Instructions for Use

380342-A

English

REF LMC403

Loopamp™ SARS-CoV-2 Detection Kit

INTENDED USE

Loopamp™ SARS-CoV-2 Detection Kit is a qualitative *in vitro* diagnostic test for the detection of nucleic acids from SARS-CoV-2 in nasopharyngeal, oropharyngeal swab samples and saliva sample from patients with signs and symptoms suggestive of COVID-19 (e.g., fever and/or symptoms of acute respiratory illness).

Results are for the detection of SARS-CoV-2 RNA that are detectable in nasopharyngeal, oropharyngeal swab samples and saliva sample during infection. Positive results are indicative of SARS-CoV-2 RNA detection, but may not represent the presence of transmissible virus.

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 medical examination, patient history, and epidemiological information.

TEST PRINCIPLES

This product is based on the nucleic acid amplification method, Loop-mediated Isothermal Amplification (LAMP) developed by Eiken Chemical Co., Ltd.

The LAMP method has the following characteristics: (1) Only one enzyme is required and the amplification reaction proceeds under isothermal conditions;[1), 2)] (2) it has an extremely high specificity because of the use of four primers recognizing six distinct regions on the target; (3) it has a high amplification efficiency and enables amplification within a short time; and (4) it produces tremendous amount of amplified product which makes simple visual detection possible.[3), 4)]

The primers provided with this product have been designed in the N and RdRp genes of SARS-CoV-2 RNA. This region has been confirmed well-conserved in the selected nucleic acid sequences of SARS-CoV-2 by alignment analysis.

RNA from clinical samples, nasopharyngeal, oropharyngeal swab samples and saliva sample are extracted using Loopamp™ Viral RNA Extraction Kit or QIAamp Viral RNA Mini Kit of QIAGEN (available for sale separately).

Sample solution and Primer Mix. 2019-nCoV (PM nCV19) containing SARS-CoV-2 specific primers are then dispensed into a reaction tube and mixed with the dried reagent for RNA amplification. Since Reverse transcriptase, Strand displacement DNA polymerase, deoxynucleotide 3 phosphate, and calcein are stored in dried form in the cap of the reaction tube (inside the rib), they are dissolved in the aforementioned mixture solution (sample solution, Primer Mix. 2019-nCoV (PM nCV19)) and incubated at 62.5°C. cDNA is synthesized by Reverse transcriptase based on SARS-CoV-2 genomic RNA in the sample solution. From this cDNA, Strand displacement DNA polymerase allow LAMP reaction.

The detection of amplified products is based on the turbidity measurement of a by-product, magnesium pyrophosphate (a white precipitate).[(3)] Also, visual judgment under UV irradiation may be used instead of turbidity measurement. During amplification the calcein contained in the dried LAMP reagent is released, generating fluorescence light that can be detected by eye.[(4)] Before the reaction, the calcein is in a quenched state due to bound manganese ions bound thereto; however, once the LAMP reaction is started, pyrophosphate ions are generated and bind out the manganese ions, and calcein becomes fluorescent.[(4)]

CONTENTS OF THE KIT

Reagents are stable until the date on the label assuming the container remains unopened at a storage temperature of 2 – 8°C.

Dried RNA Amplification Reagent ····· 1 x 48 tubes
- *Bst* DNA polymerase (*Bst* DNA pol) ※1
- Reverse transcriptase (RT) ※2
- Deoxynucleotide triphosphates
- Magnesium sulfate
- Calcein
- Manganese chloride

Primer Mix. 2019-nCoV (PM nCV19)※3 ····· 1 x 0.72 mL
Positive Control 2019-nCoV (PC nCV19)※4 ····· 1 x 0.16 mL
Negative Control (NC) ····· 1 x 0.16 mL

※ 1: *Bst* DNA pol is derived from *Bacillus stearothermophilus* and is a strand displacement DNA polymerase that lacks 5'→3' exonuclease activity.

※ 2: Reverse-transcriptase is derivered from Avian Myeloblastosis Virus.

※ 3: Primers are designed in the N gene and RNA dependent RNA polymerase (RdRp) gene of SARS-CoV-2 genome RNA and are synthesized oligonucleotides purified by HPLC.

※ 4: PC nCV19 contains RNA transcribed *in vitro* by restriction-enzyme treatment from plasmid DNA artificially synthesized and inserted with SARS-CoV-2 N or RdRp genes.

