The Fosun COVID-19 RT-PCR Detection Kit ***Instruction for Use***, ***Fact Sheet for Health Care Providers***, ***Fact Sheet for Patients*** can be downloaded from the following link: **www.fosunpharmausa.com/covid19/pcr/**

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# Instruction for Use

# Fosun COVID-19 RT-PCR Detection Kit

**Rx Only**

IVD

**For Emergency Use Authorization (EUA) only**

**PRODUCT NAME**
Fosun COVID-19 RT-PCR Detection Kit

REF
Catalog Number:
PCSYHF02-a (48 tests/kit); PCSYHF03-a (96 tests/kit).

Manufactured for:
Fosun Pharma USA Inc.
Princeton, NJ 08540
Tel: (866) 611-3762

Manufactured by:
Shanghai Fosun Long March Medical Science Co., Ltd
No.830, Chengyin Rd, Baoshan District
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## INTENDED USE

The Fosun COVID-19 RT-PCR Detection Kit is a real-time RT-PCR test intended for the qualitative detection of nucleic acid from the SARS-CoV-2 in upper and lower respiratory specimens (such as anterior nasal swabs, mid-turbinate nasal swabs, nasopharyngeal swabs, oropharyngeal swabs, sputum, lower respiratory tract aspirates, bronchoalveolar lavage, and nasopharyngeal wash/aspirate or nasal aspirate) from individuals suspected of COVID-19 by their healthcare provider. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments (CLIA) of 1988, 42 U.S.C. §263a, to perform high complexity tests.

Results are for the identification of SARS-CoV-2 RNA. The SARS-CoV-2 RNA is generally detectable in upper and lower respiratory specimens during the acute phase of infection. Positive results are indicative of the presence of SARS-CoV-2 RNA; clinical correlation with patient history and other diagnostic information is necessary to determine patient infective status. The agent detected may not be the definite cause of disease. Positive results do not rule out bacterial co-infection with other viruses. Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.

Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The Fosun COVID-19 RT-PCR Detection Kit is intended for use by qualified trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. The Fosun COVID-19 RT-PCR Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

## PRINCIPLE OF DETECTION

This product is a fluorescent probe-based Taqman RT-PCR assay system. Firstly, the RNA of SARS-CoV-2 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 SARS-CoV-2 will be detected qualitatively by FAM channel, the N gene of SARS-CoV-2 will be detected qualitatively by JOE channel, the E gene of SARS-CoV-2 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.

## WARNINGS & PRECAUTIONS

- **The contamination of laboratory environment and reagent, or cross contamination during specimen treatment may lead to false positive result.**
- **The decrease of detection effect even the false negative result may occur if there are any mistakes in the transportation, storage and operation of reagents.**
  **COVID-19 early infection or other respiratory virus infection can't be excluded in patients with negative results.**
- **For *in vitro* diagnostic use.**
- **For use under an Emergency Use Authorization (EUA) only.**
- **For prescription use only.**

- **Laboratories within the United States and its territories are required to report all positive results to the appropriate public health authorities.**
- **Handle all specimens as if infectious using safe laboratory procedures. Refer to CDC Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html**
- **Inappropriate sample collection, transfer, storage and operation may lead to inaccurate 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 in accordance with SAMPLING & HANDING.**
- **As this test involves the extraction of viral RNA and PCR amplification, please take care to avoid contamination of the amplification reaction mixture. Regular monitoring of laboratory contamination is recommended.**
- **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.**
- **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.**
- **Discard all materials in a safe and acceptable manner, in compliance with all legal requirements.**
- **If exposure to skin or mucous membranes occurs, immediately wash the area with large amounts of water. Seek medical advice immediately.**
- **Do not use components beyond the expiration the date printed on the kit boxes.**
- **Do not mix reagents from different lots.**
- **Return all components to the appropriate storage condition after preparing the working reagents.**
- **Do not interchange vial or bottle caps, as cross-contamination may occur.**
  **Reagents supplied are formulated specifically for use with this kit. Make no substitutions in order to ensure optimal performance of the kit. Further dilution of the reagents or alteration of incubation time and temperature may result in erroneous or discordant data**

**PRODUCT COMPONENTS**

The Fosun COVID-19 RT-PCR Detection Kit includes the following components:

- SARS-COV-2 Reaction Reagent
- RT-PCR Enzyme
- Positive Control of SARS-CoV-2
- Negative Control
- Internal Reference A

The Fosun COVID-19 RT-PCR Detection Kit is available in 2 pack sizes-48 tests kit and 96 tests kit. Individual components and their descriptions are listed in Table 1 below.

