WHO Emergency Use Assessment SARS-CoV-2 IVDs
PUBLIC REPORT

Product: Real-time fluorescent RT-PCR kit for detecting 2019-nCoV
Manufacturer: BGI Europe A/S
EUL Number: EUL 0498-191-00
Outcome: Accepted

The EUL process is intended to expedite the availability of in vitro diagnostics needed in public health emergency situations and to assist interested UN procurement agencies and Member States in determining the acceptability of using specific products in the context of a Public Health Emergency of International Concern (PHEIC), based on an essential set of available quality, safety and performance data. The EUL procedure includes the following:

- Quality Management Systems Review and Plan for Post-Market Surveillance: desk-top review of the manufacturer's Quality Management System documentation and specific manufacturing documents.
- Product Dossier Review: assessment of the documentary evidence of safety and performance.

The Real-time fluorescent RT-PCR kit for detecting 2019-nCoV with product code MFG030010, CE-mark regulatory version manufactured by BGI Europe A/S, Beishan Industrial area, 518000, Yantian district, Shenzhen, China was listed as eligible for WHO procurement on 7 May 2020.[1]

Report amendments and/or product changes

This public report has since been amended. Amendments may have arisen because of changes to the EUL product for which WHO has been notified and has undertaken a review. Amendments to the report are summarized in the following table, and details of each amendment are provided below.

Version	Summary of amendment	Date of report amendment
2.0	Changes in the labels and IFU to address comments raised during labelling review.	22 June 2020
3.0	Change to the catalogue number from MFG030011 to MFG030010 for consistency with CE version. Addition of new compatible PCR instruments: BioRad CFX96 PCR System, Fluorescent Quantitative PCR Detection System FQD96-A and Real-Time Quantitative Thermal Cycler MA-6000.	16 February 2022
4.0	1. Extension of shelf-life claim to 12 months.	24 June 2022

[1] EUL renewal assessment is ongoing.

	2. Changes to the IFU: Deletion of repeated statement: • Addition of two warnings and precautions related to threshold adjustment. • Addition of a quality control illustration for result interpretation.	

Intended use:

According to the claim of intended use from BGI Europe A/S, "*the Real-time fluorescent RT-PCR kit for detecting 2019-nCoV is a qualitative in vitro nucleic acid amplification assay to detect ORF1ab gene of 2019-nCoV using Reverse transcription PCR in specimen of throat swab and Bronchoalveolar Lavage Fluid (BALF) from suspects, suspicious clustering cases and others for the purpose of aiding the diagnosis of SARS-CoV-2 infection.. Definitions of suspects and suspicious clustering cases should be in line with relevant guidelines of COVID-19 diagnosis and treatment released by local authority.*

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. The kit is intended to use for assisting in COVID-19 diagnosis and epidemic control and the testing results should be used in practices in conjunction with epidemiology history, clinical manifestation, image examinations and other laboratory findings as well. It should be operated in line with relevant guidelines, such as diagnosis and treatment guideline of COVID-19 and guideline of COVID-19 prevention and control.

The Real-Time Fluorescent RT-PCR Kit for Detecting 2019-nCoV is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures. Operations of detecting Nucleic Acid of 2019-nCoV should be manipulated in professional molecular laboratory in line with related laboratory guidelines for 2019-nCoV and biosafety."

Specimen type(s) that were validated:

Throat swab and bronchoalveolar lavage fluid (BALF) specimens.

Test kit contents:

Component	**50 tests (product code MFG030010)**
2019-nCoV Reaction Mix	1 vial x 1 mL
2019-nCoV Enzyme Mix	1 vial x 80 µL
2019-nCoV Positive control	1 vial x 750 µL
2019-nCoV Blank control	1 vial x 750 µL

Items required but not provided:

Specimen collection devices:
Aseptic sampling swab manufactured by Medico Biomedical Technology Co., Ltd (Cat#：MVTM-MH-10A, MDK-98-K-3-A, 968-FMH-10(101)-3R) and Shenzhen malcolin Technology Co., Ltd (Cat#: 93050D（CE）.

Extraction/Purification:

Extraction reagents:

- TIANamp Virus RNA extraction Kit (DP315-R) manufactured by TIANGEN.
- QIAamp Viral RNA Mini Kit (52904) by QIAGEN.
- Nucleic Acid Extraction Kit (96 Preps: 1000021042, 1728 Preps: 1000021043) by Wuhan MGI Tech Co., Ltd.

Extraction platforms and consumables:

- High-throughput Automated Sample Preparation System （MGISP-960，Cat. No. 900-000165-00）or DNA Sequencing Library Preparation System（MGISP-100，Cat. No. 900-000207-00）both manufactured by Wuhan MGI Tech Co., Ltd can be used to extract nucleic acid automatically using Nucleic Acid Extraction Kit from Wuhan MGI Tech Co., Ltd.
- RNase/DNase-free microcentrifuge tube, RNase/DNase-free tips for pipettes, 8-tube strips for real-time PCR, disposable gloves.
- PCR hood, Benchtop centrifuge, Vortex mixer, Transparent Multi-well Plate 96 for Applied Biosystems® 7500 Real-Time PCR System, Adjustable calibrated pipettes.

