WHO Emergency Use Assessment Coronavirus disease (COVID-19) IVDs PUBLIC REPORT

Product: SARS-CoV-2 (2019-nCoV) qPCR Detection Kit
EUL Number: EUL-0497-190-00
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. This evaluation of limited scope is to verify critical analytical and performance characteristics.

SARS-CoV-2 (2019-nCoV) qPCR Detection Kit with product code BS-SY-WCOR-304-100, CE-mark regulatory version manufactured by Bioeksen R&D Technologies Ltd, ITU Teknokent ARI 3 Building No: 4/B 105 Reşitpaşa Mah. Katar Cd, Istanbul, Turkey, was listed as eligible for WHO procurement on 30 November 2020.

Intended use and assay description:

According to the claim of intended use from Bioeksen R&D Technologies Ltd, " *Kit is used for manual, qualitative detection of SARS-CoV-2 causing Coronavirus Disease 2019 (COVID-19). The kit is applied to nucleic acid isolates obtained from nasopharyngeal swab, oropharyngeal swab, nasopharyngeal aspirate/lavage, bronchoalveolar lavage and sputum samples from patients with signs and symptoms suggestive of COVID-19 (e.g., fever and/or symptoms of acute respiratory illness). Rapid diagnosis with the kit is achieved via one-step reverse transcription (RT) and real-time PCR (qPCR) (RT-qPCR) targeting SARS-CoV-2 specific RdRp (RNA-dependent RNA polymerase) gene fragment. The oligonucleotide set targeting human RNase P gene functions as a control of the sampling, nucleic acid extraction and inhibition.*
The test is intended for professional use by laboratory personnel specifically trained in RT-PCR in a level 2 biosafety laboratory.
A positive RT-qPCR result is recommended for at least two different targets on the COVID-19 virus genome, of which at least one target is preferably specific for COVID-19 virus in an area with no COVID-19 virus circulation. In areas where COVID-19 virus is widely spread, RT-qPCR

of a single discriminatory target such as RdRp gene is considered sufficient (Laboratory testing for 2019 novel coronavirus 2019-nCoV in suspected human cases, World Health Organization, published on March 2, 2020).
The kit is validated with RINA M14 Nucleic Acid Extraction Robot, Cat No: RINA-M14-01 and its consumables (Cat No: RN-NA-14-111-100).

The kit is validated for 10 µL and 20 µL qPCR volumes using Roche LightCycler 96, Bio-Rad CFX96 Touch, and Qiagen Rotor-Gene 5 Plex Real-Time PCR Systems. Analytical and clinical performance of the kit was determined by the "Turkish Ministry of Health, General Directorate of Public Health, Department of Microbiology Reference Laboratories and Biological Products (HSGM)".

Specimen type(s) that were validated:

Nasopharyngeal swab, oropharyngeal swab, nasopharyngeal aspirate/lavage, bronchoalveolar lavage and sputum samples.

Test kit contents:

Component	**100 tests (product code BS-SY-WCOR-304-100)**
Oligo Mix	1 vial x 250 µL
2X Prime Script Mix	1 vial x 500 µL
NTC	2 vials x 1000 µL
PC	1 vial x 250 µL

Items required but not provided:

Specimen collection and transportation

- Dacron or polyester flocked swabs for nasopharyngeal swab and oropharyngeal swab samples (it is recommended to use frangible tipped swabs to prevent contamination during sampling)
- Sterile containers for other samples
- Sample transport medium: Viral Transport Medium (VTM)- Preparation of viral transport medium, Centers for Disease Control and Prevention, SOP#: DSR-052-01
- Laboratory coat and disposable gloves; it is recommended to use powder-free nitrile gloves.

Extraction and purification platform and kits

- RINATM M14 Nucleic Acid Extraction Robot (Cat No: RINA-M14-01) and its consumables (Cat No: RN-NA-14-111-100)

Amplification and detection platforms

- Roche LightCycler 96 or
- Bio-Rad CFX96 Touch or
- Qiagen Rotor-Gene 5 Plex

General laboratory equipment and consumables

- Reaction tubes and their caps/seals compatible with the qPCR instrument and the reaction volume
- 1,5 mL or 2 mL microcentrifuge tubes
- 1-10 µL, 10-100 µL and 100-1000 µL micropipettes and the compatible filtered tips
- Quick-spin centrifuge with adaptors for PCR plates and tubes.
- Laboratory coat and disposable gloves; it is recommended to use powder-free nitrile gloves.
- UV cabinet for PCR Setup
- Cold tube rack (for microcentrifuge tubes and PCR tubes/strips).
- Vortex

Storage:
The test kit should be stored at ≤ –20°C.

Shelf-life upon manufacture:

18 months (stability studies are ongoing).

Warnings/limitations:

Please refer to the attached instructions for use.

Product dossier assessment

Bioeksen R&D Technologies Ltd submitted a product dossier for SARS-CoV-2 (2019-nCoV) qPCR Detection Kit as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_0347)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external assessor appointed by WHO.

Post listing Commitments for EUL:

As a requirement to listing, the manufacturer is required to;

1. Review the limit of detection with the WHO international standard by 30 May 2021.
2. Provide interim stability study reports on a regular basis and the final report by 31 May 2022.

Risk benefit assessment conclusion : acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, Bioeksen R&D Technologies Ltd 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 and external technical experts (assessors), it was established that sufficient information was provided by Bioeksen R&D Technologies Ltd to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid, PQDx_ 347"*.

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 status:
1. Notification to WHO of any planned changes to an 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).

Bioeksen R&D Technologies Ltd is also required to submit an annual report that details sales data and 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*".[1]

[1] Available on the web page

https://www.who.int/publications/i/item/guidance-for-post-market-surveillance-and-market-surveillance-of-medical-devices-including-in-vitro-diagnostics

Scope and duration of procurement eligibility

The SARS-CoV-2 (2019-nCoV) qPCR Detection Kit with product code BS-SY-WCOR-304-100, manufactured by Bioeksen R&D Technologies Ltd is considered to be eligible for WHO procurement for 12 months from the day of listing. The assay may be used for the detection of the 2019 novel coronavirus (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 emergency use listing as eligible for WHO procurement, Bioeksen R&D Technologies Ltd must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Bioeksen R&D Technologies Ltd is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days, and any changes made to the product.

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.

Labelling

1. **Labels**

2. **Instructions for use**[2]

[2] 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.