**Table 1. Components Provided with the Kit**

**Pack-size: 48 tests**

| Name of Component | Part# | Description | Pack Size: 48 tests kit | Labe Volume for each vial |
|---|---|---|---|---|
| SARS-CoV-2 Reaction Reagent | PZHF01B-a | dNTPs, $MgCl_2$, Primers and Probes (for ORF1ab gene, E gene and N gene of SARS-CoV-2, and Internal Reference A) | **1 vial** | 672 μL |
| RT-PCR Enzyme | PZHF02B | Taq DNA polymerase, Reverse Transcriptase, UNG enzyme | **1 vial** | 288 μL |
| Positive Control of SARS-CoV-2 | PZHFQ-a | RNA template | **1 vial** | 200 μL |
| Negative Control | PZHFY | NaCl | **1 vial** | 200 μL |
| Internal Reference A | PZHF03B | Lentivirus | **1 vial** | 240 μL |

**Pack-size: 96 tests**

| Name of Component | Part# | Description | Pack Size: 96 tests kit | Labe Volume for each vial |
|---|---|---|---|---|
| SARS-CoV-2 Reaction Reagent | PZHF01B-a | dNTPs, $MgCl_2$, Primers and Probes (for ORF1ab gene, E gene and N gene of SARS-CoV-2, and Internal Reference A) | **2 vial** | 672 μL |
| RT-PCR Enzyme | PZHF02B | Taq DNA polymerase, Reverse Transcriptase, UNG enzyme | **2 vial** | 288 μL |
| Positive Control of SARS-CoV-2 | PZHFQ-a | RNA template | **2 vial** | 200 μL |
| Negative Control | PZHFY | NaCl | **2 vial** | 200 μL |
| Internal Reference A | PZHF03B | Lentivirus | **2 vial** | 240 μL |

Note: Do not mix the components from different batches for detection. The positive control of SARS-CoV-2 and internal reference were constructed artificially, and they were not infectious.

**COMPONENTS REQUIRED BUT NOT PROVIDED WITH THE KIT:**

1. Sample collection
   - Nasopharyngeal or oropharyngeal swabs
   - Collection tubes or sputum containers
   - Transport media
2. Nucleic acid extraction (Table 2)

**Table 2. The Recommended RNA Extraction Kit (Not provided with the Kit)**

| Use | Product | Catalog No. | No. of preps | Supplier |
|---|---|---|---|---|
| RNA extraction (Recommended) | QIAamp® Viral RNA Mini Kit | 52904/52906 | 50/250 | QIAGEN® |

3. Other reagents and consumables required but not included with the test:
   - RNase/DNase free water, anhydrous ethanol
   - 96 well plates, pipette tips with filters, microcentrifuge tubes
   - Liquid waste container, solid waste bag and container
   - Double-layer latex gloves, waterproof boot covers, protective clothing, goggles, and masks with higher filtration efficiency.
4. Other instruments required but not included with the test:
   - Applied Biosystems® 7500 instrument (software version #v1.4 or v1.5)
   - PCR hood, centrifuge with rotor for 1.5 mL and 2 mL tubes, vortex mixer, metal bath, and pipette.

**STORAGE & SHELF LIFE**

All reagents should be stored at -25°C to -15°C (-13°F to 5°F) with protection from light, and the reagents are stable for 12 months when stored at the recommended condition. See label for 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.

**SAMPLING & HANDING**

Sample collection should be conducted by qualified health care providers.

(1) Oropharyngeal swab (throat swab): Wipe the bilateral pharyngeal tonsils and the posterior pharyngeal wall at the same time, immerse the swab head into the sample collection tube containing transport media, discard the tail, and tighten the tube cover.

(2) Nasopharyngeal swab: Hold the nasopharyngeal swab close to the nasal septum slowly and deeply to the back of the nasopharynx, rotate it several times to obtain secretions; quickly dip the swab into the sample collection tube containing transport media, discard the tail, and tighten the tube cap to seal to avoid drying.

(3) 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.

The sample can be stored at 2°C to 8°C for 24 hours, at -20°C for 4 days, and below -70°C for an extended time.

