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

Product: 3DMed 2019-nCoV RT-qPCR Detection Kit
EUL Number: EUL-0488-184-00
Outcome: Accepted.

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

- Quality Management Systems Review and Plan for Post-Market Surveillance: desk-top review of the manufacturer's Quality Management System documentation and specific manufacturing documents;
- Product Dossier Review: assessment of the documentary evidence of safety and performance. This evaluation of limited scope is to verify critical analytical and performance characteristics.

3DMed 2019-nCoV RT-qPCR Detection Kit with product code 3103010011, Rest-of-World regulatory version manufactured by 3D Biomedicine Science & Technology, Block A, Building 2 , No.158 Xinjunhuan Rd., Shanghai 201114, was listed as eligible for WHO procurement on 23 November 2020.

Intended use:

According to the claim of intended use from 3D Biomedicine Science & Technology, *3DMed 2019-nCoV RT-qPCR Detection Kit is a real-time reverse transcription Polymerase Chain Reaction (RT-qPCR) intended for the manual, qualitative detection of N, E and ORF 1ab genes of SARS-CoV-2 RNA in oropharyngeal swab samples from patients with signs and symptoms suggestive of COVID-19 (e.g., fever and/or symptoms of acute respiratory illness).*

The test is intended as aid in the diagnosis of SARS-CoV-2 infection. Positive results are indicative of SARS-CoV-2 RNA detection, but may not represent the presence of transmissible virus. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The test is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures in a level 2 biosafety laboratory."

Assay description:

According to the description from 3D Biomedicine Science & Technology, "*The test consists of three processes in a single tube assay:*

- *Reverse transcription of target RNA and Internal Control RNA to cDNA*
- *PCR amplification of target and Internal Control cDNA*
- *Simultaneous detection of PCR amplicons by fluorescent dye labelled probes.*

The 3DMed 2019-nCoV RT-qPCR Detection Kit is a one-step real-time reverse transcription polymerase chain reaction (RT-PCR) test for qualitative detection of N, E and ORF1ab genes of SARS-CoV-2 RNA.
The 3DMed 2019-nCoV RT-qPCR Detection Kit includes all reagents needed for RT-PCR, 2 sets of primers and probes designed to detect the SARS-CoV-2 RNA in oropharyngeal specimens and one set of primers and probes designed to detect the RNA from virus-like particles (VLPs) of bacteriophage MS2. The MS2 RNA serves as an internal control for RNA extraction, reverse transcription and PCR amplification.
The viral RNA is isolated and purified from oropharyngeal specimens collected from individuals who meet case definition of suspected case of COVID-19 disease.
The 3DMed 2019-nCoV RT-qPCR Detection Kit is a one-step RT-qPCR test in a single tube that first reverse transcribes specific RNA templates into cDNA copies and then subsequently amplified by Applied Biosystems 7500 Real-Time PCR System. 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 Applied Biosystems 7500 Real-Time PCR System."

Specimen type(s) that were validated:

Oropharyngeal swab specimens.

Test kit contents:

Component	**100 tests (product code 3103010011)**
RT-PCR Reaction Mix Reagent	1 vial x 1800µL
Enzyme Mix Reagent	1 vial x 300µL
2019-nCoV Assay*	2 vials x 200µL
Negative Control	1 vial x 200 µL
Positive Control	1 vial x 200 µL
Process Control	1 vial x1600 µL
Internal Control	1 vial x100 µL

*Note: 2019-nCoV Assay consists primers and probes for detection of E, N, and ORF 1ab genes, as well as for the detection of MS2 RNA.

Items required but not provided:

Specimen collection, storage and transportation materials:

- Swabs with synthetic tip (nylon or Dacron) and an aluminum or plastic shaft, and sterile tubes containing 3 mL of viral media.
- Disposal virus Sampling Tube (Kang Jian, Catalogue 16611), or equivalent is preferred.

Extraction and purification platform and kits

- ANDiS Viral RNA Auto Extraction & Purification Kit Cat.3103010006 (16 Test); Cat.3103010007 (64 Test); Cat.3103010008 (128 Test)
- Automated Nucleic Acid Extraction System ANDiS 350 (Cat. 3105020003 for 240 voltage, 3105020002 for 110 voltage).
- Qiagen DSP Viral RNA Mini Kit (50) Catalog number: 61904.

Amplification and detection platforms

- 7500 Real-Time PCR Instrument (Applied Biosystems; catalog # 4351105) with 7500 Software version 2.3.1.

General laboratory equipment and consumables

- Microcentrifuge, capable of 16,000 × g (Eppendorf, Part no. 5415D; or equivalent)
- Vortex mixer
- Calibrated Single- and multi-channel pipettes
- Pipette tips with filters
- 100% ethanol, ACS reagent grade or equivalent
- Nuclease-Free Water
- 1.5 mL microcentrifuge tubes (DNase/RNase free)
- 2 mL microcentrifuge tubes (DNase/RNase free)
- 0.2 mL PCR reaction plates (Applied Biosystems; catalog # 4316813 or #4326659), or equivalent
- MicroAmp Optical 8-tube Strips (Applied Biosystems; catalog #4316567), or equivalent
- MicroAmp Optical 8-cap Strips (Applied Biosystems; catalog #4323032), or equivalent
- Biological safety cabinet approved for working with infectious materials.
- Powder-free gloves
- Cold block
- Appropriate personal protective equipment, such as, but not limited to powder- free gloves, laboratory coat and eye protection.

Storage:

The test kit should be stored at -15°C to -25°C.

Shelf-life upon manufacture:

6 months (13-month real-time stability study is ongoing and will be reported on August 31, 2021).

Warnings/limitations:

Please refer to the attached instructions for use.

Product dossier assessment

3D Biomedicine Science & Technology submitted a product dossier for 3DMed 2019-nCoV RT-qPCR Detection Kit as per the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid (PQDx_0347)"*. The information (data and documentation) submitted in the product dossier was reviewed by WHO staff and external assessor appointed by WHO.

Post listing Commitments for EUL:

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

1. Review the limit of detection with the WHO international standard by 23 May 2021.
2. Provide interim stability study report on 28 February 2021 and the final report by 31 August 2021.

Risk benefit assessment conclusion: acceptable.

Quality Management Systems Review

To establish the eligibility for WHO procurement, 3D Biomedicine Science & Technology was asked to provide up-to-date information about the status of their quality management system.

Based on the review of the submitted quality management system documentation by WHO staff and external technical experts (assessors), it was established that sufficient information was provided by 3D Biomedicine Science & Technology to fulfil the requirements described in the "*Instructions for Submission Requirements: In vitro diagnostics (IVDs) Detecting SARS-CoV-2 Nucleic Acid, PQDx_347"*.

Quality management documentation assessment conclusion: acceptable.

Plan for Post-Market Surveillance

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

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

3D Biomedicine Science & Technology is also required to submit an annual report that details sales data and all categories of complaints in a summarized form. There are certain categories of complaints and changes to the product that must be notified immediately to WHO, as per the above-mentioned documents.

The manufacturer has committed to ensure that post-emergency use listing safety, quality and performance monitoring activities are in place which are in accordance with WHO guidance "*Guidance for post-market surveillance and market surveillance of medical devices, including in vitro diagnostics*".[1]

Scope and duration of procurement eligibility

The 3DMed 2019-nCoV RT-qPCR Detection Kit with product code 3103010011, manufactured by 3D Biomedicine Science & Technology is considered to be eligible for WHO procurement for 12 months from the day of listing. The assay may be used for the detection of the 2019 novel coronavirus (SARS-CoV-2) RNA. This listing does not infer that the product meets WHO prequalification requirements and does not mean that the product is listed as WHO prequalified.

As part of the on-going requirements for emergency use listing as eligible for WHO procurement, 3D Biomedicine Science & Technology must engage in post-market surveillance activities to ensure that the product continues to meet safety, quality and performance requirements. 3D Biomedicine Science & Technology is required to notify WHO of any complaints, including adverse events related to the use of the product within 7 days, and any changes made to the product.