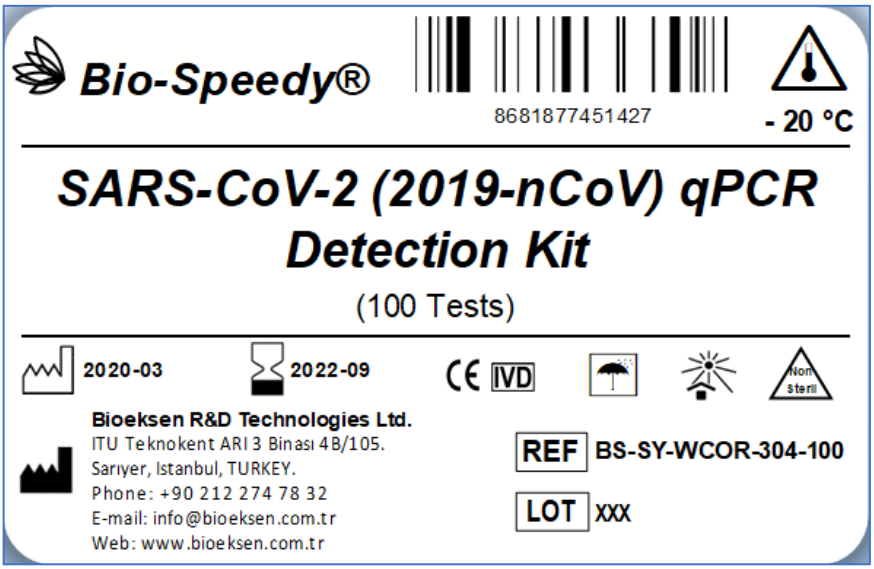

Figure 1-1: *Label of "Bio-Speedy® SARS-CoV-2 (2019-nCoV) RT-qPCR Detection Kit (outer label)*

Figure 1-2: *Label of "Oligo Mix" (component label)*

Figure 1-3: *Label of "2X Prime Script Mix" (component label)*

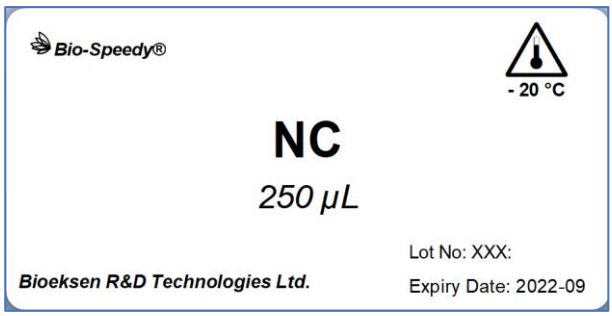

Figure 8-4: *Label of "Negative Control (NC)" (component label)*

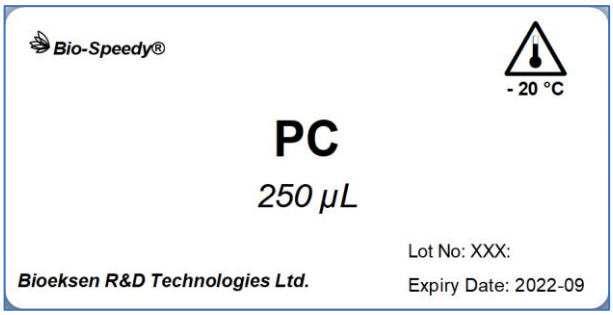

Figure 1-5: *Label of "Positive Control (PC)" (component label)*

Figure 1-6: *Image of the flattened product box*

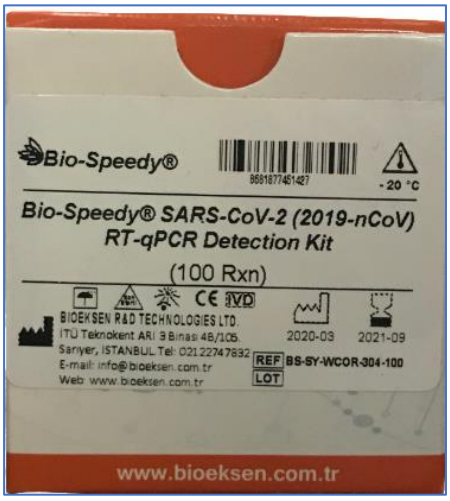

Figure 1-7: *Image of the "end product" box*

REPUBLIC OF TURKEY
MINISTRY OF HEALTH
GENERAL DIRECTORATE OF
PUBLIC HEALTH

Bio-Speedy®

SARS-CoV-2 (2019-nCoV) qPCR Detection Kit

Instructions for Use

Catalog Number: BS-SY-WCOR-304-100

IFU Version Number: 202012101310EAG

Approval Date for Use: 11.12.2020

CE IVD

For in vitro diagnostic use only

For laboratory and professional use only

About this Document

Notice to User:

Before using this product, read and understand the information in the “Warnings and Precautions” section in this document. Reading and carefully following these warnings will minimize usage errors and other possible risks and hazards.

Revision History

Version	Date	Description
01312020MK	31.01.2020	First release
202004301317EAG	30.04.2020	Additions to the "Analytical Specifications" section.
202005042342EAG	04.05.2020	• Addition of "About this Document", "General Requirements and Warnings for Good Practices on PCR and RT-PCR", "Troubleshooting", “Kit Sensitivity and Specificity", "Safety Precautions" and "Customer and Technical Support" sections. • Changes in "Application Protocol" section.
202005221342EAG	22.05.2020	• Changes in RT-qPCR Thermal Cycling Conditions • Changes in the cut-offs of the Cq values • Changes in the LoD values
202005291035EAG	29.05.2020	The terms "For in vitro diagnostic use only" and "For laboratory and professional use only" were added to the cover page.
202007290938EAG	29.07.2020	• Changes in the "Interpretation of The Assay Results" section • Addition of the "Performance Characteristics” section • Addition of the "Assay Limitations” section
202009191845EAG	19.09.2020	Changes in the specimen types.
202010091910EAG	09.10.2020	Changes made in line with WHO recommendations.
202011240831EAG	24.11.2020	Changes regarding validated extraction methods (WHO recommendations).
202012101310EAG	10.12.2020	Removal of 1000 test kit version (WHO recommendation based on EUL assessment)

Table of Contents

Product Information

Product Description

Intended Use and Test Principle

Kit is used for manual, qualitative detection of SARS-CoV-2 causing Coronavirus Disease 2019 (COVID-19). The kit is applied to nucleic acid isolates obtained from **nasopharyngeal swab, oropharyngeal swab, nasopharyngeal aspirate/lavage, bronchoalveolar lavage and sputum samples** from patients with signs and symptoms suggestive of COVID-19 (e.g., fever and/or symptoms of acute respiratory illness). Rapid diagnosis with the kit is achieved via one-step reverse transcription (RT) and real-time PCR (qPCR) (RT-qPCR) targeting SARS-CoV-2 specific *RdRp (RNA-dependent RNA polymerase)* gene fragment. The oligonucleotide set targeting human *RNase P* gene functions as a control of the sampling, nucleic acid extraction and inhibition.

The test is intended for professional use by laboratory personnel specifically trained in RT-PCR in a level 2 biosafety laboratory.

A positive RT-qPCR result is recommended for at least two different targets on the COVID-19 virus genome, of which at least one target is preferably specific for COVID-19 virus in an area with no COVID-19 virus circulation. **In areas where COVID-19 virus is widely spread, RT-qPCR of a single discriminatory target such as *RdRp* gene is considered sufficient** (Laboratory testing for 2019 novel coronavirus 2019-nCoV in suspected human cases, World Health Organization, published on March 2, 2020).

The kit is validated with *RINA™ M14 Nucleic Acid Extraction Robot, Cat No: RINA-M14-01* and its consumables *(Cat No: RN-NA-14-111-100)*.