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

**PROTOCOL**

1. Reagent Preparation

Prepare reagent with ice box, 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 SARS-CoV-2 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°C to 8°C for 3 hours after separation.

2. RNA Extraction

It is recommended to use QIAamp® Viral RNA Mini Kit (QIAGEN®) to extract RNA from samples and reference.

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 reference); the RNA elution volume is 60 μL; after RNA extraction, the extracted RNA shall be added to the reaction tubes within 10 minutes, or transferred to the centrifuge tubes and stored at -25°C to -15°C.

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°C for 15 minutes, 1 cycle;
Step2: 95°C for 3 minutes, 1 cycle;
Step3: 95°C for 5 seconds to 60°C for 40 seconds, 5 cycles;
Step4: 95°C for 5 seconds to 60°C for 40 seconds, 40 cycles. The signals of FAM, JOE, ROX and CY5 fluorescence channels will be collected at 60°C.

Note: Select "None" from the pull-down menu of the passive reference on operation interface of Applied Biosystems® 7500 RT-PCR software (v1.4, v1.5).

5. Data Analysis (Applied Biosystems® 7500RT-PCR software v1.4, v1.5)

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 (see Table 3). 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.

**Table 3. Criteria of Quality Control**

| Products of Quality Control | Requirements of Quality Control | | | |
|---|---|---|---|---|
| | FAM Channel | JOE Channel | ROX Channel | CY5 Channel |
| Positive Control of SARS-COV-2 | Ct ≤ 32 | Ct ≤ 32 | Ct ≤ 32 | No requirement |
| Negative Control | Undet | Undet | Undet | Ct ≤ 32 |

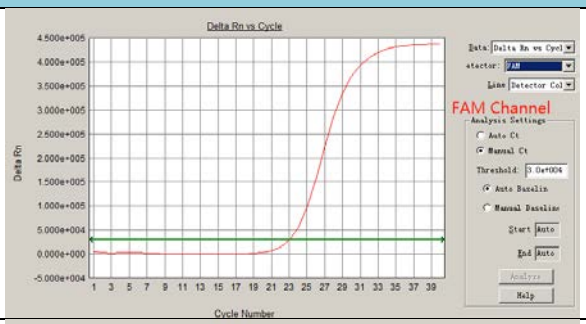
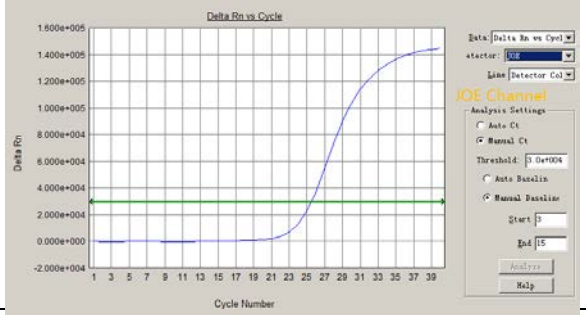
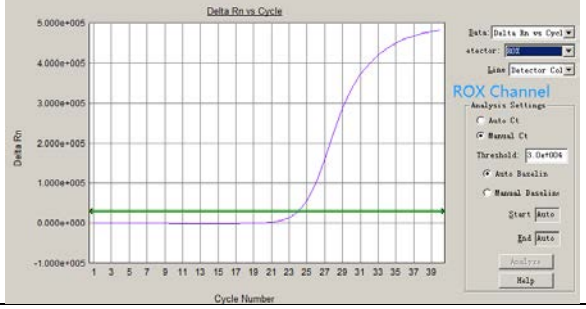
7. Interpreting Test Results (Table 4.)

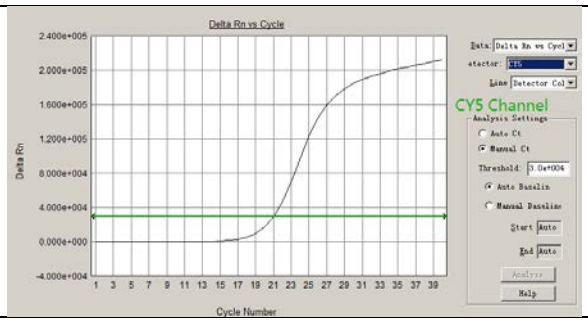
**Table 4. The Interpreting Test Results**

| 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. |

The typical S-shape amplification curves were showed in Table 5 below.

