Intended Use

BZ IsoMDx COVID-19 kit is an in vitro diagnostic using RT-LAMP(Real time Reverse Transcription Loop-mediated Isothermal Amplification) assay for qualitative detection of coronavirus disease (COVID-19) from RNA extracted from human nasopharyngeal swab, oropharyngeal swab and sputum

Introduction

The coronavirus disease (COVID-19) is the first positive single-stranded RNA coronavirus reported in 2019. The sequence is similar to the beta coronavirus found in bats. It is genetically distinct from common coronaviruses, such as Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) and Middle East Respiratory Syndrome coronavirus (MERS-CoV).

The outbreak of pneumonia caused by a coronavirus disease in December 2019 in Wuhan City, Hubei Province, China, is believed to have occurred in the Wuhan Huanan seafood wholesale market on December 12, 2019.

Symptoms of infection are fever, dry cough, and shortness of breath, and worsening symptoms can lead to pneumonia, kidney failure, or death in the case of serious infections.

In accordance with the WHO literature published on January 23, 2020, a quarter of those infected experienced severe illness, and many of the deaths showed the immune system damage including high blood pressure, diabetes, cardiovascular disease, etc.

There are no known vaccines or treatments to date, and the incubation periods is known to be 2 to 14 days which is predicted based on the incubation period of COVID-19 virus.

Principle

BZ LAMP COVID-19 kit is developed to use the real-time RT-LAMP method using Taqman probe. RNA extracted from patient specimen is converted into the complementary DNA (cDNA) by reverse-transcription and target genes are amplified by polymerase chain reaction using primers specific to two site at viral genome in order to detect N gene and RdRP gene simultaneously. In this process, the fluorescence signal decomposed from the fluorescence probe is detected by real-time RT-LAMP.

Materials Provided (100 tests/kit)

Components	Volume	Storage
2X 1 step RT-LAMP Mix	1450 µL	Below -20°C
COVID-19 primer/probe Mix	450 µL	Below -20°C
Positive control	600 µL	Below -20°C
Negative control	600 µL	Below -20°C

Materials Required but Not Provided

1. Appropriate (optical) 96-well reaction PCR plate or tube
2. Micropipette
3. Centrifuge, Vortex mixer
4. Disposable powder-free gloves
5. Any of following PCR machine
   (1) CFX96™ Dx System (Bio-Rad Laboratories, Inc.)

Warnings and Precautions

1. For Professional Use Only
2. Be careful when handling specimens as they cannot exclude infections such as unknown microorganisms or other infectious diseases.
3. Wear lab clothing and disposable rubber gloves or vinyl gloves while handling specimen and using this product.
4. (Disposable items are prohibited to reuse.)
5. Do not chat or eat while using the product.
6. Be careful not to contaminate the specimen or product when you open the tube cap or take out the contents.
7. When processing specimen and testing with the product, filter tip should be used to prevent contamination.
8. When using this product, we recommend testing in a clean bench to prevent contamination.
9. Mixing with previous lot product is prohibited.
10. Dispense the reagents and store the reagents after freezing (below -20 ℃) for long term storage.
11. Because LAMP is a very sensitive method, take care to avoid carry-over during the test.
12. Wastes generated during the experimental should be discard in the waste container and managed according to the waste management regulations.
13. It is recommended to use the commercial RNA extraction kit. [QIAamp DSP Virus RNA Mini Kit (QIAGEN, cat no. 61704)].
14. The final diagnosis should not be based solely on the results of this product. The final diagnosis should be based on a combination of different test methods and clinical results at the discretion of the physician.

Test Procedure

Specimen collection and handling

It is recommended to use the upper and lower respiratory tract specimens of people with symptoms of coronavirus disease (COVID-19) infection and store them under the following conditions.

Specimen from upper respiratory tract

1. Collect nasopharyngeal swabs and oropharyngeal swabs simultaneously and place them in one virus transport medium (VTM).
   A. Nasopharyngeal swab: scrape the secretion through the nostrils from the lower and lower nasal concha (oropharyngeal).
   B. Oropharyngeal swab : Press the tongue and scrape the secretion from the pharyngeal wall.
   ※ VTM is not provided.
2. To ensure accurate test results, immediately store the bottle containing the sample in the refrigerator (4°C) until the test.

Specimen from lower respiratory tract

1. Sputum: Collect sputum into the sterilization container (sputum cans, etc.) by inducing cough to prevent saliva contamination.
2. To ensure accurate test results, immediately store the bottle containing the sample in the refrigerator (4°C) until the test.

RNA Sample preparation and storage

The RNA sample used for the test is extracted using QIAamp DSP Virus RNA Mini Kit (QIAGEN, cat no. 61704) and it is recommended to store the extracted RNA below -20℃.

※The specimen should be stored at 4℃ up to 2 days after collection. For longer period of storage, the specimen should be stored below -70℃.

Real-time PCR Master Mix set up

1. Mix the components following the table below.

Components	Volume (1 test)
2X 1 Step RT-LAMP Mix	14.5 µL
COVID-19 primer/probe Mix	4.5 µL
Total volume of Master mixture	19 µL

2. Dispense 19 µL of the Master mixture into each well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
3. Add 6 µL of RNA sample into each well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube, and mix 2~3 times.
4. Set the PCR machine with appropriate detection channel.

