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

Product: Novel Coronavirus (2019-nCoV) RT-PCR Detection Kit
Manufacturer: Fosun Diagnostics (Shanghai) Co., Ltd[1]
EUL Number: EUL 0516-203-00
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 (2019-nCoV) RT-PCR Detection Kit with product codes PCSYHF32 , PCSYHF46 and PCSYHF96, CE mark regulatory version, manufactured by Fosun Diagnostics (Shanghai) Co., Ltd, No.830 Chengyin Road, Baoshan District, Shanghai, China was listed on 2 September 2020[2].

Intended use:

According to the claim of intended use from Fosun Diagnostics (Shanghai) Co., Ltd, *"the Novel Coronavirus (2019-nCoV) RT-PCR Detection Kit is a qualitative, manual real-time RT-PCR test intended for the qualitative detection of ORF1ab, E and N genes of nucleic acids from SARS-CoV-2 in oropharyngeal swab (throat swab) and sputum specimens from patients with signs and symptoms suggestive of COVID-19 (e.g., fever and/or symptoms of acute respiratory illness). It is used as an aid in the diagnosis of COVID-19 infection. The product is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedure in level 2 biosafety laboratory."*

Specimen type that was validated:

[1] Manufacturer name changed from Shanghai Fosun Long March Medical Science Co., Ltd to Fosun Diagnostics (Shanghai) Co., Ltd.

[2] Assessment for EUL renewal is in progress.

Oropharyngeal swab and Sputum specimens.

Test kit contents:

Component	PCSYHF32(32 tests/kit)	PCSYHF48 (48 tests/kit)	PCSYHF96(96 tests/kit)
2019-nCoV Reaction Reagent	448 µL x 1 vial	672 µL x 1 vial	672 µL x 2 vials
RT-PCR Enzyme	192 µL x 1 vial	288 µL x 1 vial	288 µL x 2 vials
Positive Control of 2019-nCoV	200 µL x 1 vial	200 µL x 1 vial	200 µL x 2 vials
Negative Control	200 µL x 1 vial	200 µL x 1 vial	200 µL x 2 vials
Internal Reference A	160 µL x 1 vial	240 µL x 1 vial	240 µL x 2 vials

Items required but not provided:

Specimen collection kits:

- Oropharyngeal swabs (Throat swabs), provided from Jiangsu Kangjie Medical Devices Ct., Ltd. ((cat. # FS1002), Jiangsu Jianyou Medical Technology Co., Ltd. (cat. # JY-0111), or Zhejiang Gene Science Co., Ltd. (cat. # GT810411)
- Collection tubes or sputum containers
- Universal Transport media (UTM), provided from Fosun Long March Medical Science Co., Ltd. (cat. # PCSYWC).

Extraction/Purification kits and platforms (systems):

- QIAamp Viral RNA Mini Kit (cat. #52904 or 52906)
- Fosun Nucleic Acid Extraction and Purification kit (cat. #PCSYMF), matching the automated TANBead extraction system
- Genolution NX-48 Viral RNA Kit (cat. #VN), matching the automated Genolution NX-48 nextractor.

Real-Time PCR equipment:

- Applied Biosystems 7500 instrument (software version 1.4 or 1.5)
- Roche LightCycler 480 instrument (software version 1.5)
- SLAN-96P instrument (software version 8.2).

General laboratory equipment and consumables

- Calibrated adjustable pipettes (10µL, 100µL, 200µL, 1000µL),
- Calibrated Adjustable Multi-channel pipette (5-50µL),
- 1.5 mL centrifuge tube shelf,
- PCR hood,

- Biosafety cabinet,
- Centrifuge with rotor for 1.5 mL and 2 mL tubes
- Vortex mixer
- Metal bath
- RNase/DNase free water, anhydrous ethanol
- 96-well PCR reaction plates, aerosol barrier pipette tips with filters, microcentrifuge tubes (DNase/RNase free)
- Liquid waste container, solid waste bag and container
- Double-layer latex gloves, waterproof boot covers, protective clothing, goggles, and masks with higher filtration efficiency.

Storage:

-25 to -15 °C, away from direct sunlight.

Shelf-life upon manufacture:

12 months.

Warnings/limitations:

Refer to the instructions for use (IFU).

Product dossier assessment

Fosun Diagnostics (Shanghai) Co., Ltd submitted a product dossier for the Novel Coronavirus (2019-nCoV) RT-PCR Detection 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 and Antigen (PQDx_0347)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external technical experts (assessors) appointed by WHO.

Post listing Commitments for EUL:

As commitments to listing, the manufacturer is required,

1. Verify limit of detection using the First WHO International Standard for SARS-CoV-2 RNA (NIBSC code: 20/146) and submit the study report to WHO after one month of completion.
2. Provide shipping stability study report to WHO by 9 August 2021. The submitted information is under review.
3. Provide shipping stability study report that includes evidence of investigation at extremes of conditions likely to be encountered during transportation of the product by 9 August 2021. The submitted information is under review.
4. Provide the real-time extended shelf life interim and final study reports within one month of study completion by 14 May 2021. The submitted information is under review.

5. Provide temperature data logger readings for the real-time extended shelf life studies conducted by 14 May 2021. The submitted information is under review.
6. Provide a full study report for the completed precision study that includes details of specimen preparation, acceptance criteria, data generated, data analysis, and the study conclusions. The submitted information is under review.

Risk benefit assessment conclusion: acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, Fosun Diagnostics (Shanghai) 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 Fosun Diagnostics (Shanghai) Co., Ltd to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid and Antigen(PQDx_347)*".

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)

Fosun Diagnostics (Shanghai) Co., Ltd is also required to report complaints related to the product. 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*"[3]

Scope and duration of procurement eligibility

Novel Coronavirus (2019-nCoV) RT-PCR Detection Kit, product codes PCSYHF32, PCSYHF46 and PCSYHF96 manufactured by Fosun Diagnostics (Shanghai) 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, Fosun Diagnostics (Shanghai) Co., Ltd must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality, and performance requirements. Fosun Diagnostics (Shanghai) Co., 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 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.