**Table 5. The Typical S-shape Amplification Curve Example**

| Fluorescent Channel | Typical S-Shape Amplification Curve Example in Applied Biosystems® 7500 |
|---|---|
| FAM |   |
| JOE |   |
| ROX |   |

| | |
|---|---|
| Cy5 |  |

According to the above channel detection results, the results interpretations are as follows (Table 6):

**Table 6. The Result Interpretation Criteria**

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

**CUT-OFF VALUE OR REFERENCE INTERVAL**

The cut-off value of COVID-19 is Ct ≤ 36.

**ASSAY LIMITATIONS**

1. The performance of the Fosun COVID-19 RT-PCR Detection Kit was established using pharyngeal swab samples and sputum samples. Anterior nasal swabs and mid-turbinate nasal swabs are also considered acceptable specimen types for use with the Fosun COVID-19 RT-PCR Detection Kit but performance has not been established. Testing of nasal and mid-turbinate nasal swabs (self-collected or collected by a healthcare provider) is limited to patients with symptoms of COVID-19. Please refer to FDA's FAQs on Diagnostic Testing for SARS-CoV-2 for additional information.

2. Samples must be collected, transported, and stored using appropriate procedures and conditions. Improper collection, transport, or storage of specimens may hinder the ability of the assay to detect the target sequences.

3. Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction kits have not been evaluated.

4. The positive result detected by this kit can't indicate whether there is virus in vivo. It is suggested to use other methods for confirmation at the same time.

5. This kit is intended for classification and detection of SARS-CoV-2. 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 treatment responses.

6. Although the detected target sequences of this kit are the conservative region of SARS-CoV-2's gene, the missed detection of coronavirus types with rare mutations in the conservative region can't be

completely avoided in theory.

7. 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.

**CONDITIONS OF AUTHORIZATION FOR THE LABORATORY**

The Fosun COVID-19 RT-PCR Detection Kit Letter of Authorization, along with the authorized Fact Sheet for Healthcare Providers, the authorized Fact Sheet for Patients, and authorized labeling are available on the FDA website: https://www.fda.gov/medical-devices/emergency-situations-medical-devices/emergency-use-authorizations.

However, to assist clinical laboratories using the Fosun COVID-19 RT-PCR Detection Kit ("your product" in the conditions below), the relevant Conditions of Authorization are listed below:

A. Authorized laboratories[1] using your product will include with result reports of your product, all authorized Fact Sheets. Under exigent circumstances, other appropriate methods for disseminating these Fact Sheets may be used, which may include mass media.

B. Authorized laboratories using your product will use your product as outlined in the Instructions for Use. Deviations from the authorized procedures, including the authorized instruments, authorized extraction methods, authorized clinical specimen types, authorized control materials, authorized other ancillary reagents and authorized materials required to use your product are not permitted.

C. Authorized laboratories that receive your product will notify the relevant public health authorities of their intent to run your product prior to initiating testing.

D. Authorized laboratories using your product will have a process in place for reporting test results to healthcare providers and relevant public health authorities, as appropriate.

E. Authorized laboratories will collect information on the performance of your product and report to DMD/OHT7-OIR/OPEQ/CDRH (via email: CDRH-EUA-Reporting@fda.hhs.gov) and You (via email: inquiry@fosunpharmausa.com or via phone: (866) 611-3762 any suspected occurrence of false positive or false negative results and significant deviations from the established performance characteristics of your product of which they become aware.

F. All laboratory personnel using your product must be appropriately trained in RT-PCR techniques and use appropriate laboratory and personal protective equipment when handling this kit and use your product in accordance with the authorized labeling.

G. You, authorized distributors, and authorized laboratories using your product will ensure that any records associated with this EUA are maintained until otherwise notified by FDA. Such records will be made available to FDA for inspection upon request.

[1] The letter of authorization refers to, "United States (U. S.) laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. §263a, to perform high complexity tests" as "authorized laboratories."

## ASSAY PERFORMANCE

### Analytical Sensitivity and Limit of Detection (LOD)

In the LoD determination study, serial dilutions of the 6 quantified SARS-CoV-2 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 300 copies/mL, and the tentative LoD from 3 sputum samples were 100 copies/mL, 300 copies/mL and 300 copies/mL, respectively. Therefore, the tentative LoD was determined to be at 300 copies/mL for both matrixes. 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.