The kit is validated for 10 µL and 20 µL qPCR volumes using *Roche LightCycler® 96*, *Bio-Rad CFX96 Touch™*, *Qiagen Rotor-Gene® 5 Plex* Real-Time PCR Systems. Analytical and clinical performance of the kit was determined by the *"Turkish Ministry of Health, General Directorate of Public Health, Department of Microbiology Reference Laboratories and Biological Products (HSGM)".*

Kit Contents and Storage

Kit Contents

Table 1. *Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit*

Component	Amount (100 Rxns)	Intended Use
Oligo Mix	1 x 250 µL	SARS-CoV-2 Detection (*RdRp* gene) (**FAM**)
		Internal Control (IC) (*RNase P* gene) (**HEX**)
2X Prime Script Mix	1 x 500 µL	DNA polymerase, dNTP mix, reaction buffer, reverse transcriptase and ribonuclease inhibitor

NTC	2 x 1000 µL	No Template (Negative) Control Test it in each run for contamination control
PC	1 x 250 µL	Positive Control Template: Synthetic SARS-CoV-2 genom fragment Test it in each run for reactive integrity control

Shelf-Life, Storage and Transport

- Storage temperature: ≤ –20°C
- Transport temperature: +2°C - +8°C
- Shelf Life: 18 months; refer to the expiration date on the box. Each reagent stored at storage temperature, may be used until the expiration date indicated on the tube. The expiration date of the kit is determined by the expiration date of the reagents.

Materials Required but Not Supplied with the Product

For Sampling and Transportation

- Dacron or polyester flocked swabs for nasopharyngeal swab and oropharyngeal swab samples (it is recommended to use frangible tipped swabs to prevent contamination during sampling)
- Sterile containers for other samples
- Sample transport medium: Viral Transport Medium (VTM)- Preparation of viral transport medium, Centers for Disease Control and Prevention, SOP#: DSR-052-01
- Laboratory coat and disposable gloves; it is recommended to use powder-free nitrile gloves.

For Nucleic Acid Extraction

- Nucleic acid extraction device and kit:
  - *RINA™ M14 Nucleic Acid Extraction Robot (Cat No: RINA-M14-01)* and its consumables (*Cat No: RN-NA-14-111-100*)
- 1.5 mL or 2 mL microcentrifuge tubes
- Vortex
- 10-100 µL and 100-1000 µL micropipettes and the compatible filtered tips.
- Laboratory coat and disposable gloves; it is recommended to use powder-free nitrile gloves.

For RT-qPCR

- Real-Time PCR (qPCR) system:
  - *Roche LightCycler® 96* or
  - *Bio-Rad CFX96 Touch™* or
  - *Qiagen Rotor-Gene® 5* Plex
- Reaction tubes and their caps/seals compatible with the qPCR instrument and the reaction volume
- 1,5 mL or 2 mL microcentrifuge tubes
- 1-10 µL, 10-100 µL and 100-1000 µL micropipettes and the compatible filtered tips
- Quick-spin centrifuge with adaptors for PCR plates and tubes.
- Laboratory coat and disposable gloves; it is recommended to use powder-free nitrile gloves.

Extra components recommended to use:

- UV cabinet for PCR Setup
- Cold tube rack (for microcentrifuge tubes and PCR tubes/strips).

Warnings and Precautions

Use Statements

- For In Vitro Diagnostic (IVD) Use only
- *Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit* is intended for use in a laboratory environment by qualified clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- The *Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit* has been developed only for the detection of nucleic acid from SARS-CoV-2, not for any other viruses or pathogens.

Safety & Hazards

General Safety

Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the “Customer and Technical Support” section in this document.

Chemical Safety

To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturers before you store, handle, or work with any chemicals or hazardous materials.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.

Biosafety

- *Positive Control:*

  The SARS-CoV-2 *RdRp* gene, used as a control material, was obtained synthetically. Infective virus was not used in the production process, it is harmless.

- *Bovine Serum Albumin:*

  All bovine serum albumin used in the reagents, originate from herds in countries declared free of Transmissible Spongiform Encephalopathies and are obtained from Transmissible Spongiform Encephalopathies-free certified manufacturers.

Biohazard

- Follow all applicable local, state/provincial, and/or national regulations and standard precautions. All patient specimens should be considered potentially infectious and handled accordingly.
- Proper personal protective equipment including lab coats, gowns, gloves, eye protection and a biological safety cabinet are recommended for manipulation of clinical specimens. Refer to Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition - CDC and World Health Organization, Laboratory Biosafety Manual, 3rd Edition, WHO/CDS/CSR/LYO/2004.11.
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/labbiosafety-guidelines.html.
- Specimen processing should be performed in accordance with national biological safety recommendations. Refer to the following:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetyMicrobiologicalBiomedicalLaboratories-2009-P.PDF
- If infection with SARS-CoV-2 is suspected based on current clinical and epidemiological screening criteria recommended by public health authorities, specimens should be collected with appropriate infection control precautions.
- Perform all manipulations of human clinical specimens within a Class II (or higher) biological safety cabinet (BSC).
- Immediately clean up any spill containing potentially infectious material with 0.5-1% (w/v) sodium hypochlorite (10-20% v/v bleach). Dispose of cleaning materials in a biohazard waste stockpot. If the spill contains guanidinium thiocyanate, do not use bleach or acidic solutions. Due to the danger of cyanide gas formation, clean with a suitable laboratory detergent and water.
- Report incident to supervisor, fill in the Accident Report and consult a physician immediately in the event that infectious materials are ingested or come into contact with mucus membranes, open lacerations, lesions or other breaks in the skin.

Waste Management

Medical Waste

Appropriate waste management and decontamination procedures should be used.

Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. All medical wastes including the IVD and its consumables used with it should be collected in transportable and sealed biohazard bags / containers that are resistant to tear, puncture, breakage in accordance with the regulations on medical wastes. The contents of medical waste bags/containers should be never compressed, removed from the bag/container, emptied and transferred to another container.

Dispose of waste in a designated matter in accordance with local, regional and federal regulations.

Molecular Waste

Nucleic acid contamination from molecular waste can be caused by dust and spreading aerosols. PCR products can be destroyed using a 3 % (mass fraction) hypochlorite solution (refer to ISO 22174:2005).

Chemical Waste

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure use of primary and secondary waste containers (a primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage).
- After emptying a waste container, seal it with the cap provided.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

General Requirements for Good Practices on PCR and RT-PCR

Laboratory Setup

To prevent contamination of the reaction mixture by previously amplified target sequences, it shall be ensured that separate work areas with their own apparatus are available. If possible, maintain separate work areas, dedicated equipment, and supplies for:

- Sample preparation
- PCR setup
- PCR amplification
- Analysis of PCR products

Rooms can be simulated using a clean bench or the UV cabinet for PCR setup. Physical separation through the use of different rooms is the most effective and preferable way of ensuring separate work areas and working facilities.

For additional information, refer to ISO 22174:2005 (section of "6.3. Laboratory setup").

Personnel

- Different sets of laboratory coats should be worn pre- and post-PCR.
- Disposable gloves should be worn at sample preparation and when setting up PCR.
- Laboratory coats and gloves should be changed at appropriate frequencies (when suspected that they are contaminated) and before leaving the work area (in passing from one work area to another).
- All personnel who perform aspects of the testing procedures should be trained to work with PCR and microbiology as appropriate.