[1] Available on the web page

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

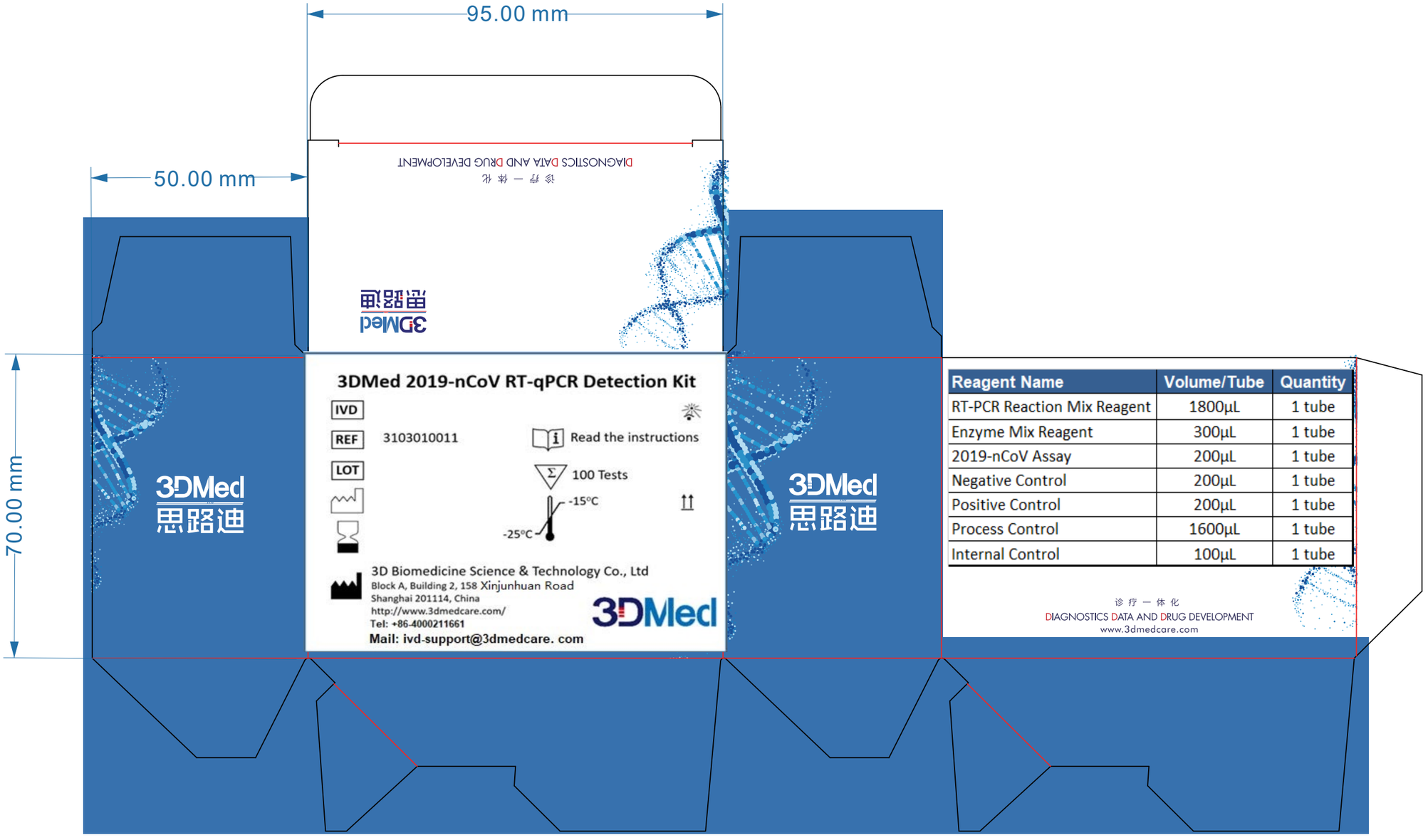
WHO reserves the right to rescind eligibility for WHO procurement, if additional information on the safety, quality, performance during post-market surveillance activities, and if new data becomes available to WHO that changes the risk benefit balance.

Labelling

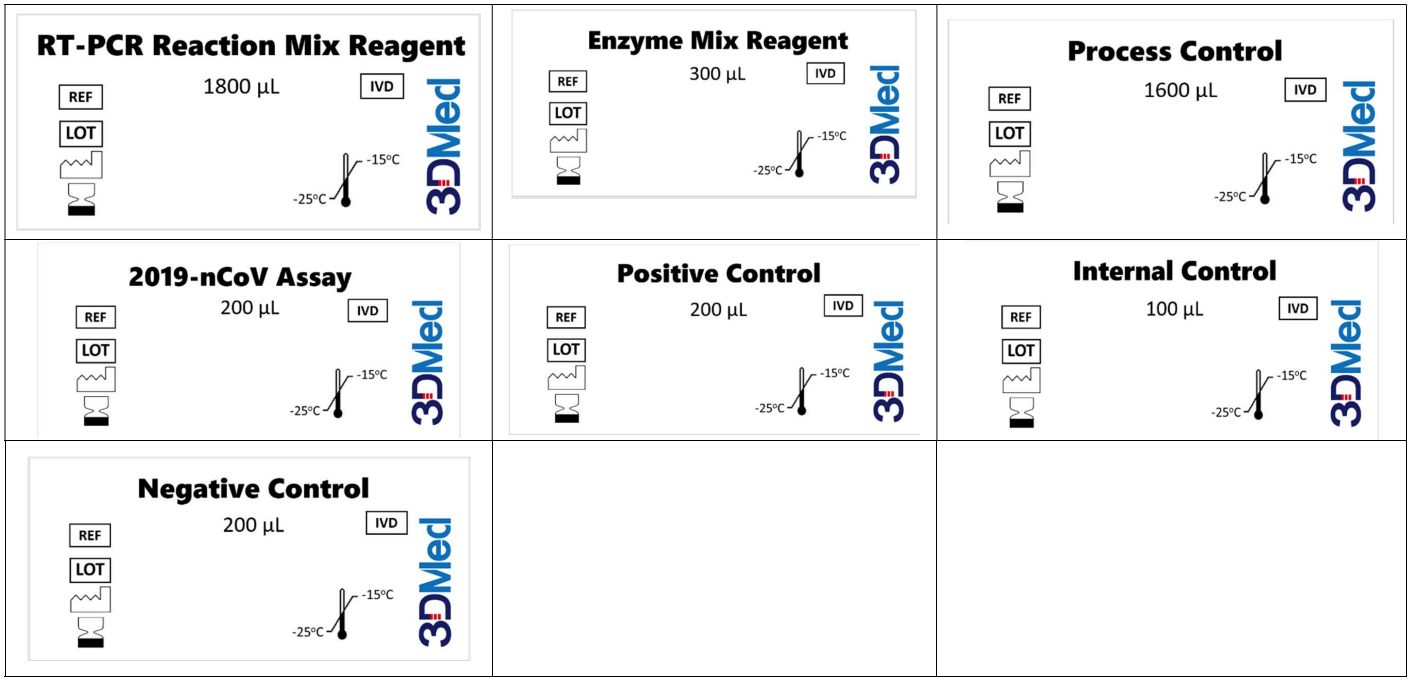
1. Labels

Label Layout of 3DMed 2019-nCoV RT-qPCR Detection Kit

1. Package Label Layout



2. Tube Label Layout



2. Instructions for Use[2]

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

3DMed 2019-nCoV RT-qPCR detection Kit

For Emergency Use Authorization Use Only

Instructions for Use
Version 01

Catalog # 3103010011

100 reactions

For *In-vitro* Diagnostic (IVD) Use

For *Prescription* Use only

3D Biomedicine Science & Technology Co., Ltd.
Block A, Building 2, No. 158 Xinjunhuan Rd.
Shanghai 201114, P.R. China
(86)-21-3469-6522

3DMed 2019-nCoV RT-qPCR detection Kit

For Emergency Use Authorization Use Only

For use with

Automated Nucleic Acid Extraction System ANDiS 350 with ANDiS Viral RNA Auto Extraction & Purification Kit.