### Inclusivity

The Fosun COVID-19 RT-PCR Detection kit has been designed to detect publicly available SARS-CoV-2 viral RNA sequences. Alignments were performed with the designed oligonucleotide primer and probe sequences of Fosun COVID-19 RT-PCR Detection kit panel with 1119 publicly available sequences of SARA-CoV-2 from NCBI and GISAID as of March 23, 2020 to demonstrate the estimated inclusivity of the Fosun COVID-19 RT-PCR Detection kit. All the alignments exhibited high homology to the available SARS-CoV-2 sequences, suggesting the potential ability of the Fosun COVID-19 RT-PCR Detection kit to detect thoseSARS-CoV-2 strains.

### Cross-Reactivity

The Fosun COVID-19 RT-PCR Detection 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 SARS-CoV-2 virus specific genome region ensuring the specific detection of the SARS-CoV-2 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 7 ) or by Direct wet lab Testing (see Table 8).

**Table 7. 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 5 | 34 | *Haemophilus influenzae-B* |
| 13 | Human adenovirus 7 | 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 | |

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. 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 COVID-19 RT-PCR kit exhibited satisfactory analytical specificity and had no cross-reactivity with virus and microbes tested (see Table 8).

**Table 8. Cross-Reactivity: List of Organisms analyzed by Wet Testing**

| Virus/Bacteria/Parasite | Strain | Source/Sample type | Concentration |
|---|---|---|---|
| Human coronavirus 229E | ATCC VR740 | Standard Strain | $\geq 1\times10^5$ $TCID_{50}$/mL |
| Coronavirus OC43 | / | Patient Sample | $4.97\times10^7$copies /mL |
| Coronavirus HKU1 | / | Patient Sample | $7.18\times10^7$copies /mL |
| Coronavirus NL63 | / | Patient Sample | $4.19\times10^7$copies /mL |
| SARS Coronavirus | / | Pseudovirus | $1\times10^8$copies /mL |
| MERS Coronavirus | / | Pseudovirus | $1\times10^8$copies /mL |
| Human adenovirus 1 | 1w3263 | Patient Sample | / |
| Human adenovirus 2 | 1w9852 | Patient Sample | / |
| Human adenovirus 3 | ATCC VR3 | Standard Strain | $\geq 1\times10^5$ $TCID_{50}$/mL |
| Human adenovirus 4 | 89860 | Patient Sample | / |
| Human adenovirus 5 | 95031 | Patient Sample | / |
| Human adenovirus 5 | 95114 | Patient Sample | / |
| Human adenovirus 7 | ATCC VR7 | Standard Strain | $\geq 1\times10^5$ $TCID_{50}$/mL |
| Human adenovirus 55 | 77653 | Patient Sample | / |
| Human metapneumovirus | D4111 | Patient Sample | / |
| Human metapneumovirus | SE14142 | Patient Sample | / |
| Human parainfluenza virus 1 | ATCC VR94 | Standard Strain | $\geq 1\times10^5$$TCID_{50}$/mL |
| Human parainfluenza virus 2 | ATCC VR92 | Standard Strain | $\geq 1\times10^5$ $TCID_{50}$/mL |
| Human parainfluenza virus 3 | ATCC VR93 | Standard Strain | $\geq 1\times10^5$ $TCID_{50}$/mL |
| Influenza A virus, H1N1 | ATCC VR1736 | Standard Strain | $\geq 1\times10^5$ $TCID_{50}$/mL |
| Influenza A virus, H3N2 | ATCC VR1811 | Standard Strain | $\geq 1\times10^5$$TCID_{50}$/mL |
| Influenza B virus | ATCC VR1735 | Standard Strain | $\geq 1\times10^5$$TCID_{50}$/mL |
| Enterovirus D68 | D1414 | Patient Sample | / |
| Human enterovirus 71 | ATCC VR1432 | Standard Strain | $\geq 1\times10^5$$TCID_{50}$/mL |
| Human respiratory syncytial virus A | ATCC VR1540 | Standard Strain | $\geq 1\times10^5$$TCID_{50}$/mL |
| Human respiratory syncytial virus B | ATCC VR955 | Standard Strain | $\geq 1\times10^5$$TCID_{50}$/mL |
| Human rhinovirus 1A | ATCC VR1559 | Standard Strain | $\geq 1\times10^5$$TCID_{50}$/mL |
| Human rhinovirus 14 | ATCC VR284 | Standard Strain | $\geq 1\times10^5$$TCID_{50}$/mL |
| Human rhinovirus 57 | ATCC VR1600 | Standard Strain | $\geq 1\times10^5$$TCID_{50}$/mL |
| Human rhinovirus 1B | ATCC VR1645 | Standard Strain | $\geq 1\times10^5$$TCID_{50}$/mL |