The abbreviations of names of the following reagents and the Lot No. are printed on the containers as below, and also manufacturer (EKN) is printed.

Reagents	Labelling on the tube	Code on the cap
Primer Mix. 2019-nCoV	PM nCV19 Lot No., EKN	PM nCV19
Positive Control 2019-nCoV	PC nCV19 Lot No., EKN	PC nCV19
Negative Control	NC Lot No., EKN	NC

WARNINGS AND PRECAUTIONS

(1) For *in vitro* diagnostic use only.
(2) This product is designed only for clinical diagnosis of SARS-COV-2 from clinical samples of human origin. Do not use for other purposes.
(3) When using this product, always follow this Instructions for Use.
(4) Do not freeze the reagents.
(5) Do not use any expired reagent.
(6) Do not mix different lots.
(7) Do not replenish any reagent.
(8) Performance of Loopamp™ SARS-CoV-2 Detection Kit is dependent on operator proficiency and adherence to procedural directions. Testing should be performed by properly trained personnel.
(9) Remove the required number of reaction tubes from the packaging just before use and seal the aluminium pouch immediately.
(10) Do not remove the desiccant from the aluminium pouch. High level of humidity may deteriorate Dried RNA Amplification Reagent in the reaction tubes.
(11) Exposure to heat and light might deteriorate the Dried RNA Amplification Reagent. Remove only the required number of reaction tubes (number of samples + number of controls) and seal any unused tubes immediately.
(12) Read the instruction manual of equipment involved incubator before use.
(13) Clinical samples pose a potential risk for infection. Take all necessary preventive measures to avoid biohazard.
(14) PM nCV19, PC nCV19 and NC contain small amount of sodium azide as preservative. Sodium azide is classified as toxic. Avoid any contact with eyes, mouth, or skin.
(15) In case of accidental contact of any reagent with eyes, mouth, or skin, immediately rinse the affected site with plenty of water and, if necessary, seek medical advice.
(16) Do not dilute or add PC nCV19 to the samples. Use PC nCV19 only as described in this Instructions for Use in order to avoid RNA contamination.
(17) Store PC nCV19 and any positive clinical samples separately from the other kit reagents.
(18) Cap of each reaction tube contains Dried RNA Amplification Reagent. Do not touch the inside of the cap.
(19) Before using the reaction tubes, check carefully to see if they have any cracks or scratches. Damaged tubes might give false results

and lead to contamination of the incubator and work area.

(20) Do not expose reaction tubes to UV light before the end of the LAMP reaction. Prolonged exposure to UV light might damage the tubes and lead to false results.

(21) When a UV light is used for visual fluorescence judgment, do not stare directly at UV light. Since UV light is harmful to the eyes, even watching for a short period would irritate eyes and cause symptoms similar to conjunctivitis. Use a glass screen or wear goggles or a protective eye mask whenever looking directly at the UV light.

(22) Refer to the manual of thermal cycler in a medical grade with a temperature accuracy ± 0.5°C. When Real-Time Turbidimeter LA-500 is used, be careful in removing the reaction tube from the Amplification Unit to avoid burns.

WASTE DISPOSAL

(1) Do not open the tubes after RNA amplification. Leave the cap closed and dispose of the used tubes as medical waste by incineration or after double bagging with sealable plastic bags.

(2) Never autoclave or re-use the reaction tubes, else amplified products will disperse and cause contamination.

(3) The main material for the reaction tubes, reagent tube is PP; for the reaction tube tray it is PET; for the aluminium pouch it is aluminium; for the kit case it is paper.

(4) Dispose of any used reagent, container, or labware in accordance with local regulations.

SPECIMEN COLLECTION

Nasopharyngeal, oropharyngeal swab samples and saliva sample

(1) For information on how to collect/transport patient specimens, see the "Laboratory testing for coronavirus disease (COVID-19) in suspected human cases" (WHO, Interim guidance, 19 March 2020 and beyond).

(2) The collected specimens should be used immediately.