[3] https://www.who.int/publications/i/item/9789240015319

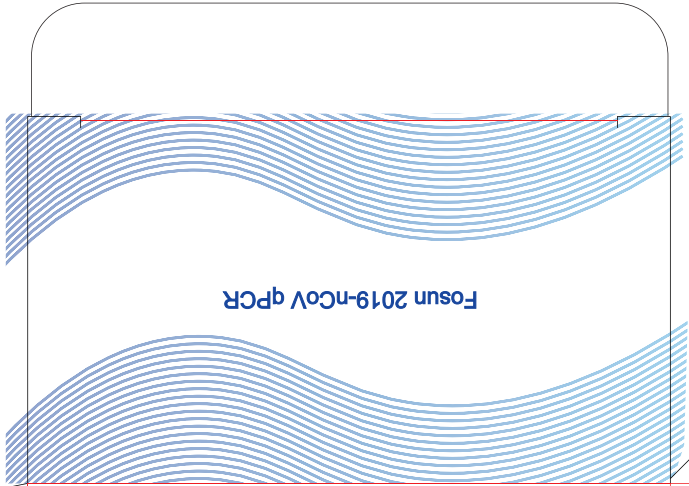
Labelling

1.0 Labels

2.0 Instructions for Use (IFU)

1.0 Product labels

1.1 Outer box design



Manufacturer:
Fosun Diagnostics (Shanghai) Co., Ltd.
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200444 Shanghai, PEOPLE'S REPUBLIC OF CHINA.
Tel: +86-21-60765888 Fax: +86-21-60765999
E-mail: diagnostics@fosundiagnostics.com
Website: http://en.lm-diagnostics.com.cn

EC REP
Obelis s.a.
Boulevard Général Wahis, 53
B-1030 Brussels
Belgium
Tel: +(32)27325954
Fax: +(32)27326003

FOSUN DIAGNOSTICS

Made in China

REF

Novel Coronavirus (2019-nCoV) RT-PCR Detection Kit

LOT

FOSUN DIAGNOSTICS

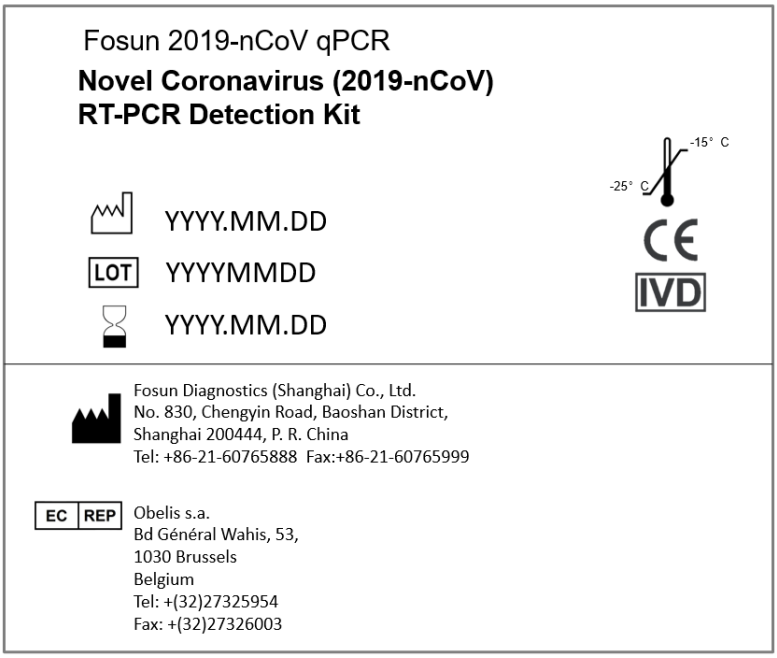
IVD

-25℃ -15℃

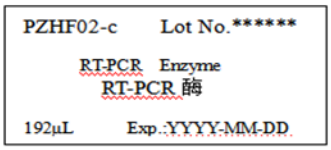
CE

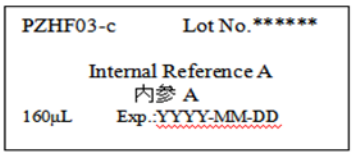
Fosun Diagnostics (Shanghai) Co., Ltd.
No.830, Chengyin Road, Baoshan District, 200444 Shanghai, PEOPLE'S REPUBLIC OF CHINA.
Contact: sales@fosundiagnostics.com Website: http://en.lm-diagnostics.com.cn Tel: +86-21-60765888 Fax: +86-21-60765999

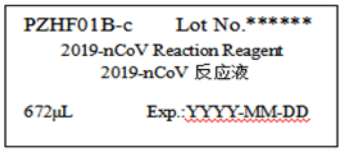
1.2 Outer box label

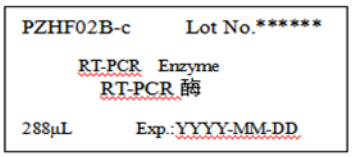


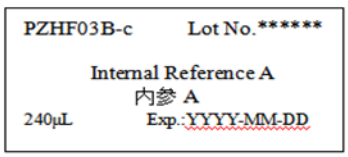
1.3. Component labels











2.0 Instructions for use[4]

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

CE IVD

Novel Coronavirus (2019-nCoV) RT-PCR Detection Kit

Instruction for Use

Label	Description
REF	Product catalog number
Σ <N>	Contains sufficient for <N> tests
LOT	Batch number
	Date of manufacturing
	Date by which the device should be used
	Manufacturer identification
CE IVD	In vitro diagnostic
	Consult instruction before use
EC REP	European Authorized Representative
	Temperature limitation

Sep 2021 — Version 1.7

【PRODUCT NAME】

Novel Coronavirus (2019-nCoV) RT-PCR Detection Kit (Commercial names: Fosun 2019-nCoV qPCR)

【SIZE】

32 tests/kit, 48 tests/kit, 96 tests/kit

【INTENDED USE】

The Fosun 2019-nCoV qPCR (Novel Coronavirus (2019-nCoV) RT-PCR Detection Kit) is a qualitative, manual real-time RT-PCR test intended for the qualitative detection of ORF1ab, E and N genes of nucleic acids from SARS-CoV-2 in oropharyngeal swab (throat swab) and sputum specimens from patients with signs and symptoms suggestive of COVID-19 (e.g., fever and/or symptoms of acute respiratory illness). It is used as an aid in the diagnosis of COVID-19 infection. The product is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedure in level 2 biosafety laboratory.

