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

**Product: Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit
EUL Number: EUL 0486-139-00
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.

Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit with product code RR-0485-02, Rest-of-World regulatory version manufactured by Shanghai ZJ Bio-Tech Co., Ltd, Building #26, 588 Xinjunhuan Road, Shanghai 201114, China was listed as eligible for WHO procurement on 22 May 2020.

Report amendments and/or product changes

This public report has since been amended. Amendments may have arisen because of changes to the product under EUL 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	In the early stage of product development, the commercial extraction kit QIAamp Viral RNA Mini Kit (Cat. No.52904/52906) manufactured by QIAGEN was selected as the only recommended extraction method. Although this QIAGEN kit is effective in extraction, the manual extraction approach has operational limitations. The additional recommended extraction reagent, Viral RNA Isolation Kit (Preloaded for Auto Extraction), utilizes magnetic particle technology for isolation and purification of pathogen's nucleic acid from biological specimens with automated nucleic acid extraction systems. Compared with QIAGEN kit, this additional extraction method not only guarantees extraction effectiveness but also minimizes manual work during extraction process.	26 April 2021

Intended use:

According to the claim of intended use from Shanghai ZJ Bio-Tech Co., Ltd, *"the Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit is an in vitro diagnostic test for manual qualitative detection of ORF 1ab, N and E genes of SARS-CoV-2 RNA in nasopharyngeal or oropharyngeal swabs and sputum specimens collected from individuals suspected of being infected with SARS-CoV-2. The kit is for aiding in the diagnosis of COVID-19 infection and it is for professional use in level 2 biosafety laboratory by laboratory personnel trained in RT-PCR."*

Specimen type(s) that were validated:

Nasopharyngeal or Oropharyngeal swabs and sputum specimens.

Test kit contents:

Component	50 tests (product code RR-0485-02)
SARS-CoV-2 Super Mix	1 vial x 513 µL
RT-PCR Enzyme Mix	1 vial x 27 µL
SARS-CoV-2 Internal Control	1 vial x 30 µL
SARS-CoV-2 Negative Control	1 vial x 400 µL
SARS-CoV-2 Positive Control	1 vial x 30 µL

Items required but not provided:

Extraction/Purification:

Extraction reagent:

- QIAamp Viral RNA Mini Kit, catalogue number 52904/52906.
- Viral RNA Isolation Kit (Preloaded for Auto-Extraction), catalogue number ME-0044.

Extraction platform:

- Automated Nucleic Acid Extraction Instrument (Liferiver, Cat. No. EX3600) manufactured by Shanghai ZJ Bio-Tech Co., Ltd

General laboratory equipment and consumables:

- Vortex mixer (Qinlinbeier ; catalog # VORTEX-5) or equivalent
- Microcentrifuge

- Desk top centrifuge with a rotor for 2ml reaction tubes (Eppendorf; catalog #5415C) or equivalent
- Micropipettes
- Multichannel micropipettes
- Racks for 1.5mL microcentrifuge tubes
- 2 x 96-well -20°C cold-blocks
- Molecular grade water, nuclease-free
- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- DNAZap (Ambion, cat. #AM9890) or equivalent
- RNAse Away (Fisher Scientific; cat. #21-236-21) or equivalent
- Disposable powder-free gloves and surgical gowns
- Aerosol barrier pipette tips
- 1.5mL microcentrifuge tubes (Axygen; catalog #MCT-150-C) or equivalent
- 8-strip tubes (Axygen; catalog #PCR-0208-C) or equivalent
- 8-strip caps (Axygen; catalog #PCR-2CP-RT-C) or equivalent
- Class II (or higher) biological safety cabinet (BSC)

Amplification and detection instruments:
- 7500 Real-time PCR Systems with SDS 2.3 software (Applied Biosystems; catalog #4351104 or #4351105)
- 7500 Fast Real-time PCR Systems with SDS 2.3 software (Applied Biosystems; catalog #4351106 or #4351107)

Storage:
Store all reagents below -20±5°C.

Shelf-life upon manufacture:

6 months, real-time stability study is ongoing.

Warnings/limitations:

Refer to the instructions for use (IFU)

Product dossier assessment

Shanghai ZJ Bio-Tech Co., Ltd submitted a product dossier for the Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit for detecting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (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 Commitment for EUL:

As a commitment to listing, the manufacturer is required to review the limit of detection with the WHO international standard by 15 February 2022.

Risk benefit assessment conclusion: acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, Shanghai ZJ Bio-Tech Co., 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, it was established that sufficient information was provided by Shanghai ZJ Bio-Tech Co., Ltd to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (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).[1]

Shanghai ZJ Bio-Tech Co., 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.

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[1] Available on the web page

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

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*" (ISBN 978-92-4-001531-9).

Scope and duration of procurement eligibility

Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit with product code RR-0485-02 manufactured by Shanghai ZJ Bio-Tech Co., 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 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, Shanghai ZJ Bio-Tech Co., Ltd must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. Shanghai ZJ Bio-Tech Co., Ltd 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.
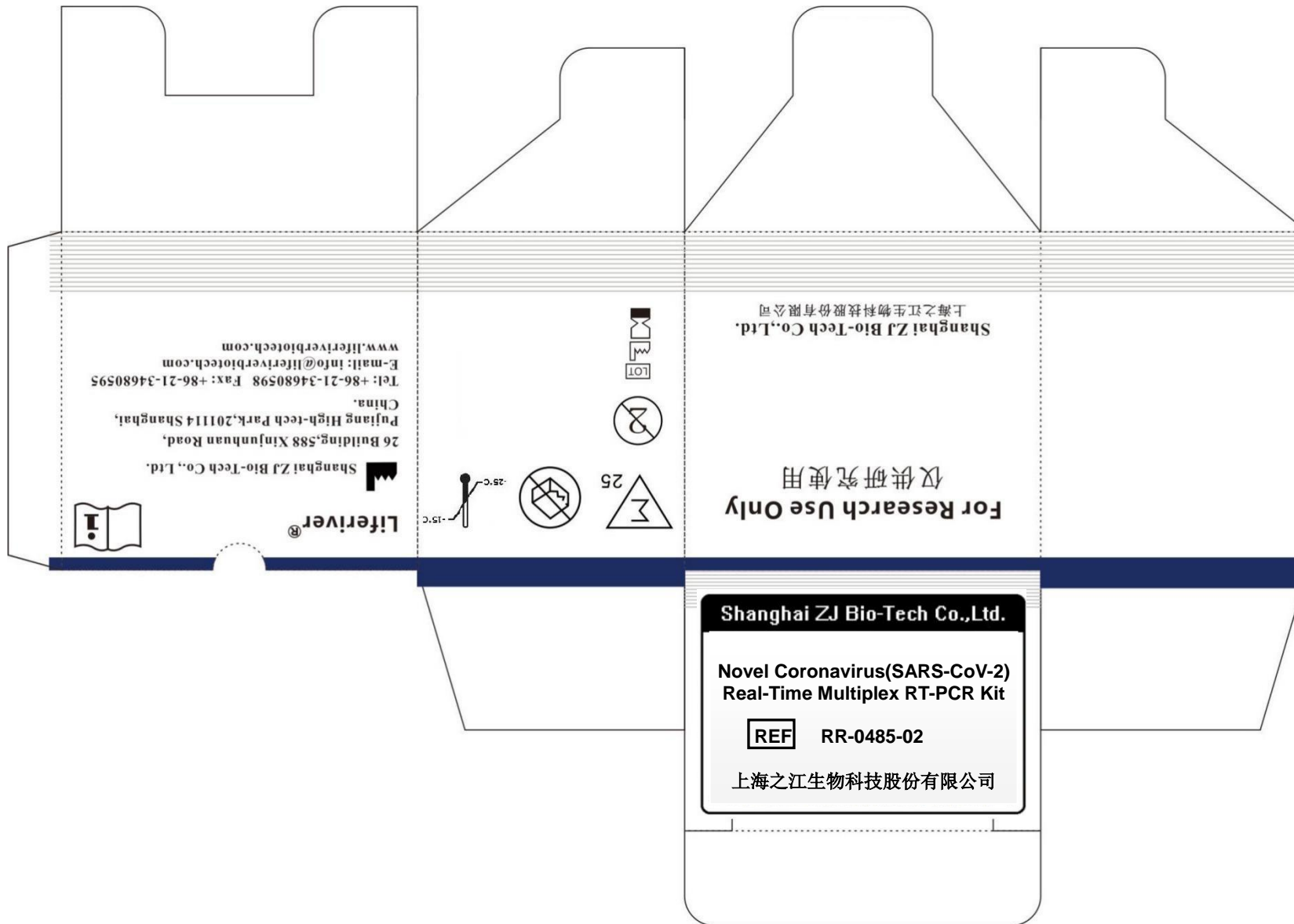
Labelling

1.0 Labels

2.0 Instructions for Use (IFU)

1.0 Outer box label and vial labels

Labels of Novel Coronavirus (SARS-CoV-2) Real Time Multiplex RT-PCR Kit (Cat. #: RR-0485-02)

Shanghai ZJ Bio-Tech Co.,Ltd.

26 Building,588 Xinjunhuan Road,
Pujiang High-tech Park,201114 Shanghai,
China.
Tel: +86-21-34680598 Fax: +86-21-34680595
E-mail: info@liferiverbiotech.com
www.liferiverbiotech.com

Liferiver®

上海之江生物科技股份有限公司
Shanghai ZJ Bio-Tech Co.,Ltd.

For Research Use Only

仅供研究使用

LOT

-25℃
-15℃

25

Shanghai ZJ Bio-Tech Co.,Ltd.

**Novel Coronavirus(SARS-CoV-2)
Real-Time Multiplex RT-PCR Kit**

REF   RR-0485-02

上海之江生物科技股份有限公司

SARS-CoV-2 Super Mix
Vol：513μL
Store at：-20±5℃
Lot：

RT-PCR Enzyme Mix
Vol：27μL
Store at：-20±5℃
Lot：

**SARS-CoV-2
Internal Control**
Vol：30μL
Store at：-20±5℃
Lot：

**SARS-CoV-2
Negative Control**
Vol：400μL
Store at：-20±5℃
Lot：

**SARS-CoV-2
Positive Control**
Vol：30μL
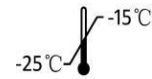Store at：-20±5℃
Lot：

2.0 Instructions for use[2]

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[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.

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Novel Coronavirus (SARS-CoV-2)

Real-Time Multiplex RT-PCR Kit

Instructions For Use

For Emergency Use Only

For *In Vitro Diagnostic* Use Only

-15℃
-25℃

Σ 25

REF **RR-0485-02**

Table of Contents

Intended Use

The Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit is an in vitro diagnostic test is for manual qualitative detection of ORF 1ab, N and E genes of SARS-CoV-2 RNA in nasopharyngeal or oropharyngeal swabs and sputum specimens collected from individuals suspected of being infected with SARS-CoV-2. The kit is for aiding in the diagnosis of SARS-CoV-2 infection and it is for professional use in level 2 biosafety laboratory by laboratory personnel trained in RT-PCR.

