+	+	+/-	Invalid result / retest
5	-	+	+/-	
6	-	-	+/-	

LAMP RT-PCR reaction takes about 30 minutes. A cut-off value for positive is less than or equal to 30 cycles.



Inconclusive Results

Problems	Possible Causes	Action
No fluorescent signal is detected in any samples, including positive control	Error in the preparation of the master mixture	Ensure the volumes of reagent dispensed during preparation of the master mixture are correct.
	Probe degradation	Use a new probe aliquot.
	Omitted components	Verify each component and repeat the PCR mixture preparation.
	Instrument settings error	Verify the rRT-PCR instrument settings are correct.
If the fluorescent signal is detected in a negative control reaction.	Carry-over contamination	Change tips between samples; clean pipettes; use filter tips.
	Tube cap not properly sealed	Ensure plates are sealed correctly.
	Contamination of the master mixture	Prepare a new master mix and retest samples from RT-PCR.
	Contamination of the extraction/preparation area	Clean surfaces and instruments with aqueous detergents, wash lab coats, and replace test tubes and tips in use.
If the fluorescent signal does not exhibit sigmoidal characteristic in the patient samples.	Poor quality of RNA samples	Extract RNA from samples and store the extracted RNA at -70 ^{0}C.
	Not enough volume of RNA samples added	Repeat samples from RT-PCR.
If the fluorescent signal does not exhibit sigmoidal characteristic in the positive control	Probe degradation	Use a new probe aliquot. Repeat samples from RT-PCR
If inconsistent Intensity of fluorescent signals appear	Pipetting error	Repeat samples from RT-PCR
	Contamination in the outer surface of PCR tubes and plate	Repeat samples from RT-PCR
	Bubbles in wells	Repeat samples from RT-PCR

10. Assay Limitations

- Validation of this test has not been reviewed by FDA - Review under the EUA program is pending.
- The use of this assay is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.
- Use of this assay is limited to personnel who are trained in the procedure. Failure to follow these instructions may result in erroneous results.
- SARS-CoV-2 may mutate in one or more of the target regions of the AllplexTM 2019-nCoV Assay. If this occurs, then SARS-CoV-2 may not be detected.
- Based on the in silico analysis, SARS-CoV and other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with the AllplexTM 2019-nCoV Assay. SARS-CoV is not known to be currently circulating in the human population, therefore is highly unlikely to be present in patient specimens.
- Samples must be collected, transported, and stored using appropriate procedures and conditions.
- False negative results may arise from improper specimen collection, handling, and degradation of the viral RNA during shipping/storage.
- Detection of viral RNA may not indicate the presence of infectious virus or that 2019-nxtraction 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.
- Avoid contamination by adhering to good laboratory practices and to the procedures specified in this package insert.
- False positive results may happen from cross- contamination between patient samples, specimen mix-up and RNA contamination during product handling.
- 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.
- A positive result indicates the detection of nucleic acid from the relevant virus. Nucleic acid may persist even after the virus is no longer viable.

11. Performance Evaluation

Validated Instruments

The following instruments were validated with Rapid COVID-19 PoaCheck.

PCR instrument	Test Items	Test result	Extraction kits	Sample type
CFX96™, bio-Rad	Clinical Evaluation	Positive concordance rate(%): 95.56% Negative concordance rate(%): 100% Overall concordance rate(%): 97.78%	QIAamp Viral RNA Mini Kit (Cat No./ID: 52906)	Based on random selection for stored residual specimens. Upper airway(Oropharyngeal / Nasopharyngeal Swab) residual specimens that are scheduled to be discarded by patients who have been examined and reported for the purpose of detecting the SARS-CoV-2 virus in the Seoul Clinical Laboratory.
CFX96™, bio-Rad	Precision (Reproducibility)	Positive matching rate: 100% Negative matching rate: 100% Total matching rate: 100%	QIAamp Viral RNA Mini Kit (Cat No./ID: 52906)	in vitro transcription RNA
Applied Biosystems™ 7500 Fast real time PCR			QIAamp Viral RNA Mini Kit (Cat No./ID: 52906)	in vitro transcription RNA
Applied Biosystems™ 7500 Fast real time PCR	Analytical sensitivity (Detection Limit)	LoD: 1 copies / μL	QIAamp Viral RNA Mini Kit (Cat No./ID: 52906)	in vitro transcription RNA
	Analytical specificity (Interference)	No difference in results due to interfering substances. Positive matching rate: 100% Negative matching rate: 100%	QIAamp Viral RNA Mini Kit (Cat No./ID: 52906)	in vitro transcription RNA