【PRODUCT DESCRIPTION】

Coronavirus is a +ssRNA virus with envelope. Its diameter is about 80-120 nm. Its genetic material is the largest of all RNA viruses. It is an important pathogen of many livestock, pets, including human diseases, and can cause many kinds of acute and chronic diseases. According to *Virus Taxonomy-- Ninth Report of the International Committee on Taxonomy of Viruses*, the Coronaviridae is divided into three genera: α, β and γ. Among them, α Coronavirus (such as human Coronavirus 229E, human Coronavirus NL63), β Coronavirus (such as human Coronavirus HKU1, human Coronavirus OC43 and SARS) can cause disease of varying degree to human.

The common signs of people infected with coronavirus are respiratory symptoms, fever, cough, shortness of breath and dyspnea. In more serious cases, infection can lead to pneumonia, severe acute respiratory syndrome, renal failure, and even death. The "2019-nCoV" was named by WHO in January 12, 2020.

According to the confirmed case request in *New Coronavirus Pneumonia Prevention and Control Program (6th ed)*, clinical diagnosis case or suspected case has the following pathogenic evidences: RT-PCR detection result of 2019-nCOV in respiratory tract or blood sample is positive. This Fosun 2019-nCoV qPCR kit is intended for the detection of 2019-nCOV and is helpful for clinical diagnosis of 2019-nCOV infection.

【PRINCIPLE OF DETECTION】

This product is a fluorescent probe-based Taqman RT-PCR assay system. Firstly, the RNA of 2019-nCov will be reverse transcribed into cDNA by reverse transcriptase, and then PCR amplification will be performed with cDNA as template. During amplification of the template, the Taqman probe will be degraded due to the 5'-3' polymerase activity and exonuclease activity of Taq DNA polymerase, then the separation of fluorescent reporter and quencher enables the fluorescent signal to be detected by instrument. The ORF1ab gene of 2019-nCoV will be detected qualitatively by FAM channel, the N gene of 2019-nCoV will be detected qualitatively by JOE channel, the E gene of 2019-nCoV will be detected qualitatively by ROX channel, and the internal reference will be detected by CY5 channel.

dUTP and UNG enzyme are used in the kit to prevent contamination of the amplified products.

Internal reference is used in the kit for quality control starting from sample collection.

【WARNING AND PRECAUTIONS】

1. The kit is only used for in vitro diagnosis.
2. Please read this manual carefully before beginning the experiment.
3. Storage instructions
   - The kit should be stored as required conditions (-15°C~-25°C with protection from light), and repeated freeze-thaw should be less than 5 times.
   - Please thaw all kit component reagents at ambient temperature from storage condition before commencing IVD.
4. Safety and handling
   - Avoid exchanging components from different lots or reagent kits, or pooling reagents (e.g. buffer vials from different lots should not be exchanged across lots)
   - All the consumables including 96-well PCR reaction plates, aerosol barrier pipette tips with filters, microcentrifuge tubes, should be DNase/RNase free in the experiment.

- Wrong sample collection, transfer, storage and operation may lead to wrong test results.
- RNA extraction shall be carried out as soon as possible after sample collection to avoid degradation. If it cannot be carried out immediately, it shall be stored for 24 hours at 2~8℃ and below -70℃ for a long time. It can also be stored in refrigerator at -20℃ temporarily..
- After the operation of the nucleic acid extractor, the used consumables shall be sealed. After the RNA extraction instrument is cleaned, turn on the ultraviolet lamp for 30 minutes.
- When using this kit, please strictly follow the instructions. The collection, storage and transfer of samples, the extraction and detection of RNA, and the interpretation of results must be carried out in strict accordance with the requirements of the kit instructions. The processes of sample preparation and addition must be carried out in the biosafety cabinet or other basic protective facilities according to the technical requirements of the clinical gene amplification laboratory.
- 2019-nCOV has strong transmission ability and high-risk coefficient. Personal protection should be a BSL-2 level. The operator must have professional skills and PCR inspection qualification. During the whole operation process, it is necessary to prevent the infection risk of aerosol pollution, and the operator must add samples and use reagents and consumables accurately.
- To prevent virus spreading, the 2019-nCOV must be detected in a BSL-2 or above laboratory. Laboratory management should strictly follow the management standard of PCR gene amplification laboratory, and the experimental operation must be strictly partitioned. The instruments, equipment, consumables, PPE used in each region must be distinguished strictly and can't be used intercross to avoid contamination.
- All test samples shall be regarded as infectious substances. During the experiment, work clothes shall be worn, disposable gloves shall be worn and replaced frequently to avoid cross contamination between samples. The operation of sample and waste shall meet the requirements of relevant laws and regulations such as *The general guidelines for biosafety of microbiological biomedical laboratories* and *The regulations on the management of medical wastes* issued by the Ministry of Health, WHO Laboratory testing of 2019 novel coronavirus (2019-nCoV) in suspected human cases: interim guidance. Laboratory biosafety guidance related to coronavirus disease (COVID-19).