Summary and Explanation

The SARS-CoV-2 is a β-coronavirus, which is enveloped non-segmented positive-sense RNA virus(subgenus sarbecovirus, Orthocoronavirinae subfamily). By June 20, 2020, SARS-CoV-2 has resulted in more than 8,690,000 confirmed human infections in a number of countries globally, including close to 461,300 deaths （https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports）.

The Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis of SARS-CoV-2 and is based on widely used real time RT-PCR technology utilizing reverse-transcriptase (RT) reaction to convert RNA into complementary DNA (cDNA) and nucleic acid amplification technology. The product contains Super Mix, RT-PCR Enzyme Mix and control material used in rRT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in respiratory specimens.
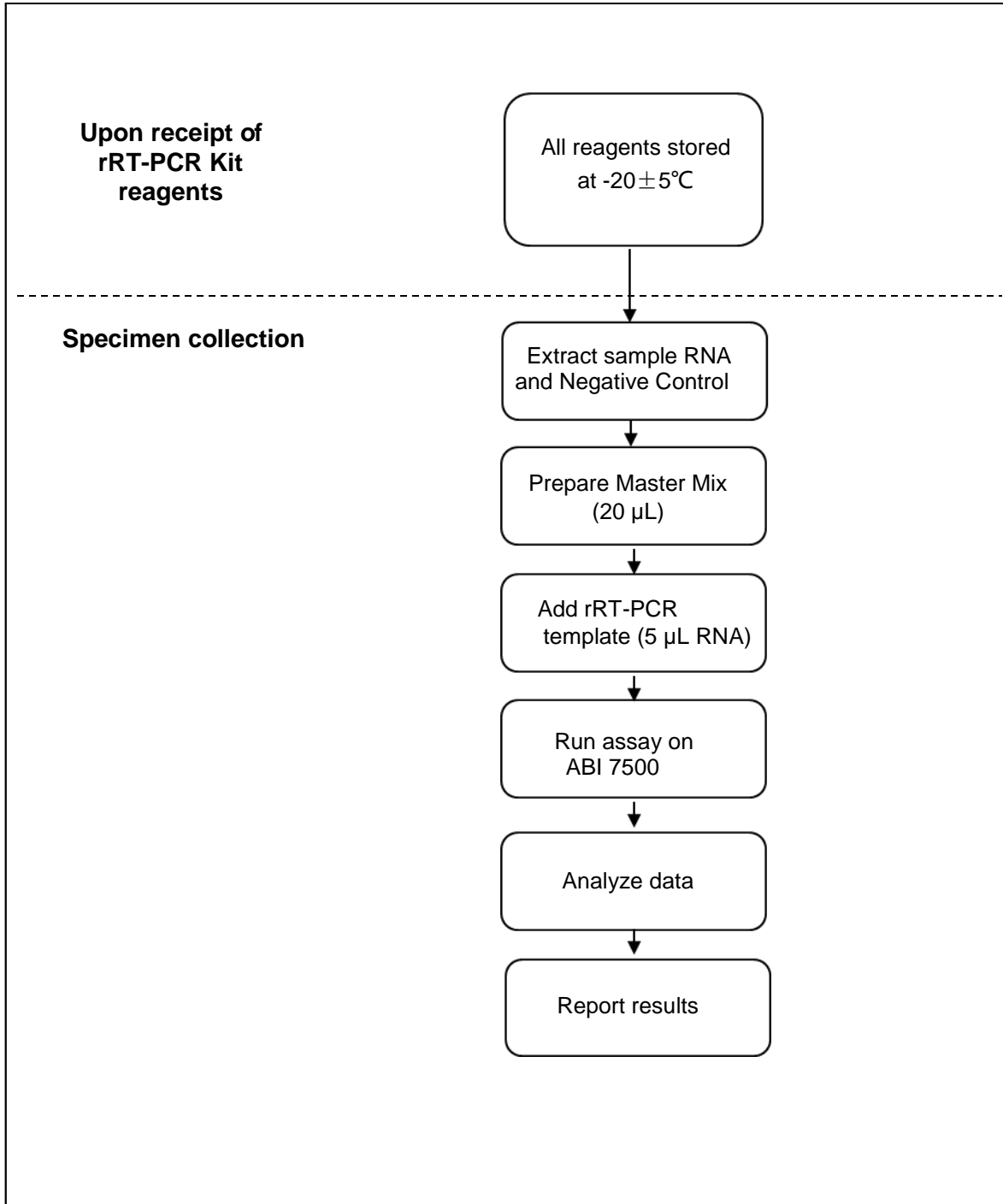
Principles of the Procedure

Three sets of oligonucleotide primers and probes for detection of SARS-CoV-2 were selected respectively from regions of the virus ORF1ab gene, N gene and E gene. An additional primer/probe set to detect the internal control gene processed with the clinical specimens is also included in the kit.

RNA isolated and purified from upper and lower respiratory specimens is reverse transcribed to cDNA and subsequently amplified in the real-time PCR instrument. In the process, the probe anneals to a specific target sequence located between the forward and reverse primers. During the extension phase of the PCR cycle, the 5′ nuclease activity of Taq polymerase degrades the probe, causing the reporter dye to separate from the quencher dye, generating a fluorescent signal. With each cycle, additional reporter dye molecules are cleaved from their respective probes, increasing the fluorescence intensity. Fluorescence intensity is monitored at each PCR cycle by real-time PCR system.

Detection of viral RNA not only aids in the diagnosis of illness but also provides epidemiological and surveillance information.

Summary of Preparation and Testing Process

Upon receipt of rRT-PCR Kit reagents

All reagents stored at -20±5℃

Specimen collection

Extract sample RNA and Negative Control

↓

Prepare Master Mix (20 μL)

↓

Add rRT-PCR template (5 μL RNA)

↓

Run assay on ABI 7500

↓

Analyze data

↓

Report results

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Materials Required (Provided)

RR-0485-02

Ref.	Type of Reagent	Quantity Sufficient for 25 Rxns
1	SARS-CoV-2 Super Mix	1 vial, 513 μL
2	RT-PCR Enzyme Mix	1 vial, 27 μL
3	SARS-CoV-2 Internal Control	1 vial, 30 μL
4	SARS-CoV-2 Negative Control	1 vial, 400 μL
5	SARS-CoV-2 Positive Control	1 vial, 30 μL

Control materials

- SARS-CoV-2 Negative Control is DEPC-water that will serve as an external negative specimen during RNA extraction procedure.
- SARS-CoV-2 Positive Control is a mixture of plasmids containing partial ORF1ab gene, N gene and E gene RNA fragment which are designed to cover the target sequence respectively to react with the real time RT-PCR reagents in this kit to indicate whether the real time RT-PCR worked.
- Internal Control (IC) is a plasmid containing non-target RNA fragment that will be added into the specimen before RNA extraction procedure to evaluate RNA extraction efficiency and identify possible PCR inhibitors. The RNA fragment in plasmid will be amplified and detected by another set of primer and probe.

Materials Required (Not Provided)

RNA Extraction Options

Manufacturer	Extraction Kit	Catalog No.	Operating Mode
QIAGEN	QIAamp Viral RNA Mini Kit	52904/52906	Manual operation
Shanghai ZJ Bio-Tech Co., Ltd.	Viral RNA Isolation Kit (Preloaded for Auto-Extraction)	ME-0044	Automated operation, used with Automated Nucleic Acid Extraction Instrument (Liferiver, Model EX3600)

Equipment and Consumables Required (Not Provided)

- Vortexmixer (Qinlinbeier; catalog # VORTEX-5) or equivalent
- Microcentrifuge
- Desk top centrifuge with a rotor for 2ml reaction tubes (Eppendorf; catalog #5415C) or equivalent
- Micropipettes
- Multichannel micropipettes
- Racks for 1.5 mL microcentrifuge tubes
- 2 x 96-well -20°C cold-blocks
- 7500 Real-time PCR Systems with SDS 2.3 software (Applied Biosystems; catalog #4351104 or #4351105) or 7500 Fast Real-time PCR Systems with SDS 2.3 software (Applied Biosystems; catalog #4351106 or #4351107)
- Automated Nucleic Acid Extraction Instrument (Liferiver, Model EX3600)
- Molecular grade water, nuclease-free

- 10% bleach (1:10 dilution of commercial 5.25-6.0% hypochlorite bleach)
- DNA*Zap*™ (Ambion, cat. #AM9890) or equivalent
- RNAse Away™ (Fisher Scientific; cat. #21-236-21) or equivalent
- Disposable powder-free gloves and surgical gowns
- Aerosol barrier pipette tips
- 1.5 mL microcentrifuge tubes (Axygen; catalog #MCT-150-C) or equivalent
- 8-strip tubes (Axygen; catalog #PCR-0208-C) or equivalent
- 8-strip caps (Axygen; catalog #PCR-2CP-RT-C) or equivalent
- Class II (or higher) biological safety cabinet (BSC)

Warnings and Precautions

- For *in vitro diagnostic* use (IVD).
- For emergency use only.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- 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/SARS-CoV-2/lab-biosafety-guidelines.html.
- Specimen processing should be performed in accordance with national biological safety regulations.
- 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 live virus specimens within a Class II (or higher) biological safety cabinet (BSC).
- Use personal protective equipment such as (but not limited to) gloves, eye protection, and lab coats when handling kit reagents, while performing this assay and handling materials including specimens, reagents, pipettes, and other equipment and reagents.
- Amplification technologies such as PCR are sensitive to accidental introduction of PCR product from previous amplification reactions. Incorrect results could occur if either the clinical specimen or the real-time reagents used in the amplification step become contaminated by accidental introduction of amplification product (amplicon). Workflow in the laboratory should proceed in a unidirectional manner.
  - Maintain separate areas for assay setup and handling of nucleic acids.
  - Always check the expiration date prior to use. Do not use expired reagent. Do not substitute or mix reagent from different kit lots or from other manufacturers.
  - Change aerosol barrier pipette tips between all manual liquid transfers.
  - During preparation of specimens, compliance with good laboratory techniques is essential to minimize the risk of cross-contamination between specimens, and the inadvertent introduction of nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with nucleic acids.

- Maintain separate, dedicated equipment (e.g., pipettes, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between specimens and whenever contamination is suspected.
- Keep reagent and reaction tubes capped or covered as much as possible.
- Super Mix and RT-PCR Enzyme Master Mix must be thawed and maintained on cold-block at all times during preparation and use.
- Do not open the reaction tubes/plates post amplification to avoid contamination with amplicons.
- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning products such as 10% bleach, "DNA*Zap*™" or "RNase AWAY™" to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- The consumables that have touched the control materials (such as tips), tubes with PCR amplification products, specimens and the residual components of the kit should be disinfected or sterilized before disposal.
- RNA should be maintained on cold-block or on ice during preparation and use to ensure stability.
- Dispose of unused kit reagents and human specimens according to local, state, and federal regulations.

Reagent Storage, Handling, and Stability

- Store all reagents at -20±5°C until thawed for use.
- Always check the expiration date prior to use. Do not use expired reagents.
- All reagents must be thawed and kept on a cold-block at all times during preparation and use.
- Repeated thaw-&-freeze for >3 times should be avoided as this may reduce the sensitivity of the assay.