| | | | |
|---|---|---|---|
| Human rhinovirus C | D2055 | Patient Sample | / |
| Human rhinovirus C | D2056 | Patient Sample | / |
| *Chlamydia pneumoniae* | ATCC 53592 | Standard Strain | ≥$1\times10^5$$TCID_{50}$/mL |
| *Haemophilus influenzae*-B | ATCC10211 | Standard Strain | $1\times10^8$ bacteria/mL |
| *Legionella pneumophila* | / | / | $1\times10^8$ bacteria/mL |
| *Mycobacterium tuberculosis* | / | Patient Sample | $10^7$copies /mL |
| *Streptococcus pneumonia*-19 | ATCC49619 | Standard Strain | $1\times10^8$ bacteria/mL |
| *Streptococcus pyogenes* | ATCC19615 | Standard Strain | $1\times10^8$ bacteria/mL |
| *Bordetella pertussis* | / | / | $1\times10^8$ bacteria/mL |
| *Mycoplasma pneumoniae* | ATCC 15531TTR | Standard Strain | ≥$1\times10^5$$TCID_{50}$/mL |
| *Candida albicans* | / | / | $1\times10^8$ bacteria/mL |
| *Pneumocystis jirovecii* (PJP) | Not yet tested | | |
| *Staphylococcus salivarius* | Not yet tested | | |

**Clinical Evaluation of Fosun COVID-19 RT-PCR Detection Kit Using Contrived Samples**

The performance evaluation of Fosun COVID-19 RT-PCR Detection Kit was tested using contrived samples. 12 positive samples at $10^3$~$10^8$ copies/mL and additional 23 positive samples at 1~2 × LoD were prepared by spiking in SARS-CoV-2 RNA to negative specimens. 32 negative samples were also included in this study. The results are shown below (Table 9).

**Table 9. Clinical Evaluation with Contrived Samples**

| No. | Type | FAM | JOE | ROX | CY5 | Rs. |
|---|---|---|---|---|---|---|
| 1 | Positive Throat Swab | 20.04 | 20.09 | 20.62 | 19.30 | + |
| 2 | | 11.08 | 11.36 | 11.83 | Undet. | + |
| 3 | | 14.55 | 15.05 | 15.36 | 20.46 | + |
| 4 | | 23.16 | 23.15 | 23.93 | 19.47 | + |
| 5 | | 21.48 | 21.53 | 22.06 | 19.39 | + |
| 6 | | 28.23 | 27.93 | 29.04 | 19.24 | + |
| 7 | Positive Sputum | 15.13 | 15.36 | 15.76 | 19.68 | + |
| 8 | | 21.25 | 21.37 | 21.87 | 19.30 | + |
| 9 | | 23.93 | 24.05 | 24.76 | 19.23 | + |
| 10 | | 23.16 | 23.27 | 23.93 | 19.30 | + |
| 11 | | 26.81 | 27.03 | 27.66 | 19.33 | + |
| 12 | | 20.30 | 20.33 | 21.02 | 19.37 | + |
| 13 | 1~2×LoD Throat Swab | 32.19 | 31.08 | 32.42 | 19.52 | + |
| 14 | | 31.12 | 30.47 | 31.43 | 19.35 | + |
| 15 | | 32.62 | 30.61 | 31.54 | 19.25 | + |
| 16 | | 30.52 | 32.60 | 32.31 | 19.27 | + |
| 17 | | 30.69 | 30.90 | 33.70 | 19.18 | + |
| 18 | | 31.40 | 32.92 | 32.46 | 19.35 | + |
| 19 | | 30.61 | 31.21 | 31.09 | 19.32 | + |
| 20 | | 31.07 | 32.67 | 32.09 | 19.39 | + |
| 21 | | 35.20 | 31.29 | 33.34 | 27.20 | + |
| 22 | | 31.46 | 31.58 | 32.70 | 19.35 | + |
| 23 | | 31.51 | 31.22 | 32.15 | 19.23 | + |
| 24 | 1~2× LoD Sputu | 34.72 | Undet. | 35.47 | 27.16 | + |
| 25 | | 32.05 | 31.23 | 32.11 | 19.52 | + |
| 26 | | Undet. | 32.43 | 34.00 | 27.96 | + |