5. Contamination and inhibition

- As this test involves the extraction of viral RNA and PCR amplification, please take care to avoid contamination of PCR product or high concentration of template.
- Regular monitoring of laboratory contamination is recommended. After each run has been set up and performed, clean work surfaces and equipment with DNA/RNA remover.
- Use DNase/RNase free disposable plasticware and pipettes reserved for DNA/RNA work to prevent cross-contamination with DNases/RNases from shared equipment.
- Use DNase/RNase free filter tips throughout procedure to prevent aerosol and liquid contamination.
- False negative results may be caused by: unsuitable collection, handling and/or storage of samples; sample outside of viraemic phase; failure to follow procedures in this manual; use of unauthorised extraction kit or PCR platform; PCR inhibition from sample or RNA missing in operation.
- False positive results may be caused by: unsuitable handling of samples containing high concentration of COVID-19 viral RNA or positive control template; unsuitable handling of amplified product.

6. If the test results of the control samples are outside the expected range, please stop and check the IVD reagents and operations. If the kit expires, please stop using it.

【PRODUCT CONTENTS】

Components	Amount			Amount per reaction	Ingredient
	32 Tests/kit	48 Tests/kit	96 Tests/kit		
2019-nCoV Reaction Reagent	448 μL	672 μL	672 μL*2	14 μL	dNTPs, $MgCl_2$, Primers (ORF1ab gene, E gene and N gene of 2019-nCOV), Probes
RT-PCR Enzyme	192 μL	288 μL	288 μL*2	6 μL	Taq DNA polymerase, Reverse Transcriptase, UNG enzyme
Positive Control of 2019-nCoV	200 μL	200 μL	200 μL*2	-	Pseudovirus
Negative Control	200 μL	200 μL	200 μL*2	-	NaCl
Internal Reference A	160 μL	240 μL	240 μL*2	5 μL	Pseudovirus

Note: Do not mix the components from different batches for detection. The positive control of 2019-nCOV and internal reference were constructed artificially, and they were not infectious.

【STORAGE & SHELF LIFE】

All reagents should be stored at -15°C~-25°C with protection from light, and the reagents are stable for 12 months (to be determined) when stored at the recommended condition. See label for production date and expiration date.

The kit should be transported by cold chain transport or sealed foam box with ice. The temperature should be controlled below -8°C and the transportation time should not exceed 4 days. Repeated freeze-thaw should be less than 5 times.

【MATERIALS REQUIRED BUT NOT PROVIDED】

1. Sample Collection
   - Oropharyngeal swabs (Throat swabs), provided from Jiangsu Kangjie Medical Devices Ct., Ltd. ((cat. # FS1002), Jiangsu Jianyou Medical Technology Co., Ltd.(cat. # JY-0111), or Zhejiang Gene Science Co., Ltd. (cat. # GT810411)
   - Collection tubes or sputum containers
   - Universal Transport media(UTM), provided from Fosun Diagnostics (Shanghai) Co., Ltd. (cat. # PCSYWC)
2. RNA Extraction/Purification
   - QIAamp® Viral RNA Mini Kit (cat. #52904 or 52906)

Alternatively,

   - Fosun Nucleic Acid Extraction and Purification kit (cat. #PCSYMF), matching the automated TANBead® extraction system; OR
   - Genolution® NX-48 Viral RNA Kit (cat. #VN), matching the automated Genolution® NX-48 nextractor.

3. Real-time PCR Instrument
   - Applied Biosystems® 7500 intrument (software version 1.4 or 1.5)

Alternatively,

   - Roche LightCycler® 480 instrument (software version 1.5); OR
   - SLAN-96P instrument (software version 8.2).

4. Other instruments required but not included with the test:
   - Calibrated adjustable pipettes (10μL, 100μL, 200μL, 1000μL)
   - Calibrated Adjustable Multi-channel pipette (5-50μL）
   - 1.5 mL centrifuge tube shelf
   - PCR hood
   - Biosafety cabinet
   - Centrifuge with rotor for 1.5 mL and 2 mL tubes
   - Vortex mixer
   - Metal bath
5. Other reagents and consumables required but not included with the test:
   - RNase/DNase free water, anhydrous ethanol

- 96-well PCR reaction plates, aerosol barrier pipette tips with filters, microcentrifuge tubes(DNase/RNase free)
- Liquid waste container, solid waste bag and container
- Double-layer latex gloves, waterproof boot covers, protective clothing, goggles, and masks with higher filtration efficiency.

【SAMPLING & HANDING】

1. Sample Collection

(1) Throat Swab: Use the plastic rod swab with polypropylene fiber head to wipe the bilateral pharyngeal tonsils and the posterior pharyngeal wall at the same time, immerse the swab head into the tube containing UTM, discard the tail, and tighten the tube cover.

(2) Sputum: Cough up the sputum in the deep part of the respiratory tract and collect it in the container. Liquefying method: add equal volume of acetylcysteine (10 g/L) into the sputum sample, shake at room temperature for 30 minutes, and then carry out RNA extraction after sufficient liquefying.

2. Sample Storage

The collected sample should be used for detection as soon as possible. If the sample need to be transferred cannot be detected immediately, please store it at low temperature.

The throat swab or sputum samples can be stored for 24 hours at 2~8℃ and for a long time below -70℃. It can also be stored in refrigerator at -20℃ temporarily.

3. Sample Transportation

Samples shall be transported at low temperature in accordance with biosafety regulations.

【PROTOCOL】

1. Reagent Preparation

Take out the kit component tubes except RT-PCR Enzyme and thaw thoroughly at ambient temperature, vortexing and centrifuge briefly. It is recommended that the RT-PCR Enzyme should be placed in an ice box to maintain low temperature, and prepare reaction reagent according to the number of reaction samples (number of reaction samples, n = number of samples to be tested + 2 control samples + 1):

Add n × 6 μL of RT-PCR enzyme and n × 14 μL of 2019-nCOV reaction reagent into the centrifuge tube, mix by shaking, and centrifugate at low speed for a few seconds, then make aliquots of 20 μL into different PCR reaction tubes. The reaction tubes can be placed at 2~8℃ for 3 hours after separation.

2. RNA Extraction

Before RNA extraction, the collected clinical samples should be inactivated by heating to 56℃ for 30 minutes firstly.