Specimen Collection, Handling, and Storage

Inadequate or inappropriate specimen collection, storage, and transport are likely to yield false test results. Training in specimen collection is highly recommended due to the importance of specimen quality. CLSI MM13-A may be referenced as an appropriate resource.

➢ Collecting Specimens
- Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (SARS-CoV-2) https://www.cdc.gov/coronavirus/SARS-CoV-2/guidelines-clinical-specimens.html
- Follow specimen collection device manufacturer's instructions for proper collection methods.
- Nasopharyngeal (NP) and oropharyngeal (OP) swab specimens should be collected using only swabs with a synthetic tip, such as nylon or Dacron®, and an aluminum or plastic shaft. Calcium alginate swabs are unacceptable and cotton swabs with wooden shafts are not recommended. Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media. NP and OP specimens should be placed in the same tube to increase the viral load.
- Have the patient rinse the mouth with water and then expectorate deep cough sputum directly 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 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 SARS-CoV-2 specimens.
- ➢ Storing Specimens
  - • Specimens can be stored at 2-8°C for up to 72 hours after collection.
  - • If a delay in extraction is expected, then store specimens at -70°C or lower.
  - • Extracted nucleic acid should be stored at -70°C or lower.

General Preparation

Equipment Preparation

Clean and decontaminate all work surfaces, pipettes, centrifuges, and other equipment prior to use. Decontamination agents should be used including 5% bleach, 70% ethanol, and *DNAzap™* or *RNase AWA™* to minimize the risk of nucleic acid contamination.

Nucleic Acid Extraction

Performance of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit is dependent upon the amount and quality of template RNA purified from human specimens. The following commercially available RNA extraction kit and procedure have been qualified and validated for recovery and purity of RNA for use with the kit:

QIAamp Virus RNA Mini Kit

Recommendation(s): Utilize 140 µL of specimen and elute with 60 µL of buffer AVE.

Viral RNA Isolation Kit (Preloaded for Auto-Extraction)

Recommendation(s): Utilize 300 µL of specimen and proceed with the Automated Nucleic Acid Extraction Instrument (Liferiver, Model EX3600), with elution volume of 75 µL.

It is noted that SARS-CoV-2 Negative Control in this kit should be extracted with the same protocol as for specimens. The Internal Control in this kit should be added into the extraction mixture with 1µl/test to monitor the whole process.

Manufacturer's recommended procedures (except as noted in recommendations above) are to be followed for specimen extraction.

Assay Setup

Reaction Master Mix

Note: Plate set-up configuration can vary with the number of specimens and work day organization. Negative Control and Positive Control must be included in each run.

1) In the reagent setup room clean hood, place Super Mix and RT-PCR Enzyme Mix on ice or cold-

block. Keep cold during preparation and use.
2) Thaw Super Mix prior to use.
3) Mix Super Mix and RT-PCR Enzyme Mix by inversion 5 times.
4) Centrifuge Super Mix and RT-PCR Enzyme Mix for 5 seconds at the speed of 5000 rpm to collect contents at the bottom of the tube, and then place the tube in a cold rack.
5) Determine the number of reactions (N) to set up per assay. It is necessary to make excess reaction mix for the Negative Control, Positive Control, and for pipetting error. Use the following guide to determine N:
   - If number of specimens (n) including controls equals 1 through 14, then N = n +1
   - If number of specimens (n) including controls is 15 or greater, then N = n +2

Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit

Step #	Reagent	Vol. of Reagent Added per Reaction
1	Super Mix	N x 19 µL
2	Enzyme Mix	N x 1 µL
	Total Volume	**N x 20.0 µL**

6) After addition of the reagents, mix reaction mixtures for 5 seconds with vortex mixer.
7) Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
8) Set up reaction strip tubes or plates in a 96-well cooler rack.
9) Dispense 20 µL of master mix into each PCR tube.
10) Cover the entire reaction plate and move the reaction plate to the specimen nucleic acid handling area.

Template Addition
1) Gently vortex nucleic acid sample including positive and extracted negative control tubes for approximately 5 seconds.
2) Centrifuge for 5 seconds to collect contents at the bottom of the tube, and then place the tube in a cold rack.
3) After centrifugation, place nucleic acid sample including positive and negative control tubes in the cold rack.
4) Carefully pipette 5.0 µL of sample including positive and negative control into each well. *Keep other sample wells covered during addition. Change tips after each addition.*
5) Securely cap the column to which the sample has been added to prevent cross contamination and to ensure sample tracking.
6) Change gloves often and when necessary to avoid contamination.
NOTE: *If using 8-tube strips, label the TAB of each strip to indicate sample position.* **DO NOT LABEL THE TOPS OF THE REACTION TUBES!**
7) Briefly centrifuge reaction tube strips for 10-15 seconds. After centrifugation return to cold rack.
NOTE: *If using 96-well plates, centrifuge plates for 30 seconds at 500 x g, 4°C.*

Create and Run an Experiment on Applied Biosystems 7500 or 7500 Fast Real-time PCR Instrument

1) Launch Applied Biosystems 7500 or 7500 Fast Real-time PCR Instrument by double clicking on the 7500 Software v2.3 icon on the desktop.
2) A new window should appear, click **Log in as Guest** to log in anonymously.
3) Choose the **New Experiment** to start an experiment (see **Figure 1**)

Figure 1. Home Window
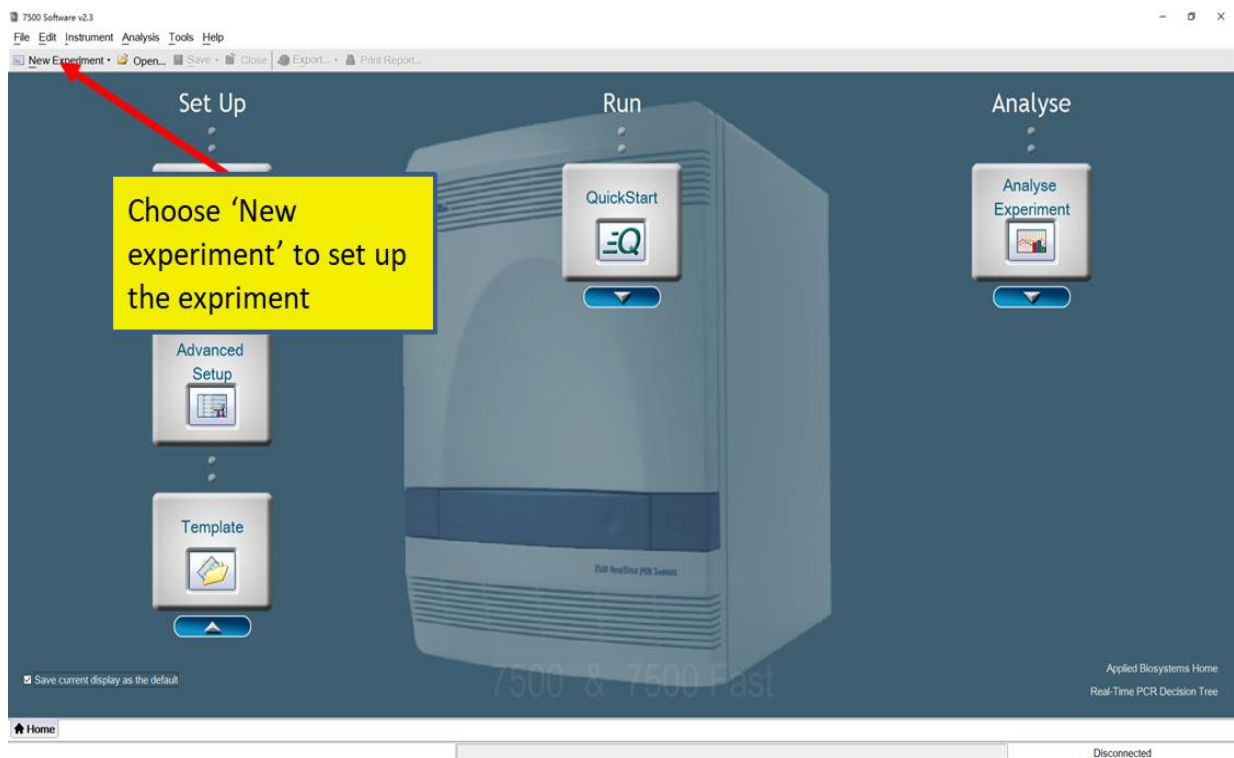
4) Set up the **Experiment Properties**. Fill in or select:
   - For 7500 Real-time PCR Systems (see **Figure 2**)
     a. Experiment name: *your own customized choice*
     b. Barcode (optional): *leave blank or your choice*
     c. User Name (optional): *leave blank or your name*
     d. Comments (optional): *leave blank or your choice*
     e. Which instrument are you using to run the experiment: **7500 (96-wells)**
     f. What type of experiment do you want to set up: **Quantitation-Standard Curve**
     g. Which reagents do you want to use to detect the target sequence: **TaqMan® Reagents**
     h. Which ramp speed do you want to use in the instrument run: **Standard (~2 hours to complete a run)**

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Figure 2. Experiment Properties Window (For 7500 Real-time PCR Systems)

- For 7500 Fast Real-time PCR Systems (see **Figure 3**)
    a. Experiment name: *your own customized choice*
    b. Barcode (optional): *leave blank or your choice*
    c. User Name (optional): ***leave blank or your name***
    d. Comments (optional): ***leave blank or your choice***
    e. Which instrument are you using to run the experiment: **7500 Fast (96-wells)**
    f. What type of experiment do you want to set up: **Quantitation-Standard Curve**
    g. Which reagents do you want to use to detect the target sequence: **TaqMan® Reagents**
    h. Which ramp speed do you want to use in the instrument run: **Standard (~2 hours to complete a run)**

Figure 3. Experiment Properties Window (For 7500 Fast Real-time PCR Systems)

5) After making selections click **Plate Setup** at the left side of the window. Then the **Define Targets and Samples** and **Assign Targets and Samples** will appear as below (see **Figure 4**).