QIAamp DSP Viral RNA Mini Kit

ABI 7500 Real-Time PCR System

IVD For in vitro diagnostic use

REF Catalog number: 3103010011

100 Test

-25°C to -15°C

Read the instructions

3D Biomedicine Science & Technology Co., Ltd. Block A, Building 2, No.158 Xinjunhuan Rd. Shanghai, 201114, China

Telephone: +86-4000211661

E-mail: ivd-support@3dmedcare.com

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Intended Use

3DMed 2019-nCoV RT-qPCR Detection Kit is a real-time reverse transcription Polymerase Chain Reaction (RT-qPCR) intended for the manual, qualitative detection of N, E and ORF 1ab genes of SARS-CoV-2 RNA in oropharyngeal swab samples from patients with signs and symptoms suggestive of COVID-19 (e.g., fever and/or symptoms of acute respiratory illness).

The test is intended as aid in the diagnosis of SARS-CoV-2 infection. Positive results are indicative of SARS-CoV-2 RNA detection, but may not represent the presence of transmissible virus. Negative results do not preclude SARS-CoV-2 infection and should not be used as the sole basis for patient management decisions. Negative results must be combined with clinical observations, patient history, and epidemiological information.

The test is intended for use by trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures in a level 2 biosafety laboratory.

Summary and Explanation

The 3DMed 2019-nCoV RT-qPCR Detection Kit is a molecular test that aids in diagnosis COVID-2019 and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers and fluorescent dye labeled probes and control material used in RT-qPCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in oropharyngeal specimens.

The qualified laboratories in which all users, analysts, and any person reporting results from use of this device should be trained to perform and interpret the results from this procedure by a competent instructor prior to use. 3D Medicine will limit the distribution of this device to laboratories whose users have successfully completed a training course provided by 3D Medicine instructors or designees.

Test Principle

The test consists of three processes in a single tube assay:

- Reverse transcription of target RNA and Internal Control RNA to cDNA
- PCR amplification of target and Internal Control cDNA
- Simultaneous detection of PCR amplicons by fluorescent dye labelled probes

The 3DMed 2019-nCov RT-qPCR Detection Kit is a one-step real-time reverse transcription polymerase chain reaction (RT-PCR) test for qualitative detection of N, E and ORF1ab genes of SARS-CoV-2 RNA.

The 3DMed 2019-nCoV RT-qPCR Detection Kit includes all reagents needed for RT-PCR, 2 sets of primers and probes designed to detect the SARS-CoV-2 RNA in oropharyngeal specimens and one set of primers and probes designed to detect the RNA from virus-like particles (VLPs) of bacteriophage MS2. The MS2 RNA serves as an internal control for RNA extraction, reverse transcription and PCR amplification.

The viral RNA is isolated and purified from oropharyngeal specimens collected from individuals who meet case definition of suspected case of COVID-19 disease.

The 3DMed 2019-nCoV RT-qPCR Detection Kit is a one-step RT-qPCR test in a single tube that first reverse transcribes specific RNA templates into cDNA copies and then subsequently amplified by Applied Biosystems 7500 Real-Time PCR System. 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 Applied Biosystems 7500 Real-Time PCR System.

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

Product Description

One box of 3DMed 2019-nCoV RT-qPCR Detection Kit contains the reagents and controls summarized in the **Table 1** and should be stored at -20°C.

Table 1: Components of 3DMed 2019-nCov RT-qPCR Detection Kit (Material Provided)

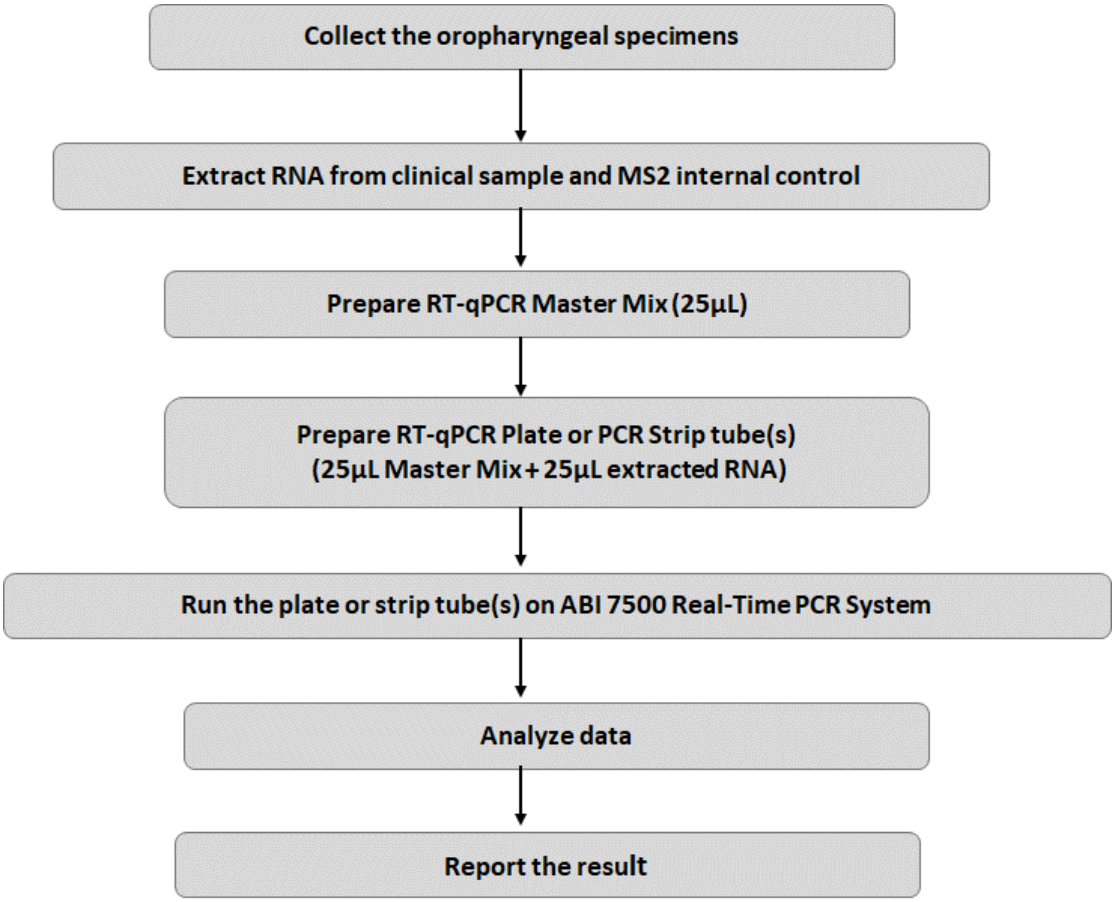
Reagent Name	Kit Size of 100 Reactions	
	Volume per tube	Quantity
RT-PCR Reaction Mix Reagent	1800µL	1 tube
Enzyme Mix Reagent	300µL	1 tube
2019-nCoV Assay*	200µL	1 tube
Negative Control	200µL	1 tube
Positive Control	200µL	1 tube
Process Control	1600µL	1 tube
Internal Control	100µL	1 tube

*Note: 2019-nCoV Assay consists primers and probes for detection of E, N, and ORF 1ab genes, as well as for the detection of MS2 RNA.

2019-nCoV assay contains three (3) sets of primers and probes. One set of primers and probe target specific regions on N gene and E gene in SARS-CoV-2 genome and the probes are labeled with fluorophore FAM, a second set of primers and probe targets specific region on ORF 1ab in SARS-CoV-2 genome and the probe is labeled with fluorophore ROX, and the third set of primers and probe targets specific nucleic acid sequence in virus-like particles of bacteriophage MS2 is labeled with the fluorophore VIC. Using probes linked to distinguishable dyes enables the parallel detection of SARS-CoV-2 specific RNA and the RNA of Internal Control particle in the corresponding detector channels of the real-time PCR System.