It is recommended to use the RNA extraction and purification reagent (general type) produced by our company, QIAamp Viral RNA Mini Kit (Qiagen) and NX-48 Viral RNA Kit (Genolution) to extract RNA from sample and reference sample.

The volume of sample to be extracted is 200 μL, and 5 μl of internal reference A will be added to each sample (including the negative and positive controls); after RNA extraction, the extracted RNA shall be added to the reaction tubes within 10 minutes, or transferred to the centrifuge tubes and stored for amplification at -15 ℃~-25 ℃ within 4 days.

3. Template Addition

Add 10 μL of extracted Negative Control, 10 μL of extracted Positive Control, and 10 μL of extracted RNA from sample to different PCR reaction tubes. Centrifuge them at low speed. Then, move them to the Real-time PCR instrument.

4. PCR Amplification

Step1: 50℃ for 15 minutes, 1 cycle；

Step2: 95℃ for 3 minutes, 1 cycle；

Step3: 95℃ for 5 seconds to 60℃ for 40 seconds, 5 cycles；

Step4: 95℃ for 5 seconds to 60℃ for 40 seconds, 40 cycles. The signals of FAM, JOE, ROX and CY5 fluorescence channels will be collected at 60℃.

Note: (1) Select "None" from the pull-down menu of the passive reference on operation interface of ABI7500 RT-PCR software. (2) no signal is being recorded in the step 3. (3) Step 4 uses a number of cycles that is in line with the instructions for result interpretation.

The corresponding relationship between the fluorescence channel and SARS-CoV-2 target genes, internal

reference/internal control (IC) is shown in the following table.

FAM	JOE	ROX	Cy5
ORF1ab gene	N gene	E gene	IC

The PCR program setting for different real-time PCR instruments are listed in the following figures.

