Extraction, Purification and Detection of SARS-CoV-2 Nucleic Acid

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[Abstract] Nucleic acid extraction and purification kit is used for isolating high-quality pathogen nucleic acids (DNA/RNA) from a variety of specimens. Nucleic acid can be directly extracted from liquid samples such as blood, serum, plasma, urine, nasopharyngeal swabs, and cell culture. And soil samples such as stool, tissue samples need to be pre- treated, obtaining a supernatant and carrying out extraction and purification to obtain the nucleic acids. The obtained nucleic acids can be directly used in related experiments such as Fluorescence quantitative PCR, RT-PCR, biochip analysis, second-generation sequencing.

The SARS-CoV-2 Nucleic Acid Detection Kit is a molecular *in vitro* diagnostic test that aids in the detection and diagnosis of 2019-nCoV and is based on widely used nucleic acid amplification technology. The product contains oligonucleotide primers and dual-labeled hydrolysis probes and control material used in RT-PCR for the *in vitro* qualitative detection of SARS-CoV-2 RNA in nasopharyngeal swab, oropharyngeal swab and sputum specimens.

The oligonucleotide primers and probes for detection of SARS-CoV-2 were selected from regions of the virus ORF1ab gene and nucleocapsid (N) gene. The panel is designed for specific detection of the SARS-CoV-2 (two primer/probe sets). An additional primer/probe set to detect the human RNase P gene (RNP) in control samples and clinical specimens is also included in the panel.

[Background] Nucleic acid extraction and purification kit is based on superparamagnetic particles technology with high adhesion. Under the condition of high concentration of ionizing agent, the nanometer magnetic particles can specifically adsorb nucleic acids through hydrogen bonding and electrostatic, while proteins or other impurities are not absorbed. The nucleic acid-adsorbed nanoparticles are washed to remove proteins, salts and other impurities on the particles surface by washing buffer. Finally, the low-salt buffer can be used to elute the purified nucleic acids on the particles. The magnetic beads with special surface modification have stronger ability of binding nucleic acid and easier elution, which can minimize the risk of cross- contamination and improve detection sensitivity and accuracy. The kit has simple operation steps in a short time, and can complete the entire extraction process at room temperature (about 25 °C).

SARS-CoV-2 Nucleic Acid Detection Kit contains the assays and controls for a real-time reverse transcription polymerase chain reaction (RT-PCR) test intended for the qualitative detection of nucleic acid from SARS-CoV-2 in nasopharyngeal swab, oropharyngeal swab, sputum specimens from individuals suspected of COVID-19. Testing is limited to laboratories certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 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 nasopharyngeal swab, oropharyngeal swab, sputum 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 infection status. Positive results are indicative of active infection. 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 2019-nCoV 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 SARS-CoV-2 Nucleic Acid Detection Kit is intended for use by qualified and trained clinical laboratory personnel specifically instructed and trained in the techniques of real-time PCR and *in vitro* diagnostic procedures. SARS-CoV-2 Nucleic Acid Detection Kit is only for use under the Food and Drug Administration's Emergency Use Authorization.

Materials and Reagents

Materials

1. Dedicated laboratory coat for each area
2. Disposable booties
3. Biohazard bag for tip and tube disposal
4. Powder-free latex, vinyl or nitrile gloves
5. 1.5 ml screw-capped microcentrifuge tubes
6. Aerosol barrier tips
7. 96-well PCR plates
8. Optical caps
9. Disposable Plasticware

   The use of sterile, disposable polypropylene tubes is recommended throughout the procedure. These tubes are generally RNase-free and do not require pretreatment to inactivate RNases
10. 20% (v/v) bleach solution (2.0% w/v sodium hypochlorite in water)
11. 70% ethanol
12. Nuclease-free water
13. Qiagen extractor EZ1 Advanced XL and EZ1 DSP Virus kit (62724)

Kits

A. Nucleic acid extraction and purification kit

Notes:

1. *Date of manufacture and expiry: see outer packing box.*
2. *Transport and store at room temperature (about 25 °C), and the validity period is 6 months.*