The workflow of the test is summarized in Figure 1.

Figure 1: Summary of test Workflow:



Material and Equipment Required (Not Provided)

- Specimen collection, storage and transportation materials:
  - Swabs with synthetic tip (nylon or Dacron) and an aluminum or plastic shaft, and sterile tubes containing 3 mL of viral media.
  - Disposal virus Sampling Tube (Kang Jian, Catalogue 16611), or equivalent is preferred.
- Real-Time RT-PCR Instrument:
  - 7500 Real-Time PCR Instrument (Applied Biosystems; catalog # 4351105) with 7500 Software version 2.3.1
- ANDiS Viral RNA Auto Extraction & Purification Kit Cat.3103010006 (16 Test); Cat.3103010007 (64 Test); Cat.3103010008 (128 Test)
- Automated Nucleic Acid Extraction System ANDiS 350 (Cat. 3105020003 for 240 voltage, 3105020002 for 110 voltage)
- Microcentrifuge, capable of 16,000 × *g* (Eppendorf, Part no. 5415D; or equivalent)
- Vortex mixer
- Calibrated Single- and multi-channel pipettes
- Pipette tips with filters
- 100% ethanol, ACS reagent grade or equivalent
- Qiagen DSP Viral RNA Mini Kit (50) Catalog number: 61904.
- Nuclease-Free Water
- 1.5 mL microcentrifuge tubes (DNase/RNase free)
- 2 mL microcentrifuge tubes (DNase/RNase free)
- 0.2 mL PCR reaction plates (Applied Biosystems; catalog # 4316813 or #4326659), or equivalent
- MicroAmp Optical 8-tube Strips (Applied Biosystems; catalog #4316567), or equivalent
- MicroAmp Optical 8-cap Strips (Applied Biosystems; catalog #4323032), or equivalent
- Biological safety cabinet approved for working with infectious materials.
- Powder-free gloves
- Cold block
- Appropriate personal protective equipment, such as, but not limited to powder- free gloves, laboratory coat and eye protection.

Warnings and Precautions

- This test is for in vitro diagnostic use only.
- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Equilibrate all the reagents except Enzyme Mix Reagent to room temperature (15^{o}C to 25^{o}C) before commencing use of IVD Product. The Enzyme Reagent Mix should be thaw on ice or cold box.
- 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 2019-nCoV https://www.cdc.gov/coronavirus/2019-nCoV/lab-biosafety-guidelines.html.
- Specimen processing should be performed in accordance with national biological safety regulation
- Perform all manipulations of live virus samples within a Class II (or higher) biological safety cabinet (BSC).
- 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.
- Performance characteristics have been determined with oropharyngeal specimens. Use of other specimen types have not been validated with this product.
- Use of this product is limited to personnel specially instructed and trained in the techniques of real-time PCR and in vitro diagnostic procedures
- Use separate and segregated working area for (1) specimen preparation, (2) reaction set-up and (3) amplification/detection activities. Workflow in the laboratory should proceed in unidirectional manner. Always wear disposable powder-free gloves in each area and change them before entering different areas
- Always check the expiration date prior to use. Do not use expired reagents.
- Avoid exchanging components from different lots or reagent kits or pooling reagents.
- Change aerosol barrier pipette tips between all manual liquid transfers.
- During preparation of samples, compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples, 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, microcentrifuge) 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 samples and whenever contamination is suspected
- Keep reagent and reaction tubes capped or covered as much as possible
- Enzyme Mix Reagent is heat sensitive and must be thawed and maintained on cold block at all times during preparation and use.
- Repeat freeze/thaw should not be more than 9 times to prevent reagent degradation.

- Work surfaces, pipettes, and centrifuges should be cleaned and decontaminated with cleaning product such as 10% bleach, “DNAZap” to minimize risk of nucleic acid contamination. Residual bleach should be removed using 70% ethanol.
- 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.
- The 2019-nCoV Assay, which contains fluorogenic probes, is light sensitive. Please avoid excessive exposure to light.
- If the outside package is damaged, but the vials remain intact upon reception, the kit can still be used without compromising performance. If the package and vials are both damaged, the kit must not be used.
- If the test result of any positive control, negative control and internal control fail to meet the predefined specification summarized in the interpretation of results and Reporting section, the test must be invalid.

Reagent Storage and Handling

- 3DMed 2019-nCoV RT-qPCR Detection Kit shall be stored at -15^{o}C to -25^{o}C.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect 2019-nCoV Assay, which contains fluorogenic probes, from light.
- Enzyme Mix Reagent must be thawed and kept on a cold block at all times during preparation and use.

Specimens 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.

- Collecting the Specimen
  - Refer to Interim Guidelines for Collecting, Handling, and Testing Clinical Specimens from Patients Under Investigation (PUIs) for 2019 Novel Coronavirus (2019-nCoV)

    https://www.cdc.gov/coronavirus/2019-nCoV/guidelines-clinical-specimens.html

  - Follow specimen collection devices manufacturer instructions for proper collection methods.
  - 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 3 ml of viral media.
- Transporting Specimens
  - Specimens must be packaged, shipped, and transported according to the current edition of the International Air Transport Association (IATA) Dangerous Goods Regulation. Follow shipping regulations for UN 3373 Biological Substance, Category B when sending potential SARS-CoV-2 specimens.
  - Store specimens at 2-8°C and ship overnight to testing facility on ice pack. If a specimen is frozen at -70°C or lower, ship overnight to testing facility on dry ice
- Storing Specimens

  - Store the specimens at -20^{o}C and ship overnight to test facility on dry ice.
  - Specimens could be stored at -20^{o}C up to 7 days after collection.
  - If a RNA extraction could not be performed with 48 hours, the specimens should be stored at -90^{o}C to -70^{o}C.
  - Extracted nucleic acid should be stored at -90^{o}C to -70^{o}C for up to 4 months.

1. RNA extraction:

1.1. RNA extraction with Automated Nucleic Acid Extraction System ANDiS 350 with ANDiS Viral RNA Auto Extraction & Purification Kit

RNA extraction is performed with ANDiS Viral RNA Auto Extraction & Purification Kit (Cat. 3103010006) on Automated Nucleic Acid Extraction System ANDiS 350 (Cat.3105020002/3105020003).

ANDiS Viral RNA Auto Extraction & Purification Kit contains the following components

- One (1) 96-well deep plate contains lysis buffer, magnetic beads, wash buffers and elution buffer
- One tube of Proteinase K
- Two magnetic rod 8-cover strips

1.1.1. Remove Internal Control tube from 3DMed 2019-nCoV RT-qPCR Detection Kit, and thaw at room temperature (15°C to 25°C).
1.1.2. Equilibrate the clinical specimen tube and Process Control sample to room temperature (15°C to 25°C).
1.1.3. Mix the samples by vortexing for 5 seconds and spin briefly.
1.1.4. Equilibrate 96-well deep plate containing all the reagents required for RNA extraction and Proteinase K to room temperature.
1.1.5. Mix the Internal Control and Proteinase K by vortexing for 5 seconds, and spin briefly to collect the content to bottom of the tube.
1.1.6. Label a 1.5 mL DNase/RNase free tube as “IC Mix”
1.1.7. Mix the internal control and Proteinase K by following the formula described in **Table 3** below:

Table 3: Formula of IC Mix

Reagent Name	Volume in µL per Reaction	Volume in µL per N Reactions
Internal Control	1	1 x (N+1)
Proteinase K	20	20 x (N+1)
Total Volume	21	21 x (N+1)

1.1.8. Mix well by vortexing for 5 seconds, spin briefly to collect the content to bottom of the tube.
1.1.9. Invert the 96-well deep plate 5 times and centrifuge the plate at 2000 rpm briefly.
1.1.10. Unsealed the 96-well deep plate carefully.
1.1.11. Add 21µL of IC Mix and 200µL of each clinical sample or 200µL of a Process Control in the well in A1 to H1 and A7 to H7 columns which containing Lysis Buffer.
1.1.12. Turn on Automated Nucleic Acid Extraction System ANDiS 350
1.1.13. Ensure the instrument is in idle, and then open the instrument door.