| No. | Type | FAM | JOE | ROX | CY5 | Rs. |
|---|---|---|---|---|---|---|
| 27 | | 31.27 | 32.33 | 31.94 | 19.38 | + |
| 28 | | 30.32 | 30.82 | 32.34 | 19.33 | + |
| 29 | | 30.82 | 31.24 | 32.23 | 19.37 | + |
| 30 | | 30.78 | 31.28 | 31.27 | 19.27 | + |
| 31 | | 33.18 | Undet. | 32.98 | 25.77 | + |
| 32 | | 30.32 | 32.31 | 31.46 | 19.46 | + |
| 33 | | 35.19 | 33.15 | Undet. | 25.58 | + |
| 34 | | 34.41 | 32.85 | Undet. | 25.53 | + |
| 35 | | 31.17 | 31.12 | 32.22 | 19.42 | + |
| 36 | Negative Throat Swab | Undet. | Undet. | Undet. | 19.37 | - |
| 37 | | Undet. | Undet. | Undet. | 19.26 | - |
| 38 | | Undet. | Undet. | Undet. | 19.22 | - |
| 39 | | Undet. | Undet. | Undet. | 19.42 | - |
| 40 | | Undet. | Undet. | Undet. | 19.42 | - |
| 41 | | Undet. | Undet. | Undet. | 19.38 | - |
| 42 | | Undet. | Undet. | Undet. | 19.48 | - |
| 43 | | Undet. | Undet. | Undet. | 19.33 | - |
| 44 | | Undet. | Undet. | Undet. | 19.27 | - |
| 45 | | Undet. | Undet. | Undet. | 19.31 | - |
| 46 | | Undet. | Undet. | Undet. | 19.19 | - |
| 47 | | Undet. | Undet. | Undet. | 19.42 | - |
| 48 | | Undet. | Undet. | Undet. | 19.36 | - |
| 49 | | Undet. | Undet. | Undet. | 19.48 | - |
| 50 | | Undet. | Undet. | Undet. | 19.48 | - |
| 51 | | Undet. | Undet. | Undet. | 19.42 | - |
| 52 | Negative Sputum | Undet. | Undet. | Undet. | 19.31 | - |
| 53 | | Undet. | Undet. | Undet. | 19.19 | - |
| 54 | | Undet. | Undet. | Undet. | 19.19 | - |
| 55 | | Undet. | Undet. | Undet. | 19.23 | - |
| 56 | | Undet. | Undet. | Undet. | 19.20 | - |
| 57 | | Undet. | Undet. | Undet. | 19.36 | - |
| 58 | | Undet. | Undet. | Undet. | 19.42 | - |
| 59 | | Undet. | Undet. | Undet. | 19.43 | - |
| 60 | | Undet. | Undet. | Undet. | 19.27 | - |
| 61 | | Undet. | 33.07 | Undet. | 19.30 | - |
| 62 | | Undet. | Undet. | 36.63 | 19.10 | - |
| 63 | | Undet. | Undet. | Undet. | 19.35 | - |
| 64 | | Undet. | Undet. | Undet. | 19.37 | - |
| 65 | | Undet. | Undet. | Undet. | 19.39 | - |
| 66 | | Undet. | Undet. | Undet. | 19.53 | - |
| 67 | | Undet. | Undet. | Undet. | 19.58 | - |

**Clinical Evaluation of Fosun COVID-19 RT-PCR Detection Kit Comparing to FDA EUA-authorized CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel**

The comparison study was conducted using 50 positive specimens and 100 negative clinical specimens (75 sputum specimens and 75 throat swab specimens) with FDA EUA-approved CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel (Tables 10-12). The percent

agreement was calculated according to the following 2x2 grid table (Table 10). The comparison results between the Fosun COVID-19 RT-PCR Detection kit and FDA authorized EUA CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel showed PPA was 100% (92.86%-100%, 95%CI) and NPA was 100% (96.30%-100%, 95%CI).