Amplification and detection instruments:

- Applied Biosystems Real time PCR system 7500/7500 Fast (software v2.0.5 or v2.0.6).
- Applied Biosystems QuantStudio 5 Real-Time PCR Systems (software v1.5.1).
- SLAN-96P PCR system (Software version 8.2.2).
- LightCycler 480 System (software v1.5.0).
- Bio-Rad Real time PCR system CFX96（Software version 3.1）
- Real-Time Quantitative Thermal Cycler MA-6000（MA-6000 V1.0.0.2）
- Fluorescent Quantitative PCR Detection system FQD-96A（FQD-96A V1.0.13）

Storage:
Store all reagents below -18°C in dark.

Shelf-life upon manufacture:
12 months, real-time stability study is ongoing.

Warnings/limitations:
Refer to the instructions for use (IFU)

Product dossier assessment

BGI Europe A/S submitted a product dossier for the Real-time fluorescent RT-PCR kit for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) nucleic acid as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid and antigen (PQDx_0347 version 4)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

Post listing Commitments for EUL:

1. Establish limit of detection using the First WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146) and submit the study report to WHO after one month of completion.

2. Provide the operating temperature study results when complete.

Risk benefit assessment conclusion: Acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, BGI Europe A/S was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation by WHO staff, it was established that sufficient information was provided by BGI Europe A/S to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid and antigen (PQDx_ 347 version 4)*".

Quality management documentation assessment conclusion: Acceptable.

Plan for Post-Market Surveillance

Post-market surveillance, including monitoring all customer feedback, detecting and acting on adverse events, product problems, non-conforming goods and processes is a critical component of minimizing potential harm of an IVD listed for emergency use.

The following post-EUL activities are required to maintain the EUL listing status:
1. Notification to WHO of any planned changes to a EUL product, in accordance with "*WHO procedure for changes to a WHO prequalified in vitro diagnostic" (document number PQDx_121*); and
2. Post-market surveillance activities, in accordance with "*Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics"* (ISBN 978-92-4-001531-9).

BGI Europe A/S is also required to submit all categories of complaints in a summarized form. There are certain categories of complaints and changes to the product that must be notified immediately to WHO, as per the above-mentioned documents.

The manufacturer has committed to ensure that post-emergency use listing safety, quality and performance monitoring activities are in place which are in accordance with WHO guidance *"Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics"*. [2]

Scope and duration of procurement eligibility

The Real-time fluorescent RT-PCR kit for detecting 2019-nCoV with product code MFG030010 manufactured by BGI Europe A/S is considered to be eligible for WHO procurement for 12[3] months from the day of listing. The assay may be used for the detection of the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) RNA. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

As part of the on-going requirements for listing as eligible for WHO procurement, BGI Europe A/S must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. BGI Europe A/S is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days.

WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality, performance during post-market surveillance activities, and if new data becomes available to WHO that changes the risk benefit balance.

[2] https://www.who.int/publications/i/item/guidance-for-post-market-surveillance-and-market-surveillance-ofmedical-devices-including-in-vitro-diagnostics.

[3] EUL renewal assessment is ongoing.

Labelling

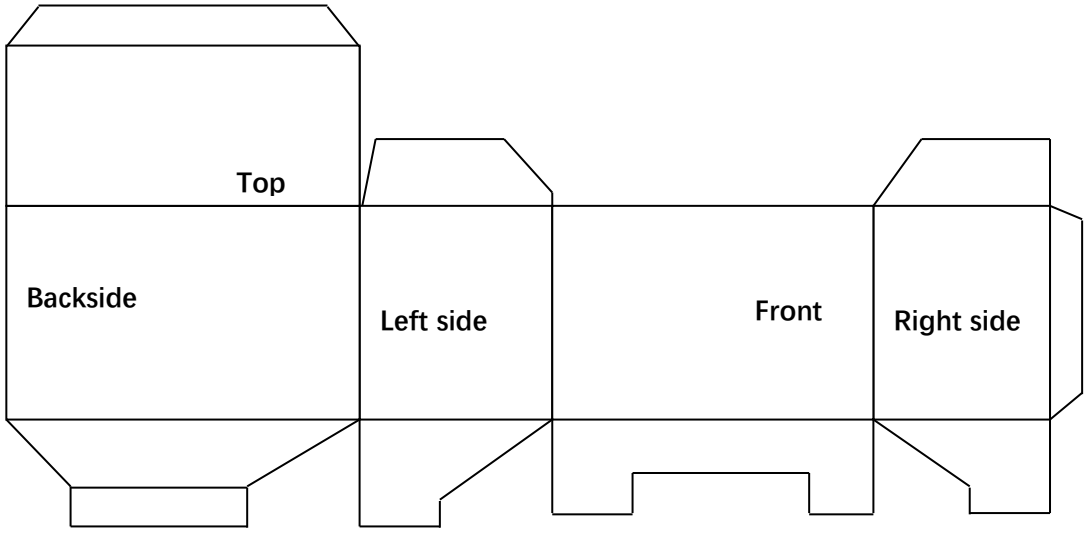
1.0 Labels

2.0 Instructions for Use (IFU)

1.0 Labels

1 Schematic diagram of Label Sample：

From top to bottom：Top→Front→Backside→Left side→Right side



2 Real-time fluorescent RT-PCR kit for detecting 2019-nCoV

Top	华大基因 BGI
Front	华大基因 BGI IVD Real-time fluorescent RT-PCR kit for detecting 2019-nCoV CE LOT Eg.ABC12 Eg.2019-10 Eg.2019-10 -18°C Σ 50 REF MFG030010
Left side	 **Table of Components**