Figure 4. Plate Setup
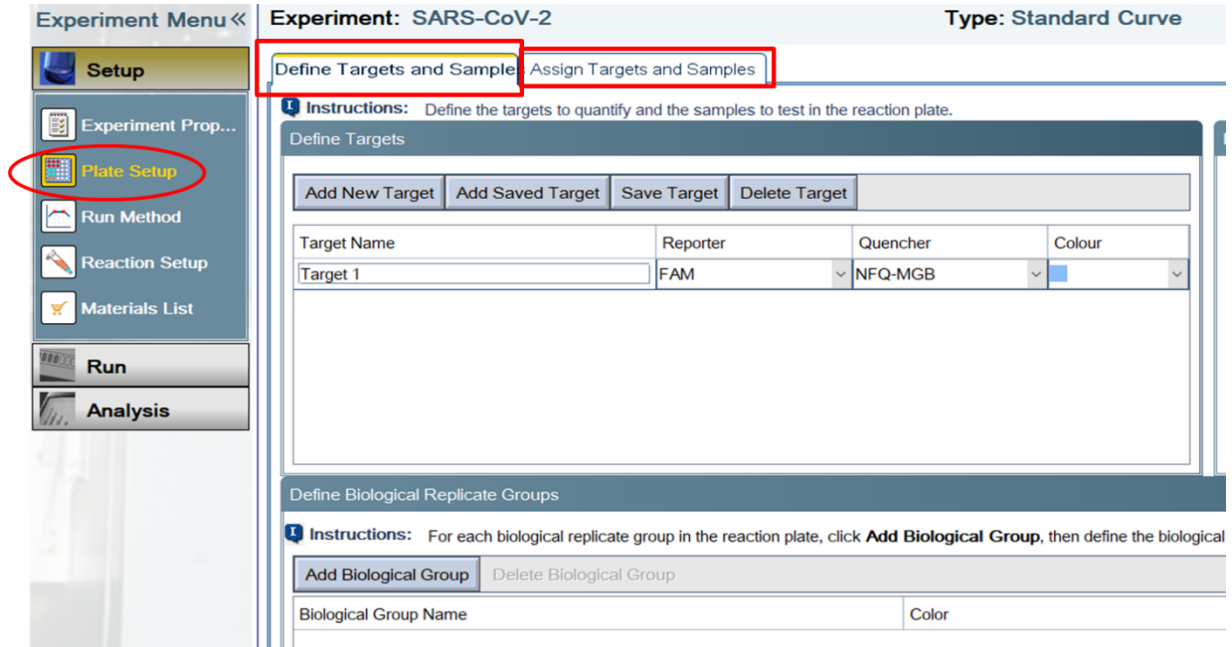
6) Define targets (see **Figure 5**). Fill in or select:
   a. Target Name: *1*
   b. Reporter: **FAM**
   c. Quencher: **None**
   d. Color: *to change the color of the detector indicator, do the following:*
      ⇒Click on the color square to reveal the color chart
      ⇒Select a color by clicking on one of the squares
   e. When necessary to add a new target or delete an exited target, click **Add New Target** or **Delete Target**.
7) Repeat Step 6 for each target in **Define Targets** window.

Name	Reporter Dye	Quencher Dye	Corresponding Gene
1	FAM	None	ORF1ab
2	VIC	None	N
3	TEXAS RED	None	E
IC	CY5	None	IC

'Target 1' represents 'ORF1ab gene', 'Target 2' represents 'N gene', and 'Target 3' represents 'E gene'.

Figure 5. Define Targets

8) Define Samples (see **Figure 6**). Fill in or select:
   a.   Sample Name: ***Your Choice***
   b. Color: *to change the color of the detector indicator do the following:*
       ⇒Click on the color square to reveal the color chart
       ⇒Select a color by clicking on one of the squares
   c. When necessary to add a new sample or delete an exited sample, click **Add New Sample** or **Delete Sample**.

Figure 6. Define Samples

9) Click **Assign Targets and Samples** (see **Figure 7**) to layout samples.
    a. Assign target(s): Select wells and assign the four targets including 'ORF1ab/N/E/IC'. Then specify the reaction well under **Task** tab (S means a standard while U represents an unknown sample and N is a negative control).
    b. Assign sample(s): Select wells and assign sample.
    c. Select the dye: **None**

Figure 7. Assign Targets and Samples

10) After finishing **Plate Setup**, proceed to **Run Method** (see **Figure 8**) in the **Experiment Menu**.
   a. Reaction Volume Per Well: *25*
   b. In First Holding Stage, Set to **10 min** at **45°C**.
   c. In First Holding Stage, Set to **3 min** at **95°C**.
   d. In Cycling Stage, Step 1 set to **15 sec** at **95°C.**
   e. In Cycling Stage, Step 2 set to **30 sec** at **58°C**.
   f. In Cycling Stage, Numbers of Cycles should be set to **45.**
   g. The icon under the time in Step 2 of Cycling Stage should be highlighted to indicate data collection.

Figure 8. Define Run Method
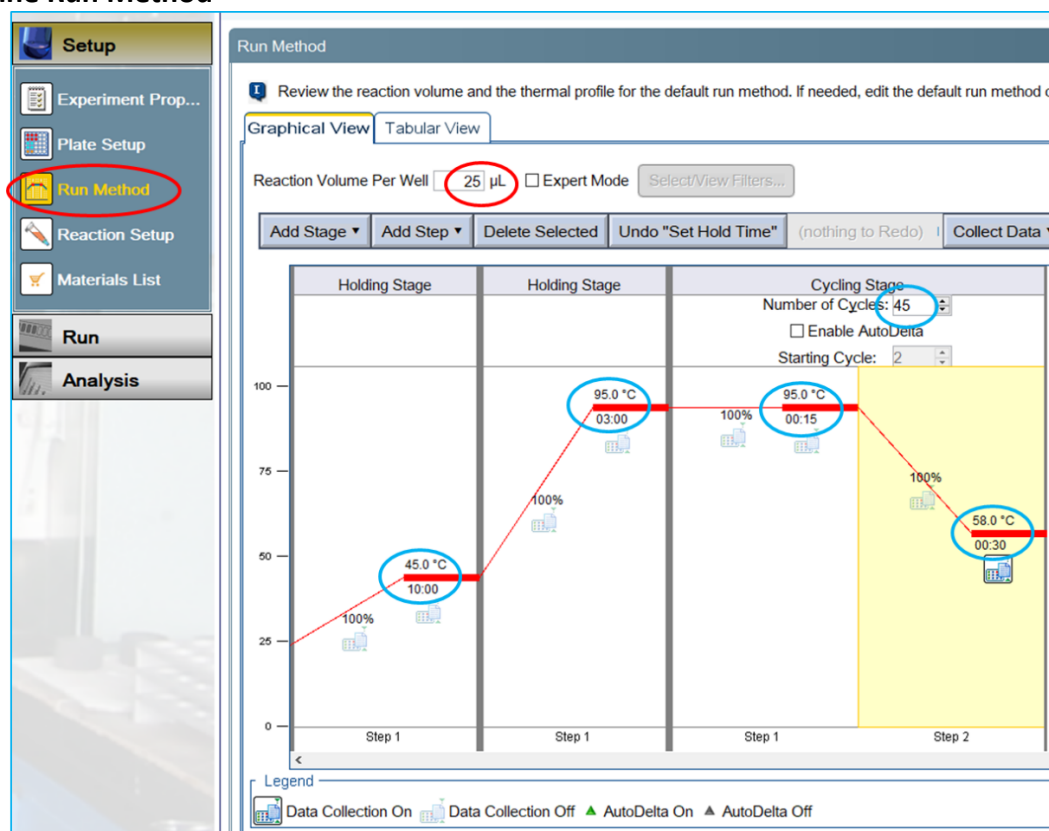
11) Before proceeding, the run file must be saved; from the main menu, select **File,** then **Save As**. Save in appropriate run folder designation.

12) Turn on ABI 7500 or 7500 Fast Real-time PCR Instrument.

13) Load the plate into the plate holder in the instrument. Ensure that the plate is properly aligned in the holder.

14) Once the run file is saved, click **Start** button. *Note: The run should take approximately 1 hour and 20 minutes to complete.*

Data Analysis

1) Once the run has completed, select the **Analysis** tab at the upper left corner of the software.

2) Select the Amplification Plot tab to view the raw data (see **Figure 9**).

3) Start by highlighting all samples from the run; to do this, click on the upper left hand box **(a)** of the sample wells (see **Figure 9**). All the growth curves should appear on the graph.

4) On the top of the window **(b)**, the **Plot Type** drop down selection should be set to **Δ Rn vs Cycle**. The **Graph Type** drop down selection should be set to **Linear (c)**.

5) Select **1** from **(d)** the Target drop down menu, using the downward arrow.
   Note: Please note that each target is analyzed individually to reflect different performance profiles of target.

6) Cancel the check of **Auto** in **Threshold (e)**.
   Note: Do not cancel the check of **Auto Baseline**.

7) Add the check of **Threshold** in **Show (f)**.

8) Using the mouse, click and drag the blue threshold line **(g)** until it lies within the exponential phase

of the fluorescence curves.

Figure 9. Amplification Plot Window
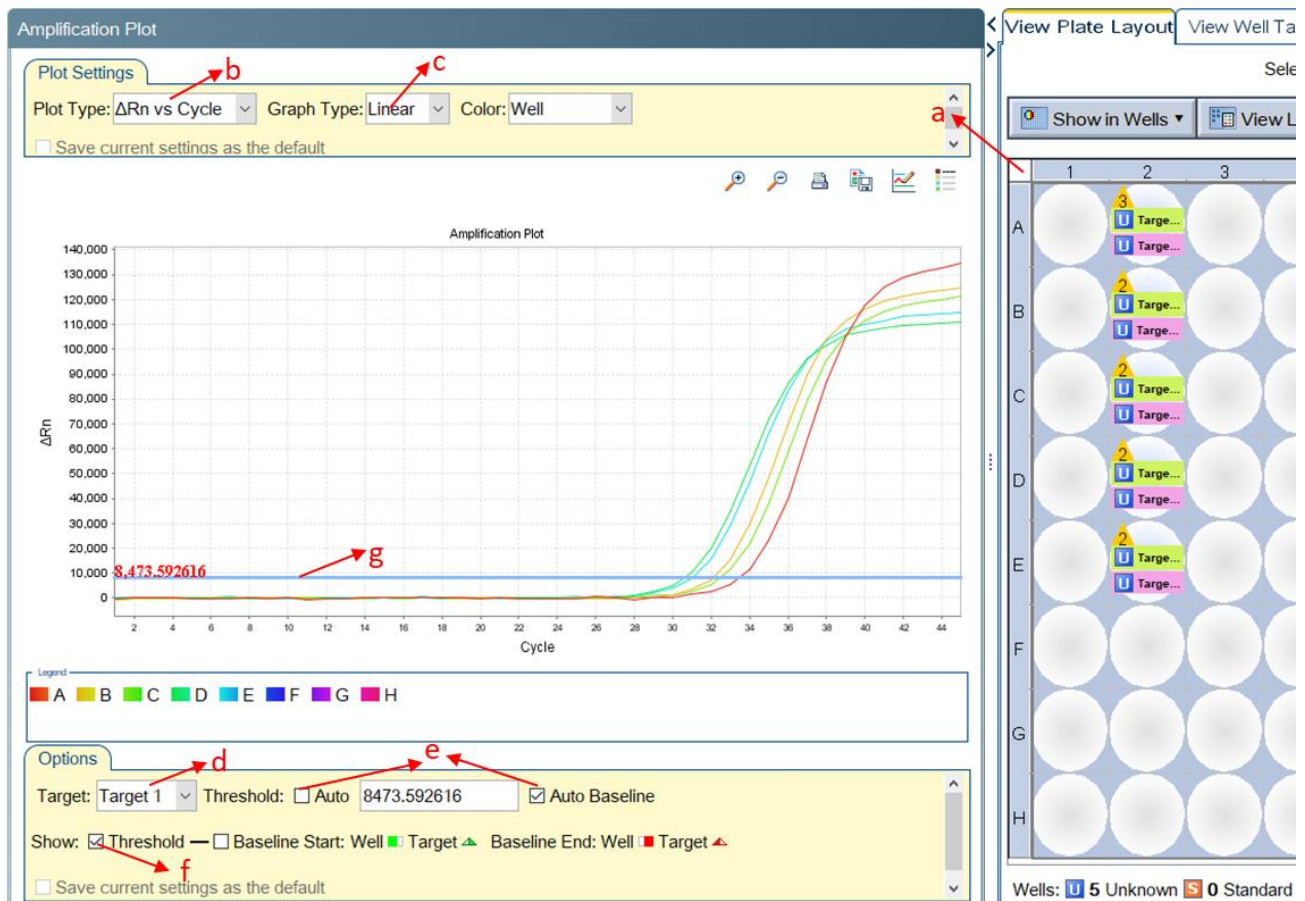
9) Click the **Reanalyse** button in the upper right corner of the window.
10) Repeat Steps 5-9 to analyze results generated for each set of markers (i.e. 1, 2, 3, etc).
11) Save analysis file by selecting **File**, then **Save As** from the main menu.
12) After completing analysis for each of the markers, click the **Export** tab, then the **Export Data** screen (see **Figure 10**) will appear. Select **Customise Export** to display the Ct values (see **Figure 10**).
13) To filter report by sample name in ascending or descending order, simply click on **Sample Name** in the table.