※ Fluorescent Reporter

Detection target	Reporter
N gene	Cy5
RdRP gene	Cy5
Internal Control ACTB (IC)	HEX

5. Perform PCR amplification step as follows.
   (Do not set up the passive reference).

Step	Amplification	Reaction termination
Temperature	60℃	80℃
Time	1 min	5 min
Cycle	30	1

Data Analysis

1. Analysis setting
   (1) Set the baseline of all PCR results using flat signal in an initiation phase.
   (2) Set up the threshold by PCR system as follows.

Instrument	Threshold
CFX96™ Dx system	300 (RFU)

2. Acceptance Criteria
   (1) Positive: Ct value of signal is 30 or less.
   (2) Negative: Ct value is not detected.

3. Interpretation of Results

(Examples of positive/Negative result)

No.	Cy5 (N gene)	Cy5/ (RdRP gene)	HEX (IC ACTB)	Results interpretation
1	Positive	Positive	Positive	COVID-19 Positive
2	Positive	Positive	Negative	COVID-19 Positive
3	Positive	Negative	Positive	COVID-19 Positive
4	Positive	Negative	Negative	COVID-19 Positive
5	Negative	Positive	Positive	COVID-19 Positive
6	Negative	Positive	Negative	COVID-19 Positive
7	Negative	Negative	Positive	Negative
8	Negative	Negative	Negative	Invalid

※ Even if the internal control is negative, it is positive if the target fluorescence is positive.

※ In the case of the test results are positive, even of the results of the test are strong and the internal control is not shown, it should be determined as positive.

※ The test results of both negative and positive controls should be valid. If either one is not valid, retest.

Performance Characteristic

Analytical sensitivity (Limit of Detection)

To determine the analytical sensitivity of BZ IsoMDx COVID-19 kit, the upper respiratory tract specimens (Nasopharyngeal swab) were diluted with internal standard material, and was tested 20 times. The concentration of 100% or more positive result was determined as the minimum detection limit.

The limit of detection is 10^2 copies/μL for the specimens from the upper respiratory tract regardless of the PCR systems including CFX96™ Dx System (Bio-Rad)..

Analytical sensitivity (Cut off Value)

The cut off value was determined as **30** based on the Ct value, which was set using the LOD (Limit of detection) test result value.

Analytical specificity (Cross Reactivity)

To evaluate the cross reactivity of BZ IsoMDx COVID-19 kit, the possible cross reactive pathogens as listed in the table below were tested 3 repeated times.

As a result, no cross reactivity was observed for the pathogens showing the similar symptoms or alpha coronavirus.

- *Mycoplasma Pneumoniae*
- *Streptococcus Pneumoniae*
- *Influenza A Virus H1*
- *Influenza A Virus H3*
- *Influenza A Virus H1N1*
- *Influenza B Virus1*
- *Human Respiratory Syncytial A Virus (RSV)*
- *Human Respiratory Syncytial B Virus (RSV)*
- *Adeno Virus*
- *Para Influenza Virus*
- *Human Corona Virus, OC43*
- *Human Corona Virus, NL63*
- *Human Corona Virus, 229E*
- *Human Boca Virus*
- *Meta Pneumoniae*
- *Huma Rhino Virus*
- *Human Entero Virus*
- *Staphylococcus Aureus Strain*
- *Middle East Respiratory Syndrome Corona Virus*
- *Human Astro Virus*

Analytical specificity (Interference)

To test the effect of the possible interfering substances BZ IsoMDx COVID-19 kit was tested 3 repeated times using specimen prepared by adding the materials listed below. (Mucin 1%, Acetyl salicylic Acid 15mg/mL, NaCl 7.4mg/mL, Oxymetazoline 20%, Hemoglobin 0.2%, Whole blood 3%)

Precision (Reproducibility)

To evaluate reproducibility of BZ IsoMDx COVID-19 kit for nasopharyngeal swab from the upper respiratory tract from the lower respiratory tract, one run of test were performed each day. Each test was repeated twice with 1 lot by two experimenters in 3 different places for 5 days.

As a result, the precision between places and between experimenters showed 100% consistency for each sample. SD and CV are below 0.5 and 2.3 in nasopharyngeal swab.

Precision (Repeatability)

To evaluate repeatability of BZ IsoMDx COVID-19 kit for nasopharyngeal swab from the upper respiratory tract from the lower respiratory tract, one run of test were performed each day. Each test was repeated twice with 3 lots for 20 days. Specimens used includes strong positive sample(3×LOD), weak positive sample(1×LOD) and negative sample.

As a result, the precision by day and lot was 100% consistent for each sample. SD and CV are below 2.35 and 9.8 in nasopharyngeal swab.

Storage condition

BZ IsoMDx COVID-19 kit components: Store below -20℃ (sealed). It is stable and can be used for 6 months from the date of manufacture.

Reference

1. https://www.who.int/emergencies/diseases/novel-coronavirus-2019
2. https://www.cdc.gov/coronavirus/2019-ncov/about/symptsym.html
3. Manual for the Laboratory Diagnosis and Virological Surveillance of Influenza, WHO 2011, page 32

Description of Symbol Used

Symbol	Description	Symbol	Description
REF	Catalogue number	⚠	Caution
LOT	Batch code		Manufacturer
	Use-by date		Consult instructions for use
	Upper limit of temperature		

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