Guideline

Chinese Expert Consensus on the Nucleic Acid Detection of SARS-CoV-2

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Abstract: The coronavirus disease 2019 (COVID-19) has already become a pandemic wherein the infection's timely diagnosis has proven beneficial to patient treatment and disease control. Nucleic acid detection has been the primary laboratory diagnostic method for the detection of SARS-CoV-2. To ensure laboratory staff safety and quality nucleic acid testing, the Chinese Society of Laboratory Medicine formulated this consensus, based on the Chinese National Recommendations and previous literature for

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nucleic acid detection. A working group comprises 34 hospital professionals experience with real-time polymerase chain reactions (PCR) testing for SARS-CoV-2 drafted guidance statements during online discussions. A modified Delphi methodology was used in forming a consensus among a wider group of hospital professionals with SARS-CoV-2 detection experience. Guidance statements were developed for four categories: (I) specimen type, priority, collecting, transportation and receiving; (II) nucleic acid isolation and amplification; (III) quality control; (IV) biosafety management and decontamination. The modified Delphi voting process included a total of 29 guidance statements and final agreement. Consensus was reached after two rounds of voting. Recommendations were established for the detection of SARS-CoV-2 using real time PCR testing based on evidence and group consensus. The manuscript was evaluated against The Appraisal of Guidelines for Research & Evaluation Instrument (AGREE II) and was developed to aid medical laboratory staff in the detection of the ribonucleic acid (RNA) of SARS-CoV-2.

Keywords: SARS-CoV-2; coronavirus disease 2019 (COVID-19); nucleic acid; polymerase chain reaction (PCR)

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Introduction

Since the outbreak of COVID-19, the number of infected people has been increasing rapidly worldwide (1-4); hence, rapid and effective laboratory diagnostic testing has been essential for a timely diagnosis of confirmed and suspected patients. The detection of SARS-CoV-2 using nucleic acid provides direct evidence for the diagnosis (5,6). Because transmission routes and the underlying pathogenicity of SARS-CoV-2 have not yet been clarified, laboratory staff face a high risk of infection during the testing process. In addition, because the gene structure of SARS-CoV-2 is different from that of other RNA viruses, the testing process, quality control, and biosafety measures have been adjusted accordingly. In order to guide laboratory testing, this consensus was developed on the recommendations of the National Health Commission of China, literature, and expert opinion in Wuhan, China.

This consensus was developed for use in laboratories using nucleic acid in the detection of SARS-CoV-2, especially those using real-time polymerase chain reaction (PCR).

The following article was drafted in accordance with the AGREE II reporting checklist (available at http://dx.doi.org/10.21037/atm-20-4060).

Methods

Development of guidance statements

The Consensus of Nucleic Acid Detection of SARS-CoV-2 was formulated to fight against the COVID-19 epidemic and provide suggestions for medical laboratories. At the beginning of the outbreak, there was limited information regarding SARS-CoV-2; therefore, a working group comprised of experienced molecular testing professionals was convened to formulate this consensus. The group included molecular testing experts in China specializing in biosafety and quality management. Issues were discussed online and a list of questions developed, then grouped into four areas of clinical focus (*Table 1*, Table S1). It should be noticed that the guidance statements in this consensus were based on both literature and clinical experience. The working group also deemed it necessary to provide additional clarity in the supporting text and footnotes to supplement their statements, as new testing information became available.

Guidance statements were developed based on both relevant literature, the regulations of The National Health Commission of the People's Republic of China and supplemented with appropriate expert opinion. To maintain independence from commercial organizations, none were

Table 1 Areas of clinical focus

Specimen type and priority, collecting, transportation and receiving (*Table 2*)
Nucleic acid isolation and amplification (*Table 3*)
Quality control (*Table 4*)
Biosafety management and decontamination (*Table 5*)

invited to participate in the development of this consensus. To ensure the applicability of the statements, a review was conducted by the Chinese Society of Laboratory Medical Experts. Specific advice is detailed in the discussion.

Literature review methodology

The literature review examined what specimens could be used in the detection of SARS-CoV-2 along with what priority a particular specimen should be given; furthermore, deactivation of the virus and biosafety management in specimen handling were also examined. Given the limited availability of published data during the research period, especially for real-time PCR testing in the detection of SARS-CoV-2, and as this consensus was not related to the benefits and side-effects of patients, the evidence regarding such was not assessed.