Item（50 tests/kit）	Specifications	Quantity
2019-nCoV Reaction Mix	1mL/ vial	1 vial
2019-nCoV Enzyme Mix	80μL/vial	1 vial
2019-nCoV Positive Control	750μL/vial	1 vial
2019-nCoV Blank Control	750μL/vial	1 vial

Back side	**【Warning and Precautions】** • FOR IN VITRO TEST ONLY. Please read the package insert carefully before your operation. The appropriate operations from specimen collection, storage and transportation, and laboratory test should be strictly manipulated in line with relevant regulations of biosafety and molecular laboratory management; • The false positive or negative testing result can be led by poor quality of specimen, incorrect operations in sample collection, transportation or laboratory processing, or limitation of the technology. Operator should understand well the principles of the procedures and its limitation in performance in advance and avoid any potential mistakes intentionally • All contents in the package are prepared dedicatedly for the intended testing purpose and validated. Replacing any of them will affect the testing performance of the kit. Components contained within a kit are intended to be used together. Do not mix components from different kit lots. • Thaw all kit components thoroughly and centrifuge them briefly before starting an assay. Avoid repeated thaw-freeze cycle. • 8-tube strips for real time PCR capped fasten and transferred to specimen processing area immediately after addition of Nucleic Acid reaction Mix. • To prevent the contamination from exogenous RNA, sample addition should follow the sequence of negative control, specimen RNA and positive control. Filtered tips should be prepared and used separately in preparing reagent and sample addition. • Ensure to pipette the samples exactly into the reaction mix in PCR tubes and avoid sticking the samples to the inside tube wall. The tubes should be capped fasten immediately after the addition. • After the protocol of amplification is done, remove PCR tubes from the thermal cycler and discard them in a sealable plastic bag for autoclave and decontamination. • Ensure no foam or bubbles present in the tubes when aliquoting nucleic acid Mix. All PCR tubes capped fasten before loading them into the thermal cycler to avoid any possible leakage and contamination. • The workbench and lab supplies should be cleaned and disinfected regularly using 75% ethanol or UV light. All pipette tips and centrifuge tubes in the assay should be DNase/RNase-free. The used centrifuge tubes and pipette tips should be discarded in waste bin with Clorox (84) disinfectant and disposed with other laboratory wastes after decontamination
Right side	 BGI Europe A/S, Ole Maaløes Vej 3, DK-2200 Copenhagen N, Denmark Manufacturing Site: BGI Biotechnology (Wuhan) Co.,Ltd. Site Address: Building B2, Zone B/C/D, Wuhan National Bioindustry Base, NO.666 Gaoxin Avenue, East Lake High-tech Development Zone, Wuhan Please contact :BGI Europe A/S Service hotline: Copenhagen, Denmark: 0045-80300800/ 0045-70260806 Website: http://www.bgi.com

REAL-TIME FLUORESCENT RT-PCR KIT FOR DETECTING SARS-CoV-2

MFG030010

2019-nCoV Enzyme Mix

LOT eg.ABC123

IVD

eg.2017-09

volume:80μL

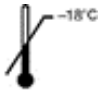
华大基因 BGI

REAL-TIME FLUORESCENT RT-PCR KIT FOR DETECTING SARS-CoV-2 MFG030010

2019-nCoV Reaction Mix

IVD

LOT eg.ABC123



eg.2017-09

volume:1mL

华大基因 BGI

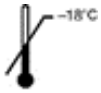
REAL-TIME FLUORESCENT RT-PCR KIT FOR DETECTING SARS-CoV-2

MFG030010

2019-nCoV Positive Control

LOT eg.ABC123

IVD



eg.2017-09

volume: 750μL

华大基因 BGI

REAL-TIME FLUORESCENT RT-PCR KIT FOR DETECTING SARS-CoV-2

MFG030010

2019-nCoV Blank Control

LOT eg.ABC123 IVD

eg.2017-09 −18°C

volume: 750 μL 华大基因 BGI

1.0 Instructions for use[4]

[4] English version of the IFU was the one that was assessed by WHO. It is the responsibility of the manufacturer to ensure correct translation into other languages.

Real-time fluorescent RT-PCR kit for detecting 2019-nCoV

【Generic product name】

Real-time fluorescent RT-PCR kit for detecting 2019-nCoV

【Package Specifications】

50 tests/kit

【Catalogue Number】

MFG030011

【Intended use】

The Real-time fluorescent RT-PCR kit for detecting 2019-nCoV is a qualitative in vitro nucleic acid amplification assay to detect ORF1ab gene of 2019-nCoV using Reverse transcription PCR in specimen of throat swab and Bronchoalveolar Lavage Fluid (BALF) from suspects, suspicious clustering cases and others for the purpose of diagnosis and differential diagnosis. Definitions of suspects and suspicious clustering cases should be in line with relevant guidelines of COVID-19 diagnosis and treatment released by local authority.

The kit is intended to use for assisting in COVID-19 diagnosis and epidemic control and the testing results should be used in practices in conjunction with epidemiology history, clinical manifestation, image examinations and other laboratory findings as well. It should be operated in line with relevant guidelines, such as diagnosis and treatment guideline of COVID-19 and guideline of COVID-19 prevention and control. Operations of detecting Nucleic Acid of 2019-nCoV should be manipulated in line with related laboratory guidelines for 2019-nCoV and biosafety.