Figure 10. View Well Table
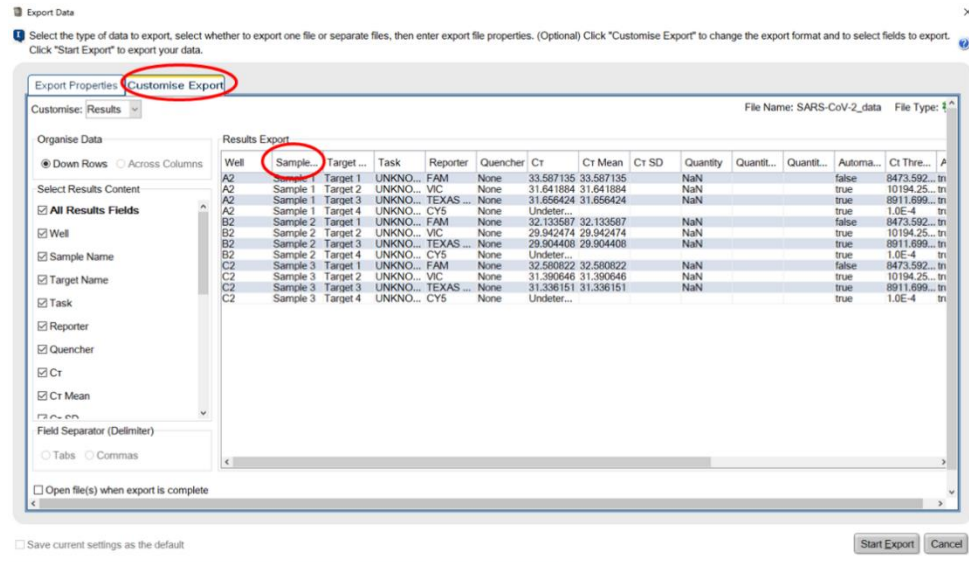
Interpretation of Results and Reporting

Expected Performance of Controls Included in Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit

Control Name	Detection Target			
	ORF1ab	N	E	IC
Positive Control	≤35Ct	≤35Ct	≤35Ct	None detected
Negative Control	None detected	None detected	None detected	Standard 'S' Amplification Curve

If any of the above controls do not exhibit the expected performance as described, the assay may have been set up and/or executed improperly, or reagent or equipment malfunction could have occurred. Invalidate the run and re-test.

SARS-CoV-2 Real-Time Multiplex RT-PCR Results Interpretation Guide

The table below lists the expected results for the SARS-CoV-2 Real-Time Multiplex RT-PCR Kit. If results are obtained that do not follow these guidelines, re-extract and re-test the specimen. If repeat testing yields similar results, contact Liferiver for consultation.

Ct Value				Result Interpretation[a]
ORF1ab	N	E	IC	
+	+	+	/	All target results are valid. SARS-CoV-2 RNA is detected.
+	-	+	/	All target results are valid. SARS-CoV-2 RNA is detected. Target ORF1ab and E are both positive andTarget N is negative, suggesting 1) a sample at concentrations near or below LoD of the test, 2) a mutation in Target N, target region, or 3) other factors.
+	+	-	/	All target results are valid. SARS-CoV-2 RNA is detected. Target ORF1ab and N results are both positive and Target E is negative, suggesting 1) a sample at concentrations near or below LoD of the test, 2) a mutation in Target E, target region, or 3) other factors.
+	-	-	/	All target results are valid. SARS-CoV-2 RNA is detected. Target ORF1ab result is positive and Target N and E are both negative, suggesting 1) a sample at concentrations near or below LoD of the test, 2) a mutation in Target N and Target E, target region, or 3) other factors.
-	+	+	/	All target results are valid. SARS-CoV-2 RNA is detected. Target ORF1ab result is negative and Target N and E are both positive, suggesting 1) a sample at concentrations near or below LoD of the test, 2) a mutation in Target ORF1ab, target region, or 3) other factors.
-	-	-	+	All target results are valid. SARS-CoV-2 RNA is not detected[b].
-	-	-	-	All target results are invalid. Sample should be retested; if the result is still invalid, a new specimen should be obtained.
-	-	+	/	All target results are valid. SARS-CoV-2 RNA is presumptive positive. Sample should be retested. For sample with a repeated presumptive positive result, additional confirmatory test may be performed if it is necessary to differentiate between SARS-CoV-2 and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans for epidemiological purposes or patient management.
-	+	-	/	

'+' represents a positive detection signal, which is defined as Ct≤41;
'-' represents a negative detection signal, which is defined as Ct>41;
'/' represents no requirement. Detection of Internal Control is not required if result positive in any of the other three detection channels.

Note:
[a]**Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system.**
[b]**Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined.**
Collection of multiple specimens from the same patient may be necessary to detect the virus.

Quality Control

- 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.
- Quality control procedures are intended to monitor reagent and assay performance.
- Test all positive controls prior to running diagnostic specimens with each new kit lot to ensure all reagents and kit components are working properly.
- Good Laboratory Practice (GLP) recommends a positive extraction control in each nucleic acid isolation batch.
- The internal control must be extracted and processed with each specimen at the same time to monitor the process of extraction.
- Always include negative control and positive control in each amplification and detection run.

Limitations

- All users, analysts, and any person reporting diagnostic results should be trained to perform this procedure by a competent instructor. They should demonstrate their ability to perform the test and interpret the results prior to performing the assay independently.
- Performance of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit has only been established in upper and lower respiratory specimens (includingnasopharyngeal or oropharyngeal swabs and sputum).
- Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for treatment or other patient management decisions. Optimum specimen types and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple specimens (types and time points) from the same patient may be necessary to detect the virus.
- A false negative result may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are present in the specimen or if inadequate numbers of organisms are present in the specimen. Additionally, false negative results may also occur if there's a mutation or deletion in target gene.
- Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely when prevalence is moderate to low.
- Do not use any reagent past the expiration date.
- If the virus mutates in the rRT-PCR target region, SARS-CoV-2 may not be detected or may be detected less predictably. Inhibitors or other types of interference may produce a false negative result.
- Test performance can be affected because the epidemiology and clinical spectrum of infection caused by SARS-CoV-2 is not fully known. For example, clinicians and laboratories may not know the optimum types of specimens to collect, and when during the course of infection these specimens are most likely to contain levels of viral RNA that can be readily detected.
- Detection of viral RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.

- The performance of this test has not been established for monitoring treatment of SARS-CoV-2 infection.
- The performance of this test has not been established for screening of blood or blood products for the presence of SARS-CoV-2.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Due to the relatively fast molecular revolution of RNA viruses, there is an inherent risk for any RT-PCR based test system that accumulation of mutations over time may lead to false negative results.

Performance Characteristics

Limit of Detection (LOD):

The LOD is defined as the lowest amount of analyte in a specimen that is detected with a 95% probability, and it was determined by probit analysis.

1). QIAGEN QIAamp Virus RNA Mini Kit

A dilution series consisting of 6 different dilutions levels of specimens, starting with 2,500 copies/ml (ORF1ab), was used. Since no positive specimens of the SARS-CoV-2 were available, assays designed for detection of the SARS-CoV-2 were tested with characterized stocks of nucleic acid spiked into SARS-CoV-2 negative specimens.

For LOD of nasopharyngeal swabs (NPS), the test was performed with one SARS-CoV-2 positive nucleic acid, mixed with SARS-CoV-2 NPS. Each dilution was extracted in 8 replicates. Specimens were tested with one lot of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit.

For LoD of oropharyngeal swabs (OPS), the test was performed with one SARS-CoV-2 positive nucleic acid, mixed with SARS-CoV-2 OPS. Each dilution was extracted in 8 replicates. Specimens were tested with one lot of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit.

For LOD of sputum, the test was performed with another SARS-CoV-2 positive nucleic acid, mixed with SARS-CoV-2 negative sputum specimens. Each dilution was extracted in 8 replicates. Specimens were then tested with two lots of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit.

A probit regression with SPSS Software was performed and the 95% LOD value was determined. The results for NPS,OPS and sputum specimens are shown in Table 1-12.

Table 1. Positive rate of NPS with Lot 1 (Target: ORF1ab)

SARS-CoV-2 concentration (copies/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
2,500	8	8	100%
791	8	8	100%
250	8	6	75%
79	8	1	13%
25	8	0	0%
8	8	0	0%

Table 2. Positive rate of NPS with Lot 1 (Target: N)

SARS-CoV-2 concentration (copies/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
4,986	8	8	100%
1,578	8	8	100%
499	8	8	100%
158	8	6	75%
50	8	2	25%
16	8	0	0%

Table 3. Positive rate of NPS with Lot 1 (Target: E)

SARS-CoV-2 concentration (copies/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
8,317	8	8	100%
2,632	8	8	100%
833	8	8	100%
264	8	6	75%
83	8	4	50%
26	8	0	0%

Table 4. LoD of NPS with Lot 1 by Probit

	Target	95% LOD by Probit	Lower 95% CI	Upper 95% CI
SARS-CoV-2 concentration (copies/mL)	ORF1ab	324	249	550
	N	213	156	447
	E	365	251	1,030

Table 5. Positive rate of OPS with Lot 1 (Target: ORF1ab)

SARS-CoV-2 concentration (copies/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
2,500	8	8	100%
791	8	8	100%
250	8	6	75%
79	8	3	38%
25	8	0	0%
8	8	0	0%

Table 6. Positive rate of OPS with Lot 1 (Target: N)

SARS-CoV-2 concentration (copies/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
4,986	8	8	100%
1,578	8	8	100%
499	8	7	88%

158	8	6	75%
50	8	1	13%
16	8	1	13%

Table 7. Positive rate of OPS with Lot 1 (Target: E)

SARS-CoV-2 concentration (copies/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
8,317	8	8	100%
2,632	8	7	88%
833	8	6	75%
264	8	4	50%
83	8	2	25%
26	8	0	0%

Table 8. LoD of OPS with Lot 1 by Probit

	Target	95% LOD by Probit	Lower 95% CI	Upper 95% CI
SARS-CoV-2 concentration (copies/mL)	ORF1ab	327	240	613
	N	239	162	549
	E	262	175	692

Table 9. Positive rate of sputum with Lot 2 and Lot 3 (Target: ORF1ab)

SARS-CoV-2 concentration (copies/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
2,500	16	16	100%
791	16	15	94%
250	16	10	63%
79	16	5	31%
25	16	4	25%
8	16	2	13%

Table 10. Positive rate of sputum with Lot 2 and Lot 3 (Target: N)

SARS-CoV-2 concentration (copies/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
5,133	16	16	100%
1,624	16	16	100%
514	16	13	81%
163	16	7	44%
51	16	7	44%
16	16	6	38%

Table 11. Positive rate of sputum with Lot 2 and Lot 3 (Target: E)

SARS-CoV-2 concentration (copies/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate(%)
5,800	16	16	100%
1,835	16	16	100%
581	16	15	94%
184	16	11	69%
58	16	6	38%
18	16	4	25%

Table 12. LoD of sputum with Lot 2 and Lot 3 by Probit

	Target	95% LOD by Probit	Lower 95% CI	Upper 95% CI
SARS-CoV-2 concentration (copies/mL)	ORF1ab	746	545	1,251
	N	853	554	2,437
	E	552	392	1,041

Based on above results, the LOD of the Kit was determined to be 1,000 copies/mL for NPS and sputum specimens across all three targets (ORF1ab, N and E).