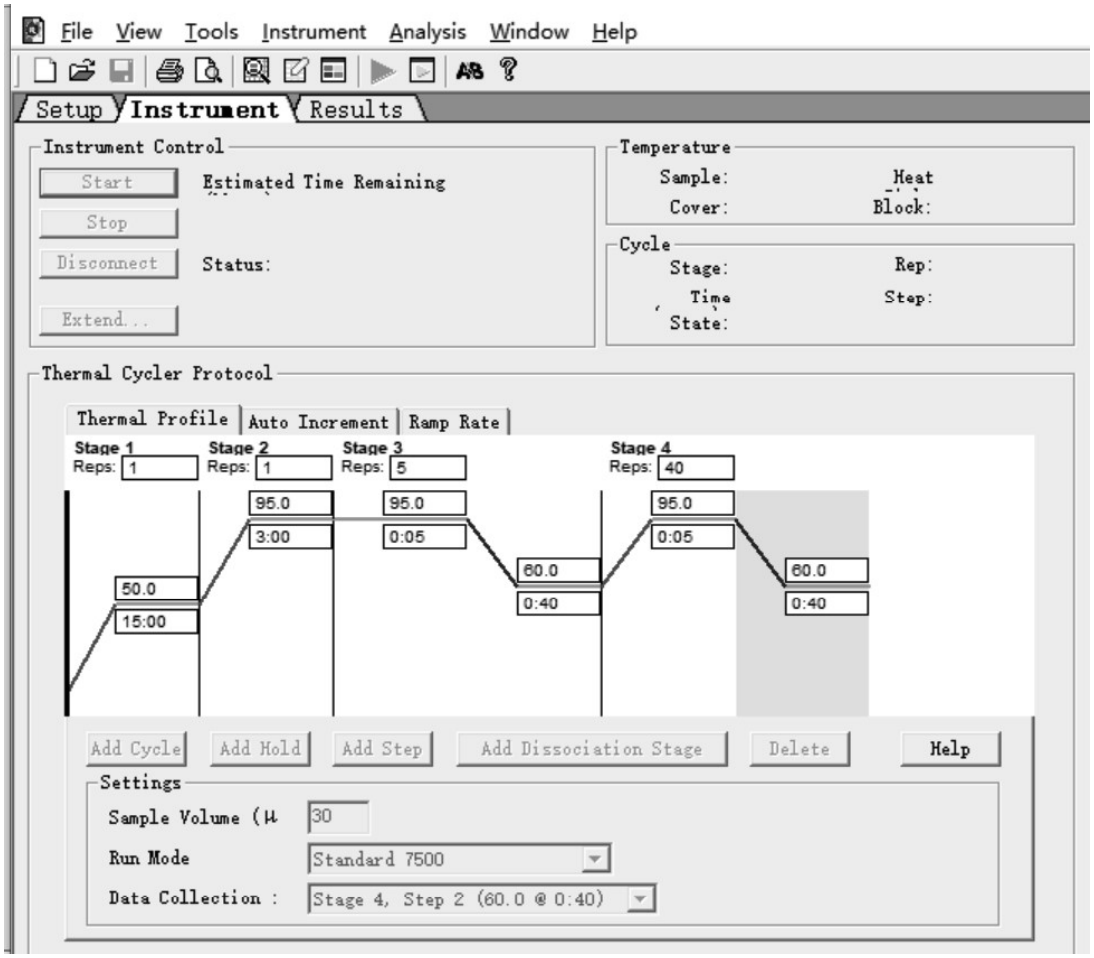


Figure1. PCR Program Setting for Applied Biosystems® 7500 instrument

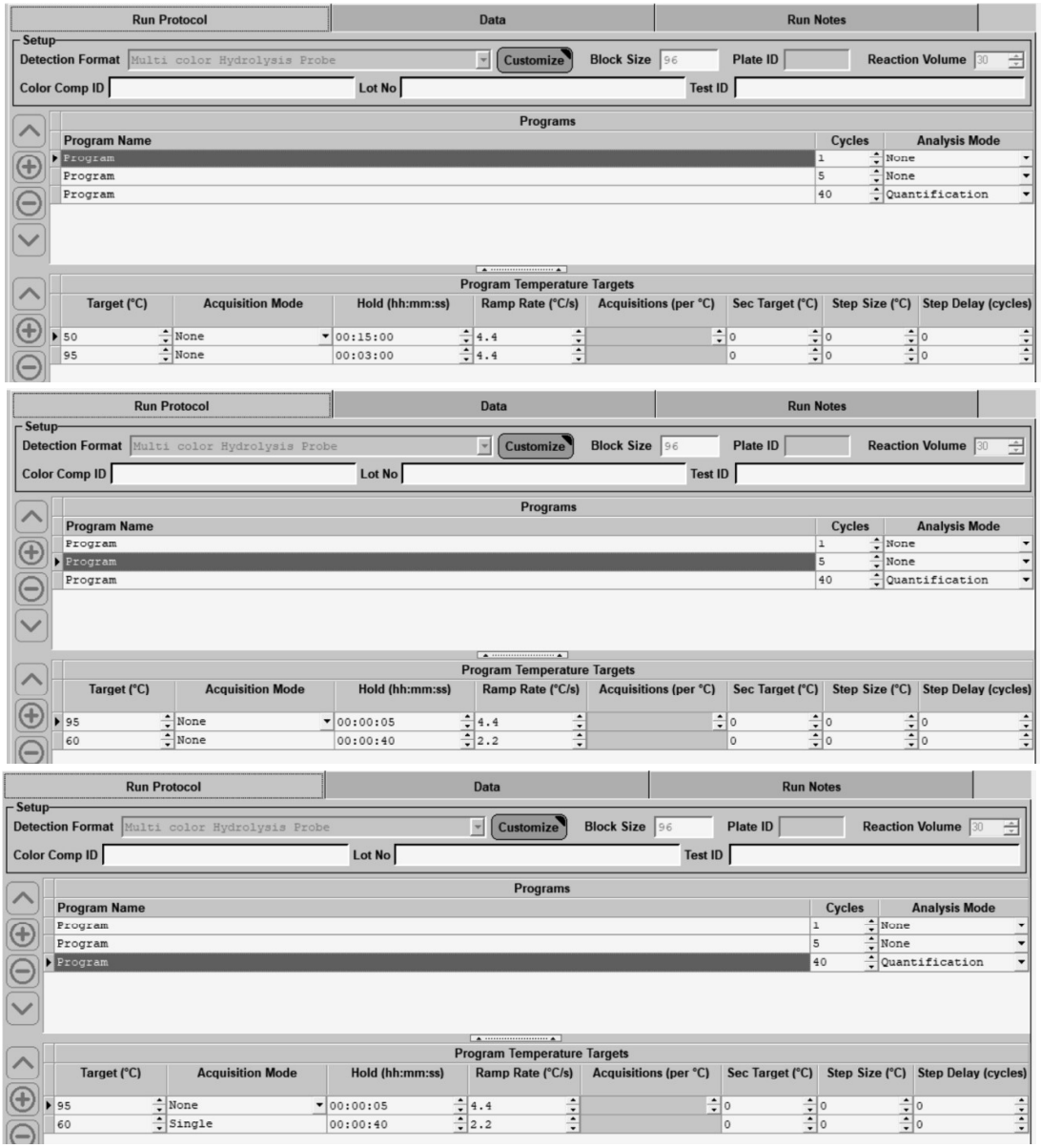


Figure 2. PCR Program Setting for LightCycler® 480 instrument

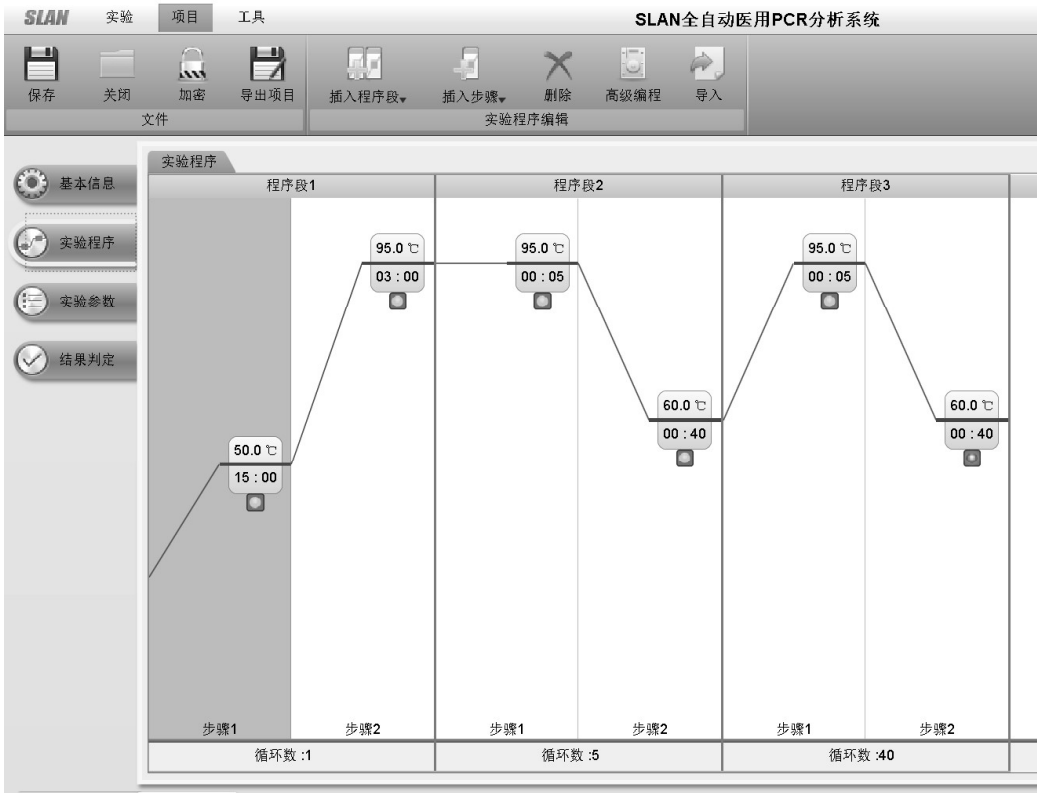


Figure 3. PCR Program Setting for SLAN 96P instrument

5. **Data Analysis**

Test data file need to be saved after PCR reaction. Please set the parameters and analysis the results of FAM, JOE, ROX and CY5 channels respectively.