Protection of Product Performance and Analysis Efficiency

- The components in the kit should not be mixed with components with different lot numbers or chemicals of the same name but from different manufacturers.
- Master stock reagents should be kept on the cold block during the PCR setup; if possible, the PCR setup should be performed on the cold block.
- Kit components should be mixed by gently shaking before use.
- Maintenance/ calibration interval should be determined for all instruments and equipment used with the kit.
- Sampling should be carried out by personnel with sufficient knowledge and experience.
- For collection of nasopharyngeal/ oropharyngeal swabs, polyester flocked swabs are preferred. Sterile dacron swabs with plastic or flexible metal handles may also be used. Cotton or calcium alginate swabs or swabs with wooden sticks should not be used since they may contain substances that inactivate some viruses and inhibit PCR.
- Dry swab samples should be analyzed within a maximum of 1 hour after the sampling; otherwise these samples should not be accepted by the laboratory.
- For the efficiency of Real-Time PCR analysis and the reliability of the results, it is recommended that the nucleic acid concentration be in the range of 10-250 ng/reaction. If the concentration of nucleic acid isolate is too high, it should be diluted 10 or 100 times with molecular grade / nuclease-free water.
- The kit is intended for use in a laboratory environment by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.

Preventing Contamination

- The kit should be stored away from nucleic acid sources and qPCR amplicons.
- The micropipettes used for pipetting qPCR mixes and template nucleic acids should be separate. Filtered and nuclease-free pipette tips should be used.
- All sample tubes should be opened and closed carefully. Should be tried not to splash or spray PCR samples.
- Template nucleic acid and positive control tubes should always be kept closed, except for fluid transfers.
- Amplified products should not be brought into the reaction setup area. To avoid false positives due to amplified material, the qPCR completed reaction tubes should be disposed of before opening in the laboratory (PCR products can be destroyed using a 3% -mass fraction- hypochlorite solution; refer to ISO 22174:2005).
- To avoid false positives due to cross contamination, the positive control should not be included unless required. If a positive control is necessary, all unknown sample tubes should be closed before pipetting the positive control.
- It is recommended to use frangible tipped swabs to prevent contamination during sampling.
- The wipeable surfaces of the rooms, benches and devices where the analysis is performed should be cleaned regularly with freshly diluted 10% bleach solution (0.5% sodium hypochlorite).

Plate Layout Suggestions

- In multi-targeted PCR runs, separate different targets by a row or by a column if enough space is available.

- If possible, put at least one well between unknown samples and controls.
- Separate negative and positive controls by one well if possible.
- Place replicates of one sample for the same target next to each other.
- Start with the unknown samples and put controls at the end of the row or column.
- If possible, put positive controls in one of the outer rows or columns.
- Consider that caps for PCR tubes come in strips of 8 or 12.

Quality Control

Quality Control Requirements

- Quality control requirements must be performed in conformance with local, state, and federal regulations or accreditation requirements and the user's laboratory's standard quality control procedures. For further guidance on appropriate quality control practices, refer to 42 CFR 493.1256.
- Quality control procedures are intended to monitor reagent and assay performance.
- Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all reagents and kit components are working properly.
- Good laboratory practice (cGLP) recommends including a positive extraction control in each nucleic acid isolation batch.
- The negative extraction control must proceed through nucleic acid isolation per batch of specimens to be tested.
- Always include a negative control (NTC), and the appropriate positive control (PC) in each amplification and detection run. All clinical samples should be tested for human *RNase P* (RP) gene to control for specimen quality and extraction.

Description of Controls

No Template (Negative) Control/ Extraction Negative Control (NTC)

The NTC is molecular grade, DNase and RNase-free water, used in place of sample nucleic acid as a no template (negative) control in each PCR run. NTC also serves as an extraction negative control and it is used in place of respiratory sample in each extraction batch/run. No Template (Negative) Control/ Extraction Negative Control should give a negative result (Cq not detected) for both the oligo mixes targeting *RdRp* (SARS-CoV-2, FAM channel) and *RNase P* (IC, HEX channel). Otherwise, it shows that there is a contamination problem. In this case, it is recommended to repeat the analysis by paying attention to the "Warnings and Precautions" section.

Positive Control

The Positive Control (PC) includes synthetic SARS-CoV-2 RNA that contains *RdRp* sequence and total nucleic acid extract from human blood which contains *RNase P* gene. The positive control should give positive results (Cq<38.0) for both the oligo mixes targeting *RdRp* (SARS-CoV-2, FAM channel) and *RNase P* (IC, HEX channel). Otherwise, it indicates that there is a reagent stability problem. In this case, it is recommended to contact the manufacturer and renew the reagents and repeat the analysis.

Internal/ Extraction Control

Detection of *RNase P* in extracted nucleic acid serves as an extraction, inhibition and sampling control for each sample. Nucleic acid extracted from each specimen, should yield a positive result in the HEX

channel, with a Cq value < 38.0. If the *RNase P* assay is negative on a clinical sample, it is interpreted as follows:

- If the *RdRp* assay is positive with a negative *RNase P* result, it is considered that there is no inhibition, extraction or sampling problem and the run is valid. In this case, the result is interpreted as "SARS-CoV-2 Positive" as long as there is no sigmoidal amplification curve in the No Template (Negative) Control and the Positive Control is valid.
- If the *RdRp* assay is negative along with a negative *RNase P*, the specimen result is considered invalid and should be repeated. If residual specimen is available, nucleic acid is re-extracted from the specimen and test is performed again. If the re-tested sample does not give a positive result in the HEX channel, a new specimen should be collected from the patient.

Application Protocol

Before PCR

Collection, Storage and Shipment of Clinical Specimens

Collecting the Specimen

Nasopharyngeal (NP) or oropharyngeal (OP) swab samples, NP aspirate or lavage, bronchoalveolar lavage (BAL) and sputum samples shall be collected by a healthcare provider in accordance with the updated version of CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 (https://www.cdc.gov/coronavirus/2019-ncov/lab /guidelines-clinical-specimens.html).

Swabs (dacron or polyester flocked) should be placed immediately into a sterile transport tube containing 2-3 mL of viral transport medium (VTM) (Preparation of viral transport medium, Centers for Disease Control and Prevention, SOP#: DSR-052-01).

Nasopharyngeal (NP) aspirate or lavage and nasal wash samples should be transferred into sterile containers containing 2-3 mL of VTM (in case of immediate analysis, these samples can be taken into sterile containers by healthcare providers).

Bronchoalveolar lavage (BAL) and sputum samples should be collected 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

Transporting Specimens

Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential 2019-nCoV specimens. Store specimens at 2-8°C and ship overnight to the laboratory on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to the laboratory on dry ice.

Storing Specimens

Specimens can be stored at 2-8ºC for up to 72 hours after collection. If a delay in extraction is expected, store specimens at -70ºC or lower in accordance with the CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19. Extracted nucleic acid should be stored at -70ºC or lower.

Preparation of Nucleic Acid Isolates

The kit is applied to nucleic acid isolates from nasopharyngeal swab, oropharyngeal swab, nasopharyngeal aspirate/lavage, bronchoalveolar lavage and sputum samples. Nucleic acid isolates should be prepared with a method that allows processing of these samples. It is recommended to use the method which the kit is validated with:

- *RINA™ M14 Nucleic Acid Extraction Robot, Cat No: RINA-M14-01* and its consumables *(Cat No: RN-NA-14-111-100)*

RNA extraction should be performed according to the manufacturer's instructions for use.