The LOD was then verified at a concentration of 1,000copies/mL with 3 lots of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit by diluting SARS-CoV-2 positive nucleic acid, which was mixed with NPS, OPS or sputum specimens. Each specimen was extracted in 7 replicates and tested with three lots of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit. The positive rate of each type of three specimens was 100% (21/21). The result demonstrated that the LOD of the Kit was verified 1,000copies/mL for NPS, OPS and sputum specimens.

2). Viral RNA Isolation Kit(Preloaded for Auto-Extraction)

A dilution series consisting of 6 different dilution levels was used, starting with 0.04 $TCID_{50}$/mL cultured virus (SARS-CoV-2/ZJU-02/Human/2020, inactivated) spiked into SARS-CoV-2 negative NPS, OPS or sputum specimens. Each specimen was extracted with 20 replicates and tested with three lots of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit. A probit regression with SPSS Software was performed and the 95% LoD value was determined. The results for NPS,OPS and sputum specimens are shown in Tables 13-24.

Table 13. Positive rate of NPS (Target: ORF1ab)

SARS-CoV-2 RNA concentration ($TCID_{50}$/ml)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.04000	20	20	100%
0.01333	20	17	85%
0.00444	20	13	65%
0.00148	20	5	25%
0.00049	20	2	10%
0.00016	20	0	0%

Table 14. Positive rate of NPS (Target: N)

SARS-CoV-2 RNA concentration (TCID$_{50}$/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.0400	20	20	100%
0.01333	20	17	85%
0.00444	20	10	50%
0.00148	20	5	25%
0.00049	20	1	5%
0.00016	20	0	0%

Table 15. Positive rate of NPS (Target: E)

SARS-CoV-2 RNA concentration (TCID$_{50}$/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.0400	20	20	100%
0.01333	20	19	95%
0.00444	20	6	30%
0.00148	20	3	15%
0.00049	20	1	5%
0.00016	20	0	0%

Table 16. LoD of NPS by Probit

	Target	95% LoD by Probit	Lower 95% CI	Upper 95% CI
SARS-CoV-2 RNA concentration (TCID$_{50}$/mL)	ORF1ab	0.022	0.013	0.058
	N	0.025	0.014	0.055
	E	0.023	0.010	0.298

Table 17. Positive rate of OPS (Target: ORF1ab)

SARS-CoV-2 RNA concentration (TCID$_{50}$/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.0400	20	20	100%
0.01333	20	17	85%
0.00444	20	5	25%
0.00148	20	4	20%
0.00049	20	3	15%
0.00016	20	1	5%

Table 18. Positive rate of OPS (Target: N)

SARS-CoV-2 RNA concentration (TCID$_{50}$/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.0400	20	20	100%
0.01333	20	16	80%
0.00444	20	7	35%

0.00148	20	3	15%
0.00049	20	1	5%
0.00016	20	0	0%

Table 19. Positive rate of OPS (Target: E)

SARS-CoV-2 RNA concentration (TCID$_{50}$/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.0400	20	20	100%
0.01333	20	19	95%
0.00444	20	7	35%
0.00148	20	3	15%
0.00049	20	2	10%
0.00016	20	1	5%

Table 20. LoD of OPS by Probit

	Target	95% LOD by Probit	Lower 95% CI	Upper 95% CI
SARS-CoV-2 RNA concentration (TCID50/mL)	ORF1ab	0.022	0.016	0.036
	N	0.033	0.019	0.087
	E	0.015	0.012	0.022

Table 21. Positive rate of sputum (Target: ORF1ab)

SARS-CoV-2 RNA concentration (TCID$_{50}$/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.0400	20	20	100%
0.01333	20	15	75%
0.00444	20	7	35%
0.00148	20	6	30%
0.00049	20	0	0%
0.00016	20	0	0%

Table 22. Positive rate of sputum (Target: N)

SARS-CoV-2 RNA concentration (TCID$_{50}$/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.0400	20	20	100%
0.01333	20	19	95%
0.00444	20	7	35%
0.00148	20	5	20%
0.00049	20	1	5%
0.00016	20	1	5%

Table 23. Positive rate of sputum (Target: E)

SARS-CoV-2 RNA concentration (TCID$_{50}$/mL)	Number of replicates tested (N)	Number of positives detected	Positive rate (%)
0.0400	20	20	100%
0.01333	20	17	85%
0.00444	20	8	40%
0.00148	20	3	15%
0.00049	20	2	10%
0.00016	20	1	5%

Table 24. LoD of sputum by Probit

	Target	95% LOD by Probit	Lower 95% CI	Upper 95% CI
SARS-CoV-2 RNA concentration (TCID$_{50}$/mL)	ORF1ab	0.035	0.019	0.096
	N	0.015	0.012	0.021
	E	0.022	0.016	0.036

Precision

1). QIAGEN QIAamp Virus RNA Mini Kit

Within-laboratory precision

The precision of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit was determined by testing a 9-member panel: 3 negative specimens (NPS, OPS, sputum), 3 specimens of different specimen types (NPS, OPS, sputum) with a moderate concentration (P2, approximately 3-9xLoD), and 3 specimens of different specimen types (NPS, OPS, sputum) with a concentration of approximately 40-100xLoD (P1). The P1 and P2 of 9-member panels were prepared with SARS-CoV-2 nucleic acid spiked into SARS-CoV-2 negative NPS/OPS/sputum specimens respectively. Each panel member was evaluated in three replicates for 5 days, with one run by one operator per day. A total of 3 different lots of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit were used for the study. A total of 2 different operators performed the test. The coefficient of variation (%CV) of Ct and negative percent agreement (NPA) was analyzed.

The results showed that CVs were all lower than 5% for samples of P1 and P2 in variability of the within-laboratory precision. Please refer to Tables 25-27 for detailed data.

Table 25. Within-laboratory precision (%CV) of each lot (P1[a])

Type of specimens	N of tests[b]	Lot 1			Lot 2			Lot 3		
		ORF1ab	N	E	ORF1ab	N	E	ORF1ab	N	E
NPS	30	1.06%	1.09%	1.64%	0.91%	1.10%	1.81%	0.98%	1.17%	1.75%
OPS	30	0.88%	1.08%	1.63%	0.74%	1.06%	1.63%	0.85%	1.17%	1.75%
Sputum	30	0.79%	1.13%	1.66%	0.89%	1.03%	1.63%	0.96%	1.13%	1.85%

Note:

[a]The nominal value of P1 is 4.00E+04 copies/mL (ORF1ab), 1.00E+05 copies/mL (N), and 1.15E+05 copies/mL (E).

Table 26. Within-laboratory precision (%CV) of each lot (P2ᵃ)

Type of specimens	N of testsᵇ	Lot 1			Lot 2			Lot 3		
		ORF1ab	N	E	ORF1ab	N	E	ORF1ab	N	E
NPS	30	1.28%	0.87%	1.50%	1.08%	1.00%	1.69%	0.99%	1.09%	1.77%
OPS	30	1.00%	0.96%	1.62%	1.06%	0.98%	1.63%	1.25%	1.00%	1.69%
Sputum	30	1.30%	0.90%	1.54%	0.98%	0.91%	1.56%	1.33%	0.98%	1.47%

Note:

ᵃThe nominal value of P2 is 3.00E+03 copies/mL (ORF1ab), 7.50E+03 copies/mL (N), and 8.64E+03copies/mL (E).

ᵇIndicating the total of replicates tested by two operators in 5 days with 1 lot.

Table 27. Within-laboratory precision (%CV) of all lots

Type of specimens	N of testsᵃ	All lots, %CV					
		P1ᵇ			P2ᶜ		
		ORF1ab	N	E	ORF1ab	N	E
NPS	90	0.99%	1.12%	1.72%	1.12%	0.99%	1.64%
OPS	90	0.83%	1.12%	1.65%	1.10%	0.98%	1.63%
Sputum	90	0.88%	1.10%	1.70%	1.21%	0.93%	1.52%

Note:

ᵃIndicating the total of replicates tested by two operators in 5 days with 3 lots.

ᵇThe nominal value of P1 is 4.00E+04 copies/mL (ORF1ab), 1.00E+05 copies/mL (N), and 1.15E+05 copies/mL (E).

ᶜThe nominal value of P2 is 3.00E+03 copies/mL (ORF1ab), 7.50E+03 copies/mL (N), and 8.64E+03copies/mL (E).

In Table 28 below, the negative percent agreement (NPA) for Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit on negative panel member results was 100%.

Table 28. Negative percent agreement on negative panel member of all lots

Type of specimens	Expected SARS-CoV-2 Concentration	No. of testsᵃ	Positive results	Negative results	NPAᵇ	95%CIᶜ
NPS	Negative	90	0	90	100%	(95.91%, 100.00%)
OPS	Negative	90	0	90	100%	(95.91%, 100.00%)
Sputum	Negative	90	0	90	100%	(95.91%, 100.00%)

Note:

ᵃIndicating the total of replicates tested by two operators in 5 days with 3 lots.

ᵇNPA = (number of negative results/total number of valid tests in negative panel member) * 100%.

ᶜCalculated using score confidence interval.

Multi-Site Reproducibility Study

The reproducibility of the Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit was determined following the recommendations of the CLSI Guideline EP05-A3 by testing a three-member panel: specimens with a concentration of approximately 2-3xLoD, which were prepared with SARS-CoV-2 RNA spiked into SARS-CoV-2 negative NPS/OPS/sputum specimens respectively. A run, consisting of five replicates of the panel members, was performed daily with three lots for five different days at each of the three sites. The coefficient of variation (%CV) of Ct was analyzed.

The results showed that CVs were all lower than 5% for samples with all lots in Multi-Site Reproducibility Study. Please refer to Tables 29-32 for detailed data.