【Principle of the procedures】

The kit is based on in vitro RT-PCR combining fluorescent probing. Primers and a sequence-specific fluorescence probes were designed tailored to high conservative region in 2019-nCoV genome. The probes are oligonucleotide attached fluorophores at the 5' end with FAM as reporter and 3' end with quencher. In a meantime, specific primers and probes on basis of human housekeeping gene beta-actin were developed as internal reference with fluorophores VIC/HEX attached at 5' end as reporter. During the PCR procedures, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye when the probes hybridize to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. Monitoring the fluorescence intensities during Real Time allows the qualitative detection of 2019-nCoV in specimens.

【Key contents】

Item（50 tests/kit）	Specification	Quantity	Description
2019-nCoV Reaction Mix	1mL /vial	1 vial	Composed of reagent for amplification and probes and primers of target gene and internal reference
2019-nCoV Enzyme Mix	80μL /vial	1 vial	Taq polymerase, Reverse transcriptase and UDG
2019-nCoV Positive control	750μL/vial	1 vial	Mix solution of pseudo-viruses with target virus genes and internal reference
2019-nCoV Blank control	750μL/vial	1 vial	DNase/RNase free water

Materials required but not provided

- Reagents：TIANamp Virus RNA extraction Kit (DP315-R) manufactured by TIANGEN, QIAamp Viral RNA Mini Kit (52904) by QIAGEN, or Nucleic Acid Extraction Kit (96 Preps: 1000021042, 1728 Preps: 1000021043) by Wuhan MGI Tech Co., Ltd.

- Equipment (Optional): High-throughput Automated Sample Preparation System （MGISP-960，Cat. No. 900-000165-00）or DNA Sequencing Library Preparation System（MGISP-100，Cat. No. 900-000207-00）both manufactured by Wuhan MGI Tech Co., Ltd can be used to extract nucleic acid automatically using Nucleic Acid Extraction Kit from Wuhan MGI Tech Co., Ltd.

- RNase/DNase-free microcentrifuge tube, RNase/DNase-free tips for pipettes, 8-tube strips for real-time PCR, disposable gloves.

- PCR hood, Benchtop centrifuge, Vortex mixer, Transparent Multi-well Plate 96 for Applied Biosystems® 7500 Real-Time PCR System, Adjustable calibrated pipettes.

- Notes：Components contained within a kit are intended to be used together. Do not mix components from different kit lots.

【Storage and shelf-life】

- The RT-PCR Kit should be stored at temperature lower than -18°C in dark. It is stable with shelf-life at temperature lower than -18°C for 12 months(tentative). Unpacked kit should avoid repeated thaw-freeze (within 4 times).

- The PCR Kit can be transported at -18°C in dark stable for 5 days. The manufacture date and shelf life would be provided in the labelling.

【Applicable instruments】

Applied Biosystems™ Real time PCR system 7500/7500 Fast (software v2.0.5 or v2.0.6).

Applied Biosystems QuantStudio 5 Real-Time PCR Systems (software v1.5.1).

SLAN-96P PCR system (Software version 8.2.2).

LightCycler® 480 System (software v1.5.0).

【Specimen】

The recommendation of the specimen collection, storage and transportation is made by referencing the Interim Guidelines for Collecting, Handling and Testing Clinical Specimens from Persons under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19) at https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html [4]. Local regulations pertaining to sample handling may be considered to apply.

Specimen collection

- Collect fresh specimen of throat swabs and BALF from suspects. The operation of specimen should avoid possible contamination in collection, storage and transportation. The specimen should be presumed contagious and be collected according to related regulations.

- **Throat swabs**:
    - Only synthetic fiber swabs with plastic shafts should be used. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing. Place swabs immediately into sterile tubes containing 2-3 mL of viral transport media.
    - Carefully take out the swab from package and quickly insert it into the posterior pharynx and tonsillar areas. Rub swab over both tonsillar pillars and posterior oropharynx a few times applying pressure to collect as much secretions as possible. Avoid touching tongue, teeth, and gums. Break the swab stick and put the head into sampling solution in specimen tubes. Screw the tube cap tightly to ensure no leakage.
- **BALF**: Collect not less than 3ml of unprocessed BALF in sterile, dry and clean DNase/RNase free Cryotubes. Screw the tube cap tightly to ensure no leakage and seal the tube with film.

Storage

- The specimen should be kept in proper condition. The specimen may be tested immediately after collection, or it may be stored at 2-8°C for up to 72 hours before testing. If a delay in testing or shipping is expected, the specimen may be stored at -18°C for no longer than 1 week or at -70°C for no longer than 6 months.
- Frozen specimens should be thawed thoroughly by leaving them at room temperature until they are thoroughly thawed and equilibrate to room temperature while avoiding repeated thaw-freeze cycle more than 4 times.

Transportation

- Freshly collected specimens must be transported at 2-8°C on ice packs. Frozen specimen shipped at -18°C on dry ice for 5 days.