(3) **Aerosols generated during specimen collection may cause the virus or its RNA to disperse into the test environment and cause contamination. Therefore, sample collection should be performed in a separate room from the room where this product is used.**

MATERIALS REQUIRED BUT NOT PROVIDED

1) Swabs for sampling: FLOQ swabs (with tubes) from Copan Italia S.p.A. or equivalent
2) Reagents for RNA extraction QIAGEN's QIAamp Viral RNA Mini Kit, or reagents for nucleic acid extraction with equivalent performance
3) Microcentrifuge (with 2 mL rotor, 20,000 x g) ※5
4) Low-adsorption 1.5-mL-tube ※5 of nucleic acid for extract recovery
5) Pipette (0.5 – 10 µL, 10 – 100 µL, 100 – 1000 µL) ※5 and tip with filter
6) Ethanol (96 – 100%) ※5
7) Cooling racks of aluminum and ice (crash ice) or equivalent
8) Bench top centrifuge
9) Simple Centrifuge for 8-strip microtube
10) Real-Time turbidity measuring device (dedicated to the LAMP method, wavelength: 600 – 700 nm, amplified temperature: 62.5°C)
11) Vortex mixer ※5
12) Loopamp™ Viral RNA Extraction Kit

※ 5: Used for QIAamp Viral RNA Mini Kit. When other reagents for nucleic acid extraction are used, reagents, instruments, etc. should be prepared and used according to their instructions for use. In addition, the precautions for use and handling should be followed in accordance with this Instructions for Use.

For Visual Fluorescence Detection

- Incubator (temperature accuracy: ±0.5°C; with hot bonnet)
- Heating block
- UV light (wavelength: 240 – 260 nm, and 350 – 370 nm)
- Goggles and a protective eye mask

For Real-Time Turbidity Detection

- Real-Time Turbidimeter LA-500
- Centrifuge for 8-strip microtube

PREPARATION OF REAGENTS

1) Dried RNA Amplification Reagent
   (1) Leave Dried RNA Amplification Reagent stored in the refrigerator (2 – 8°C) for 5 minutes at room temperature while it is placed in the aluminium pack.
   (2) Take out the required number (number of samples + 2) of Dried RNA Amplification Reagent and store on ice. Immediately seal the remaining reagents in the original aluminium pack and place them in a refrigerator (2 – 8°C).
2) PM nCV19
   Spin down before use.
3) PC nCV19
   Spin down before use. It should be measured once a day.
4) NC
   Spin down before use. Measurements should be made each time.

MEASUREMENT PROCEDURE

RNA Extraction

Preparation of sample solution (Preparation of RNA extraction)

RNA solution extracted using Loopamp™ Viral RNA Extraction Kit or QIAamp Viral RNA Mini Kit, etc. shall be used as sample solution.

Reagent and Sample Mixing (on ice)

(1) Turn on the incubator or Real-Time Turbidimeter LA-500.
(2) Dispense 15 µL of PM nCV19 into reaction tubes by pipette.
(3) Dispense 10 µL of the sample solution to make a total volume of 25 µL, and close the cap.
(4) Dispense 10 µL of NC instead of the sample solution, and close the cap.
(5) Dispense 10 µL of PC nCV19 instead of the sample solution, and close the cap.
(6) Spin down and make sure that all solutions are added, using the line of the reaction tubes (lower line of the two).
(7) Reconstitute the dried reagents in the cap by inverting the reaction tubes and collecting the solutions in the cap. Leave the tubes standing upside down for 2 minutes to reconstitute the dried reagents.
(8) Invert the reaction tubes 5 times to mix the content. Make sure that the dried reagents in the cap are fully dissolved.
(9) Spin down the reaction tubes with a centrifuge.

Amplification

A. Real-Time Turbidity Detection (Standard Method)

(1) Configure Real-Time Turbidimeter LA-500 for detection with this product.
(2) Check if the displayed temperature reaches 62.5°C (allow the turbidimeter to warm up for 20 minutes before use).
(3) Load the reaction tubes and start the measurement.
(4) Watch the display of the turbidimeter to check the positive and negative controls for any increase in turbidity. If the turbidity increases in PC nCV19 but doesn't in NC, amplification reaction is proceeding properly (Fig 1). If any other situation occurs, however, amplification reaction may be proceeding in a wrong way. In such a case, retest affected samples from reagent preparation.
(5) Confirm the completion of polymerase inactivation (automatically completed by the turbidimeter). Take all reaction tubes out of Real-Time Turbidimetry and discard them without opening.