1.1.14. Load the 96-well deep plate by placing the plate on the heating stand with A1 position in upper left corner.
1.1.15. Load the 8-cover strip to a magnetic rod cover holder and ensure the 8-cover strip fit in the holder firmly.
1.1.16. Close the instrument door
1.1.17. In a display, click on “Program Management”，select “create new program”，enter the new program name as “SARS-CoV-2 RNA extraction”，Click “Enter” to create a new program with the parameters described in **Table 4**.

Table 4: RNA extraction Parameters

Step	Well Position	Action	Mixing Time (min)	Bead Collection time (Sec)	Holding time (Min)	Volume in µL	Mixing Speed (1 to 3)	Temperature
1	3	Transfer beads	1	20	0	900	3	15°C to 25°C
2	1	Lysis	20	20	0	900	3	
3	2	Wash 1	2	20	0	900	3	
4	3	Wash 2	2	20	0	900	3	
5	6	Elution	6	20	2	100	1	60°C
6	3	Discard Beads	1	0	0	900	3	15°C to 25°C

1.1.18. If the “SARS-CoV-2 RNA extraction” program is existed, click on the program icon to open the program parameter. Ensure the program match the parameters described in **Table 4**
1.1.19. Start the instrument.
1.1.20. After the program completed, transfer approximately 100µL extracted RNA to a clean 1.5mL DNase/RNase free tube labeled with sample ID.
1.1.21. Store the extracted RNA at -90°C to -70°C.
1.1.22. Discard the used 96-well deep plate properly.

1.2. RNA extraction with Qiagen QIAamp DSP Viral RNA Mini Kit (50) Cat. 61904

1.2.1. Recommendation (s): Utilize 200µL of clinical specimens or a Process Control sample to 800µL of Buffer AVL containing carrier RNA and Internal Control, and elute with 100µL of Buffer AVE.
1.2.2. Follow the instructions described in the manual of QIAamp DSP Viral RNA Mini Kit (50) except as noted in the recommendations above for RNA extraction. A Process Control should be included in each batch from RNA extraction to RT-qPCR (from start to the end of testing process).

2. RT-PCR

2.1. Equilibrate all the reagents and controls except Enzyme Mix Reagent to room temperature (15°C to 25°C)
2.2. Thaw the Enzyme Mix Reagent in biocooler or on ice

2.3. Mix all the reagents and Controls except Enzyme Mix Reagent by vortex for 10 seconds, centrifuge briefly to make homogenous mixture.
2.4. Mix the Enzyme Mix Reagent by flick 5 times and centrifuge briefly to make homogenous mixture.
2.5. For the extracted RNA containing Internal Control after RNA extraction, preparation of RT-qPCR Master Mix according to the formula described in the **Table 5** below

Table 5: Formula of RT-qPCR Master Mix for RNA containing Internal Control

Reagent Name	Volume in µL per Reaction	Volume in µL per N Reactions
RT-PCR Reaction Mix Reagent	18	18 x (N+1)
Enzyme Mix Reagent	3	3 x (N+1)
2019-nCoV assay*	2	2 x (N+1)
Nuclease-free water	2	2 x (N+1)
Total Volume	25	25 x (N+1)

*Note: 2019-nCoV Assay consists primers and probes for detection of E, N, and ORF 1ab genes, as well as for the detection of MS2 RNA.

2.6. For the positive and negative controls, preparation of RT-qPCR Master Mix according to the formula is described in the **Table 6** below

Table 6: Formula of RT-qPCR Master Mix for Positive and Negative Controls

Reagent Name	Volume in µL per Reaction	Volume in µL per N Reactions
RT-PCR Reaction Mix Reagent	18	18 x (N+1)
Enzyme Mix Reagent	3	3 x (N+1)
2019-nCoV assay*	2	2 x (N+1)
Internal Control	0.5	0.5 x (N+1)
Nuclease-free water	1.5	1.5 x (N+1)
Total Volume	25	25 x (N+1)

*Note: 2019-nCoV Assay consists primers and probes for detection of E, N, and ORF 1ab genes, as well as for the detection of MS2 RNA.

2.7. Add 25µL of RT-PCR Master Mix into each required well of an appropriate optical 96-well reaction plate or optical -8 tube strip.
2.8. Add 25µL of extracted RNA sample or 25µL of the Control (Positive and Negative Control) into each well or tube containing 25µL of RT-PCR Master Mix.
2.9. Mix the extracted RNA sample and control with RT-PCR Master Mix thoroughly by pipette up and down 10 times.

2.10. Seal the optical 96-well reaction plate with optical adhesive file or cap the optical 8-tube strip with optical cap.
2.11. Spin the optical 96-well reaction plate or optical 8-tube strip briefly to make a homogenous mixture.
2.12. Ensure one Positive Control and one Negative Control are used in each run.

3. Programming 7500 Real-Time PCR System with 7500 Software version 2.3.1:

3.1. Define the general setting:

Settings	
Reaction volume per well (tube)	50 µL
Ramp Rate	default
Passive Reference	None

***Note**: "None" should be selected in the "Select the dye to use for passive reference" since the default is "ROX".*

3.2. Define the Fluorescent Detectors (Dye)

Table 7: Define the target with fluorescent dye

Detection	Reporter Dye	Quencher
SARS-CoV-2 specific RNA (E gene)	FAM	None
SARS-CoV-2 specific RNA (N gene)		
SARS-CoV-2 specific RNA (ORF1ab)	ROX	None
Internal Control	VIC	None

3.3. Set up RT-PCR Thermal Cycle profile:

Table 8: Define the Thermal Cycling Parameter

Stage	Temperature	Time	Cycle number
RT	50°C	10 minutes	1
Hold	95°C	2 minutes	1
PCR	95°C	5 seconds	45
	60°C	35 seconds	

Note: Collect fluorescent signal at 60°C step.

4. Data Analysis:

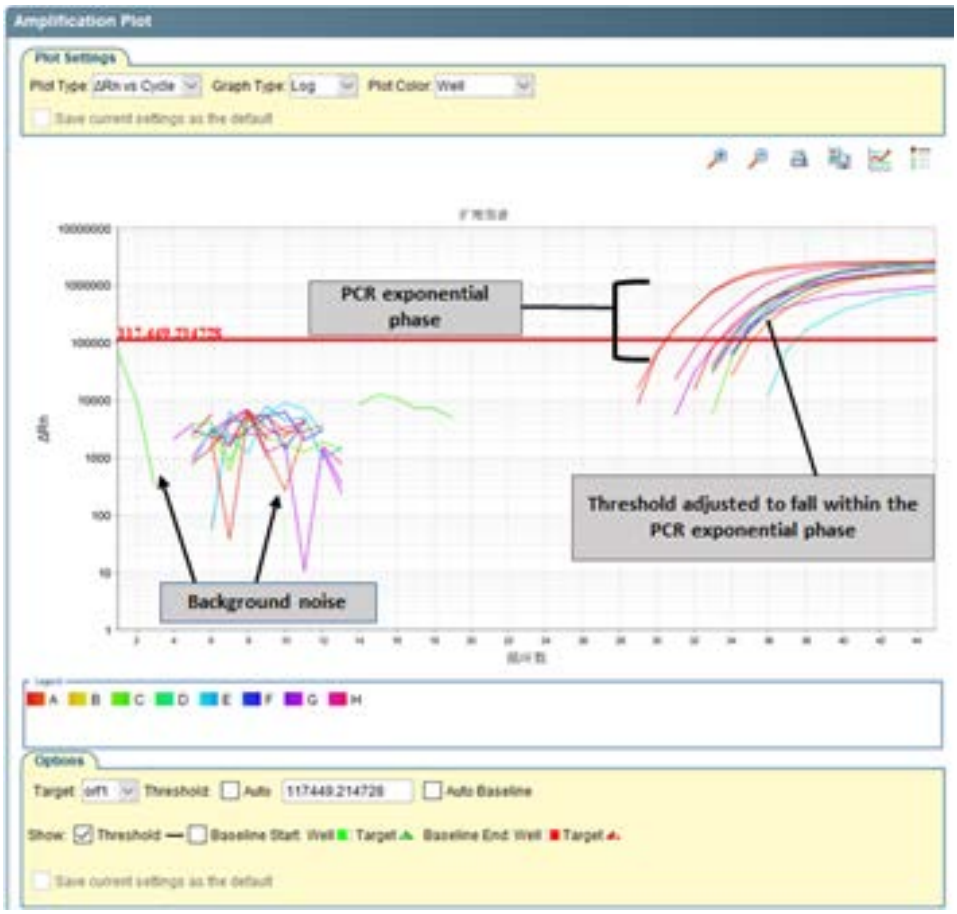
4.1. Analyze the data using the following Ct settings in 7500 Software version 2.3.1 for 7500 RT-PCR System.