【Warning and precautions】

- FOR IN VITRO TEST ONLY. Please read the package insert carefully before your operation. The appropriate operations from specimen collection, storage and transportation, and laboratory test should be strictly manipulated in line with relevant regulations of biosafety and molecular laboratory management.
- The package is intact and liquid contents are clear, transparent and no sediments. All contents are in correct quantity as the package insert listed.
- The false positive or negative testing result can be led by poor quality of specimen, incorrect operations in sample collection, transportation or laboratory processing, or limitation of the technology. Operator should understand well the principles of the procedures and its limitation in performance in advance and avoid any potential mistakes intentionally.
- Separate laboratory areas are recommended to performing predefined procedures of the assay. (1) 1st Area：Preparation Area—Prepare testing reagent. (2) 2nd Area：Sample processing—Process the specimen and controls. And, (3) 3rd Area：Amplification Area—PCR conducted.
- All materials used in one area should always be remained in the area and should not be moved or used in other areas. After the assay procedures, the workbench and lab supplies should be cleaned and disinfected timely.

- All contents in the package are prepared dedicatedly for the intended testing purpose and validated. Replacing any of them will affect the testing performance of the kit. Components contained within a kit are intended to be used together. Do not mix components from different kit lots.

- The kit should be stored and transported in claimed conditions. Thaw all kit components thoroughly and centrifuge them briefly before starting an assay. Avoid repeated thaw-freeze cycle.

- To prevent the contamination from exogenous RNA, sample addition should follow the sequence of negative control, specimen RNA and positive control. Filtered tips should be prepared and used separately in preparing reagent and sample addition.

- 8-tube strips for real time PCR capped fasten and transferred to specimen processing area immediately after addition of Nucleic Acid reaction Mix. Ensure to pipette the samples exactly into the reaction mix in PCR tubes and avoid sticking the samples to the inside tube wall. Mineral oil should be added immediately, and the tubes should be capped fasten immediately after the addition.

- After the protocol of amplification is done, remove PCR tubes from the thermal cycler and discard them in a sealable plastic bag for autoclave and decontamination.

- The workbench and lab supplies should be cleaned and disinfected regularly using 75% ethanol or UV light.

- All pipette tips and centrifuge tubes in the assay should be DNase/RNase-free. The used centrifuge tubes and pipette tips should be discarded in waste bin with Clorox (84) disinfectant and disposed with other laboratory wastes after decontamination.

- Operator should receive professional training including result interpretation and threshold adjustment before operating.

- Please contact BGI sales for the most up-to-date information in the event of damage to the protective packaging.

- In case of later amplification in Blank control, the user should investigate to determine whether contamination has occurred. If contamination can be ruled out the threshold for FAM and VIC should be adjusted manually and separately against the curve in Blank Control following the IFU. If the user has any questions about the threshold adjustment, please contact BGI for detail communication.

【Laboratory procedures】(Please read the procedures carefully before your operation)

Specimen processing

- The fresh specimen should be collected to ensure the qualified RNA in terms of quality and quantity for the assay. Whether the specimen should be inactivated upon receipt of specimen in laboratory or how to inactivate them, it should be determined based on local relevant guidance, viral transport medium used in specimen transportation, and available lab infrastructure, etc.

- RNA of specimen should be extracted using Nucleic Acid extracting Kit in line with the manufacturer's instructions. Equivalent volumes of positive control and blank control should also be processed simultaneously for nucleic Acid extraction. The assay was validated by the recommended RNA extraction kits by TIANGEN (DP315-R), QIAGEN (52904) and MGI. 140μL specimen is used by extraction kits from TIANGEN and QIAGEN.

160μL specimen is needed by kit from MGI to extract nucleic acid manually or automatically using High-throughput Automated Sample Preparation System （MGISP-960，Cat. No. 900-000165-00）or DNA Sequencing Library Preparation System（MGISP-100）.

- The extracted RNA should be tested immediately or stored at temperature lower than -70°C to test later.

Reagent preparation

- Take out all the kit contents and thaw them thoroughly at ambient temperature. Vortex 5 seconds and centrifuge at 2000rpm for 10 seconds. The Enzyme Mix should be kept in ice continuously.

- Estimate the number of reactions (N) in the test, which includes the Blank control (1 tube), Positive control (1 tube), and specimens prepared. Prepare 8-tube strips for PCR based on the estimated N of reactions and develop the PCR mix as ingredients in following table. Pipette 20μL PCR Mix per tube into the 8-tube strips. Cap them tightly and transfer them to specimen processing Area. The remaining Nucleic acid reaction Mix and Enzyme Mix should be stored at temperature lower than -18°C immediately.

	2019-nCoV Reaction Mix(μL)	2019-nCoV Enzyme Mix(μL)
PCR-Mix (μL)	18.5×N	1.5×N

Add specimen

- Add 10μL the extracted RNA of specimens, Blank control and Positive control respectively into the 8-tube strips prefilled with PCR Mix. Cap them tightly and centrifuge at 2000rpm for 10 seconds.

Real time PCR

- Set the fluorescent channels：Please refer to the manufacturer's instructions of thermocycler for detailed information on channel setting.

FAM channel (Reporter: FAM, Quencher: None) for RNA of 2019-nCoV.

VIC/HEX channel (Reporter: VIC/HEX, Quencher: None) for internal reference (Beta-actin).

Reference Dye：None (only for ABI PCR system);

Reaction Volume: 30μL.

- Configure PCR protocol

Step	Cycle	Temperature	Duration	Fluorescence measured(Y/N?)
1	1 cycle	50℃	20 minutes	N
2	1 cycle	95℃	10 minutes	N
3	40cycles	95℃	15 seconds	N
		60℃	30 seconds	Y

Data analysis

- ABI 7500 PCR system/ABI 7500 Fast PCR system

Baseline starting point at 3 and ending at 15

The threshold of each fluorescent channel should be set separately. In setting the threshold for a channel, the blank control should be selected firstly and click off the Automatic standard curve by changing the option from "☑ Auto"to "□Auto". Set the threshold manually just above the maximum level of blank control curve (random noise curve) at FAM channel.

