ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit

Instructions for Use

Catalog # (3103010051，50 tests)

Catalog # (3103010052，100 tests)

For *In-vitro* Diagnostic (IVD) Use Only

3D Biomedicine Science & Technology Co., Limited.
Block A, Building 2, No. 158 Xinjunhuan Rd.
Shanghai, 201114, China
(+86)-400-021-1661

ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit

For use with

ANDiS 350 Automated Nucleic Acids Extraction System
ANDiS Viral RNA Auto Extraction & Purification Kit

QuantStudio™ 5 Real-Time PCR System

CE

IVD For in vitro diagnostic use

REF Catalog number: 3103010051, 3103010052

50Tests, 100 Tests

-25℃ to -15℃

Read the instructions

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

EC REP Caretechion EC-REP GmbH
Niederrheinstr 71, 40474 Duesseldorf, Germany

Table of Contents

Intended Use

ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit is a real-time reverse transcription polymerase chain reaction (RT-qPCR) test intended for qualitative detection of nucleic acid from the SARS-CoV-2 in oropharyngeal swabs.

Results are the identification of SARS-CoV-2 RNA. The RNA is generally detective in oropharyngeal swab during infection. Positive results are indicative of active infection with SARS-CoV-2 but not rule out bacterial infection or co-infection with other virus.

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.

The level of SARS-CoV-2 that would be present in oropharyngeal swabs from individuals with early infection is unknown. Therefore, negative results must be combined with clinical observations, patient history, and epidemiological information.

Summary and Explanation

The novel coronavirus belong to the β genus. COVID-19 is an acute respiratory infectious disease. People are generally susceptible. Currently, the patients infected by the novel coronavirus are the main source of infection; asymptomatic infected people can also be an infectious source. Based on the current epidemiological investigation, the incubation period is 1 to 14 days, mostly 3 to 7 days. The main manifestations include fever, fatigue and dry cough. Nasal congestion, runny nose, sore throat, myalgia and diarrhea are found in a few cases.

The ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit aids in diagnosis COVID-19 and is a real-time reverse transcription polymerase chain reaction test. The product contains oligonucleotide primers, fluorescent dye labeled probes and control material used in RT-qPCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in oropharyngeal swabs.

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 ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit is a one-step real-time reverse transcription polymerase chain reaction (RT-qPCR) test for qualitative detection of SARS-CoV-2 specific RNA.

The ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit includes all reagents needed for RT-qPCR, 2 sets of primers and probes designed to detect the SARS-CoV-2 RNA in oropharyngeal swabs 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 ANDiS FAST SARS-CoV-2 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 QuantStudio™ 5 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 QuantStudio™ 5 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

The ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit is a one-step real-time reverse transcription polymerase chain reaction (RT-qPCR) test for qualitative detection of SARS-CoV-2 specific RNA.

One box of ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit contains the reagents and controls summarized in the **Table 1** and is stored at -20±5℃ for no more than 12 months.

Table 1: Components of ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit

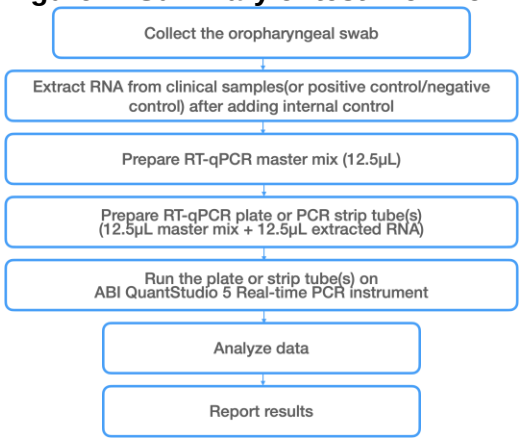
Reagent Name	Kit Size of 50 Tests		Kit Size of 100 Tests	
	Volume per tube	Quantity	Volume per tube	Quantity
RT-qPCR Reaction Mix Reagent	550μL	1 tube	1100μL	1 tube
Enzyme Mix Reagent	75μL	1 tube	150μL	1 tube
Negative Control	400μL	1 tube	400μL	1 tube
Positive Control	400μL	1 tube	400μL	1 tube
Internal Control	500μL	1 tube	1000μL	1 tube

The test is based on real-time RT-qPCR technology, utilizing reverse transcriptase reaction to convert RNA into complementary DNA (cDNA) with gene specific primers, polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. The probes are labelled with fluorescent reporter dyes.