For the efficiency of Real-Time PCR analysis and the reliability of the results, it is recommended that the nucleic acid concentration be in the range of 10-250 ng/reaction.

Things to Consider Before Starting the Assay

- The kit was validated only for the template nucleic acid volume that is 25% of the total qPCR volume.
- The kit can not be used with real-time PCR instruments without the periodic maintenance records.
- Both white and clear 0.1 mL qPCR plates can be used for the assay, while slightly better performance can be obtained using the white plates for *Bio-Rad CFX96 Touch™* and *Roche LightCycler® 96* instruments.
- 0.1 mL and 0.2 mL clear qPCR tubes can be used for the assay, while slightly better performance can be obtained using the 0.1 mL tubes for *Qiagen Rotor-Gene® 5 Plex* instrument.
- Both 10 µL and 20 µL qPCR volumes can be used for the assay on *Bio-Rad CFX96 Touch™* and *Roche LightCycler® 96* instruments.
- 10 µL qPCR volumes can be used on *Qiagen Rotor-Gene® 5 Plex* instruments (72-well rotor) with 0.1 mL clear qPCR tubes.
- 20 µL qPCR volumes can be used on *Qiagen Rotor-Gene® 5 Plex* instruments (both 36- and 72-well rotor) with both 0.1 mL and 0.2 mL clear qPCR tubes.

Real-Time RT-PCR

Planning of the PCR plate & PCR Setup

- Determine the number of reactions and create the PCR plate plan.
- Plan to include the following reactions:
  - Reactions for each test sample and extraction negative control,
  - Control reactions:
    - Positive Control (included in the kit),
    - No Template (Negative) Control (included in the kit).
- Thaw all reagents.
- Vortex all reagents to mix thoroughly.
- Centrifuge the reagent tubes briefly (3 to 5 seconds at 2000 x g) to bring the contents to the bottom and place on cold rack / ice.
- Combine the following components for the number of reactions required plus 10% overage to compensate for pipetting errors:

Table 2. *Reaction set-up*

Component	Volume per reaction [1]	Volume for 96 reactions [2]
2X Prime Script Mix	5 µL	528 µL
Oligo Mix	2.5 µL	264 µL

[1] *These amounts are valid for a reaction volume of 10 µL. For 20 µL reaction volume, these values should be multiplied by 2.*

[2] *Includes 10% overage to compensate for pipetting errors.*

- Mix the solution by vortexing gently, then centrifuge briefly (3 to 5 seconds at 2000 x g) to collect liquid at the bottom of the tube.
- Distribute the solution 7.5 µL (distribute 15 µL for the reaction volume of 20 µL) to each reaction well or tube.
- Add 2.5 µL (add 5 µL for the reaction volume of 20 µL) of Nucleic Acid sample, Negative Control and Positive Control to the appropriate wells.
- Seal the plate or close the tubes, centrifuge briefly to bring the contents to the bottom and place into the Real-Time PCR device.

Programing and Running the Real-Time PCR Instrument

- The kit is validated for 10 µL and 20 µL qPCR volumes using *Roche LightCycler® 96, Bio-Rad CFX96 Touch™, Qiagen Rotor-Gene® 5 Plex* Real-Time PCR Systems. It is recommended to use one of these instruments.
- Program the Real-Time PCR instrument as in *Table 3*. Please refer to the user manual of each device before programming.

Table 3. *qPCR program details*

<table>
<tr><th>Reaction Volume[1]</th><th>Cycle Number</th><th>Step</th><th>Temperature</th><th colspan="2">Duration</th></tr>
<tr><td rowspan="8">10 µL or 20 µL</td><td>1</td><td>Reverse Transcription</td><td>52 °C</td><td colspan="2">5 min</td></tr>
<tr><td>1</td><td>Hold</td><td>95 °C</td><td colspan="2">10 sec</td></tr>
<tr><td rowspan="6">40</td><td>Denature</td><td>95 °C</td><td colspan="2">1 sec</td></tr>
<tr><td>Anneal / Extend</td><td>55 °C</td><td colspan="2">30 sec</td></tr>
<tr><td rowspan="4">Detection (Reading)</td><td>Instrument:</td><td>RdRp (SARS-CoV-2)</td><td>RNase P (Internal Control)</td></tr>
<tr><td>Bio-Rad</td><td>FAM</td><td>HEX</td></tr>
<tr><td>Roche</td><td>FAM (470/514)</td><td>HEX (533/572)</td></tr>
<tr><td>Qiagen[2]</td><td>Green (470/510)</td><td>Yellow (530/555)</td></tr>
</table>

[1] Both 10 µL and 20 µL PCR volumes can be used for the assay on *Bio-Rad CFX96 Touch™, Roche LightCycler® 96,* and *Qiagen Rotor-Gene® 5 Plex* (72-well rotor) instruments. 10 µL PCR volume is recommended for high test capacity on these instruments. But, 20 µL PCR volume should be used on Qiagen Rotor-Gene® 5 Plex instruments with 36-well rotor.

[2] When using Qiagen Rotor-Gene® 5 Plex, please perform "Gain Optimization" on the channels to be read according to the reaction positions. To do this, select the "Perform Optimization Before 1st Acquiring" option.

- Define the plate set-up.
- Start the run.

Interpretation of the Assay Results

Assessment of clinical specimen test results must be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted.

- Examine the shape of the amplification curves obtained in the FAM/HEX channels and record non-sigmoidal curves as negative.
- Calculate the number of threshold cycles (Cq). The recommended threshold levels to calculate the number of threshold cycles (Cq) for both 10 µL and 20 µL reactions are 0.05, 200 and 0.02 RFU for *Roche LightCycler® 96, Bio-Rad CFX96 Touch™* and *Qiagen Rotor-Gene® 5 Plex*, respectively.
- Record the result as negative if there is no sigmoidal curve.
- Record the result as positive if Cq<38.
- Repeat the analysis with the same nucleic acid extract if Cq≥38, if the result is Cq≥38 again, ask a new sample collected from the patient.
- Interpret results as follows:

Table 4. *Positive and No Template (Negative) Control Interpretation*

Positive Control		**Negative Control**		**Results**
***RdRp* (FAM)**	***RNase P* (HEX)**	***RdRp* (FAM)**	***RNase P* (HEX)**	
Positive (Cq<38.0)	Positive (Cq<38.0)	Negative (No Cq)	Negative (No Cq)	VALID (Continue to result interpretation of patient specimens)
Any of them is Negative (Cq not detected)		Not considered		INVALID (Reagent stability problem)
Not considered		Any of them is Positive (Cq<38.0)		INVALID (Contamination problem)

Table 5. *Interpretation of Patient Samples*

***RdRp* / FAM (positive for Cq < 38)**	***RNase P* / HEX (positive for Cq < 38)**	**Results Interpretation**	**Action**
Positive (+)	Positive (+)	Results are VALID, SARS-CoV-2 RNA is **Detected**	Report as POSITIVE
Positive (+)	Negative (-)	Results are VALID, SARS-CoV-2 RNA is **Detected**	Report as POSITIVE
Negative (-)	Positive (+)	Results are VALID, SARS-CoV-2 RNA is **Not Detected**	Report as NEGATIVE
Negative (-)	Negative (-)	Results are **INVALID**	If residual specimen is available, re-extract nucleic acid from the specimen and perform the test again. If the result is still invalid, a new specimen should be obtained. If additional clinical sample is unavailable, report as INVALID