- SLAN-96P PCR system

The starting and ending points of baseline should be set as 6 and 12 respectively.

The threshold of each fluorescent channel should be set separately. In setting the threshold for a channel, change the configuration of baseline optimization in basic parameter from automatic to manual. Then, manually set the threshold just above the maximum level of blank control curve (random noise curve) at FAM.

- Light Cycler® 480 Real time PCR system

Baseline is set as default.

Threshold: In most cases, the auto threshold line function yields satisfying results. In some cases, it can be adjusted through slightly improving the standard curve error value by manually moving the threshold line up or down, fitting the line to the exponential portion of the amplification curve, higher than while horizontally paralleling the amplification curve of Blank control. Click [Analysis] to get results and [Report] to present them.

- Applied Biosystems™ QuantStudio®5 Real time PCR system

Baseline is set as default.

Threshold: In most cases, the auto threshold line function yields satisfying results. In some cases, it can be adjusted manually. In setting threshold, click[Show Plot Setting], select the target gene to view and the "Show: Threshold" as ☑. Adjust the threshold through dragging it by mouse or inputting values directly, then, click [Analyze].

Quality control

- Positive control is positive at both FAM and VIC/HEX channel in testing while blank control is negative at both channels.
    - Blank control: Ct values at FAM and VIC/HEX channels are 0 or no data available.
    - Positive control： Standard curves at channel FAM and VIC/HEX channels are in S-shape with Ct values not higher than 32.
- Testing specimen (internal control): Standard curves at VIC/HEX channel is in S-shape with Ct not higher than 32.
- Above requirements should be met in a single test. Otherwise, the test is invalid. Please operate the retest strictly in line with the package insert.

Quality control metrics	VIC /HEX(observation)	FAM (observation)	Interpretation

Blank control	No S-shape standard curve or Ct value is >38 with later tail*.	No S-shape standard curve or Ct value is >38 with later tail*	Pass; proceed to sample analysis
Positive control	S-shape standard curve and Ct value is ≤32.	S-shape standard curve and Ct value is ≤32.	
Blank control	S-shape standard curve and Ct value is ≤38.	S-shape standard curve and Ct value is ≤38.	Fail; repeat run before proceeding to sample analysis.
Positive control	No S-shape standard curve or Ct value is >32.	No S-shape standard curve or Ct value is >32.	Fail; Repeat extraction and RT-PCR operation before proceeding to sample analysis

*If Ct higher than 38 with later amplification in blank control, the threshold for FAM and VIC in specimen should be adjusted manually following the IFU in data analysis.

【Threshold and reference range】

- Cut-off value of the kit was determined based on the Receiver Operating characteristic curve from testing clinical specimens. Ct value for positive 2019-nCoV by the kit is not higher than 38.

【Testing result interpretation】

IF the test was valid through assessing the quality control matrix including positive control, blank control and internal reference in testing specimen, the testing results of specimen for 2019-nCoV should be interpreted as follows.

- **The specimen is Positive 2019-nCoV if standard curves at both FAM and VIC/HEX channels are in S-shape with Ct value not higher than 38 at FAM and not higher than 32 for VIC/HEX.**
- **The specimen is Negative 2019-nCoV if standard curve at FAM channel is not in S-shape with Ct at FAM as 0 or no data available while Ct at VIC/HEX not higher than 32.**
- **The test result is Indeterminate if standard curve at FAM is in S-shape with Ct higher than 38. The specimen should be retested and reported on basis of retesting results as positive 2019-nCoV if standard curve at FAM is in S-shape regardless of Ct value, or as negative 2019-nCoV if standard curve at FAM is not in S-shape without Ct and Ct of internal reference is not higher than 32 at VIC/HEX.**
- **The test is Invalid if the standard curve at FAM is not in S-shape with Ct value as 0 or no data available and Ct value of the internal control at VIC/HEX is higher than 32 or no data available. The specimen should be retested.**

Please refer to following table for example of interpreting test results for Real-Time Fluorescent RT-PCR Kit for Detecting 2019-nCoV.

	VIC/HEX (Observation)	**FAM (Observation)**	**Interpretation**
Sample 1	S-shape standard curve and Ct value is ≤32.	S-shape standard curve and Ct value is ≤38.	Positive for 2019-nCoV. Amplification detected in both

			channels and Ct is below threshold.
Sample 2	Sigmoidal amplification curve and Ct value is ≤32.	No Ct data without S-shape standard curve.	Negative for 2019-nCoV.
Sample 3	S-shape standard curve and Ct value is ≤32.	S-shape standard curve and Ct value is >38.	Repeat RT-PCR
Sample4	No Ct or Ct>32	Any	Repeat extraction and RT-PCR operation.

【Limitation of the assay】

- The Results of the test is just for information in clinical practices to assess infection condition of patients combining with clinical presentations and other laboratory markers.
- The incorrect result can be caused by incorrect operations in sample collection, transportation or processing, very low concentration of target virus in the specimens, mutations within the viral genome covered by the kit's primers and/or probe, and unproved external interference factors, such as PCR inhibitor.