4.1.1. Unselect the box for “Use Default Settings”

4.1.2. For baseline setting: Use “Baseline Start Cycle 5 End Cycle 22”

4.1.3. For Threshold setting: In the “Amplification Plot” display window, manually drag the threshold line until it lies within the exponential phase of the fluorescence curve and above any background signal.

Figure 2: Define the target with fluorescent dye



4.2. Determine the cycle threshold (Ct) values and standard deviation (if applicable) for each assay. Export the run data as an excel file which contains Ct value.

4.3. Assess the test results of the clinical specimens after positive, negative and internal controls have been examined and determined to be valid.

4.4. Interpret the positive and negative results by comparing the Ct value from each fluorescent channel to its respective expected Ct value.

4.5. Interpret the results according to the criteria listed in **Table 9**.

Interpretation of Results and Reporting

Table 9: Expected performance of Controls Included in 3DMed 2019-nCoV RT-qPCR Detection Kit

		2019-nCoV		Internal Control	
Control Type	**Name of the Reagent**	**FAM**	**ROX**	**VIC**	**Expected Ct**
Positive	Positive Control	POS	POS	POS	FAM Ct < 39.50 ROX Ct < 39.50 VIC Ct < 35.00
Negative	Negative Control	Neg	Neg	POS	FAM Ct ≥ 39.50 ROX Ct ≥ 39.50 VIC Ct < 35.00
Process	Process Control	POS	POS	POS	FAM Ct < 35.00 ROX Ct < 35.00 VIC Ct < 35.00

Note: *POS means "Positive", and Neg means "Negative"*

If any of the above controls do not exhibit the expected performance as described in **Table 9**, the test is invalid. The test shall be repeated.

- **Internal Control (Extraction Control)**
  - All clinical samples should exhibit VIC Threshold Cycle (Ct) less than 35.00 (< 35.00) which indicated the present of Internal Control.
  - Failure to detected Internal Control (VIC Ct value is great and equal to 35.00) in any clinical samples may indicate:
    - Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.
    - Improper test set up and execution
    - Reagent or equipment malfunction.
  - If Internal Control signal is negative for the specimen, the result should be considered invalid for the specimen. If the residual specimen is available, repeat the extraction procedure and RT-PCR test. If Internal Control signal remains negative after re-test, report the results as invalid and a new specimen should be collected if possible.

- **For SARS-CoV-2 gene specific markers**
  - When all controls exhibit the expected performance, a specimen is considered as negative if all the SARS-CoV-2 specific markers (FAM and ROX) have Ct value greater than its respective Ct cutoff value.
  - When all controls exhibit the expected performance, a specimen is considered as positive for SARS-CoV-2 if the SARS-CoV-2 specific markers (FAM and ROX) and Internal Control (VIC) have Ct value less than its respective Ct cutoff value.
  - When all controls exhibit the expected performance, a specimen is considered as inconclusive if one of SARS-CoV-2 gene specific marker and Internal Control have Ct value less than its respective Ct cutoff value
  - 3DMed 2019-nCoV RT-qPCR Detection Kit Interpretation Guide

If the results are obtained that do not follow these guidelines, re-extract and re-test the specimen from original specimen stock tube. If the original specimen stock tube is no longer available, or if the results still do no follow the guidelines upon retesting of the original specimen, extract and test a recollected specimen from the same patient. If the repeat testing yields similar results, contact 3D Medicine for consultation.

The interpretation guide is summarized in Table 10 below:

Table 10: Interpretation Guidance:

Sample ID	3DMed 2019-nCoV RT-qPCR Detection Kit Interpretation Guide					
	FAM Detection Channel	ROX Detection Channel	VIC Detection Channel (Internal Control)	Result Interpretation	Report	Action
A	Positive	Positive	Positive	SARS-CoV-2 detected	Positive for SARS-CoV-2	Report the results to the appropriate health authorities and sender. .
B	If only one of the two targets is positive		Positive	Inconclusive	Inconclusive	The specimen needs to be retested from RNA extraction. If the test result remains inconclusive, collect a new specimen.
C	Negative	Negative	Positive	SARS-CoV-2 not detected	The sample does not contain detectable amount SARS-CoV-2 specific RNA	Report results to sender. Consider testing for other respiratory viruses
D	Negative	Negative	Negative	Invalid	Repeat test	The specimen needs to be retested from RNA extraction. If the result remains invalid after retesting, collect a new specimen.

Note: *Positive: FAM Ct < 39.50 and ROX Ct < 39.50,*
Negative: FAM Ct ≥ 39.50 and ROX Ct ≥ 39.50.
Inconclusive: 1) FAM Ct < 39.50 and ROX Ct ≥ 39.50; or
2) FAM Ct ≥ 39.50 and ROX Ct < 39.50

Limitations

- All user, 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 test independently.
- 3D Biomedicine Science and Technology Co will limit the distribution of this Kit to only those users who have proficient by 3D Biomedicine Science and Technology instructors or designees.
- Performance of 3D 2019-nCoV RT-qPCR Detection Kit has only been established in the specimens collected with oropharyngeal swabs.
- Negative results (SARS-CoV-2 not detected) do not preclude infection of SARS-CoV-2 and should not be used as the sole basis for treatment or other patient management decision. Optimum specimen type and timing for peak viral levels during infections caused by SARS-CoV-2 have not been determined. Collection of multiple specimens (types or time point of infection) from the same patient may be necessary to detect the virus.
- A false negative result (SARS-CoV-2 not detected) may occur if a specimen is improperly collected, transported or handled. False negative results may also occur if amplification inhibitors are in the specimen or if inadequate numbers of organisms are present in the specimen.
- A false positive (SARS-CoV-2 detected) result may be observed if cross contamination occurred during the specimen handling or preparation.
- Based on the design of the primers and probes, testing of specimens containing SARS-CoV-2 sequences corresponding to EPI_ISL-413752 or EPI-ISL-414015 sequences, or of specimens containing SARS CoV-2 strains with mutations in the N gene assay target region, may result in 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 understood.
- Detection of SARS-CoV-2 RNA does not rule out other causative agents for the 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 present of SARS-CoV-2.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.
- Based on the information described in Interpretation Guide, the result for a sample with single channel positive will be reported as inconclusive, therefore, the retest rate will be approximately 15%. After retest, all the samples will be reported a test result as positive or negative according the instruction described in the Interpretation Guide for inconclusive result.

Performance Characteristics

Analytical Performance

- ***Limit of Detection (LoD)***

LoD study determine the lowest detectable concentration of SARS-CoV-2 at which approximated 95% of all (expected positive) replicates test positive. The LoD was determined by limited dilution study with contrived positive samples.

The analytical sensitivity of the real-time RT-PCR assays containing in the 3DMed 2019-nCoV Detection Kit were determined in Limit of Detection study. Since no qualified virus isolation of SARS-CoV-2 are currently available, assays designed for detection of SARS-CoV-2 RNA were tested with a contrived positive sample built with RNA extracted from a positive clinical specimen spike into clinical matrix.