PCR instrument	Test Items	Test result	Extraction kits	Sample type
		Total matching rate: 100%		
	Analytical specificity (Cross)	Rapid COVID-19 PoaCheck showed Ct of 17.70, 17.88, and 17.98 in the positive control group as the target virus strain, and the target nucleic acid was not amplified or detected in other bacteria and virus standard strains.	QIAamp Viral RNA Mini Kit (Cat No./ID: 52906)	in vitro transcription RNA
	Precision (Repeatability)	Positive matching rate: 100% Negative matching rate: 100% Total matching rate: 100%	QIAamp Viral RNA Mini Kit (Cat No./ID: 52906)	in vitro transcription RNA
	Accuracy	All 3LOTs did not deviate from the 95% confidence interval.	QIAamp Viral RNA Mini Kit (Cat No./ID: 52906)	in vitro transcription RNA
	Cross contamination	Rapid COVID-19 PoaCheck proved to be a very stable diagnostic reagent with no cross contamination.	QIAamp Viral RNA Mini Kit (Cat No./ID: 52906)	in vitro transcription RNA
	Shipping unopened kit, stability data	Packaging option capable of maintaining refrigeration temperatures between 2℃ and 8℃ for up to 108 hours. We confirmed that the product performance was	QIAamp Viral RNA Mini Kit (Cat No./ID: 52906)	in vitro transcription RNA

PCR instrument	Test Items	Test result	Extraction kits	Sample type
		not affected by transportation.		
	In-use/opened kit stability data	It was confirmed that there is no abnormality in the performance of the product in use and is stable.	QIAamp Viral RNA Mini Kit (Cat No./ID: 52906)	in vitro transcription RNA
	Inverted kit stability data	The stability of the product was confirmed in the Inverted kit stability test.	QIAamp Viral RNA Mini Kit (Cat No./ID: 52906)	in vitro transcription RNA

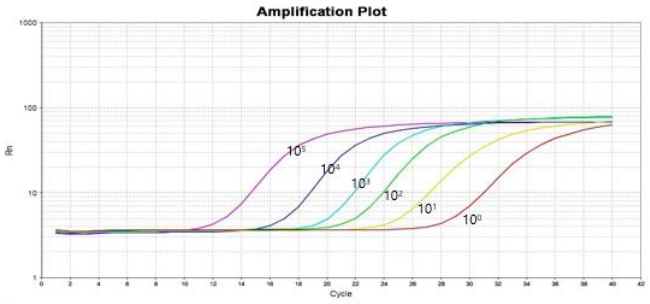
Limit of Detection (LoD) - Analytical Sensitivity:

- Materials
  - Sample material used: in vitro transcribed viral RNA
  - Sample material source: nasopharyngeal swab
  - Negative control: molecular grade distilled water
  - Positive control: SARS-CoV-2 Orf1a plasmid DNA
- RNA preparation and extraction
  - Sample viral mRNA was synthesized via in vitro transcription. Concentration was adjusted by diluting the synthesized mRNA with distilled water, and diluted to a concentration ranging from 100,000 copies/µL to 1/µL.
  - Diluted samples were extracted by Qiagen or Roche RNA extraction kits (described above). There was no observable differences between the samples extracted by the two kits. Extraction was performed in accordance with the manufacturer's recommended protocol.
- Testing method
  - Prepared 20 samples as 1 x 10^5 copies/µL and each sample was serially diluted to 1 copies/µL.
  - LAMP RT-PCR assay was performed per instruction for use.
- Selection criteria
  - Negative control: no detectable amplification
  - Positive control: Ct. value of 20±2 cycles
  - LOD: 95% positive detection
  - CV value: must be less than 10%
- Test result