SARS-CoV-2 Assay contains three (3) sets of primers and probes. One set of primers and probe target specific regions on N gene in SARS-CoV-2 genome and the probe is labeled with fluorophore ROX, the second set of primers and probe targets specific region on ORF 1ab in SARS-CoV−2 genome and the probe is labeled with fluorophore FAM, the third set of primers and probe targets specific nucleic acid sequence in virus like particles 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



Materials Required (Provided)

Table 2: ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit

Reagent Name	Kit Size of 50 Tests		Kit Size of 100 Tests	
	Volume per tube	Quantity	Volume per tube	Quantity
RT-qPCR Reaction Mix Reagent	550μL	1 tube	1100μL	1 tube
Enzyme Mix Reagent	75μL	1 tube	150μL	1 tube
Negative Control	400μL	1 tube	400μL	1 tube
Positive Control	400μL	1 tube	400μL	1 tube
Internal Control	500μL	1 tube	1000μL	1 tube

Material and Equipment Required but not Provided

- RT-qPCR Instruments:
  - QuantStudio™ 5 Real-Time PCR Instrument (Applied Biosystems; catalog # A28573)
- ANDiS 350 Automated Nucleic Acids Extraction System（Cat.3105020003）
- ANDiS Viral RNA Auto Extraction & Purification Kit (Cat. 3103010024,16 Tests; Cat. 3103010025,64 Tests; Cat. 3103010026,128 Tests)
- Microcentrifuge, capable of 16,000 × *g* (Eppendorf, Part no. 5415D; or equivalent)
- Vortex mixer
- Single- and multi-channel pipettes
- Pipette tips with filters
- 100% ethanol, ACS reagent grade or equivalent
- 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

Warnings and Precautions

- Follow standard precautions. All patient specimens and positive controls should be considered potentially infectious and handled accordingly.
- Do not eat, drink, smoke, apply cosmetics or handle contact lenses in areas where reagents and human specimens are handled.
- Handle all specimens as if infectious using safe laboratory procedures. Refer to Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with SARS-CoV-2 https://www.cdc.gov/coronavirus/SARS-CoV-2/lab-biosafety-guidelines.html.
- Specimen processing should be performed in accordance with national biological safety 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 swabs collected from individuals who meet the US Center for Disease Control and Prevention (CDC) clinical and epidemiologic criteria
- 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 of this product is limited to specified laboratories and clinical laboratory personnel who have been trained on authorized instruments.
- Results need to be interpreted in conjunction with clinical signs, symptoms and travel history of the patient or contact information
- 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 gloves in each area and change them before entering different areas
- Dedicate supplies and equipment to the separate working areas and do not move them from one area to another
- Always check the expiration date prior to use. Do not use expired reagents.
- Change aerosol barrier pipette tips between all manual liquid transfers.
- During preparation of samples, compliance with good laboratory techniques 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, microcentrifuges) and supplies (e.g., microcentrifuge tubes, pipette tips) for assay setup and handling of extracted nucleic acids.
- Wear a clean lab coat and powder-free disposable gloves (not previously worn) when setting up assays.
- Change gloves between samples and whenever contamination is suspected
- Keep reagent and reaction tubes capped or covered as much as possible

- Enzyme Mix Reagent must be thawed and maintained on cold block at all times during preparation and use.
- 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.

Reagent Storage and Handling

- ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit shall be stored at -25℃ to -15℃.
- Always check the expiration date prior to use. Do not use expired reagents.
- Protect SARS-CoV-2 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. Avoid more than 10 cycles of freeze-thaw.

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 (SARS-CoV-2)

    https://www.cdc.gov/coronavirus/SARS-CoV-2/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℃ and ship overnight to testing facility on ice pack. If a specimen is frozen at -70℃ or lower, ship overnight to testing facility on dry ice

- Storing Specimens
  - Specimens can be stored at 2-8℃ for up to 48 hours after collection.
  - If a RNA extraction could not be performed with 48 hours, the specimens should be stored at -70℃or lower.
  - Extracted nucleic acid should be stored at -70℃ or lower

Instruction for Use

1. RNA extraction:

Specimens, positive control and negative control should be extracted before PCR amplification.

