Intended Use

BZ QPCR COVID-19 Kit is an *in vitro* diagnostic medical device for qualitative detection of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2, *aka* 2019-nCoV) from RNA extracted from human nasopharyngeal and oropharyngeal swabs using real-time RT-PCR (Reverse Transcription-Polymerase Chain Reaction).

※ The BZ QPCR COVID-19 Kit has been validated but FDA's independent review of this validation is pending.

Introduction

The coronavirus disease 2019 (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 SARS-CoV-2.

Principle

BZ QPCR COVID-19 Kit was developed to use the real-time RT-PCR 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-PCR.

Materials Provided (100 tests / kit)

Components	Volume	Storage
4X 1 step RT-PCR Mix	500 µL	Below -20 ℃
COVID-19 primer / probe Mix	500 µL	Below -20 ℃
Positive control	600 µL	Below -20 ℃
Nuclease free water	600 µL	Below -20 ℃

Materials Required but Not Provided

1. 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 PCR 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) 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 ℃) 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 ℃) until the test.

RNA Sample Preparation and Storage

The RNA samples used for the tests were 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 ℃.

※ The RNA extraction kit is not provided.

Real-time PCR Master Mix Setup

1. Mix the components following the table below.

Components	Volume (/ test)
4X 1 Step RT-PCR Mix	5 µL
COVID-19 primer / probe Mix	5 µL
Total volume of Master mixture	10 µL

2. Dispense 10 µL of the Master mixture into each well of an appropriate optical 96-well reaction plate or an appropriate optical reaction tube.
3. Add 10 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 Reporters

Detection target	Reporter
N gene	FAM
RdRP gene	Cy5 / Quasar670
Internal Control GAPDH (IC)	ROX

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

UDG incubation	cDNA synthesis	Pre-denaturation	Amplification	
25 ℃	55 ℃	94 ℃	94 ℃	58 ℃
2 min	10 min	3 min	15 sec	30 sec
1 cycle	1 cycle	1 cycle	45 cycles	

Data Analysis

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

Instrument	Threshold
CFX96™ Dx system	300 (RFU)

2. Acceptance Criteria
(1) Positive: Ct value of signal is 43 or less.
(2) Negative: Ct value is not detected.

3. Interpretation of Results
(Examples of Positive / Negative results)

No.	FAM (N gene)	Cy5 / Quasar670 (RdRP gene)	ROX (IC)	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	Positive	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 QPCR COVID-19 Kit, the upper respiratory tract specimens (Nasopharyngeal swab) and the lower respiratory tract specimens (Sputum) were diluted with internal standard material, and was tested 20 times. The concentration of 95% or more positive result was determined as the minimum detection limit.

Human negative RNA was prepared from negative specimens (human nasopharyngeal swab and sputum) using QIAamp® DSP Virus RNA Mini Kit (QIAGEN, Cat. No. 61704). The RNA standard materials include CVR (RdRP gene, Lot # CVR-20B13) and CVN (N gene, Lot # CVN-20B13), which were serially diluted to generate six concentration points (1000 to 1 copies/µL) of spiking RNA as specified in the following table. As an internal control of the RT-PCR, GAPDH RNA was also monitored along with the target genes RdRP and N. The positive control used in the study is an RNA mix comprising 10^5 copies/µL of RdRP, N and GAPDH each. As a negative control (no template control), nuclease-free water was employed.

Specimen		Nasopharyngeal Swab		
Target	Copies / µL	Mean Ct	Result in Agreement	% Agreement
N gene	1000	28.2	20/20	100%
	100	31.7	20/20	100%
	50	32.7	20/20	100%
	10	35.5	20/20	100%
	5	37.1	15/20	75%
	1	N/A	2/20	10%
Target	Copies / µL	Mean Ct	Result in Agreement	% Agreement
RdRP gene	1000	28.3	20/20	100%
	100	31.8	20/20	100%
	50	32.7	20/20	100%
	10	35.5	20/20	100%
	5	36.9	17/20	85%
	1	N/A	3/20	15%

Specimen		Sputum		
Target	Copies / µL	Mean Ct	Result in Agreement	% Agreement
N gene	1000	28.2	20/20	100%
	100	31.7	20/20	100%
	50	32.6	20/20	100%
	10	35.3	20/20	100%
	5	37.0	15/20	75%
	1	N/A	3/20	10%
Target	Copies / µL	Mean Ct	Result in Agreement	% Agreement
RdRP gene	1000	28.2	20/20	100%
	100	31.8	20/20	100%
	50	32.6	20/20	100%
	10	35.5	20/20	100%
	5	36.9	16/20	80%
	1	N/A	3/20	15%

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

Analytical Sensitivity (Cut-off Value)

The cut-off value was determined as 43 based on the Ct value which was set using the LOD (Limit of detection) test result.

Analytical Specificity (Cross Reactivity)

To evaluate the cross reactivity of BZ QPCR 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.