- LOD: 1 copies/μL

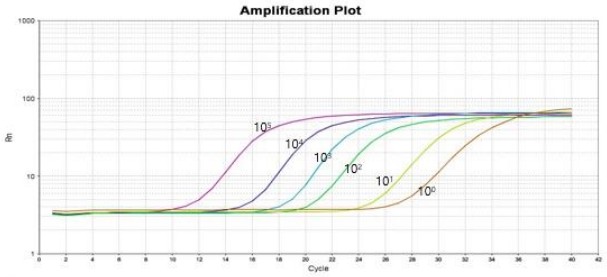
PCR instrument	Target	Positive Rate	Limit of Detection	Unit
Applied Biosystems™ 7500 Fast real time PCR	ORF1a	20/20	1 X 10^0	copies/μL

Result of 1st test on June 8, 2020



1st	1x10⁵	1x10⁴	1x10³	1x10²	1x10¹	1x10⁰
Ct	11.87	16.64	19.67	21.66	24.61	28.32

Result of 20th test on June 11, 2020



20th	1x10⁵	1x10⁴	1x10³	1x10²	1x10¹	1x10⁰
Ct	11.47	15.82	18.59	20.54	24.96	27.34

Cross-reactivity (Analytical Specificity):

Cross-reactivity study was performed using RNAs of viral strains and DNAs of bacterial strains obtained from Centers for Disease Control and Prevention at Chonbuk National University Hospital.

NO.	Name	Results	Concentration
1	human coronavirus HKU1	Negative (-)	10^5 pfu/mL
2	human coronavirus OC43	Negative (-)	10^5 pfu/mL
3	human coronavirus NL63	Negative (-)	10^5 pfu/mL
4	Human parainfluenza virus 2	Negative (-)	10^5 pfu/mL
5	Adenovirus	Negative (-)	10^5 pfu/mL
6	Human Metapneumovirus (MPV)	Negative (-)	10^5 pfu/mL
7	human respiratory syncytial virusB	Negative (-)	10^5 cfu/mL
8	Human rhinovirus A/B	Negative (-)	10^5 cfu/mL
9	Brevibacterium casei	Negative (-)	10^5 cfu/mL
10	Micrococcus luteus	Negative (-)	10^5 cfu/mL
11	Streptococcus pyogenes	Negative (-)	10^5 cfu/mL
12	Streptococcus mitis/oralis	Negative (-)	10^5 cfu/mL
13	Serratia marcescens	Negative (-)	10^5 cfu/mL
14	Entrobacter aerogenes	Negative (-)	10^5 cfu/mL
15	Klebsiella oxytoca	Negative (-)	10^5 cfu/mL
16	Staphylococcus warneri	Negative (-)	10^5 cfu/mL
17	Proteus mirabilis	Negative (-)	10^5 cfu/mL
18	Citrobacter freundii	Negative (-)	10^5 cfu/mL
19	Enterococcus faecalis	Negative (-)	10^5 cfu/mL
20	Streptococcus agalactiae	Negative (-)	10^5 cfu/mL

NO.	Name	Results	Concentration
21	Staphylococcus epidermidis	Negative (-)	10^5 cfu/mL
22	Enterobacter cloacae ssp cloacae	Negative (-)	10^5 cfu/mL
23	Propionibacterium acnes	Negative (-)	10^5 cfu/mL
24	Dermabacter hominis	Negative (-)	10^5 cfu/mL
25	Stenotrophomonas maltophilia	Negative (-)	10^5 cfu/mL
26	Acinetobacter baumanii	Negative (-)	10^5 cfu/mL
27	Pseudomonas aeruginosa	Negative (-)	10^5 cfu/mL
28	Streptococcus equi	Negative (-)	10^5 cfu/mL
29	Escherichia coli	Negative (-)	10^5 cfu/mL
30	Corynebacterium striatum	Negative (-)	10^5 cfu/mL
31	Klebsiella pneumoniae	Negative (-)	10^5 cfu/mL
32	COVID-19 (Positive Control)	Positive (+)	

Inclusivity

Blastn sequence alignment was conducted against known published sequences with test kit's amplicon sequence (565 nucleotides). Results show 100% match to all SARS-CoV-2 sequences deposited in NCBI Nucleotide Sequence Bank. See attached in silico data analysis.

In addition, test kits primer sequences was aligned to 718 Orf1a genes deposited in EpiCoV database (managed by GISAID) using ClustalW. Results show 100% to all targets. See attached in silico data analysis.