B. Incubator and Thermal Cycler

For Visual Fluorescence Detection

(1) Set the temperature of the incubator (with hot bonnet; temperature accuracy: ± 0.5°C) at 62.5°C. Wait until the displayed temperature reaches the set value.
(2) Load the reaction tubes, and then start the amplification reaction (for 35 minutes at 62.5°C).
(3) 35 minutes later, inactivate the polymerase using the heating block (for 5 minutes at 80°C, or for 2 minutes at 95°C) to terminate the reaction.

Amplification Plots

（Analyzer：Real-Time Turbidimeter LA-500）

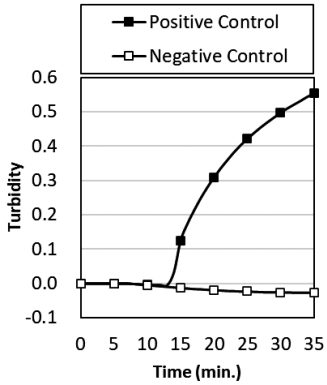

Fig1：Amplification plots for controls

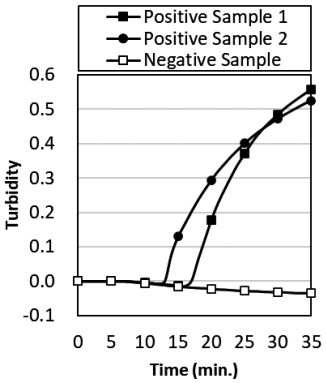

Fig2：Amplification plots for samples

Precautions for Measurement

1) The LAMP reaction is very sensitive and poses a risk to lead to erroneous results if samples are contaminated with amplified products even trace amounts. To avoid such contamination, sampling should be performed in a separate room from the room in which the reagent is used or in a separate area by dividing the testing area. If necessary, take measures to prevent contamination by using clean benches or by wearing gloves or isolation gowns.
2) The RNA molecule is very unstable and therefore must be handled with caution. It is easily degraded, particularly by RNA-degrading enzymes (RNase). RNase may also be contaminated by samples, test instruments, reagents, or saliva or perspiration from the personnel involved in the test, and may not be completely inactivated by autoclaving due to heat. DNA-degrading enzymes (DNase) has also been found to adversely affect the amplified response. Therefore, it is important to prevent contamination of RNase and DNase as much as possible, and therefore, the following caution is required.
   - Distinguishing the test table or instrument for RNA testing from others.
   - Test personnel should always wear gloves and masks to prevent contamination of RNase, DNase from the laboratory personnel's own saliva and sweat.
3) Blood-rich samples should be avoided because they may affect the results of measurements.
4) Sample solutions should, in principle, be used immediately. When unavoidably stored, store at 2 – 8°C and use within 24 hours.
5) If bubbles remain in the reaction solution after mixing the sample solution, turbidity measurement may be disturbed, which may cause erroneous determination, so be careful not to generate bubbles. If bubbles remain, spin down to remove the bubbles.
6) The reconstitution of Dried RNA Amplification Reagent should be performed reliably. Insufficient dissolution may result in inadequate performance such as decreased sensitivity.
7) The tube of PC nCV19 and NC should be spinned down before opening the cap, and the time when the cap is open should also be minimized.
8) PC nCV19 is high-copy-number. To avoid contamination to other samples, close the lids of all reaction tubes except the positive control reaction tubes and finally add the PC nCV19.
9) After reconstitution of the reagents, the reagents should be rapidly subjected to the amplification reaction.
10) In Real-Time Turbidimeter LA-500, an enzyme deactivation process is automatically performed.
11) Never open the lid of the tube after the reaction. Especially when the reaction tube is removed from the device, the tube lid should be carefully removed so that it does not open. In addition, handling of amplification products (e.g., electrophoresis) should be avoided.
12) Fluorescence visual judgement shall be performed after enzyme deactivation treatment (80°C for 5 minutes or 95°C for 2 minutes) in order to avoid misjudgment.
13) When using an UV irradiator, pass through a glass plate or apply a wide range of spectacles or protective surfaces. Do not see the UV lamp directly during lighting