Important: Add 10μL of internal control to every 200μL specimen/positive control/negative control before extraction.

1.1. RNA extraction with ANDiS 350 Automated Nucleic Acids Extraction System

RNA extraction is performed with ANDiS Viral RNA Auto Extraction & Purification Kit on ANDiS 350 Automated Nucleic Acids Extraction System.

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

- Four (4) 96-well deep plate contains lysis buffer, magnetic beads, wash buffer and elution buffer
- One tube of Proteinase K
- Eight (8) 8-strip rod combs

1.1.1. Remove Internal Control tube from ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit, and thaw at room temperature.

1.1.2. Equilibrate the clinical specimen tube to room temperature.

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 Test	Volume in μL per N Tests
Internal Control	10	10 x (N+1)
Proteinase K	20	20 x (N+1)
Total Volume	30	30 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 30μL of IC Mix and 200μL of each clinical sample in the well in A1 to H1 and A7 to H7 columns which containing Lysis Buffer.

1.1.12. Turn on ANDiS 350 Automated Nucleic Acids Extraction System.
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-strip rod combs to a magnetic rod cover holder and ensure the 8-strip rod combs 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	off
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℃
6	3	Discard Beads	1	0	0	900	3	off

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 -70℃ or lower.
1.1.22. Discard the used 96-well deep plate properly.

2. RT-qPCR

2.1. Equilibrate all the reagents and controls except Enzyme Mix Reagent to room temperature.

2.2. Thaw the Enzyme Mix Reagent in cooler or on ice.

2.3. Mix all the reagents and Controls except Enzyme Mix Reagent by vortex for 10 seconds, centrifuge briefly to collect the content to the bottom of the tube.

2.4. Mix the Enzyme Mix Reagent by flick 5 times and centrifuge briefly to collect the contents to the bottom of tube.

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 with Internal Control

Reagent Name	Volume in µL per Reaction	Volume in µL per N Reactions
RT-qPCR Reaction Mix Reagent	11	11 x (N+1)
Enzyme Mix Reagent	1.5	1.5 x (N+1)
Total Volume	12.5	12.5 x (N+1)

2.7. Add 12.5µL of RT-qPCR Master Mix into each required well of an appropriate optical 96-well reaction plate or optical -8 tube strip.

2.8. Add 12.5µL of extracted RNA sample or 12.5µL of the Control (Positive and Negative Control) into each well or tube containing 12.5µL of RT-qPCR Master Mix.

2.9. Mix the extracted RNA sample and control with RT-qPCR 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 collect the content to the bottom of the tube.

2.12. Ensure one Positive Control and one Negative Control are used in each run.

3. Programming QuantStudio™ 5 Real-Time PCR System with QuantStudio™ 5 Software :

3.1. Define the general setting:

Settings	
Reaction volume per well (tube)	25 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 (N gene)	ROX	None
SARS-CoV-2 specific RNA (ORF1ab)	FAM	None
Internal Control	VIC	None

3.3. Set up RT-qPCR Thermal Cycle profile:
"Experiment Properties" - "Run mode" choose "Fast".

Table 8: Define the target with fluorescent dye

Stage	Temperature	Time	Cycle number	Heating/cooling rate
RT	50℃	2 minutes	1	3.19℃/s
Hold	95℃	2 seconds	1	3.19℃/s
PCR	95℃	1 seconds	41	3.19℃/s
	60℃	13 seconds		2.45℃/s

Note: Collect fluorescent signal at 60℃ step.

4. Data Analysis:

4.1. Analyze the data using the following Ct settings in QuantStudio™ 5 software for QuantStudio™ 5 RT-qPCR System.

4.1.1. Unselect the box for "Use Default Settings".

4.1.2. For baseline setting: Use "Baseline Start Cycle 3~15 End Cycle 5~22".

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.