12. Clinical Evaluation

Sample matrix tested.

Samples were collected from upper airway(oropharyngeal, nasal or nasopharyngeal swab).

Sample type

Specimens collected from patients who have been examined and reported for the purpose of detecting the SARS- CoV-2 virus in the Seoul Clinical Laboratory(SCL).

The comparator method used

Clinical validity assessment performed by a single-center, randomized, single-blind and retrospective to compare the performance of "COVID-19 PoaCheck" with EUA approved "Allplex™ 2019-nCoV Assay" (Seegene).

Test were carried out on the collected specimens using a test reagent(COVID-19 PoaCheck) and compared with the results of an RT-PCR(control reagent: Allplex™ 2019-nCoV Assay) test on the same specimens.

The Instrument used during the validation

The CFX96™, bio-Rad was used for the clinical evaluation.

The extraction method used during the validation

The QIAamp Viral RNA Mini Kit (Cat No./ID: 52906) was used.

Results

Of the 45 positive specimens, 43 specimens were judged to be positive due to the agreement between the test reagent and the control reagent test, and 45 specimens were also judged to be negative due to the agreement between the test reagent and the control reagent results. However, two of the 45 positive specimens were positive in the control reagent, but the test reagent showed negative results and determined to be negative.

To evaluate the clinical efficacy of SARS-CoV-2 of COVID-19 PoaCheck, an upper airway specimen was used to compare the results of the emergency use approval device Allplex™ 2019-nCoV Assay(Permit No. 20-119) with the results of COVID-19 PoaCheck.

Performance Test Results		Contrast reagent (*Allplex*™ 2019-nCoV Assay)		Total
		Positive	Negative	
Test reagent (COVID-19 PoaCheck)	Positive	43	0	43
	Negative	2	45	47
Total		45	45	90

Concordance rate

- Positive concordance rate (%) = a / (a+c) × 100(%)
- = 43 / (43+2) × 100(%) = 95.56%
- (The 95% confidence interval 0.8517 – 0.9877)
- Negative concordance rate(%) = d / (b+d) × 100
- = 45 / (0+45) × 100(%) = 100%
- (The 95% confidence interval 0.9213 – 1.0000)
- Overall concordance rate(%) = (a+d) / (a+b+c+d) × 100
- = (43+45) / (43+0+2+45) × 100(%) = 97.78%
- (The 95% confidence interval 0.9226 – 0.9939)

The results showed a positive concordance rate of 95.56%(95% confidence interval 0.8517~0.9877), a negative concordanse rate of 100.00% (95% confidence interval 0.9226~0.9939), and the conformity degree assessment showed that capa=0.9556(95% confidence interval 0.9488~0.9615) was near perfect matching(good).

13. Description of Symbols

Symbol	Description
	**In vitro diagnostic medical device**
	**Manufacturer**
	**Temperature limit**
	**Batch code**
	**Contains sufficient for <n> tests**
	**Use-by date**
	**Instructions for use**
	**Do not reuse**
	**For prescription use only**
	**Fulfill the requirements of Directive 98/79/EC on in vitro diagnostic medical devices**
	**Authorized representative in the European Community**
	**Caution**

14. Ordering Information

Cat. No.	Name	Size
POA-nCoV-LAMP-100	Rapid COVID-19 PoaCheck™	100 reactions/Kit

BioPOA Co., Ltd.

593-26, Dongtangiheung-ro, Hwaseeong-si, Gyeonggi-do, 18469, Republic of Korea

Web: www.biopoa.co.kr

Email: biopoa@biopoa.co.kr

Tel: +82-31-375-8304

Fax:+82-31-375-8305

15. Technical Support

For technical support, please contact our dedicated technical support team on:

Phone: +82-10-8621-2676

Email: hwlee@biopoa.co.kr

Manufactured by:

BioPOA Co., Ltd.

Web: www.biopoa.co.kr Tel: +82-31-375-8304 Fax:+82-31-375-8305

593-26, Dongtangiheung-ro, Hwaseeong-si, Gyeonggi-do, 18469,

Republic of Korea

European Representative:

KTR Europe GmbH

Mergenthalerallee 77, Eschborn, 65760, Germany