1. Nucleic acid extraction and purification kit (25 reactions) (Shanghai BioGerm Medical Technology Co., LTD, catalog number: TQ-BG001-025)
   a. Beads* (Reagent number: BG001-PA1, 0.8 ml)
   b. Lysate (Reagent number: BG001-PA2, 15 ml)
   c. Washing buffer I** (Reagent number: BG001-PA3, 6.6 ml)
      Note: Need add 8.4 ml anhydrous ethanol before use.
   d. Washing buffer II** (Reagent number: BG001-PA4, 6 ml)
      Note: Need add 24 ml anhydrous ethanol before use.
   e. Eluate buffer (Reagent number: BG00 1-PA5, 10 ml)
2. Nucleic acid extraction and purification kit (50 reactions) (Shanghai BioGerm Medical Technology Co., LTD, catalog number: TQ-BG001-050)
   a. Beads* (Reagent number: BG001-PD1, 1.7 ml)
   b. Lysate (Reagent number: BG001-PD2, 30 ml)
   c. Washing buffer I** (Reagent number: BG00 1-PD3, 17 ml)
      Note: Need add 22 ml anhydrous ethanol before use.
   d. Washing buffer II** (Reagent number: BG00 1-PD4, 15 ml)
      Note: Need add 60 ml anhydrous ethanol before use.
   e. Eluate buffer (Reagent number: BG001-PD5, 25 ml)
3. Nucleic acid extraction and purification kit (100 reactions) (Shanghai BioGerm Medical Technology Co., LTD, catalog number: TQ-BG001-100)
   a. Beads* (Reagent number: BG001-PB1, 3.3 ml)
   b. Lysate (Reagent number: BG001-PB2, 50 ml)
   c. Washing buffer I** (Reagent number: BG001-PB3, 33 ml)
      Note: Need add 42 ml anhydrous ethanol before use.
   d. Washing buffer II** (Reagent number: BG00 1-PB4, 25 ml)
      Note: Need add 100 ml anhydrous ethanol before use.
   e. Eluate buffer (Reagent number: BG001-PB5, 50 ml)
4. Nucleic acid extraction and purification kit (200 reactions) (Shanghai BioGerm Medical Technology Co., LTD, catalog number: TQ-BG001-200)
   a. Beads* (Reagent number: BG001-PC1, 6.6 ml)
   b. Lysate (Reagent number: BG001-PC2, 100 ml)
   c. Washing buffer I** (Reagent number: BG001-PC3, 66 ml)
      Note: Need add 84 ml anhydrous ethanol before use.
   d. Washing buffer II** (Reagent number: BG00 1-PC4, 50 ml)
      Note: Need add 200 ml anhydrous ethanol before use.
   e. Eluate buffer (Reagent number: BG001-PC5, 100 ml)

Tips：

1. *"*" For the first use, the magnetic beads must be thoroughly mixed ensuring the magnetic beads is fully dispersed.*

2. *"**" Please add suitable anhydrous ethanol into washing buffer I and washing buffer II before use, and store at about 25 °C.*
3. *Please prepare anhydrous ethanol by yourself.*

B. SARS-CoV-2 Nucleic Acid Detection Kit

Notes:

1. *Store all the kit components at -15~-25 °C.*
2. *Always check the expiration date prior to use. Do not use expired reagents.*
3. *Protect SARS-CoV-2 reaction mix from light.*
4. *RT-PCR Reaction Mix, RT-PCR Enzyme Mix, and SARS-CoV-2 reaction mix must be thawed and kept on cold block at all times during preparation and use.*

1. RT-PCR Reaction Mix (600 µl)
   Tris-HCl
   KCl
   $MgCl_2$
   dNTPs
2. RT-PCR Enzyme Mix (200 µl)
   DNA polymerase
   Reverse transcriptase
   RNase inhibitor
3. SARS-CoV-2 reaction mix (200 µl)
   ORF1ab
   N gene
   RNase P specific Primers and probes
4. Positive Control (500 µl)
   ORF1ab specific fragment pseudovirus particle
   N gene specific fragment pseudovirus particle
   RNase P virus-like particles
5. Negative Control (500 µl)
   0.9% saline solution