Table 29. The result of NPS with each lot

Lot	Target	Concentration (copies/mL)	No. of tests[a]	Repeatability		Within-laboratory		Reproducibility	
				SD[b]	%CV	SD	%CV	SD	%CV
Lot 1	ORF1ab	7.00E+02	75	0.62	1.74%	0.72	2.00%	0.73	2.04%
	N	6.33E+02	75	0.81	2.22%	0.84	2.31%	0.85	2.34%
	E	5.15E+02	75	0.91	2.53%	0.92	2.56%	0.96	2.65%
Lot 2	ORF1ab	7.00E+02	75	0.69	1.91%	0.81	2.27%	0.82	2.30%
	N	6.33E+02	75	0.69	1.92%	0.80	2.22%	0.80	2.22%
	E	5.15E+02	75	0.98	2.70%	1.04	2.86%	1.04	2.86%
Lot 3	ORF1ab	7.00E+02	75	0.50	1.40%	0.65	1.82%	0.65	1.82%
	N	6.33E+02	75	0.59	1.63%	0.68	1.87%	0.68	1.87%
	E	5.15E+02	75	0.93	2.57%	1.08	3.00%	1.08	3.00%

[a]Indicating the total of replicates tested in 5 days with 1 lots at three sites.

[b]SD=standard deviation.

Table 30. The result of OPS with each lot

Lot	Target	Concentration (copies/mL)	No. of tests[a]	Repeatability		Within-laboratory		Reproducibility	
				SD[b]	%CV	SD	%CV	SD	%CV
Lot 1	ORF1ab	7.00E+02	75	0.71	1.98%	0.74	2.05%	0.80	2.22%
	N	6.33E+02	75	0.72	2.00%	0.80	2.20%	0.81	2.23%
	E	5.15E+02	75	0.94	2.60%	0.95	2.62%	0.95	2.62%
Lot 2	ORF1ab	7.00E+02	75	0.93	2.58%	0.94	2.61%	0.96	2.67%
	N	6.33E+02	75	0.67	1.84%	0.78	2.14%	0.82	2.26%
	E	5.15E+02	75	1.04	2.87%	1.08	2.98%	1.08	2.98%
Lot 3	ORF1ab	7.00E+02	75	0.73	2.05%	0.78	2.18%	0.82	2.29%
	N	6.33E+02	75	0.77	2.13%	0.92	2.52%	0.92	2.52%
	E	5.15E+02	75	0.95	2.61%	0.95	2.64%	0.96	2.66%

[a]Indicating the total of replicates tested in 5 days with 1 lot at three sites.

[b]SD=standard deviation.

Table 31. The result of sputum with each lot

Lot	Target	Concentration (copies/mL)	No. of tests[a]	Repeatability		Within-laboratory		Reproducibility	
				SD[b]	%CV	SD	%CV	SD	%CV
Lot 1	ORF1ab	1.00E+03	75	0.78	2.17%	0.78	2.17%	0.83	2.32%
	N	9.05E+02	75	0.66	1.82%	0.78	2.15%	0.78	2.16%
	E	7.35E+02	75	0.96	2.65%	0.98	2.70%	0.98	2.70%
Lot 2	ORF1ab	1.00E+03	75	0.69	1.92%	0.79	2.19%	0.79	2.19%
	N	9.05E+02	75	0.73	2.01%	0.90	2.48%	0.91	2.50%
	E	7.35E+02	75	0.80	2.22%	1.03	2.85%	1.03	2.85%
Lot 3	ORF1ab	1.00E+03	75	0.65	1.82%	0.68	1.91%	0.75	2.11%
	N	9.05E+02	75	0.68	1.88%	0.71	1.97%	0.71	1.97%
	E	7.35E+02	75	1.09	3.01%	1.13	3.11%	1.13	3.11%

[a]Indicating the total of replicates tested in 5 days with 1 lots at three sites.

[b]SD=standard deviation.

Table 32. The result of %CV between batches

Type of specimens	Target	Concentration (copies/mL)	No. of tests[a]	All lots	
				SD[b]	%CV
NPS	ORF1ab	7.00E+02	225	0.73	2.03%
	N	6.33E+02	225	0.77	2.13%
	E	5.15E+02	225	1.01	2.80%
OPS	ORF1ab	7.00E+02	225	0.85	2.37%
	N	6.33E+02	225	0.83	2.28%
	E	5.15E+02	225	0.99	2.74%
Sputum	ORF1ab	1.00E+03	225	0.77	2.15%
	N	9.05E+02	225	0.79	2.20%
	E	7.35E+02	225	1.03	2.84%

[a]Indicating the total of replicates tested in 5 days with 3 lots at three sites.

[b]SD=standard deviation.

2). Viral RNA Isolation Kit(Preloaded for Auto-Extraction)

Multi-Site Reproducibility Study

The precision of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit was determined by testing a 9-member panel: 3 negative specimens (NPS, OPS, sputum), 3 specimens of different types (NPS, OPS, sputum) with a moderate concentration (P2,approximately 2-3xLoD), and 3 specimens of different types (NPS, OPS, sputum) with a concentration of approximately 5-7xLoD $TCID_{50}$/mL (P1). The P1 and P2 of 6-member panels were prepared with SARS-CoV-2 cultured virus (SARS-CoV-2/ZJU02/Human/2020,inactivated) spiked into SARS-CoV-2 negative NPS/OPS/sputum specimens respectively. A run, consisting of five replicates of the panel members, was performed daily with three lots for five different days at each of the three sites. The coefficient variant (CV) of Ct and the negative percent agreement (NPA) was analyzed.

The results showed that CVs were all lower than 5% for samples of P1 and P2 in Multi-Site Reproducibility Study. Please refer to Tables 33-40 for detailed data.

Table 33. The result of NPS with each lot (P1[a])

Lot	Target	N. of tests[b]	Repeatability		Within-Laboratory		Reproducibility	
			SD[c]	%CV	SD	%CV	SD	%CV
Lot 1	ORF1ab	75	0.39	1.14%	0.50	1.45%	0.50	1.45%
	N	75	0.28	0.80%	0.46	1.34%	0.46	1.34%
	E	75	0.30	0.88%	0.49	1.45%	0.49	1.45%
Lot 2	ORF1ab	75	0.33	0.97%	0.46	1.34%	0.46	1.34%
	N	75	0.38	1.11%	0.55	1.60%	0.55	1.60%
	E	75	0.33	0.98%	0.49	1.45%	0.49	1.45%
Lot 3	ORF1ab	75	0.39	1.12%	0.48	1.38%	0.49	1.40%
	N	75	0.35	1.00%	0.50	1.44%	0.50	1.44%
	E	75	0.30	0.90%	0.41	1.23%	0.42	1.23%

Note:

[a]The nominal value of P1 is $0.09 TCID_{50}/mL$.

[b]Indicating the total of replicates tested in 5 days with 1 lot at three sites.

[c]SD=standard deviation.

Table 34. The result of NPS with each lot (P2[a])

Lot	Target	N. of tests[b]	Repeatability		Within-Laboratory		Reproducibility	
			SD[c]	%CV	SD	%CV	SD	%CV
Lot 1	ORF1ab	75	0.50	1.40%	0.56	1.59%	0.58	1.63%
	N	75	0.44	1.24%	0.46	1.31%	0.50	1.41%
	E	75	0.48	1.39%	0.54	1.56%	0.55	1.59%
Lot 2	ORF1ab	75	0.44	1.25%	0.55	1.54%	0.56	1.58%
	N	75	0.38	1.07%	0.43	1.21%	0.46	1.30%
	E	75	0.42	1.19%	0.67	1.91%	0.67	1.92%
Lot 3	ORF1ab	75	0.47	1.32%	0.56	1.59%	0.56	1.59%
	N	75	0.52	1.46%	0.58	1.63%	0.59	1.65%
	E	75	0.48	1.38%	0.56	1.60%	0.56	1.60%

Note:

[a]The nominal value of P2 is $0.045 TCID_{50}/mL$.

[b]Indicating the total of replicates tested in 5 days with 1 lot at three sites.

[c]SD=standard deviation.

Table 35. The result of OPS with each lot (P1[a])

Lot	Target	N. of tests[b]	Repeatability		Within-Laboratory		Reproducibility	
			SD[c]	%CV	SD	%CV	SD	%CV
Lot 1	ORF1ab	75	0.34	0.98%	0.40	1.16%	0.40	1.16%
	N	75	0.35	1.00%	0.45	1.30%	0.45	1.30%

			Repeatability		Within-Laboratory		Reproducibility	
	E	75	0.31	0.93%	0.42	1.24%	0.42	1.24%
	ORF1ab	75	0.35	1.03%	0.47	1.36%	0.47	1.36%
Lot 2	N	75	0.32	0.94%	0.48	1.39%	0.48	1.39%
	E	75	0.35	1.04%	0.44	1.30%	0.45	1.32%
	ORF1ab	75	0.28	0.80%	0.47	1.35%	0.47	1.35%
Lot 3	N	75	0.34	0.98%	0.44	1.26%	0.44	1.26%
	E	75	0.27	0.81%	0.41	1.21%	0.41	1.21%

Note:

[a]The nominal value of P1 is $0.09 TCID_{50}/mL$.

[b]Indicating the total of replicates tested in 5 days with 1 lot at three sites.

[c]SD=standard deviation.

Table 36. The result of OPS with each lot (P2[a])

Lot	Target	N. of tests[b]	Repeatability		Within-Laboratory		Reproducibility	
			SD[c]	%CV	SD	%CV	SD	%CV
Lot 1	ORF1ab	75	0.41	1.16%	0.53	1.49%	0.53	1.49%
	N	75	0.42	1.18%	0.48	1.33%	0.49	1.36%
	E	75	0.41	1.19%	0.65	1.86%	0.65	1.86%
Lot 2	ORF1ab	75	0.51	1.45%	0.60	1.69%	0.60	1.69%
	N	75	0.55	1.53%	0.57	1.59%	0.59	1.64%
	E	75	0.57	1.64%	0.65	1.87%	0.65	1.87%
Lot 3	ORF1ab	75	0.57	1.60%	0.64	1.80%	0.73	2.05%
	N	75	0.48	1.35%	0.57	1.61%	0.57	1.61%
	E	75	0.48	1.39%	0.58	1.66%	0.58	1.66%

Note:

[a]The nominal value of P2 is $0.045 TCID_{50}/mL$.

[b]Indicating the total of replicates tested in 5 days with 1 lot at three sites.

[c]SD=standard deviation.

Table 37. The result of sputum with each lot (P1[a])

Lot	Target	N. of tests[b]	Repeatability		Within-Laboratory		Reproducibility	
			SD[c]	%CV	SD	%CV	SD	%CV
Lot 1	ORF1ab	75	0.32	0.91%	0.48	1.40%	0.48	1.40%
	N	75	0.34	0.98%	0.46	1.34%	0.46	1.34%
	E	75	0.29	0.85%	0.42	1.25%	0.42	1.25%
Lot 2	ORF1ab	75	0.43	1.26%	0.60	1.75%	0.60	1.75%
	N	75	0.34	0.98%	0.51	1.49%	0.51	1.49%
	E	75	0.39	1.16%	0.50	1.49%	0.50	1.49%
Lot 3	ORF1ab	75	0.43	1.24%	0.57	1.65%	0.57	1.65%
	N	75	0.35	1.02%	0.47	1.36%	0.47	1.36%
	E	75	0.29	0.86%	0.46	1.36%	0.46	1.36%

Note:

[a]The nominal value of P1 is $0.09 TCID_{50}/mL$.