【Performance characteristics】

- The package is intact and liquid contents are clear, transparent and no sediments. All contents are in correct quantity as the package insert listed.
- Positive control is positive at both FAM and VIC/HEX channel in testing while blank control is negative at both channels.
- Analytical sensitivity
    - The kit was validated by Chinese national sensitivity standards S1-S3. The Chinese national sensitivity standards were developed by Chinese National Institutes for Food and Drug control from throat swabs positive 2019-nCOV. S1-S3 were all positive for 2019-nCoV by the kit satisfying the criterion.
    - The kit was validated by Chinese national positive standards P1-P7. The Chinese national positive standards were developed by Chinese National Institutes for Food and Drug control from throat swabs positive 2019-nCoV for P1-P6 and recombinant plasmid of N gene for P7. P1-P7 were tested by the kit with Coincidence rates 100%.
    - Study data demonstrates that Real-time fluorescent RT-PCR kit for detecting 2019-nCoV can detect 2019-nCoV with detection rate higher than 95% at concentration higher than 100 copies/mL. Limit of Detection (LOD) of Real-time fluorescent RT-PCR kit for detecting 2019-nCoV was determined as 100 copies/mL Please refer to Table below for detailed data.

	Concentration by ddPCR (Copies/mL)	Test results (positive/tests)	Detection rate
Pseudo-virus	500	20/20	100%

	300	20/20	100%
	150	20/20	100%
	100	20/20	***100%***
	75	15/20	75%
Throat swab	500	20/20	100%
	300	20/20	100%
	150	20/20	100%
	100	19/20	***95%***
	75	15/20	75%
BALF1	500	20/20	100%
	300	20/20	100%
	150	20/20	100%
	100	20/20	***100%***
	75	10/20	50%
BALF2	500	20/20	100%
	300	20/20	100%
	150	20/20	100%
	100	19/20	***95%***
	75	6/20	30%

- Repeatability (within-lot) and reproducibility (between-lot, days, operators and labs) precision

  Precision of the kit was determined as within-lot variability (variability within one production lot), between-lot variability (variability between different production lots), between-day variability (variability between experiments in different days), and between operators and labs. The manufacturer's repeatability references (CV1and CV2), LOD reference and negative specimen was tested in 20 replicates. Coefficient of Variation (CV) and coincidence rate was analyzed. The results showed that CVs were all lower than 5% for samples of repeatability references CV1 and CV2 in variability of within-lot, between-lot, between-day, between-operator and between-lab. Coincidence rates of LOD and negative samples in all variability tests were 100%.

- Analytical Specificity

  - The kit was validated by Chinese national negative standards N1-N22. The Chinese national negative standards were developed by Chinese National Institutes for Food and Drug control from culture isolates of microorganisms as table below. N1-N22 were all negative for 2019-nCoV by the Real-time fluorescent RT-PCR kit for detecting 2019-nCoV with Coincidence rates 100%.

Chinese Negative standards	Description
N1	Legionella pneumophila
N2	Klebsiella pneumoniae
N3	Streptococcus pneumoniae
N4	Haemophilus influenzae
N5	Adenovirus 3
N6	Mycoplasma pneumoniae

N7	Chlamydia pneumoniae
N8	Parainfluenza I
N9	Respiratory syncytial virus A
N10	Bordetella pertussis
N11	Coronavirus OC43
N12	Coronavirus NL63
N13	Coronavirus HKU-1
N14	Coronavirus 229E
N15	Avian influenza H7N9
N16	Avian influenza H5N1
N17	Influenza B(Victoria)
N18	Influenza A H1N1(2009)
N19	Influenza A H3N2
N20	EB virus
N21	Recombinant MERS (orf1ab+N+RdRp)
N22	RNA from throat swabs

- Cross-reactivity was assessed through in silico sequence comparison analyses and in vitro specimen wet testing. Upon in silico analysis the Real-time fluorescent RT-PCR kit for detecting 2019-nCoV was found to detect all 2019-nCoV virus strains and exhibited no cross reactivity with non-2019-nCoV species. For wet testing, specimens from Chinese National Institutes for Food and Drug control, BGI Biotechnology (Wuhan) Co., Ltd and Beijing Union Medical College Hospital were prepared and tested by Real-time fluorescent RT-PCR kit for detecting 2019-nCoV. The tested pathogens include human Coronavirus (SARSr-CoV, MERSr-CoV,HCoV-OC43, HCoV-229E, HCoV-HKU1,HCoV-NL63), Novel influenza A H1N1 (2009), seasonal influenza A (H1N1,H3N2,H5N1,H7N9), Influenza B (Yamagata, Victoria), Respiratory syncytial virus A/B, Parainfluenza virus(1,2 and 3), Rhinovirus (A,B,C), Adenovirus (1,2,3,4,5,7,55), Enterovirus (A,B,C,D), HMPV,EB virus, Measles virus, Human Cytomegalovirus, Rotavirus, Norovirus, Mumps virus, Varicella zoster virus, Mycoplasma pneumoniae, Chlamydia pneumoniae, Legionella, Bordetella pertussis, Haemophilus influenzae, Staphylococcus Aureus, Streptococcus pneumoniae, Pyogenic streptococcus, Klebsiella pneumoniae, Mycobacteria Tuberculosis, Aspergillus fumigatus, Candida albicans, Candida glabrata and Cryptococcus neoformans.

  2019-nCoV was not detected in all the specimens, indicating no cross-reactivity between Real time fluorescent RT-PCR for detecting 2019-nCoV and the tested pathogens and human gene.