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 ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit

Control Type	Name of the Reagent	SARS-CoV-2		Internal Control	Expected Ct
		FAM	ROX	VIC	
Positive	Positive Control	POS	POS	/	FAM Ct ⩽ 39.5 ROX Ct ⩽ 39.5
Negative	Negative Control	Neg	Neg	POS	FAM Ct ＞39.5 ROX Ct ＞39.5 VIC Ct ⩽ 38.0

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

"/" : Not required

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)**
  - If the result for a specimen is SARS-Cov-2 RNA not detected, the Ct value of internal control must be ≤38.0, otherwise the result of that specimen is invalid.
  - If the result for a specimen is SARS-Cov-2 RNA detected, the Ct value of the internal control is not required to be considered valid.
- **For the clinical specimen**
  - When all controls exhibit the expected performance, a specimen is considered as negative if all the SARS-CoV-2 specific markers (FAM, 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 marker (FAM or ROX) have Ct value less than its respective Ct cutoff value.

ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit Interpretation Guide

The interpretation guide is summarized in **Table 10** below:

If the results are obtained that do not follow these guidelines, re-extract and re-test the specimen. If the repeat testing yields similar results, contact 3D Medicine for consultation.

Table 10: Interpretation Guidance

Sample ID	ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit Interpretation Guide					
	FAM Detection Channel	ROX Detection Channel	VIC Detection Channel (internal control)	Result Interpretation	Report	Action
A	If any one of the two targets is Positive		/	SARS-CoV-2 detected	Presumptive positive SARS-CoV-2	Report results to appropriate public health authority and sender. Contact appropriate public health authority immediately for instructions for transfer of the specimen to public health authority for additional testing and further guidance.
B	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

Note: Positive: FAM Ct≤39.5 and/or ROX Ct≤39.5.
Negative: FAM Ct＞39.5 and ROX Ct ＞39.5.

"/": If Any SARS-CoV-2 gene is positive, Internal control(IC)'s result is not required. Amplification of target genes at high concentrations may cause IC amplification to be inhibited.

Quality Control

- Quality Control requirements must be performed in conformance with local and member state regulation or accreditation requirement and the user's laboratory's standard quality control procedures.
- Quality control procedures are intended to monitor reagent and assay performance.
- Test all positive controls prior to running diagnostic samples with each new kit lot to ensure all the kit components are working properly.
- Always included a negative control, and the appropriate positive control in each amplification and detection run. All clinical specimens should be tested with a spiked in internal control to control for the extraction of clinical specimen.

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 ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit have only been established in the specimens collected with oropharyngeal swabs.
- Negative results 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 be 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 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 result may be observed if cross contamination occurred during the specimen handling or preparation.
- If the virus mutates in the RT-qPCR target region, SARS-CoV-2 may not be detected or may be detected less predictively.
- Test performance can be affected because the epidemiology and clinical spectrum of infection caused by SARS-CoV-2 is not fully understand.
- Detective of virus RNA may not indicate the presence of infectious virus or that SARS-CoV-2 is the causative agent for clinical symptoms.
- The performance of this test has not been established for monitoring treatment of SARS-CoV-2 infection.
- The performance of this test has not been established for screening of blood or blood products for the present of SARS-CoV-2.
- This test cannot rule out diseases caused by other bacterial or viral pathogens.

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 analytical sensitivity of the ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit were determined in Limit of Detection study.

  Quantified SARS-CoV-2 pseudo virus was serially diluted in oropharyngeal swabs. The determination of LoD was conducted by testing four (4) target concentrations. Then two nucleic acid of clinical specimens previously tested positive were serially diluted in oropharyngeal swabs, respectively. The validation of LoD was conducted by testing four (4) target concentrations. Each concentration was tested 20 replicates. The results of LoD is 200 copies/mL. The verification of LOD was conducted by three qualified batches of ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit. For each lot, three (3) positive nucleic acid of clinical specimens diluted to LoD level with oropharyngeal swabs were tested. The test results showed that the detection rate of SARS-CoV-2 virus is 95-100% at 200 copies/mL.

- Specificity

  Cross reactivity testing

  Cross-reactivity of the ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit was evaluated by testing whole organisms. As listed in **Table 11**, a panel of multiple unique sub-species of microorganisms were tested. The data summarized in the **Table 11** has demonstrate that no observation of cross reaction with other organism are obtained when tested each organism with the ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit.