PROCEDURAL NOTES

(1) Clean benches with over 0.5% sodium hypochlorite before performing the test.
(2) Separate the sample collection and the LAMP testing areas.
(3) Take all measures necessary to avoid contamination, in particular change gloves after transferring the samples or if the gloves come into contact with the RNA solution.
(4) Flick (or spin) down PC nCV19 tube before opening it, in order to collect the contents at the top of the tube. Close the tube immediately after dispensing PC nCV19.
(5) Never open the reaction tubes once the LAMP reaction has started or after completion. Be particularly careful when unloading the reaction tubes from the incubator to avoid opening the tubes accidentally.
(6) Do not reuse any amplified product in the tubes for electrophoresis or other applications.
(7) For other Real-Time Turbidimeters or incubators, when visual fluorescence judgment is chosen, inactivate the polymerase (for 5 minutes at 80°C, or for 2 minutes at 95°C) before judgment or false judgment will be caused.
(8) For Real-Time Turbidimeter LA-500 or other incubator, when UV light is used, do not stare directly at UV light. Use a glass board or wear goggles or a protective eye mask whenever looking at the UV light.

For Incubator Using UV Light

If bubbles are present, flick down the tubes to get rid of them.

For Real-Time Turbidimeter LA-500

Since bubbles in reaction solution will interfere with turbidity measurement and cause false judgment, try not to cause any bubble when mixing reagent and sample solution. If bubbles are present, spin down to get rid of the bubbles.

Dried RNA Amplification Reagent should be fully dissolved. Any undissolved portion may influence performance, such as causing a decrease in sensitivity.

Polymerase inactivation is automatically performed.

INTERPRETATION OF RESULTS

For Visual Fluorescence Detection

For Incubator Using UV Light

Irradiate the bottom of each reaction tube from the side through goggles or any other protective equipment.

For a valid run, the following results must be obtained:

- Positive Control: Green fluorescent light is emitted.
- Negative Control: No fluorescent light is emitted.

If any control is invalid, all samples in the run should be reported as invalid and the test should be repeated.

After confirming that the run is valid, evaluate samples as follows:

- Positive Sample: Green fluorescent light is emitted.
- Negative Sample: No fluorescent light is emitted.

For Real-Time Turbidimeter LA-500

After confirming that the turbidity increases with PC nCV19 but doesn't in NC, evaluate samples in accordance with the following criteria (Fig 1 and 2).

- Positive: Some increase is observed in turbidity.
- Negative: No increase is observed in turbidity.

Notes:

(1) The limit of detection of this product is 60 genomes equivalent per test. Even with a negative test, patients with any persisting symptom indicative of an infection by SARS-CoV-2 should undergo re-examination.
(2) Although primers have been designed to target a region containing a relatively small number of variations, SARS-CoV-2 may possibly acquire further variations in this region and become less sensitive to this product. Therefore, a negative test does not always rule out an infection by SARS-CoV-2.
(3) Test results may be affected by specimen collection and transport, specimen preparation, inhibitors and other laboratory procedural errors. A negative test does not exclude the presence of SARS-CoV-2 from the specimen. In making a clinical diagnosis, take into account the patient's clinical condition and all other available laboratory results.
(4) This product is a kit for a qualitative detection; it is not designed for a quantitative measurement. The intensity of fluorescent light observed or the rise time of turbidity measured by Real-Time Turbidimeter does not correlate with the number of template RNA.

INTERFERING SUBSTANCES

Interfering Substances and Interfering Drugs

In-house studies have revealed that measurement was not affected by the presence of free bilirubin (77.6 mg/dL), conjugated bilirubin (81.2 mg/dL), milk bis (formazin turbidity 5,640), hemolyzed hemoglobin (1,964 mg/dL), or saliva.

With regard to drugs, in-house studies have revealed that measurement was not affected by the presence of minocycline (2.40 µg/mL), erythromycin (1.64 µg/mL), piperacillin (52.00 µg/mL), moxifloxacin (8.26 µg/mL), and clindamycin (26.00 µg/mL).

PERFORMANCE CHARACTERISTICS

1. Sensitivity and Accuracy

In testing the following samples:

- Negative Sample (concentration: 0 genome/test)
- Positive Sample 1 (250 genomes/test)
- Positive Sample 2 (1×10^4 genomes/test)

The negative sample shall test negative and the positive samples 1 and 2 shall test positive.

2. Within-run Reproducibility

In testing five negative and positive samples simultaneously, the negative sample shall test negative throughout, and the positive sample shall test positive throughout.