A. The preliminary LoD was conducted with two RNA extraction methods with a serial dilution of a contrived positive sample built with RNA extracted from a positive clinical specimen spike into clinical matrix. The RNA extraction with worst LoD was used for LoD confirmation study, and the worst LoD value was used as tentative LoD in confirmation study.

B. A confirmation of LoD study was conducted with three contrived positive samples and each contrived positive sample was diluted to 2X LoD, 1.5X LoD, 1X LoD and 0.5X LoD, and twenty replicates were tested for each dilution level per sample. The LoD was determined as the lowest concentration where ≥ 95% (19/20) of the replicates were positive. The results of LoD confirmation study using three contrived positive specimens are summarized in Table 11 to Table 13.

Table 11: LoD Confirmation study result for contrive positive sample 77

Viral RNA titer (copy/uL)	Replicates	Positive/total tested	% of detection
2X LoD	20	20/20	100
1.5X LoD	20	20/20	100
1X LoD	20	20/20	100
0.5X LoD	20	18/20	90

Table 12: LoD Confirmation study result for contrive positive sample 82

Viral RNA titer (copy/uL)	Replicates	Positive/total tested	% of detection
2X LoD	20	20/20	100
1.5X LoD	20	20/20	100
1X LoD	20	19/20	95
0.5X LoD	20	14/20	70

Table 13: LoD Confirmation study result for contrive positive sample 532

Viral RNA titer (copy/uL)	Replicates	Positive/total tested	% of detection
2X LoD	20	20/20	100
1.5X LoD	20	20/20	100
1X LoD	20	19/20	95
0.5X LoD	20	5/20	25

Table 14: Limit of Detection of 3DMed 2019-nCoV RT-qPCR Detection Kit:

RNA Extraction Method	Limit of Detection (LoD)
3DMed RNA Extraction Method	5.00 copies per PCR reaction
Qiagen RNA Extraction Method	

- ***Inclusivity (analytical sensitivity)***

  - Sequence alignment was performed with the oligonucleotide primer and probe sequences of the 3DMed 2019-nCoV RT-qPCR Detection Kit with all publicly available nucleic acid sequences for 2019-nCoV in GenBank as of February 20, 2020 to demonstrate the predicted inclusivity of the 3DMed 2019-nCoV RT-qPCR Detection Kit. All the alignments show 100% identity of the 2019-nCoV Assay to the available 2019-nCoV sequences. The alignment of the 2019-nCoV Assay includes additional sequences for SARS, MERS, and other Bat coronaviruses to show that other than SARS viruses, the alignment shows low identities and would not predict significant reactivity.

- The inclusivity study was conducted *in silico* by mapping the assays to all analyzed SARS-CoV-2 sequences in NCBI and GISAID database as March 15, 2020. The mapping results concluded as following and the data is available per request.
  - Primer and probe sequences for 2019-nCoV ORF 1ab assay had 100% homology to all analyzed SARS-CoV-2 sequences.
  - Primer and probe sequences for 2019-nCoV E gene assay had 100% homology to all analyzed SARS-CoV-2 sequences, with four exceptions such as EPI_ISL_408487 (hCoV-19/He0n/IVDC-HeN-002/2020) and EPI_ISL_408486 (hCoV-19/France/RA739/2020) showed no alignment with primer probe in 2019-nCoV E gene assay, The potential root cause may be the quality or the lengths of the reference sequences in the database. In addition, EPI_ISL-413752 (hCoV-19/Chi0/WF0023/202) showed 4 mismatched in the probe and no alignment with E gene reverse primer, and EPI_ISL_414015 (hCoV-19/Brazil/SPBR-06/2020) showed 61% homology with forward primer of E gene assay.
  - LIMITATIONS:
    - These mismatch indicated that a potential false negative result will be reported for a specimen containing the sequence as EPI_ISL-413752 or EPI-ISL-414015.
    - The mapping results for primer and probe in N gene assay showed less than 90% homology with multiple strains of SARS-CoV-2 sequence, therefore, a potential false negative results will be reported.

- ***Cross-reactivity (Analytical Specificity)***

A. *In silico* analysis for primers and probes

a) BLAST analysis queries of the 2019-nCoV RT-qPCR assay primers and probes were performed against publicly available nucleotide sequences. The database search parameters were as follows: 1) The entire nucleotide collection consists of GenBank+EMBL+DDBJ+PDB+RefSeq sequences; 2) The search parameters automatically adjust for short input sequences and the expected threshold was 1000; 3) The match and mismatch scores were 1 and -3, respectively; 4) The penalty to create and extend a gap in an alignment was 5 and 2 respectively.

b) 2019-nCoV_ORF1AB Assay

The probe sequence of 2019-nCoV ORF1AB assay showed high sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. However, both forward and reverse primers showed no sequence homology with SARS coronavirus and Bat SARS-like coronavirus genome. Combining primers and probe, there was no significant homologies with human genome, other coronaviruses or human microflora that would predict potential false positive RT-qPCR results.

c) 2019-nCoV_E Assay:
The forward primer, reverse primer and probe sequences of 2019-nCoV_E assay showed high sequence homology to Bat SARS- like coronaviruses. However, these primer and probe sequences showed no significant homology with human genome, other coronaviruses or human microflora. Combining primers and probe, there was a prediction of potential false positive RT-qPCR results in the presence of human SARS coronavirus and bat SARS coronavirus in the samples

d) 2019-nCoV_N Assay:
Analysis of the forward and reverse primer and probe sequences of 2019-nCoV N assay showed significant homology only to human SARS coronavirus and bat SARS coronavirus. No significant homology with human genome, other coronaviruses or human microflora was observed. We predict potential false positive RT-qPCR results in the presence of human SARS coronavirus and bat SARS coronavirus in samples.

e) In summary, the 2019-nCoV ORF1AB assay, designed for the specific detection of 2019-nCoV, showed no significant combined homologies with human genome, other coronaviruses, or human microflora that would predict potential false positive RT-qPCR results. The 2019-nCoV E and N assays were designed for universal detection of 2019-nCoV, human SARS coronavirus and bat SARS coronavirus.

B. *In silico* analysis for microorganisms:
a) An *in silico* analysis for all the available strains of organisms listed in the table below:

Organisms List	
Human coronavirus 229E	**Rhinovirus**
Human coronavirus OC43	***Chlamydia pneumoniae***
Human coronavirus HKU1	***Haemophilus influenzae***
Human coronavirus NL63	***Legionella pneumophila***
SARS-coronavirus	***Mycobacterium tuberculosis***
MERS-coronavirus	***Streptococcus pneumoniae***
Adenovirus (e.g. C1 Ad. 71)	***Streptococcus pyogenes***
Human Metapneumovirus (hMPV)	***Bordetella pertussis***
Parainfluenza virus 1-4	***Mycoplasma pneumoniae***
Influenza A & B	***Pneumocystis jirovecii* (PJP)**
Enterovirus (e.g. EV68)	**Pooled human nasal wash - *to represent diverse microbial flora in the human respiratory tract***
Respiratory syncytial virus	***Candida albicans***
Staphylococcus epidermis	***Pseudomonas aeruginosa***
Staphylococcus salivarius	

b) Among the tested organisms, none of the tested organisms showed the homology for primers and probe of N gene. *Candida glabrate*, *Cryptococcus neoformans*, and SARS coronavirus showed > 80% homology with forward primer of ORF 1ab gene, 55% homology with reverse primer and 37% to 60% homology with probe. Therefore, the risk of non-specific amplification is low. SARS coronavirus showed 100% homology with forward primer and reverse primer and probe for E gene, therefore, a potential false positive result may be reported for a clinical specimen containing SARS-coronavirus. A statement of "The test cannot rule out diseases caused by other bacterial or viral pathogens is included in limitation section".