(1) Baseline setting: the baseline can be set automatically or adjusted according to the shape of amplification curve.

(2) Threshold setting: the threshold value should be higher than the highest fluorescence value of negative control in this kit.

6. Quality Control

Negative control and positive control provide the calibration for the kit, and shall be set for each test. The result is valid if ALL the below criteria is met. Otherwise, the test is invalid. In this case, the errors of instruments, reagents, amplification conditions, etc. shall be checked, and the experiment shall be repeated.

Products of Quality Control	Requirements of Quality Control			
	FAM (ORF1ab)	JOE (N gene)	ROX (E gene)	CY5 (IC)
Positive Control of 2019-nCoV	Ct ≤ 32	Ct ≤ 32	Ct ≤ 32	No requirement
Negative Control	Undet	Undet	Undet	Ct ≤ 32

7. Interpreting Test Results

Interpreting Test Results of each channels
FAM has amplification signal, Ct ≤ 36, and amplification curve is typical S shape, then ORF1ab gene (+); otherwise, ORF1ab gene (-).
JOE has amplification signal, Ct ≤ 36, and amplification curve is typical S shape, then N gene (+); otherwise, N gene (-).
ROX has amplification signal, Ct ≤ 36, and amplification curve is typical S shape, then E gene (+); otherwise, E gene (-).
If the Ct of FAM, JOE and ROX is more than 36 or no value; and the Ct of CY5 is more than 32 or no value, then there is a problem with the sample or operation, which needs to be retested.

According to the above channel detection results, the judgment results are as follows:

Test Results	Interpreting Test Results
ORF1ab gene (+), N gene (+), E gene (+); OR ORF1ab gene (+), N gene (+), E gene (-); OR ORF1ab gene (+), N gene (-), E gene (+); OR ORF1ab gene (-), N gene (+), E gene (+).	2019-nCoV (+)
Only ORF1ab gene (+)	Test again, and if repeated: 2019-nCoV (+)
Only N gene (+) or E gene (+)	2019-nCoV (-)
ORF1ab gene (-), N gene (-), E gene (-)	2019-nCoV (-)

【CUT-OFF VALUE OR REFERENCE INTERVAL】

The cut-off value of 2019-nCoV is Ct ≤ 36.

【ASSAY LIMITATIONS】

1. This kit is intended for the qualitative detection of 2019-nCoV. The result is only for clinical reference, and the clinical management of patients should be considered in combination with their symptoms/signs, history, other laboratory tests and epidemiological data.

2. Although the detected target sequences of this kit are the conservative region of 2019-nCoV's gene, the missed detection of coronavirus types with rare mutations in the conservative region can't be completely avoided in theory.

3. The contamination of laboratory environment and reagent, or cross contamination during specimen treatment may lead to false positive result.

4. The decrease of detection effect even the false negative result may occur if there is any mistakes in the transportation, storage and operation of reagents.

5. 2019-nCOV early infection or other respiratory virus infection can't be excluded in patients with negative results. If conditions permit, it is recommended to collect more sensitive samples such as sputum for retest.

【PERFORMANCE SPECIFICATIONS】

1. Analytical Sensitivity and Limit of Detection (LOD)

In the LoD determination study, serial dilutions of the 6 quantified 2019-nCoV specimens (3 throat-swab and 3 sputum specimens) were prepared with negative specimens and then tested in 20 replicates. The tentative LoD determined from 3 throat-swab samples were 100 copies/mL, 300 copies/mL and 500 copies/mL, and the tentative LoD from 3 sputum samples were 100 copies/mL, 300 copies/mL and 500 copies/mL, respectively. Therefore, the tentative LoD was determined to be at 300 copies/mL for both matrices. In the LoD confirmation study, the LoD of the kit was then confirmed by testing 20 replicates at the tentative limit of detection concentration (300 copies/mL). The final LoD of each test was determined to be the lowest concentration resulting in positive detection of 19/20. The confirmation study results showed the kit LoD was 300 copies/mL for both throat swab and sputum samples.

2. Analytical Specificity

2.1 Cross-Reactivity

The Fosun 2019-nCoV qPCR kit has been designed to detect all publicly available SARS-CoV-2 strains. At the same time, the primers and probes were designed in the 2019-nCoV virus specific genome region ensuring the specific detection of the 2019-nCoV viral RNA. *In silico* analysis of the SARS-CoV-2 assay design were performed and compared to common respiratory flora and other viral pathogens from the same genetic family as SARS-CoV-2 according to the Recommended List of Organisms to be analyzed *in silico* (see Table 1) or by Direct wet lab Testing.

Table 1. List of microorganisms tested for cross-reactivity by *in silico* analysis

No.	Microorganism	No.	Microorganism
1	Human coronavirus 229E	23	Enterovirus D68
2	Coronavirus OC43	24	Human enterovirus 71
3	Coronavirus HKU1	25	Human respiratory syncytial virus A
4	Coronavirus NL63	26	Human respiratory syncytial virus B
5	SARS Coronavirus	27	Human rhinovirus 1A
6	MERS Coronavirus	28	Human rhinovirus 14
7	Human adenovirus 1	29	Human rhinovirus 57
8	Human adenovirus 2	30	Human rhinovirus 1B
9	Human adenovirus 3	31	Human rhinovirus C
10	Human adenovirus 4	32	Human rhinovirus C
11	Human adenovirus 5	33	*Chlamydia pneumoniae*
12	Human adenovirus 7	34	*Haemophilus influenzae-B*
13	Human adenovirus 71	35	*Legionella pneumophila*
14	Human adenovirus 55	36	*Mycobacterium tuberculosis*
15	Human metapneumovirus	37	*Streptococcus pneumonia*-19
16	Human metapneumovirus	38	*Streptococcus pyogenes*
17	Human parainfluenza virus 1	39	*Bordetella pertussis*
18	Human parainfluenza virus 2	40	*Mycoplasma pneumoniae*
19	Human parainfluenza virus 3	41	*Candida albicans*
20	Influenza A virus, H1N1	42	*Pneumocystis jirovecii* (PJP)
21	Influenza A virus, H3N2	43	*Staphylococcus salivarius*
22	Influenza B virus	44	*Staphylococcus aureus*
23	Influenza C virus	45	*Coxiella burnetiid* (Q fever)
24	Parechovirus	46	*Chamydia psittaci*
25	*Corynebacterium diphtheriae*	47	*Leptospira*
26	*Legionella non-pneumophila*	48	*Neisseria elongate*
27	*Bacillus anthracis* (Anthrax)	49	*Neisseria meningitidis*
28	*Moraxella cararrhalis*	50	