3. Limit of Detection

60 genomes equivalent/test

4. Cross Reactivity

SARS Frankfurt-1 did not cross-react until 4×10^6 copies/test, but slight cross reactivity was observed above 4×10^7 copies/test. For other coronaviruses, other respiratory disease-causing viruses, and organisms listed in the following tables, genomic RNA or DNA was extracted and purified and measured using 1×10^5 – 4×10^8 copies/test equivalent for viruses and 1 ng/test for organisms, all results were negative and no cross-reactions were observed.

Virus name	Virus name
Middle East respiratory Syndrome coronavirus EMC	Human coronavirus 229E_VR-740
Human coronavirus HKU1_Tokyo/SGH-15/2014	Human coronavirus 229E_Sendai-H/1121/04
Human coronavirus HKU1_Tokyo/SGH-18/2016	Human coronavirus 229E_Niigata/01/08
Human coronavirus OC43_VR-1558	Influenza A virus (A/Texas/50/2012(H3N2))
Human coronavirus OC43_Tokyo/SGH-36/2014	Influenza A virus (A/Narita/1/2009(H1N1))
Human coronavirus OC43_Tokyo/SGH-61/2014	Influenza B virus (B/Massachusetts/2/2012
Human coronavirus OC43_Tokyo/SGH-6/2015	Influenza B virus (B/Brisbane/60/2008)
Human coronavirus OC43_Tokyo/SGH-65/2016	Influenza B virus (B/Texas/2/2013)
Human coronavirus NL63_Amsterdam I	Respiratory syncytial virus- A2
Human coronavirus NL63_Tokyo/SGH-15/2017	Respiratory syncytial virus- B1
Human coronavirus NL63_Tokyo/SGH-24/2018	

Name of fungus	Name of fungus
Haemophilus influenzae	*Staphylococcus aureus*
Streptococcus pneumoniae	*Staphylococcus epidermidis*
Klebsiella pneumoniae Subsp. pneumoniae	*Streptococcus agalactiae* (group B)
Pseudomonas aeruginosa	*Moraxella catarrhalis*
Legionella pneumophila Subsp. pneumophila	*Mycoplasma pneumoniae* ATCC 15531
Escherichia coli	*Mycoplasma pneumoniae* ATCC 29342
Bordetella pertussis Tohama	

5. Information about Calibration

The performance test for this product used transcript RNA from plasmid DNA containing the N gene and RdRp gene of the genome RNA of SARS-CoV-2 as a calibrator.

6. Standard Sample Performance

Specimens from National Institute of Infectious Diseases (Japan), 10 positive and 15 negative samples, processed according to "National Institute of Infectious Diseases pathogen detection manual 2019-nCoV Ver. 2.6" were measured by turbidity detection using Real-Time Turbidimeter LA-500 and visual fluorescence detection. The results showed that the positive concordance rate was 90% (9/10), the negative concordance rate was 100% (15/15), and the overall concordance rate was 96% (24/25) for both turbidity detection and visual detection. Samples that tested positive by PCR and tested negative by LAMP had concentrations below the detection limit of the product.

		Products (LAMP)		
		Positive	Negative	Total
Reference product (PCR assay)	Positive	9	1	10
	Negative	0	15	15
	Total	9	16	25

Saliva samples, 10 positive samples and 19 negative samples, prepared at the concentrations described in "Evaluation of performance of genetic testing for new coronavirus (2019-nCoV)" at National Institute of Infectious Diseases were measured similarly by turbidity detection using Real-Time Turbidimeter LA-500 and visual fluorescent detection. The results showed that the positive concordance rate was 90% (9/10), the negative concordance rate was 100% (19/19), and the overall concordance rate was 97% (28/29) for both turbidity detection and visual detection. The negative samples were below the detection limit of the product.