- *Avian Coronavirus, Massachusetts (formerly Avian Infectious Bronchitis Virus)*: 2.8 x 10^7 $CEID^{50}$ / mL
- *Human Astrovirus Type 2, Oxford*: 1.6 x 10^{10} $TCID^{50}$ / mL
- *Human Coronavirus (HCoV), NL63*: 1.6 x 10^6 $TCID^{50}$ / mL
- *Klebsiella pneumoniae, Isolate 1*: 10^5 copies / µL
- *Canine Coronavirus, UCD1*: 1.6 x 10^6 $TCID^{50}$ / mL
- *Klebsiella oxytoca, Strain MIT 10-5244*: 10^5 copies / µL
- *Middle East Respiratory Syndrome Coronavirus (MERS-CoV), EMC/2012, Heat-Inactivated*: 8.9 x 10^6 $TCID^{50}$ / mL
- *Leptospira interrogans, Strain HAI0156 (Serovar Copenhageni)*: 10^5 copies / µL
- *SARS-CoV, Gamma-Irradiated and Sucrose-Purified*: 10^5 copies / µL
- *Mycobacterium abscessus, Strain #103*: 10^5 copies / µL
- *Kilbourne F113: A/England/42/1972 (HA, NA) x A/Puerto Rico/8/1934 (H3N2), Reassortant X-37*: 1.4 x 10^7 $CEID^{50}$ / mL
- *Mycobacterium avium subsp.avium, Strain 2285 Smooth*: 10^5 copies / µL
- *Influenza B Virus, B/Florida/4/2006 (Yamagata Lineage)*: 2.81 x 10^8 $CEID^{50}$ / mL
- *Mycobacterium intracellulare, Strain 1956*: 10^5 copies / µL
- *Human Respiratory Syncytial Virus, A2000/3-4*: 2.8 x 10^5 $TCID^{50}$ / mL
- *Staphylococcus aureus strain AIS*: 32 µg / mL
- *Measles Virus, Edmonston*: 2.8 x 10^4 $TCID^{50}$ / mL
- *Staphylococcus aureus strain M0200 (MRSA)*: 10^5 copies / µL
- *Human Astrovirus Type 1, Oxford*: 2.8 x 10^7 $TCID^{50}$ / mL
- *Streptococcus pneumoniae, Strain TCH8431*: 10^5 copies / µL

Analytical Specificity (Interference)

To test the effect of the possible interfering substances BZ QPCR COVID-19 Kit was tested 3 repeated times using specimen prepared by adding the materials listed below (Mucin 1%, Acetyl salicylic Acid 15 mg/mL, NaCl 7.4 mg/mL, Oxymetazoline 20%, Hemoglobin 0.2%, Whole blood 5%).

It was confirmed that BZ QPCR COVID-19 Kit is not affected by potential endogenous or exogenous interfering substances potentially present in specimen or specimen storage buffer.

Precision (Reproducibility)

To evaluate reproducibility of BZ QPCR COVID-19 Kit for nasopharyngeal swab from the upper respiratory tract

and sputum from the lower respiratory tract, two runs 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 0.8 and 2.1 in nasopharyngeal swab, and below 0.8 and 2.1 in sputum, respectively.

Precision (Repeatability)
To evaluate repeatability of BZ QPCR COVID-19 Kit for nasopharyngeal swab from the upper respiratory tract and sputum from the lower respiratory tract, two runs 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 0.5 and 1.3 in nasopharyngeal swab, and below 0.5 and 1.3 in sputum, respectively.

Inclusivity
An alignment was performed with the oligonucleotide primer and probe sequences of the BZ QPCR COVID-19 Kit with all publicly available nucleic acid sequences for SARS-Cov-2 in GenBank as of May 11, 2020 to demonstrate the predicted inclusivity of the BZ QPCR COVID-19 Kit.

2324 alignments showed 100% identity of N gene primer set of the BZ QPCR COVID-19 Kit to the available 2019-nCoV sequences with the exception of one nucleotide mismatch in 8 strain sequences (3 forward primers, 2 reverse primers and 3 probes).
3230 alignments showed 100% identity of RdRP gene primer set of the BZ QPCR COVID-19 Kit to the available SARS-Cov-2 sequences with the exception of one nucleotide mismatch in 3 strain sequences (1 forward primer and 2 probes).
The risk of a single mismatch resulting in a significant loss in reactivity and false negative result is low, as the primers and probes were designed with the melting temperature over 60 ℃ and the annealing temperature at 60 ℃ to tolerate one to two mismatches.

Clinical Evaluation
BZ QPCR COVID-19 Kit successfully detected target genes N and RdRP in clinical specimens even in the lowest concentration of $1/10^4$ diluted template RNA, where the effective range of Ct value was 20.56 ~ 40.61.

The clinical specimens tested in the study are unaltered original samples collected from the patients, each of which was predefined as 'positive' and 'negative' according to the test results by the Reference Kits Allplex™ 2019-nCoV Assay (Seegene) and PowerCheck™ 2019-nCov Real-Time PCR Kit (Kogenebiotech).

Positive (2)		Negative (5)		Total
NP Swab	Sputum	NP Swab	Sputum	
2	0	5	0	7
Comparator #1 (PowerCheck™ 2019-nCov Real-Time PCR Kit)				

From the side-by-side tests with PowerCheck™ 2019-nCov Real-Time PCR Kit (Kogenebiotech), the clinical sensitivity of BZ QPCR COVID-19 Kit was 100% (2/2) and the clinical specificity was 100% (5/5).

- Positive % agreement
  : 100.00% (95% CI : 15.81% to 100.00%)
- Negative % agreement
  : 100.00% (95% CI : 47.82% to 100.00%)

Positive (33)		Negative (30)		Total
NP Swab	Sputum	NP Swab	Sputum	
29	4	30	0	63
Comparator #2 (Allplex™ 2019-nCoV Assay)				

From the side-by-side tests with Allplex™ 2019-nCoV Assay (Seegene), the clinical sensitivity of BZ QPCR COVID-19 Kit was 96.97% (32/33) and the clinical specificity was 100% (30/30).

- Positive % agreement
  : 96.97% (95% CI : 84.24% to 99.92%)
- Negative % agreement
  : 100.00% (95% CI : 88.43% to 100.00%)

In the clinical settings, BZ QPCR COVID-19 Kit showed equivalent performance (sensitivity and specificity) in detecting the SARS-Cov-2, compared with other validated comparator assays including the Allplex™ 2019-nCoV Assay and the PowerCheck™ 2019-nCov Real-Time PCR Kit.

Storage Condition

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

References

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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