Equipment

1. Biological safety cabinet
2. Refrigerated microcentrifuge
3. Cold blocks
4. Vortex mixer
5. Dedicated adjustable pipettes

6. Applied Biosystems 7500 Fast DX Real-Time PCR Instrument (Pub. No. 4406991)
7. Magnetic separator

Procedure

Part I: Extraction and Purification of Nucleic Acid

A. Adsorption
1. Add 400 μl lysate, 30 μl magnetic beads suspension and 200 μl sample into centrifuge tube.
2. Swirl and mix 15 s with a vortex oscillator, and leave at room temperature for 15 min (with moderate blending for 2 to 3 times).
3. Then collecting the magnetic bead with magnetic separator (30 s) and discard supernatant.

B. Washing I
1. Add 500 μl washing buffer I.
2. Swirl and mix by a vortex oscillator for 15 s.
3. Then collecting the magnetic bead with magnetic separator (30 s) and discard supernatant.

C. Washing II
1. Add 500 μl washing buffer II
2. Swirl and mix by a vortex oscillator for 15 s
3. Then collecting the magnetic bead with magnetic separator (30 s) and discard supernatant.

D. Washing III
1. Add 500 μl washing buffer II.
2. Swirl and mix by a vortex oscillator for 15 s.
3. Then collecting the magnetic bead with magnetic separator (30 s) and discard supernatant.

Tips：If low temperature storage causes precipitation of lysate and washing buffer, please dissolve them in a 37 degree water bath before use.

E. Drying

Drying at room temperature for 3~5 min to evaporate residual ethanol.

F. Eluting
1. Add 100 μl eluate buffer and moderate blending 15 s.
2. And then, waiting for 2~3 min and instant centrifugal.
3. Adsorb the magnetic bead with magnetic separator and collect nucleic acid eluent into RNase-free centrifuge tube for subsequent testing or storage below -20 °C.

Tips: If the nucleic acid concentration is too low, 50 μl of eluent can be added for elution, and concentrated concentration extraction is performed.

Part II: Detection of SARS-CoV-2 Nucleic Acid (Video 1)

Video 1. COVID-19 Nucleic acid Detection

A. Sample Preparation

The quality of the RNA from the sample extraction is essential for the performance of SARS-CoV-2 Nucleic Acid Detection Kit. The extraction protocol should be performed following manufacturer's instructions or an internally validated protocol. The suitability of the nucleic acid extraction procedure for use with SARS-CoV-2 Nucleic Acid Detection Kit must be validated by the user.

Select appropriate nucleic acid extraction kit to extract nucleic acid from specimens, positive control and negative control. Operate according to the corresponding kit instructions.

B. Prepare the Reaction Mix

When preparing Reaction Mix, clean all working surfaces with a fresh 10% bleach solution followed by 70% ethanol, or another equivalent method of cleaning that disinfects and degrades nucleic acids. Prepare sufficient quantity of the following reagent mix for the number of samples and controls being tested. All volumes include 10% overage for pipette error.

Reagent	Volume/Sample (μl)	Volume for N Sample plus 2 controls (μl)
RT-PCR Reaction Mix	12	12×（N+2）μl
RT-PCR Enzyme Mix	4	4×（N+2）μl
SARS-CoV-2 reaction mix	4	4×（N+2）μl
Total volume	20	-

C. Set up the reaction plate

1. Add 20 μl of the Reaction Mix prepared in Procedure B into each well of a Plate or tubes.
2. Then combine with the Sample or the Control according to the following table:

Coponent	Volume/reaction (μl)		
	Sample reaction	Purified Positive Control	Purified Negative Control
Reaction Mix	20	20	20
Purified Sample nucleic acid	5	-	-
Purified Positive Control	-	5	-
Purified Negative Control	-	-	5
Total volume	25	25	25

D. Set up and run the reaction

See the Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument Reference Guide for detailed instructions. The instrument must be calibrated for FAM, HEX/VIC, ROX. And confirm the run settings:

1. Assay: Standard Curve (Absolute Quantitation);
2. Run mode: Standard 7500;
3. Passive reference: None;
4. Sample volume: 25 μl.