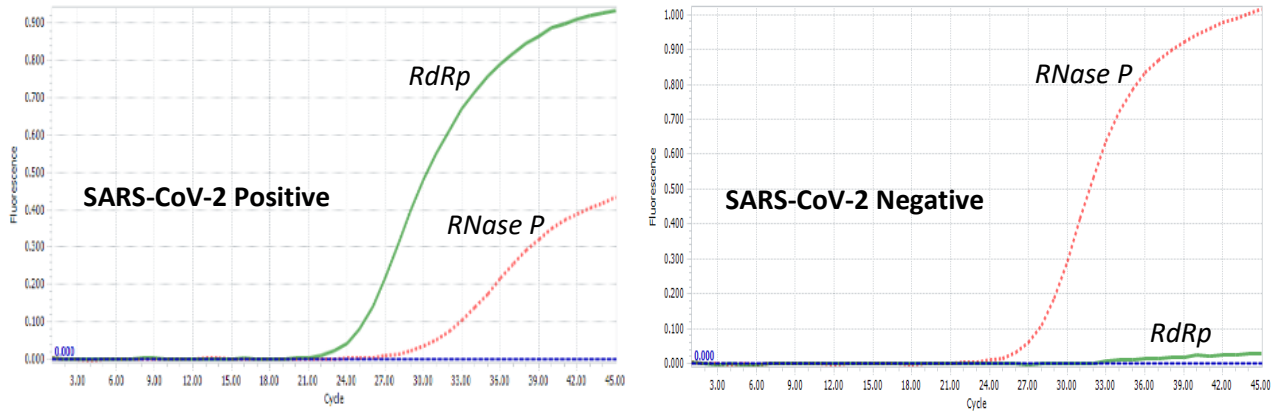

Figure 1. *Examples of the amplification curves.*

Performance Characteristics

Limit of Detection (LoD)

Limit of detection (LoD) studies determine the lowest detectable concentration of SARS-CoV-2 at which greater or equal to 95% of all (true positive) replicates test positive.

In LoD studies, a cultured SARS-CoV-2 virus of an isolate from a patient (provided by Republic of Turkey, Ministry of Health, General Directorate of Public Health) was used for artificial contamination. Dilutions of synthetic SARS-CoV-2 *RdRp* gene partial RNA were used as a quantification standard in real-time PCR to determine SARS-CoV-2 viral load in the cell culture. The cultured virus was serially diluted in VTM (viral transport medium).

For each sample type, a total of 4 concentration levels with 1.5, 2 and 3-fold dilutions between the levels, were tested with a total of 120 replicates (20 replicates in 3 different instruments; *Roche LightCycler® 96, Bio-Rad CFX96 Touch™, Qiagen Rotor-Gene® 5 Plex* and at 2 different reaction volumes; 10 µL and 20 µL) per concentration, with an additional 60 replicates of blank samples (negative clinical specimens for SARS-CoV-2).

Limit of detection (LoD) of the *Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit* using *RINA™ M14 Nucleic Acid Extraction Robot, Cat No: RINA-M14-01* and its consumables *(Cat No: RN-NA-14-111-100)* is 20 copies /mL for all the sample types. The summary of the LoD study stratified per specimen type is provided in Table 6.

Table 6. *The summary of LoD study results for each specimen type*

Sample Type	Concentration of Viral RNA (copies/mL)	Number Tested	SARS-CoV-2 *(RdRp)*			Internal Control *(RNase P)*		
			Positive	Cq (Avg)	SD	Positive	Cq (Avg)	SD
Nasopharyngeal Aspirate (NPA)	30	120	120 (100.0%)	36.99	0.08	120 (100.0%)	30.13	0.10
	20	**120**	**120 (100.0%)**	**37.56**	**0.08**	**120 (100.0%)**	**30.12**	**0.10**
	15	120	57 (47.5%)	38.02	0.17	120 (100.0%)	30.14	0.09
	10	120	3 (2.5%)	38.60	0.21	120 (100.0%)	30.13	0.09
	0	60	0 (0.0%)	-	-	60 (100.0%)	30.12	0.10
Bronchoalveolar Lavage (BAL)	30	120	120 (100.0%)	37.07	0.09	120 (100.0%)	31.92	0.09
	20	**120**	**118 (98.3%)**	**37.67**	**0.11**	**120 (100.0%)**	**31.93**	**0.10**
	15	120	20 (16.7%)	38.08	0.08	120 (100.0%)	31.92	0.12
	10	120	0 (0.0%)	38.67	0.10	120 (100.0%)	31.94	0.11
	0	60	0 (0.0%)	-	-	60 (100.0%)	31.92	0.10

Sample Type	Concentration of Viral RNA (copies/mL)	Number Tested	SARS-CoV-2 *(RdRp)*			Internal Control *(RNase P)*		
			Positive	Cq (Avg)	SD	Positive	Cq (Avg)	SD
Nasopharyngeal Dacron Swab (NPDS)	30	120	120 (100.0%)	37.08	0.09	120 (100.0%)	32.23	0.11
	20	**120**	**115 (95.8%)**	**37.68**	**0.13**	**120 (100.0%)**	**32.23**	**0.10**
	15	120	21 (17.5%)	38.08	0.08	120 (100.0%)	32.23	0.10
	10	120	0 (0.0%)	38.68	0.09	120 (100.0%)	32.22	0.11
	0	60	0 (0.0%)	-	-	60 (100.0%)	32.23	0.11
Nasopharyngeal Polyester Flocked Swab (NPFS)	30	120	120 (100.0%)	37.01	0.08	120 (100.0%)	30.52	0.09
	20	**120**	**120 (100.0%)**	**37.58**	**0.08**	**120 (100.0%)**	**30.53**	**0.09**
	15	120	49 (40.8%)	38.02	0.09	120 (100.0%)	30.52	0.11
	10	120	2 (1.7%)	38.59	0.13	120 (100.0%)	30.54	0.10
	0	60	0 (0.0%)	-	-	60 (100.0%)	30.51	0.11
Oropharyngeal Dacron Swab (OPDS)	30	120	120 (100.0%)	37.06	0.09	120 (100.0%)	31.81	0.10
	20	**120**	**116 (96.7%)**	**37.66**	**0.11**	**120 (100.0%)**	**31.84**	**0.11**
	15	120	24 (20.0%)	38.07	0.08	120 (100.0%)	31.82	0.10
	10	120	0 (0.0%)	38.65	0.10	120 (100.0%)	31.83	0.11
	0	60	0 (0.0%)	-	-	60 (100.0%)	31.82	0.11
Oropharyngeal Polyester Flocked Swab (OPFS)	30	120	120 (100.0%)	37.05	0.08	120 (100.0%)	31.11	0.10
	20	**120**	**119 (99.2%)**	**37.63**	**0.09**	**120 (100.0%)**	**31.13**	**0.09**
	15	120	47 (39.2%)	38.03	0.09	120 (100.0%)	31.13	0.11
	10	120	2 (1.7%)	38.60	0.13	120 (100.0%)	31.12	0.10
	0	60	0 (0.0%)	-	-	60 (100.0%)	31.14	0.11
Sputum	30	120	120 (100.0%)	37.11	0.09	120 (100.0%)	32.62	0.10
	20	**120**	**114 (95%)**	**37.69**	**0.11**	**120 (100.0%)**	**32.61**	**0.09**
	15	120	14 (11.7%)	38.10	0.08	120 (100.0%)	32.63	0.09
	10	120	0 (0.0%)	38.70	0.11	120 (100.0%)	32.62	0.10
	0	60	0 (0.0%)	-	-	60 (100.0%)	32.63	0.11

The effect of qPCR volume on test performance was also examined during LoD studies. Average Cq values of 10 µL and 20 µL qPCRs in LoD concentrations for each sample type were given in Table 7. These results showed that both 10 µL and 20 µL qPCRs can be used for the assay.

Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit was validated only for the template nucleic

acid volume that is 25% of the total qPCR volume.

Table 7. *The effect of qPCR volume on test performance (with RINA™ M14 Nucleic Acid Extract. Robot)*

Sample Type	Viral RNA copies/mL (LoD)	SARS-CoV-2 *(RdRp)*				Internal Control *(RNase P)*			
		20 µL		10 µL		20 µL		10 µL	
		Cq (Avg)	SD	Cq (Avg)	SD	Cq (Avg)	SD	Cq (Avg)	SD
NPA	20	37.54	0.08	37.58	0.08	30.09	0.09	30.15	0.11
BAL	20	37.64	0.11	37.69	0.10	31.90	0.10	31.95	0.10
Sputum	20	37.67	0.11	37.72	0.10	32.59	0.09	32.64	0.10
NPDS	20	37.64	0.11	37.72	0.14	32.20	0.10	32.26	0.10
NPFS	20	37.55	0.07	37.61	0.08	30.51	0.09	30.55	0.09
OPDS	20	37.62	0.10	37.69	0.12	31.81	0.10	31.86	0.11
OPFS	20	37.60	0.08	37.66	0.10	31.11	0.09	31.16	0.09

Inclusivity (Analytical Sensitivity)

The inclusivity was tested wet with 38 different clinical samples confirmed SARS-CoV-2 positive by DNA sequence analysis and tested in silico with SARS-CoV-2 whole genomes from 42 different geo locations. Repeatability of the kit is 100%. The reproducibility is 100% at concentrations over the LOD, and 67-83% at concentrations below the LOD.

Wet Tests

The kit was applied to 38 SARS-CoV-2 positive clinical samples in VTM. Viral load in the 38 positive clinical specimens were determined by using the dilutions of synthetic SARS-CoV-2 *RdRp* gene partial RNA as a quantification standard in Real-Time PCR.

The quantified samples were diluted to 1000 viral copies/mL in the sample mixture which is qPCR negative for SARS-CoV-2. The positive results were obtained for all the 38 positive clinical specimens containing 1000 viral copies/mL.

In-Silico Tests

All SARS-CoV-2 nucleotide sequences in available nucleotide databases *(NCBI; https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)* were searched against the SARS-CoV-2 targeted oligonucleotide sequences. The results are summarized in Table 8. 99.7% and 99.9% of the available 15324 SARS-CoV-2 nucleotide sequences resulted in 100% identity for the forward and reverse primers respectively; 99.8% of the available 15324 SARS-CoV-2 nucleotide sequences resulted in 100% identity for the probe sequence. The maximum number of mismatches is 2 for the 25-base long probe, 1 for the 22-base long forward primer, and 1 for the 28-base long reverse primer.

Table 8. *Inclusivity test results (in-silico)*

Oligo Name	Minimum Identity[1]	Maximum Mismatches[2]	Number of Total Alignments	Number of Alignments with Mismatches	Number of Alignments with 100% Identity
RdRp Primer-1	95.5%	1	15324	41	15283 (99.7%)
RdRp Primer-2	96.4%	1	15324	13	15311 (99.9%)

RdRp Prob	92.0%	2	15324	23	15301 (99.8%)

[1] Minimum identity%= [(base long of the oligonucleotide - number of maximum mismatches) / base long of the oligonucleotide] x 100.

[2] The decrease in Tm values when there are 1 base mismatch in the primer sequences or 2 base mismatches in the probe sequence does not prevent the primers and probe from remaining attached during the amplification step.

Exclusivity/ Cross-reactivity (Analytical Specificity)

Wet Tests

The exclusivity was tested wet with 43 respiratory pathogens at clinically relevant concentrations (10^5 genome copies/mL) and a pooled nasal wash from 20 different people, following extraction by using *RINA™ M14 Nucleic Acid Extraction Robot, Cat No: RINA-M14-01* and its consumables *(Cat No: RN-NA-14-111-100)*. All pathogens were tested in duplicate and none produced any detectable reactivity with the *Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit*.

The wet tests showed that the kit does not cross-react with the other respiratory pathogens or the microbial flora in the human respiratory tract.

In-Silico Tests

In silico tests were carried out using Primer Blast tool of NCBI (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) by entering the tested cross-reacting organism/strain and oligonucleotide sequences into the relevant fields. BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome) was also used for comparing the whole target region on SARS-CoV-2 Ref Seq Genome (NC_045512, 15431-15532) with the genomes of cross reacting organisms/strains. SARS-CoV-2 taxon was excluded in the BLAST searches while the cross-reacting organisms/strains were included.

The in-silico tests showed that the kit does not cross-react with any organism/strain except some SARS-CoV strains other than SARS-CoV-2. The blast search showed that the target region on SARS-CoV-2 genome resembles more than 90% to some SARS-CoV strains. Since the prevalence of these strains is very low, the cross-reactivity with them is irrelevant.

Table 9. *The exclusivity (cross-reactivity) test results*

Organism	In-Silico Analysis	Laboratory Testing (Wet-Tested)			
		RdRp		*RNAse P*	
		Cq (Avg)[1]	SD	Cq (Avg) [1]	SD
Human Coronavirus 229E	Not detected	No Cq	-	30.03	0.06
Human Coronavirus OC43	Not detected	No Cq	-	30.08	0.02
Human Coronavirus NL63	Not detected	No Cq	-	30.12	0.03
Human Coronavirus HKU1	Not detected	No Cq	-	30.24	0.03
MERS-coronavirus	Not detected	No Cq	-	30.06	0.09
SARS CoV strain Frankfurt 1	Not detected	No Cq	-	30.13	0.05
Influenza A H1	Not detected	No Cq	-	29.79	0.02