**Table 10. SARS-CoV-2 Comparison Detection of Two Kits for All 150 Specimens**

| Comparator kit / Fosun kit | Positive | Negative | Total |
|---|---|---|---|
| Positive | 50 | 0 | 50 |
| Negative | 0 | 100 | 100 |
| Total | 50 | 100 | 150 |

| SARS-CoV-2RT-PCR | Value Ratio | Percentage(95%CI) |
|---|---|---|
| Positive percent agreement (PPA) | 50/50 | 100% (92.86%-100%) |
| Negative percent agreement (NPA) | 100/100 | 100% (96.30%-100%) |

**Table 11. SARS-CoV-2 Comparison Detection of Two Kits for 75 Sputum Specimens**

| Comparator kit / Fosun kit | Positive | Negative | Total |
|---|---|---|---|
| Positive | 11 | 0 | 11 |
| Negative | 0 | 64 | 64 |
| Total | 11 | 64 | 75 |

| SARS-CoV-2RT-PCR | Value Ratio | Percentage(95%CI) |
|---|---|---|
| Positive percent agreement(PPA) | 11/11 | 100% (74.12%-100%) |
| Negative percent agreement(NPA) | 64/64 | 100% (94.34%-100%) |

**Table 12. SARS-CoV-2 Comparison Detection of Two Kits for 75 Throat Swab Specimens**

| Comparator kit / Fosun kit | Positive | Negative | Total |
|---|---|---|---|
| Positive | 39 | 0 | 39 |
| Negative | 0 | 36 | 36 |
| Total | 39 | 36 | 75 |

| SARS-CoV-2RT-PCR | Value Ratio | Percentage(95%CI) |
|---|---|---|
| Positive percent agreement(PPA) | 39/39 | 100% (91.03%-100%) |
| Negative percent agreement(NPA) | 36/36 | 100% (90.36%-100%) |

**Clinical Evaluation with a SARs-CoV-2 RT-PCR Kit**

Clinical evaluation of the Fosun COVID-19 RT-PCR Detection 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 13-15). 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 13. SARS-CoV-2 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%) |

**Table 14. SARS-CoV-2 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-2RT-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%) |

**Table 15. SARS-CoV-2 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%) |

**FDA SARS-CoV-2 Reference Panel Testing**

The evaluation of sensitivity and MERS-CoV cross-reactivity was performed using reference material (T1), blinded samples and a standard protocol provided by the FDA. The study included a range finding study and a confirmatory study for LoD. Blinded sample testing was used to establish specificity and to

confirm the LoD. The extraction method and instrument used were Qiagen® QIAamp® Viral RNA Mini Kit and Applied Biosystems® 7500 Real-Time PCR System. The results are summarized in Table 16.

**Table 16. Summary of LoD Confirmation Result using the FDA SARS-CoV-2 Reference Panel**

| Reference Materials Provided by FDA | Specimen Type | Product LoD | Cross-Reactivity |
|---|---|---|---|
| SARS-CoV-2 | Nasopharyngeal Swab | $6.0x10^2$ NDU/mL | N/A |
| MERS-CoV | | N/A | ND |

NDU/mL = RNA NAAT detectable units/mL
N/A: Not applicable
ND: Not detected

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1. Applied Biosystems® 7500 Real-Time PCR Systems
2. Qiagen® QIAamp® Viral RNA Mini Kit
3. ATCC® CRL-1586™ (Vero E6)

**SYMBOLS USED IN PACKAGING**

| Symbol | Description | Label | Description |
|---|---|---|---|
| IVD | *in vitro* Diagnostic Device |  | Consult Instructions for Use |
| REF | Catalog Number |  | Use-by Date |
| LOT | Batch Code |  | Temperature Limitation |
| **Rx only** | For Prescription Use Only | | |

**ANNEX KIT VERIFICATION REQUIREMENTS**

**INSTRUCTIONS AND PROCEDURE**
Refer to the **SAMPLING & HANDLING** and **PROTOCOL** sections for instruction about running the test on the acquired viral material and specimen.

**EXPECTED RESULTS**
≥ 90% of test results should be in agreement with the expected results. If test results are less than 90% in agreement with expected results, contact at inquiry@fosunpharmausa.com.

**QUESTIONS**
Please send questions or comments by email to inquiry@fosunpharmausa.com.

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