- Interfering substances: The endogenous and exogenous interfering substances in specimens were evaluated on the extraction and detection of 2019-nCoV. Specimens with elevated levels of interfering substances do not influence the kit performance at virus concentration higher than Limit of Detection. The tested interfering substances included mucoprotein (60mg/ml), 10%(V/V) human blood, Benfluline (2mg/mL), Oxymetazoline(2mg/mL), Sodium Chloride with preservatives(20mg/mL), Beclomethasone(20mg/mL), Dexamethasone(20mg/mL), Flunisolide(20ug/mL), Triamcinolone(2mg/mL), Budesonide(1mg/mL), Mometasone(2mg/mL), Fluticasone(2mg/mL), Histamine hydrochloride(5mg/mL), α-interferon(800IU/mL), Zanamivir(20mg/mL), Ribavirin(10mg/mL), Oseltamivir(60ng/mL), Paramivir (1mg/mL),Lopinavir(500mg/mL) and Ritonavir(60mg/mL), Mupirocin(20mg/mL), Azithromycin(1mg/mL), Cephalosporin(40μg/mL), Meropenem(200mg/mL), Levofloxacin(10μg/mL), and Tobramycin(0.6mg/mL).

- Clinical evaluation

The test kit was validated by 384 clinical specimens of BALF and throat swabs from cases observed and close contacts of confirmed cases or suspects. Compared to the clinical diagnosis of COVID-19, RT-PCR of 2019-nCoV showed high diagnostic power in detecting 2019-nCoV with a positive percent agreement of 88.89% (95% CI: 83.4%, 94.3%), a negative percent agreement of 100% (95% CI: 98.5%, 100%), and overall percent agreement rate 96.35% (95% CI: 94.5%, 98.2%). Please refer to table below for the data summary of clinical evaluation.

14 discrepant specimens were retested with Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (PCR-Fluorescence Probing) manufactured by Sansure Biotech Inc. and found to be negative.

RT-PCR	Comparator (Clinical diagnosis)	
	COVID-19	Not-COVID-19
Positive	112	0
Negative	14	258
Total	126	258
	Positive Percent Agreement (PPA)	Negative Percent Agreement (NPA)
	88.89%	100%
	Overall Percent Agreement (OPA)	
	96.35%	

【References】

[1] LU Rou-jian, ZHANG Ling-lin, TAN Wen-jie, ZHOU Wei-min, WANG Zhong, PENG Kun, RUAN Li. Development and Comparison of Real-Time and Conventional RT-PCR Assay for Detection of Human Coronavirus NL63 and HKU1[J]. CHINESE JOURNAL OF VIROLOGY, 2008(4).

[2] NIU P, LU R, LAN J, LIU G, WANG W, TAN W. Development of Novel Multiplex Real-time RT-PCR Assays for Detection of MERS-CoV Infection[J]. CHINESE JOURNAL OF VIROLOGY, 2016(3).

[3] CHEN Yu-jing. Development of two-panel reactions of real-time PCR for detection of 18 types/subtypes of respiratory viruses[D]. 2015

[4] Interim Guidelines for Collecting, Handling and Testing Clinical Specimens from Persons under Investigation (PUIs) for Coronavirus Disease 2019 (COVID-19). https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html.

【Contact details】

Manufacturer: BGI Europe A/S

Manufacturer Address: Ole Maaløes Vej 3, DK-2200 Copenhagen N, Denmark

Manufacturing Site: BGI Biotechnology (Wuhan) Co.,Ltd

Site Address: Building B2, Zone B/C/D, Wuhan National Bioindustry Base, NO.666 Gaoxin Avenue, East Lake High-tech Development Zone, Wuhan

Please contact:

BGI Europe A/S

Service hotline:

Copenhagen, Denmark: 0045-80300800/ 0045-70260806

Website：http://www.bgi.com

【Language edition】

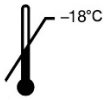
For the requirements of Instruction for Use in other languages, please contact BGI Europe A/S.

【Revision history of IFU】

Document Number	Doc Revision version	Revision Date	Description
BGI-STP-WHO-01-14	V 1.0	2020-05-08	New document publishing
BGI-STP-WHO-01-14	V 2.0	2020-06-04	Literally revised as requested.
BGI-STP-WHO-01-14	V 2.0	2020-06-19	Revise the version number in the head from V1.0 to V2.0
BGI-STP-WHO-01-14	V 3.0	2020-11-17	Revise according to the requirement of WHO.
BGI-STP-WHO-01-14	V 4.0	2021-01-13	Delete a repetition statement of the intended use. Revised the shelf life of this kit.
BGI-STP-WHO-01-14	V5.0	2021-05-14	Revise the wording of the warning/precaution relating to later amplification as WHO recommended.

【Key to symbols used】

	**IN VITRO DIAGNOSTIC MEDICAL DEVICE**
	**MANUFACTURER**
	**USE BY DATE**
	**BATCH CODE**
	**DATE OF MANUFACTURE**

	**CATALOGUE NUMBER**
	**CAUTION**
	**UPPER LIMIT OF TEMPERATURE**
	**CE MARK**
	**CONSULT INSTRUCTIONS FOR USE**
	**KEEP AWAY FROM SUNLIGHT**
	**KEEP DRY**
	**DO NOT RE-USE**
	**POSITIVE CONTROL**
	**CONTAINS SUFFICIENT FOR N TESTS**