	Number of studies	Number of positives
Positive samples	10	9
Negative samples	19	0

PRECAUTIONS FOR USE AND HANDLING

Precautions for Handling (hazard prevention)

1) Specimens should be handled with caution as being at risk of infection, and necessary biohazard measures should be implemented.
   Refer to the latest National Institute of Infectious Diseases Safety Control Regulations.
2) PM nCV19, PC nCV19 and NC contain small amount of sodium azide as a preservative. Since sodium azide is toxic, care should be taken not to enter the eyes or mouth and not to adhere to the skin.
3) If the reagent accidentally comes into contact with the eyes, mouth, or skin, immediately wash thoroughly with plenty of water and consult a doctor if necessary.
4) UV radiation emitted by the lamp (bactericidal radiation) is harmful when the UV radiation device is used for visual fluorescence detection. Staring at the light source even for a short-term may cause eye pain and symptoms similar to conjunctivitis. Avoid looking directly at the UV light. If it is necessary to see the lamp while lighting, it should be determined by passing through a glass plate or applying a wide range of spectacles or protective surfaces.

Precautions for Use

1) The reagents should be stored in the specified storage method. Cryopreservation should be avoided to maintain quality.
2) To prevent deterioration of reagents, only the necessary reagents should be removed from the kit case when used.
3) To avoid contamination of test environments, PC nCV19 should never be used (e.g. dilutes or additions to samples) other than the operating procedures described in this package insert.
4) PC nCV19 and suspected positive samples should be kept apart from other reagents.
5) Do not use reagents that have passed their expiry date.
6) Do not use in combination with other lots. Also, do not replace reagents.

Handling of Dried RNA Amplification Reagent

1) Reagents removed from refrigerator (2 – 8°C) should be returned to room temperature before opening the aluminum pack.
2) Ensure that Dried RNA Amplification Reagent is dried and retained

on the lid of the reaction tube (inside the rib). Care should be taken to avoid excessive impact on the lid. Also, caution should be exercised not to touch directly the inside of the lid.

3) Dried RNA Amplification Reagent is deteriorated by heat, light, and humidity. The required number of samples (number of samples + 2) should be removed, immediately returned to the original aluminum pack, sealed, and stored in a refrigerator (2 – 8°C).
4) Care should be taken when handling Dried RNA Amplification Reagent because the reaction tubing is susceptible to breakage. Prior to use, visually check that the reaction tube is free of scratches and cracks, etc. Not only can the test be done correctly if there is damage on the tube, but it may also contaminate the equipment due to tube breakage.
5) Sterilization by UV irradiation should not be performed because discoloration by UV irradiation may lead to erroneous results.

ORDERING INFORMATION

Product Code	Product Name	Contents
LMC403	***Loopamp™ SARS-CoV-2 Detection Kit***	***48 tests***
LMC801	Loopamp™ Viral RNA Extraction Kit	48 tests
MVL300	LA-500	1 control unit 1 amplification unit

REFERENCES

1) Notomi T., et al.: Nucleic Acids Research, 28(12), e63, 2000.
2) Nagamine K., et al.: Clin. Chem., 47(9), 1742-1743, 2001.
3) Mori Y., et al.: Biochem. Biophys. Res. Commun., 289(1), 150-154, 2001.
4) Tomita N., et al.: Nat Protoc., 3(5), 877-882, 2008

Flow chart

Operating procedures for real-time turbidity detection

<Preparation of sample solution>

Prepare Specimens

⇩

Use RNA solution extracted using Loopamp™ Viral RNA Extraction Kit or QIAamp Viral RNA Mini Kit, etc. as sample solution. (Must be handled on ice)

<Reagent preparation>

Leave Dried RNA Amplification Reagent for 5 minutes at room temperature. Afterwards, take a required number of reaction tubes.

(For samples, negative and positive control)

<Mixing Reagents and Sample Solutions **(on ice)**>

Dispense 15 µL of PM nCV19 into the reaction tubes.

⇩

Add 10 µL of the sample solution or each control.

(25 µL total as LAMP solution)

Use NC as negative control.
Use PC nCV19 as positive control.
(**Positive control should be prepared last.**)

⇩

Invert the reaction tubes to collect the solution on the cap. Left for 2 minutes on cooling racks.

⇩

Invert the reaction tubes five times to mix the contents and spin them down.

⇩

Amplification

Load the reaction tubes onto the reaction block of the turbidimeter.

⇩

As directed in the instructions for use of the turbidimeter, start reaction and measure and evaluate the turbidity (for 35 minutes at 62.5 °C).

Confirm the completion of polymerase inactivation (for 5 minutes at 80°C, or for 2 minutes at 95°C). Take all reaction tubes out of the turbidimeter and discard them without opening. Be careful not to damage the tubes.

IVD CE

EC REP

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