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[b]Indicating the total of replicates tested in 5 days with 1 lot at three sites.

[c]SD=standard deviation.

Table 38. The result of sputum with each lot (P2[a])

Lot	Target	N. of tests[b]	Repeatability		Within-Laboratory		Reproducibility	
			SD[c]	%CV	SD	%CV	SD	%CV
Lot 1	ORF1ab	75	0.41	1.16%	0.53	1.49%	0.53	1.49%
	N	75	0.42	1.18%	0.48	1.33%	0.49	1.36%
	E	75	0.41	1.19%	0.65	1.86%	0.65	1.86%
Lot 2	ORF1ab	75	0.51	1.45%	0.60	1.69%	0.60	1.69%
	N	75	0.55	1.53%	0.57	1.59%	0.59	1.64%
	E	75	0.57	1.64%	0.65	1.87%	0.65	1.87%
Lot 3	ORF1ab	75	0.57	1.60%	0.64	1.80%	0.73	2.05%
	N	75	0.48	1.35%	0.57	1.61%	0.57	1.61%
	E	75	0.48	1.39%	0.58	1.66%	0.58	1.66%

Note:

[a]The nominal value of P2 is 0.045TCID$_{50}$/mL.

[b]Indicating the total of replicates tested in 5 days with 1 lot at three sites.

[c]SD=standard deviation.

Table 39. The result of %CV between batches (P1[a])

Type of specimens	target	N. of tests[b]	All lots	
			SD[c]	%CV
sputum	ORF1ab	225	0.52	1.52%
	N	225	0.46	1.34%
	E	225	0.44	1.30%
NPS	ORF1ab	225	0.48	1.39%
	N	225	0.49	1.42%
	E	225	0.45	1.34%
OPS	ORF1ab	225	0.43	1.25%
	N	225	0.44	1.26%
	E	225	0.41	1.23%

Note:

[a]The nominal value of P1 is 0.09TCID$_{50}$/mL.

[b]Indicating the total of replicates tested in 5 days with 3 lots at three sites.

[c]SD=standard deviation.

Table 40. The result of %CV between batches (P2[a])

Type of specimens	target	N. of tests[b]	All lots	
			SD[c]	%CV
sputum	ORF1ab	225	0.60	1.70%
	N	225	0.54	1.51%
	E	225	0.60	1.74%
NPS	ORF1ab	225	0.56	1.57%

	N	225	0.55	1.54%
	E	225	0.58	1.68%
	ORF1ab	225	0.56	1.57%
OPS	N	225	0.52	1.46%
	E	225	0.58	1.68%

Note:

[a]The nominal value of P2 is 0.045$TCID_{50}$/mL.

[b]Indicating the total of replicates tested in 5 days with 3 lots at three sites.

[c]SD=standard deviation.

In Table 41 below, the negative percent agreement (NPA) for Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit on negative panel member results was 100%.

Table 41. Negative percent agreement on negative panel member with all lots

Type of specimens	Expected SARS-CoV-2 Concentration	No. of tests[a]	Positive results	Negative results	NPA[b]	95%CI[c]
NPS	Negative	75	0	75	100%	(95.13%, 100.00%)
OPS	Negative	75	0	75	100%	(95.13%, 100.00%)
Sputum	Negative	75	0	75	100%	(95.13%, 100.00%)

Note:

[a]Indicating the total of replicates tested in 5 days with 3 lots at three sites.

[b]NPA = (number of negative results/total number of valid tests in negative panel member) * 100%.

[c]Calculated using score confidence interval.

Analytical Specificity (Cross-reactivity)

The analytical specificity was evaluated by testing the cross-reactivity of a panel of different pathogens consisting of 18 viruses, 4 fungi, 2 chlamydia and mycoplasma, 1 protozoa, 8 bacteria and 1 pooled human nasal wash. The organisms selected were clinically relevant organisms (colonizing the respiratory tract or causing respiratory symptoms), common skin flora or laboratory contaminants, or microorganisms for which much of the population may have been infected with. Each organism was tested with 3 lots of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit.

For SARS-coronavirus, the result of target ORF1abwas negative, and the results of target E and target N were positive.

For the rest of 33 pathogens, there were no false positive or invalid results tested (Table 42).

Table 42. Analytical specificity

Pathogen	Lot1	Lot2	Lot3
Human coronavirusHKU1	-	-	-
Human coronavirus OC43	-	-	-
Human coronavirus NL63	-	-	-

Human coronavirus 229E	-	-	-
MERS-coronavirus	-	-	-
Influenza A	-	-	-
Influenza B	-	-	-
Respiratory syncytial virus	-	-	-
Parainfluenza virus , type 1	-	-	-
Parainfluenza virus, type 2	-	-	-
Parainfluenza virus , type 3	-	-	-
Parainfluenza virus , type 4	-	-	-
Rhinovirus	-	-	-
Human adenovirus	-	-	-
enterovirus71	-	-	-
coxsackie virus ,type16	-	-	-
Human metapneumovirus(hMPV)	-	-	-
Mycoplasma pneumoniae	-	-	-
chlamydia pneumoniae	-	-	-
Legionella pneumophila	-	-	-
Bordetella pertussis	-	-	-
Haemophilus influenzae	-	-	-
Staphylococcus aureus	-	-	-
Streptococcus pneumoniae	-	-	-
Streptococcus pyogenes	-	-	-
Klebsiella pneumoniae	-	-	-
Mycobacterium tuberculosis	-	-	-
Aspergillus fumigatus	-	-	-
Candida albicans	-	-	-
Candida glabrata	-	-	-
Cryptococcus neoformans	-	-	-
Pneumocystis yersii(PJP)	-	-	-
Pooled human nasal wash	-	-	-

Note: "–" means negative for target ORF1ab, N and E.

Interfering Substances

The potentially interfering substances were spiked into NPS/OPS/sputum specimens in the presence of a concentration near the LOD of SARS-CoV-2. Specimens were then tested with Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit. Specimens containing potentially interfering substances, which were tested in 3 replicates, were compared to NPS/OPS/sputum specimens containing no spiked interfering substance, which were tested in 3 replicates of each interfering substance. The test concentrations of interfering substances are shown in Table 43.

Table 43. Interfering substances

Potential interfering substance	Test concentration
Whole blood	5%（v/v）
Mucoprotein	1mg/mL

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Bepanthen Meerwasser Nasenspray	5%（v/v）
Budesonide Nasal Spray	5%（v/v）
Nasal cold compress gel	5%（v/v）
Bepanthen Augen-undNasensalbe	0.1g/mL
Throat lozenges	2.7g/ mL
Oseltamivir	390ng/mL
Ibuprofen Sustained Release Capsules	90mg/L
Amoxicillin and Clavulanate PotassiumTablets	16.8mg/L

All tested interfering substance of said concentrations showed no influence on the performance of Novel Coronavirus (SARS-CoV-2) Real-Time Multiplex RT-PCR Kit.

Diagnostic Evaluation

According to the requirements of clinical evidence in *Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid*, relevant clinical studies have been conducted in China.

In this clinical study, *CDC 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Diagnostic Panel*, developed by American CDC, was selected to be the comparator method.

A total of 189 specimens were tested at Guangdong Provincial Center for Disease Control & Prevention using evaluated reagent and comparator reagent. Due to repeated specimens taken from some subjects, 51 positive specimens from 36 subjects and 116 negative specimens from 108 subjects were collected. The number of specimens of various types is shown in the table below (Table 44).

Table 44. Summary of clinical study specimen types

Specimen type	Specimen Total			
	Positive	Negative	Inconclusive	Total
Nasopharyngeal swab	30	108	21	159
Oropharyngeal swab	9	5	1	15
Sputum	12	3	0	15
Total	51	116	22	189

The coincidence rate with 95% confidence intervals and kappa analysis were computed for all specimens and each specimen type.

Table 45. Clinical study coincidence for all specimens

		Comparator reagent (CDC Assay)			Total
		Positive	Negative	Inconclusive	
Evaluated reagent (Liferiver Assay)	Positive	46	2	7	55
	Negative	5	114	15	134
Total		51	116	22	189

Table 46. Coincidence rate with 95% confidence intervals for all specimens

	Coincidence rate	95% confidence intervals
Positive coincidence rate	90.20%	78.59%~96.74%
Negative coincidence rate	98.28%	93.91%~99.79%
Overall coincidence rate	95.81%	91.55%~98.30%
Kappa	0.8995	0.8286~0.9723

With each claimed specimen type, the coincidence rate with 95% confidence intervals were calculated as follows:

Table 47. Clinical study coincidence for nasopharyngeal swabs

		Comparator reagent (CDC Assay)			Total
		Positive	Negative	Inconclusive	
Evaluated reagent (Liferiver Assay)	Positive	26	2	7	35
	Negative	4	106	14	124
Total		30	108	21	159

Table 48. Clinical study coincidence for oropharyngeal swabs

		Comparator reagent (CDC Assay)			Total
		Positive	Negative	Inconclusive	
Evaluated reagent (Liferiver Assay)	Positive	9	0	0	9
	Negative	0	5	1	6
Total		9	5	1	15

Table 49. Clinical study coincidence for sputum specimens

		Comparator Reagent (CDC Assay)			Total
		Positive	Negative	Inconclusive	
Evaluated reagent (Liferiver Assay)	Positive	12	0	0	12
	Negative	0	3	0	3
Total		12	3	0	15

The coincidence rate with 95% confidence intervals of each specimen type are as follows:

Table50. Coincidence rate with 95% confidence intervals for all specimens

	Positive coincidence rate	Negative coincidence rate	Overall coincidence rate	Kappa
Nasopharyngeal swab	86.67% (69.28%~96.24%)	98.15% (93.47%~99.78%)	95.65% (90.78%~98.39%	0.8691 (0.7670~0.9712)
Oropharyngeal swab	100%	100%	100%	~
Sputum	100%	100%	100%	~

Disposal

Dispose of hazardous or biologically contaminated materials according to the practices of your institution.

References

1. Ballew, H. C., *et al*. "Basic Laboratory Methods in Virology," DHHS, Public Health Service 1975 (Revised 1981), Centers for Disease Control and Prevention, Atlanta, Georgia30333.

2. Clinical Laboratory Standards Institute (CLSI), "Collection, Transport, Preparation and Storage of Specimens for Molecular Methods: Proposed Guideline,"MM13-A

3. Lieber, M., *et al*. "A Continuous Tumor Cell Line from a Human Lung Carcinoma with Properties of Type II Alveolar Epithelial Cells."*International Journal of Cancer* 1976, 17(1),62-70.

4. National Health Commission of the People's Republic of China, "Technical Guidance on Laboratory Test of Novel Coronavirus Infected Pneumonia (current edition).''

5. National Health Commission of the People's Republic of China, "The Surveillance Protocol for Novel Coronavirus Infected Pneumonia Cases (current edition)."

6. Centers for Disease Control and Prevention (CDC), "Instructions for Use of CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. "

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