C. In addition to the *in silico* analysis, several organisms listed in **Table 15** were extracted and tested with the 3DMed 2019-nCoV RT-qPCR Detection Kit on the Applied Biosystems™ 7500 Real-Time PCR system. The results summarized in **Table 15** has demonstrated the analytical specificity and exclusivity.

Table 15: **Cross-reaction between SARS-CoV-2 and microorganisms by 3DMed 2019-nCoV RT-qPCR Detection Kit.**

Virus/Bacteria/Parasite	Strain	Source/ Sample type	Concentration	Result
Influenza B	B/Victoria	Inactivated culture	1.0×10^6 $TCID_{50}$/mL	Negative
Influenza B	B/Yamagata	Inactivated culture	7.5×10^7 $TCID_{50}$/mL	Negative
Influenza A	H1N1	Inactivated culture	1.0×10^7 $TCID_{50}$/mL	Negative
Influenza A	H3N2	Inactivated culture	1.0×10^8 $TCID_{50}$/mL	Negative
Neisseria meningitidis	N/A	isolate	10^6 PFU/mL	Negative
Haemophilus influenzae	N/A	isolate	10^6 PFU/mL	Negative
Staphylococcus aureus	N/A	isolate	10^6 PFU/mL	Negative
Streptococcus pneumoniae	N/A	isolate	10^6 PFU/mL	Negative
Rubella virus	N/A	isolate	10^6 PFU/mL	Negative
Mumps virus	N/A	isolate	10^6 PFU/mL	Negative
Adenovirus 3	N/A	isolate	10^6 PFU/mL	Negative
Adenovirus 7	N/A	isolate	10^6 PFU/mL	Negative
Respiratory syncytial virus, type B	N/A	isolate	10^6 PFU/mL	Negative
Parainfluenza 2	N/A	isolate	10^6 PFU/mL	Negative
Influenza B	B/Victoria	Culture	1.0×10^6 $TCID_{50}$/mL	Negative
Influenza B	B/Yamagata	Culture	7.5×10^7 $TCID_{50}$/mL	Negative

Influenza A	H1N1	Culture	1.0×10^7 $TCID_{50}$/mL	Negative
Influenza A	H3N2	Culture	1.0×10^8 $TCID_{50}$/mL	Negative
MERS-coronavirus	EMC	cDNA	10^5 copies/mL	Negative
SARS-coronavirus	Urbani	cDNA	10^5 copies/mL	Negative
Streptococcus pyrogenes	BNCC 186346	Culture	10^6 $TCID_{50}$/mL	Negative
Bordetella pertussis	BNCC 337541	Culture	10^6 $TCID_{50}$/mL	Negative
Streptococcus pneumoniae	BNCC 337114	Culture	10^6 $TCID_{50}$/mL	Negative
Candida albicans	BNCC 186382	Culture	10^6 $TCID_{50}$/mL	Negative
Pseudomonas aeruginosa	BNCC 125486	Culture	10^6 $TCID_{50}$/mL	Negative
Staphylococcus epidermis	BNCC 102555	Culture	10^6 $TCID_{50}$/mL	Negative
Haemophilus influenzae	BNCC 337544	Culture	10^6 $TCID_{50}$/mL	Negative

- ***Endogenous Interference Substance Studies***

  The Endogenous Interference Substances Study was conducted with contrived positive sample at 1.5X LoD and 2X LoD.

  The endogenous interference substance was spiked into the contrived with concentration listed in Table 16. The RNA extraction was conducted on Automated Nucleic Acid Extraction System ANDiS 350 with ANDiS Viral RNA Auto Extraction and Purification Kit. The RT-PCR was conducted with one lot of 3DMed 2019-nCoV RT-PCR Detection Kit on one 7500 RT-PCR System. The results summarized in **Table 16** demonstrated that the performance of 3DMed 2019-nCoV RT-PCR Detection Kit is not be impacted by the potential endogenous interference substances in the clinical specimens at the concentration listed in **Table 16.**

Table 16: Results summary for Endogenous interference study:

Potential Interfering Substance	Concentration (µg/mL)	(1.5×LoD) Results	(2×LoD) Results
Mucin: bovine submaxillary gland, type I-S	2.5	Positive	Positive
Nasal sprays or drops	100	Positive	Positive
Nasal corticosteroids	100	Positive	Positive
Homeopathic allergy relief medicine	200	Positive	Positive
Anti-viral drugs	25	Positive	Positive
Antibacterial, systemic	100	Positive	Positive

- ***Clinical Valuation***

A. As of February 21, 2020, 3D Biomedicine Science & Technology Co., Ltd. had tested 282 oropharyngeal swabs specimens collected from individuals under investigation in China using the 3DMed 2019-nCoV RT-PCR Detection Kit. The RNA extraction and RT-qPCR test with 3DMed 2019-nCoV RT-qPCR Detection Kit on 7500 RT-PCR System were conducted at 3D Biomedicine facility with qualified operators. All the test runs were valid. A total of 281 specimens had valid results, and one specimen had invalid result because it failed the Internal Control specification with VIC Ct reported as "Undetermined". The data of the invalid specimen was excluded from the data analysis. The test results are summarized in **Table 1**7

Table 17: Clinical Evaluation Information

Sample testing site	Positive	Negative	Inconclusive	Percentage %
3D Biomedicine Science and Technology	92	147	42	Positive = 32.7% Inconclusive = 15.0% Negative = 52.3%

B. Clinical performance confirmation study with Next Generation Sequencing was conducted with 111 out of 282 clinical specimens summarized in Table 18.

a) In a set of 111 oropharyngeal specimens, 51 specimens with negative RT-qPCR results were randomly selected, and 49 specimens with positive RT-qPCR results included inconclusive were selected form their Ct ranges from 21.00 to 39.50.

b) This set of 111 oropharyngeal specimens were send to RealBio Library Prep Technology to conduct a library construction with their Whole Microbe Genome Library Prep reagent

c) The library of each oropharyngeal specimen was run on Illumina NovaSeq 6000 with S2 reagent at 22G per sample.

d) The PPA and NAP analysis was conducted to evaluate the concordance of NGS results with the results of 3DMed 2019-nCoV RT-PCR Detection Kit. The results were summarized in the **Table 18**

Table 18: Results summary for PPA and NPA analysis

3DMed	NGS		Total
	Positive	Negative	
Positive	49	0	49
Negative	2	49	51
Total	2	49	100

PPA (%) = 49/(49+2) = 96%

NPA (%) = 49/(49+0) =100%

C. A Performance Comparison of 3DMed vs. CDC 2019-nCoV Assays was conducted with 40 oropharyngeal swabs collected from patients with signs and symptoms suggestive of COVID-19. Of 40, there were 20 positive samples selected based on their Ct values and 20 negative samples randomly chosen from a set of clinical samples.

a) The RNA extraction for 40 clinical specimens was conducted with QIAamp DSP Viral RNA Mini Kit. For a given extracted RNA, 5μL of extracted RNA was tested with CDC 2019-nCoV assays and 25μL of extracted RNA was tested with 3DMed 2019-nCoV RT-qPCR Detection Kit.

b) 36 out of 40 clinical specimens had testing results in agreement. 4 out of 40 clinical specimens had test results reported as “inconclusive” when tested with CDC assays, and reported as negative with “undetermined” Ct value when tested with 3DMed assays. These 4 samples were not included in the PPA and NPA analysis. The results are summarized in **Table 19.**

c) This study result has demonstrated that the performance of both CDC and 3DMed assays are comparable.

Table 19: Comparison results of 3DMed vs. CDC 2019-nCoV Assays

		CDC Assay		
		No. Positives	**No. Negatives**	**Total**
3DMed 2019-nCoV RT-qPCR Assay	**No. Positives**	**20**	**0**	**20**
	No. Negatives	**0**	**16**	**16**
	Total	**20**	**16**	**36**

Note: Four (4) inconclusive samples called by the CDC assay were not included in the analysis

Positive Predictive Agreement (PPA) % = 100% x 20/(20+0) = **100**%

Negative Predictive Agreement (NPA) % =100% x 16/(0+16) = **100**%