Results of *in silico* analysis demonstrates that there is significant homology between the SARS-coronavirus (MT007544 and MT123292) and our assay forward primer and probe for ORF1ab, N gene and E gene, however, they will not lead false positive results because of an significantly difference between the reverse primers of the three genes and the SARS-coronavirus (MT007544 and MT123292) . All other homologies were not significant for the pair of primers and probes, therefor a false positive result is not likely.

For Wet Testing, the test is performed on contrived throat swab and sputum samples containing related pathogens and microbes at infection-related medical decision level. Each sample was tested 3 times with kits from 3 different lots of detection kit. The result of wet testing showed that the Fosun 2019-nCoV qPCR kit exhibited satisfactory analytical specificity and had no cross-reactivity with virus and microbes tested.

2.2 Interfering Substances

The potentially interfering substances were spiked into throat swab/sputum samples with a concentration of 2 x LOD of SARS-CoV-2. Samples were then tested with Fosun 2019-nCoV qPCR Kit. Samples containing potentially

interfering substances, which were tested in 3 replicates, were compared to throat swab/sputum samples 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 2. All tested interfering substance of said concentrations showed no influence on the performance of Fosun 2019-nCoV qPCR kit.

Table 2 Concentration of Interfering Substances in samples

No.	Interfering Substance	Working concentration	No.	Interfering Substance	Working concentration
1	Mucin	25mg/mL	19	Paramivir	25%（V/V）
2	Blood(human)	25%（V/V）	20	Lopinavir	2mg/mL
3	Benfurin	1mg/mL	21	Ritonavir	0.5mg/mL
4	Oxymetazoline	25%（V/V）	22	Abidol	1mg/mL
5	Sodium Chloride	25%（V/V）	23	Levofloxacin	5mg/mL
6	Beclomethasone	0.05g	24	Azithromycin	25%（V/V）
7	Dexamethasone	25%（V/V）	25	Ceftriaxone	25%（V/V）
8	Flunisolide	2mg/mL	26	Meropenem	25%（V/V）
9	Triamcinolone	25%（V/V）	27	Tobramycin	25%（V/V）
10	Budesonide	25%（V/V）	28	Albumin	60mg/mL
11	Momisson	25%（V/V）	29	Hemoglobin	24mg/mL
12	Fluticasone	25%（V/V）	30	Nasal spray	25%（V/V）
13	Histamine hydrochloride	10μg/mL	31	Vacomycin	20mg/mL
14	α-interferon	0.05g	32	probiotics	≥1.5 亿 CFU
15	Zanamivir	10mg	33	Loratadine	25%（V/V）
16	Ribavirin	2.25mg/mL	34	Throat lozenges	0.1g/mL
17	Ribavirin	20.89mg/mL	35	Oral anaesthetic	50%
18	Oseltamivir	75μg/mL	36	Oral analgesic	0.0325g/mL

3. Clinical Evaluation with a SARs-CoV-2 RT-PCR Kit

Clinical evaluation of the Fosun 2019-nCoV qPCR kit was conducted with 597 clinical specimens (305 sputum specimens and 292 throat swab specimens) including 204 positive and 393 negative samples, comparing with a validated SARS-CoV-2 molecular assay (Tables 3-5). The percent agreement was calculated according to the following 2x2 grid table (Table 13). Compared with the comparator method, the PPA was 99.51% (97.30%- 99.99%, 95%CI), and the NPA was 96.44% (94.10% - 98.04%, 95%CI).

Table 3. Comparison Detection of Two Kits for All 597 Specimens

Comparator kit / Fosun kit	Positive	Negative	Total
Positive	203	14	217
Negative	1	379	380
Total	204	393	597

SARS-CoV-2RT-PCR	Value Ratio	Percentage(95%CI)
Positive percent agreement (PPA)	203/204	99.51% (97.30% - 99.99%)
Negative percent agreement (NPA)	379/393	96.44% (94.10% - 98.04%)
Overall percent agreent	582/597	97.49% (95.90% - 98.47%)

Table 4. Comparison Detection of Two Kits for 305 Sputum Specimens

Comparator kit / Fosun kit	Positive	Negative	Total
Positive	124	14	138
Negative	1	166	167
Total	125	180	305

SARS-CoV-2 RT-PCR	Value Ratio	Percentage(95%CI)
Positive percent agreement(PPA)	124/125	99.20% (95.62% - 99.98%)
Negative percent agreement(NPA)	166/180	92.22% (87.29% - 95.68%)
Overall percent agreement	290/305	95.08%(92.05% - 97.00%)

Table 5. Comparison Detection of Two Kits for 292 Throat Swab Specimens

Comparator kit / Fosun kit	Positive	Negative	Total
Positive	79	0	79
Negative	0	213	213
Total	79	213	292

SARS-CoV-2RT-PCR	Value Ratio	Percentage(95%CI)
Positive percent agreement(PPA)	79/79	100% (95.36% - 100.00%)
Negative percent agreement(NPA)	213/213	100% (98.23% - 100.00%)
Overall percent agreement	292/292	100% (98.70% - 100.00%)

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【GENERAL INFORMATION】

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