Using the Detector Manager in the tools menu create the following detectors with the quencher set as none. The detector name must be an exact match with the names shown in the table below.

Reporter dye	detector
FAM	ORF1ab
VIC/HEX	N gene
ROX	RNase P

Program with the cycling conditions below:

Step	Temperature (°C)	Time	Cyclers
Reverse transcription	50	10 min	1
Activation	95	5 min	1
Denaturation	95	10 s	45
Anneal/extension	55	40 s	

Data analysis

1. Quality Control

   Positive control: the amplification curve was S shaped, and Ct value ≤ 30.

Negative control: Ct value>38 or not detected.

Negative Control and Positive Control must be performed correctly; otherwise the sample results are invalid.

2. Data Analysis and Interpretation

Detection Channel(Target Gene)			Results Analysis
FAM(ORF1ab)	VIC/HEX(N)	ROX (RNase P)	
Ct≤38	Ct≤38	Ct≤38 or undetermined	SARS-CoV-2 positive
Ct>38 or Undetermined	Ct>38 or Undetermined	Ct≤38	SARS-CoV-2 negative
Ct>38 or Undetermined	Ct≤38	Ct≤38	Re-extract and repeat RT-PCR*
Ct≤38	Ct>38 or Undetermined	Ct≤38	
Ct>38 or Undetermined	Ct>38 or Undetermined	Ct>38 or Undetermined	Invalid

*Strongly recommend to collect samples from lower respiratory tract such as sputum specimens. If the repeat result remains inconclusive, additional confirmation testing should be conducted if clinically indicated.

Notes

For Nucleic Acid Extraction and Purification Kit:

1. Please read this manual carefully before extraction and operate strictly in accordance with the requirements.
2. Laboratory operations were performed in accordance with the "*Administrative Measures for Clinical Gene Expansion Laboratory of Medical Institutions*". The experimental operation must be strictly partitioned, and the instruments, equipment, consumables and work clothes used in each area must be dedicated to avoid cross contamination.
3. Samples should be handled in strict accordance with bio safety.
4. This kit is only for researching and development, not for clinical diagnosis.
5. The solution contains guanidine salt protein denaturant, if accidentally spilled the skin, please flush the skin with plenty of water.

For SARS-CoV-2 Nucleic Acid Detection Kit:

1. The use of this assay as an In vitro diagnostic under the FDA Emergency Use Authorization (EUA) is limited to laboratories that are certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA), 42 U.S.C. § 263a, to perform high complexity tests.

2. The SARS-CoV-2 Nucleic Acid Detection Kit performance was established using nasopharyngeal swab, oropharyngeal swab, sputum specimens only. Other specimen types have not been evaluated and should not be tested with this assay.
3. 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.
4. Extraction and amplification of nucleic acid from clinical samples must be performed according the specified methods listed in this procedure. Other extraction approaches and processing systems have not been evaluated.
5. False-negative results may arise from:
   a. Improper sample collection
   b. Degradation of the viral RNA during shipping/storage
   c. Specimen collection after nucleic acid can no longer be found in the specimen matrix
   d. Using unauthorized extraction or assay reagents
   e. The presence of RT-PCR inhibitors
   f. Mutation in the SARS-CoV-2 virus
   g. Failure to follow instructions for use
6. False-positive results may arise from:
   a. Cross contamination during specimen handling or preparation
   b. Cross contamination between patient samples
   c. Specimen mix-up
   d. RNA contamination during product handling
7. The impacts of vaccines, antiviral therapeutics, antibiotics, chemotherapeutic or immunosuppressant drugs have not been evaluated. The SARS-CoV-2 Nucleic Acid Detection Kit cannot rule out diseases caused by other bacterial or viral pathogens.
8. Negative results do not preclude infection with SARS-CoV-2 virus, and should not be the sole basis of a patient management decision.
9. Laboratories are required to report all positive results to the appropriate public health authorities.