  Table 11: Cross Reactivity Testing Results

Microorganism	Negative samples Result (No.Positive/ No.Tested)	Final Result (cross-reactivity)
Influenza A virus(H1N1)	0/3	NO
Influenza A virus H1N1(2009)	0/3	NO
Influenza A virus(H3N2)	0/3	NO
Influenza A virus(H5N1)	0/3	NO
Influenza A virus(H7N9)	0/3	NO
Influenza B virus (Victoria)	0/3	NO
Influenza B virus (Yamagata)	0/3	NO
Human Coronavirus (229E)	0/3	NO
Human Coronavirus (HKUI)	0/3	NO
Human Coronavirus (OC43)	0/3	NO
Human Coronavirus (NL63)	0/3	NO
Human Coronavirus (SARS)	0/3	NO
Human Coronavirus (MERS)	0/3	NO
Parainfluenza 1	0/3	NO
Parainfluenza 2	0/3	NO
Parainfluenza 3	0/3	NO
Rhinovirus A	0/3	NO
Rhinovirus B	0/3	NO
Rhinovirus C	0/3	NO

Adenovirus 1	0/3	NO
Adenovirus 2	0/3	NO
Adenovirus 3	0/3	NO
Adenovirus 4	0/3	NO
Adenovirus 5	0/3	NO
Adenovirus 7	0/3	NO
Adenovirus 55	0/3	NO
Human genome	0/3	NO
Respiratory syncytial virus	0/3	NO
Enterovirus A	0/3	NO
Enterovirus B	0/3	NO
Enterovirus C	0/3	NO
Enterovirus D	0/3	NO
EB virus	0/3	NO
Measles virus	0/3	NO
Human cytomegalovirus	0/3	NO
Norovirus	0/3	NO
Varicella-zoster virus	0/3	NO
rotavirus	0/3	NO
Mumps virus	0/3	NO
Human metapneumovirus	0/3	NO
Chlamydia pneumoniae	0/3	NO
Mycoplasma pneumoniae	0/3	NO
Legionella	0/3	NO
Bacillus pertussis	0/3	NO
Haemophilus influenzae	0/3	NO
Mycobacterium tuberculosis	0/3	NO
Staphylococcus aureus	0/3	NO
Streptococcus pneumoniae	0/3	NO
Streptococcus pyogenes	0/3	NO
Klebsiella pneumoniae	0/3	NO
Aspergillus fumigatus	0/3	NO
Candida albicans	0/3	NO
Candida glabrata	0/3	NO
Cryptococcus neoformans	0/3	NO

In silico analysis

BLAST queries of the ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit primers and probes were performed against all publically available nucleotide sequences of the pathogens listed in **Table 12**. All two sets of primers and probes showed no significant homologies with the genomic sequences of pathogens listed in **Table 12**, predicting no cross-reaction with these pathogens.

Table 12: Pathogen used for *In Silico* Cross-reactivity Analysis

Pathogen	Strain
Aspergillus fumigatus	Af293
Bacillus anthracosis (Anthrax)	Ames Ancestor; A2084

Bordetella pertussis	BP 165
Candida albicans	SC5314
Candida glabrata	CBS 138
Chlamydia pneumoniae	CWL029
Chlamydia psittaci	6BC
Corynebacterium diphtheriae	NCTC11397
Coxiella burneti (Q-Fever)	RSA 493
Cryptococcus neoformans	JEC21
Haemophilus influenza	Rd KW20
Human Adenovirus 1	N/A
Human Adenovirus 2	N/A
Human Adenovirus 5	N/A
Human Adenovirus 54	Kobe-H
Human Adenovirus 7	N/A
Human Adenovirus A	Huie
Human Adenovirus B1	GB
Human Adenovirus B2	Slobitski
Human Adenovirus C	N/A
Human Adenovirus D	N/A
Human Adenovirus D	Hicks; NIAID V-209-003-014
Human Adenovirus F	Dugan
Human Adenovirus type 35	N/A
Human alphaherpesvirus 3 (Varicella-zoster virus)	Dumas
Human betaherpesvirus 5 (Human cytomegalovirus)	Merlin
Human coronavirus	229E
Human coronavirus	NL63
Human coronavirus	OC43
Human coronavirus	HKU1
Human Enterovirus 68	Fermon
Human Enterovirus A	N/A
Human Enterovirus B	N/A
Human Enterovirus D	Enterovirus 70
Human gammaherpesvirus 4 (EB virus)	B95-8
Human metapneumovirus (hMPV)	00-1
Human poliovirus 1	Mahoney
Influenza A	H5N1
Influenza A	H7N9
Klebsiella pneumonia	HS11286
Legionella pneumophila	NCTC12273
Leptospira interrogans	FMAS_AW1
Measles virus	Ichinose-B95a
MERS-coronavirus	HCoV-EMC
Moraxella cararrhalis	BBH18
Mumps virus	Miyahara
Mycobacterium tuberculosis	H37Rv