Influenza A H3	Not detected	No Cq	-	30.10	0.04
Influenza B	Not detected	No Cq	-	30.15	0.12
Parainfluenza 1	Not detected	No Cq	-	30.08	0.03
Parainfluenza 2	Not detected	No Cq	-	30.02	0.07
Parainfluenza 3	Not detected	No Cq	-	29.97	0.06
Parainfluenza 4	Not detected	No Cq	-	30.02	0.07
Human Metapneumovirus (hMPV)	Not detected	No Cq	-	29.98	0.03
Rhinovirus	Not detected	No Cq	-	30.03	0.04
Respiratory syncytial virus (RSV) A	Not detected	No Cq	-	30.07	0.09
Respiratory syncytial virus (RSV) B	Not detected	No Cq	-	30.11	0.08
Bocavirus (BoV)	Not detected	No Cq	-	30.09	0.06
Enterovirus	Not detected	No Cq	-	30.10	0.11
Adenovirus	Not detected	No Cq	-	30.09	0.05
Legionella pneumophila	Not detected	No Cq	-	30.07	0.03
Chlamydia pneumoniae	Not detected	No Cq	-	30.02	0.05
Mycobacterium tuberculosis	Not detected	No Cq	-	30.04	0.05
Haemophilus influenzae	Not detected	No Cq	-	30.06	0.10
Streptococcus pneumoniae	Not detected	No Cq	-	30.12	0.02
Mycoplasma pneumoniae	Not detected	No Cq	-	30.05	0.03
Streptococcus pyogenes	Not detected	No Cq	-	30.07	0.04
Bordetella pertussis	Not detected	No Cq	-	30.09	0.06
Pneumocystis jirovecii	Not detected	No Cq	-	30.07	0.07
Candida albicans	Not detected	No Cq	-	30.08	0.04
Legionella bozemanii	Not detected	No Cq	-	30.08	0.05
Legionella micdadei	Not detected	No Cq	-	30.09	0.04
Corynebacterium diphtheriae	Not detected	No Cq	-	30.07	0.03
Bacillus anthracis	Not detected	No Cq	-	30.11	0.02
Moraxella catarrhalis	Not detected	No Cq	-	30.07	0.08
Neisseria meningitidis	Not detected	No Cq	-	30.10	0.13
Pseudomonas aeruginosa	Not detected	No Cq	-	29.98	0.05
Staphylococcus epidermidis	Not detected	No Cq	-	30.14	0.08
Coxiella burneti	Not detected	No Cq	-	30.10	0.07
Staphylococcus aureus	Not detected	No Cq	-	30.05	0.03
Streptococcus salivarius	Not detected	No Cq	-	30.15	0.05
Leptospira interrogans	Not detected	No Cq	-	30.05	0.02
Chlamydia psittaci	Not detected	No Cq	-	30.20	0.09
Pooled human nasal wash - *to represent diverse microbial flora in the human respiratory tract*	-	No Cq	-	27.29	0.11

[1] Average Cq values of dublicate qPCRs in 10^5 copies/mL concentrations for each organisms in VTM:Sample (1:1) mixtures.

Endogenous Interference Substances Studies

Robotic extraction method *(RINA™ M14 Nucleic Acid Extraction Robot, Cat No: RINA-M14-01* and its consumables, *Cat No: RN-NA-14-111-100)* was tested for inhibition with interfering substances. Mucin, blood and nasal sprays at >50% (v/v), nasal corticosteroids and gels at >10% (v/v), throat lozenges and anti-virals at >10% (w/v), antibiotics at >1% (w/v) may interfere with the *Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit*.

Clinical Evaluation

Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit (465 µL sample input) was applied to 500 (387 positives and 113 negatives according to comparator) clinical samples *(nasopharyngeal swabs, oropharyngeal swabs, nasopharyngeal aspirates, sputum and bronchoalveolar lavage samples)* in VTM concurrently with another RT-qPCR kit authorized by WHO (500 µL sample input) in a blinded fashion. Samples were obtained from individuals suspected of COVID-19 (47%) or from individuals having COVID-19 (53%). No restrictions were placed on age, gender, medications or known pharmaceutical therapies. 500 individuals in the intensive care unit (ICU) (46%) and non-ICU settings (54%) were enrolled in this study. DNA sequence analysis was applied when the assays were not in agreement. The overall tests resulted in 378 true positives and 113 true negatives. Sensitivity and specificity of the *Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit* are 97.7% and 100% respectively.

Assay Limitations

- *Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit* is intended for use in a laboratory environment by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures.
- The clinical specimens shall be collected by a healthcare provider in accordance with the updated version of CDC Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens for COVID-19 (https://www.cdc.gov/coronavirus/2019-ncov/lab/guidelines-clinical-specimens.html).
- A false negative result may occur if a specimen is improperly collected, transported or handled.
- Performance of the *Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit* has only been established in nasopharyngeal swab, oropharyngeal (throat) swab, nasopharyngeal aspirate or lavage and bronchoalveolar lavage samples.
- The use of cotton or calcium alginate swabs or swabs with wooden sticks can lead to false negative results since they may contain substances that inactivate some viruses and inhibit PCR. Flocked (polyester) or dacron swabs are recommended for collection of nasopharyngeal/ oropharyngeal swab samples.
- Mutations within the target regions of the *Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit* could affect primer and/or probe binding resulting in failure to detect the presence of virus.
- Inhibitors or other types of interference may produce a false negative result. False negative results may also occur if inadequate numbers of organisms are present in the specimen. Mucin, blood and nasal sprays at >10% (w/v), nasal corticosteroids and gels at >1% (v/v), throat lozenges and anti-virals at >0.1% (w/v), antibiotics at >0.01% (w/v) may interfere with the *Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit*.
- Detection of SARS-CoV-2 RNA may be affected by patient factors (e.g., presence of symptoms), and/or stage of infection.
- Based on the in-silico analysis, other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 may cross-react with the *Bio-Speedy® SARS-CoV-2 (2019-nCoV) qPCR Detection Kit*. Other SARS-like coronaviruses in the same subgenus (Sarbecovirus) as SARS-CoV-2 are not known to be currently circulating in the human population, therefore are highly unlikely to be present in patient specimens.

Troubleshooting

Observation: In the Positive Control wells, no target-specific and no Internal Control (IC) signals are detected.

Possible Cause: PCR amplification failure

Recommended Action: Check that the thermal cycler settings and amplification program are correct. If there is no error in these, there may be a reagent problem; contact the manufacturer, renew the reagents, and repeat the reaction

Observation: In the Negative Control wells, target-specific and/or IC signals are detected.

Possible Cause: Contamination of the PCR.

Recommended Action: Contamination may be due to errors in sample handling, reagent contamination, or environmental contamination.

- Decontaminate benchtop surfaces and other equipment where PCR is performed with 10% bleach solution.
- Use fresh reagents and repeat the PCR.
- Set up the Positive Control reactions last to avoid cross-contamination.
- Pay attention to the issues in the "General Requirements and Warnings for Good Practices on PCR and RT-PCR" section.

Observation: In unknown wells (sample wells), no IC signal is detected, but target-specific signal is detected.

Possible Cause: A high copy number of target nucleic acid (NA) exists in samples, resulting in preferential amplification of the target-specific NA.

Recommended Action: No action is required. The result is considered positive.

Observation: In unknown wells (sample wells), no IC and no target-specific signal is detected

Possible Cause: Inhibition Problem:

- **Recommended Action:** Dilute the nucleic acid isolate 1/10 and repeat the PCR. If the diluted sample does not give a positive result in the IC channel, request for a new sample and repeat the NA extraction. If the problem persists, contact Technical Support.

Possible Cause: Extraction Problem

- **Recommended Action:** Repeat the NA extraction and the PCR. If the problem persists, contact Technical Support.

Possible Cause: Sampling Problem

- **Recommended Action:** Request for a new sample, repeat the NA extraction and the PCR. If the problem persists, contact Technical Support.

Customer and Technical Support

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