Mycoplasma californicum	ST-6
Mycoplasma floccular	Ms42
Mycoplasma pneumoniae	FH
Neisseria elongata	ATCC 29315
Neisseria meningitidis	NCTC10025
Parainfluenza virus 1	Washington 1964
Parainfluenza virus 2	N/A
Parainfluenza virus 3	N/A
Parainfluenza virus 4	M-25
Parechovirus A	Gregory
Parechovirus B	87-012
Pneumocystis jirovecii	RU7
Primate Norovirus	SimianNoV-nj
Pseudomonas aeruginosa	PAO1
Respiratory syncytial virus	S2 ts1C
Rhinovirus A	ATCC VR-1559
Rhinovirus B	N/A
Rhinovirus C	NAT001
Rotavirus A	RVA/Simian-tc/ZAF/SA11-H96/1958/G3P5B[2]

- Interference Substances

  Endogenous and exogenous interference studies were conducted to assess potential interference effects on the ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit from substances that may be present in oropharyngeal swabs.

  Interfering substances in **Table 13** were tested, which are naturally present or artificially introduced, which were tested at the highest medically relevant concentration. The positive specimens were diluted in SARS-CoV-2 negative oropharyngeal swabs to achieve a concentration of 1.25 × LoD. RNase-free water was used as a control. Three batches of reagents were included in the study and the specimens were tested. Results showed that these substances did not interfere with the detection of negative and weak positive specimens by ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit.

  Table 13: Interfering Substances

No.	Exogenous interfering substances	Concentration（μg/mL）
1	Human blood	50%
2	Mucin	0.1mg/mL
3	Phenylephrine	10
4	Oxymetazoline	1
5	Sodium chloride	1000
6	Beclomethasone	1000
7	Dexamethasone	500
8	Flunisolide	10
9	Triamcinolone acetonide	1
10	Budesonide	150
11	Mometasone	1

12	Fluticasone	1
13	Histamine hydrochloride	25
14	α-Interferon	1000
15	Zanamivir	10
16	Ribavirin	300
17	Oseltamivir	75
18	Peramivir	10
19	Lopinavir	100
20	Ritonavir	1
21	Arbidol	200
22	Levofloxacin	750
23	Tobramycin	1.5
24	Azithromycin	500
25	Ceftriaxone	200
26	Meropenem	20

- Specimen Stability

  To increase the ability to detect infection with ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit, 3DMed recommends collection of oropharyngeal swabs for testing. Specimens should be collected as soon as possible once a PUI is identified regardless of symptom onset. Maintain proper infection control when collecting specimens. Based on the study of specimen stability at 2-8℃, , it is recommended not to exceed 1 day. For the specimen stability study at -20±5℃, it is recommended not to exceed 7 days. For the specimen freeze-thaw stability study, it is recommended not to exceed 6 times. Label each specimen tube with patient ID number, unique specimen ID such as laboratory requisition number, specimen type and date that specimen was collected.

- Clinical Evaluation

  Real patient oropharyngeal samples were randomly selected and tested simultaneously alongside a CE authorized RT-qPCR comparator assay. The results show that ANDiS FAST SARS-CoV-2 RT-qPCR Detection Kit had high consistency with comparartive reagent. Positive percent agreement is 97.2%, negative percent agreement is 93.1%, total percent agreement is 94.7%. The clinical study included positive control and negative control with every heat cycle. All controls performed as expected.

Disposal

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

Contact and support information

For more information about 3D Biomedicine Sciences & Technologies Co., Limited, please visit our website at http://www.3dmedcare.com or contact at E-mail: ivd-support@3dmedcare.com .

For detailed technical questions regarding the use of the 3D Biomedicine Sciences & Technologies Real-Time qPCR Kits, please contact our Technical Support at E-mail: ivd-support@3dmedcare.com .