The keyword search incorporated Medical Subject Headings and free-text keywords, listed in Table S2. The literature search was conducted using PubMed as the primary database, along with WANFANG med online, CNKI, VIP databank, and other online sources which included the Chinese Health Commission website (http://www.nhc.gov.cn; http://www.samr.gov.cn/), Selected articles related to SARS-CoV-2 or other RNA viruses, included both articles in Chinese and English. Search strategies are outlined in Table S3. The evidence for the questions were reviewed by the members of the working group and used to develop guidance statements. Evidence levels were assessed using Oxford Centre for Evidence-Based Medicine criterion (see Table S4a).

Consensus process

A modified Delphi methodology was applied among the working group's members to develop a consensus on the guidance statements. Statements were put forward based on both literature and expert opinions. They were sent to members of the working group from the 34 hospitals in China for discussion (Tables S4 and S5). Consensus was defined as an agreement of >75% on a specific statement. If consensus was not reached, it would be revised by the working group for a maximum of three rounds before a decision of "no agreement". Based on comments received during the first round of voting, the working group made a decision to update and revote on statements that reached a 75–85% consensus to improve their clarity (7,8).

Results

A total of 29 guidance statements were voted on in the modified Delphi framework. The final statements are listed in *Tables 2-5* and Tables S5,S6. A quick reference guide to all statements can be found in Table S7. In the first round of voting, 36 responses were received from 34 hospitals/institutions. While consensus was reached on 36/36 statements (Table S4b), four statements were at the threshold achieving 75–85% agreement. The working group opted to revise the four statements to improve clarity. They were sent for a second-round of voting, in which 36 responses were received from 33 hospitals/institutions. Consensus was achieved on all four statements (Table S5b); thus, a third-round was not required. Please refer to Tables S4a,S4b for evidence levels assigned for the publications used to develop each of the guidance statements. These statements apply to the screening and diagnosis of patients with a suspected SARS-CoV-2 infection.

Specimen type, priority, collecting, transportation and handover

Specimen type and priority

Optimal specimen choice was examined to improve the accuracy of detection. Sputum and bronchoalveolar lavage fluid (BALF) have proven to be the most suitable choice (9-11), but the patients with COVID-19 often do not have sputum and taking BALF has proven difficult. In addition, medical staff may face an increased risk infection when collecting sputum and BALF samples. It is recommended that in obtaining an acceptable specimen the order of priority should be given to a nasopharyngeal swab, followed by an oropharyngeal swab, sputum then BALF. Feces can be tested, controlling the source of infection (12). Blood tests

Table 2 Guidance statements: specimen type and priority, collecting, transportation and handover

Guidance statement(s)	Evidence grade
Specimen type and priority	
Following specimens can be selected: nasopharyngeal swab, oropharyngeal swab, sputum and bronchoalveolar lavage fluid (BALF); feces can be tested, controlling the source of infection; the blood tests for diagnosed patients can be used to monitor therapeutic effects (further research support is required)	B
Collect both one nasopharyngeal swab and one oropharyngeal swab at the same time and place them into a single specimen collection tube	C
Specimen collecting	
The use of lysate is recommended (supplied in the nucleic acid extraction kit) to replace the existing specimen preservation solution	C
It is recommended that proteinase K (1 g/L) is used to homogenize the sputum and BALF, and it can be added in the collection container in advance	C
Transportation and handover	
Specimens should be sent to a qualified SARS-CoV-2 nucleic acid testing laboratory, approved by the health administrative organization	A
The specimen transportation container should be water-proof, breakage-proof, leak-proof, and both resistant to high pressure along with high and low temperatures	
Two individuals should be sent to accompany the specimen transportation. If conditions permit, it should be equipped with a specimen transfer monitoring device	A
Both the specimen delivery personnel and the receiving personnel should sign during specimen handed over	D

Table 3 Guidance statements and references: nucleic acid isolation and amplification

Guidance statement(s)	Evidence grade
Virus inactivation	
The virus can be inactivated by heating to a temperature of 56 ℃ for 30 min or 60–65 ℃ for 20 min. The specimen preservation solution should contain an RNA protectant	C
Nucleic acid isolation	
Nasopharyngeal swab and oropharyngeal swab with cell lysate can be used directly for nucleic acid isolation. If necessary, virus inactivation steps may be added	D
Sputum is incubated for 15 min at 55 ℃ for homogenization. If proteinase K is not pre-added to the sputum collection cup, this step should be performed after virus inactivation	B
Automated nucleic acid extraction methods are recommended	D
Amplification reagents	
The amplification reagent should contain at least two sites of the SARS-CoV-2 gene (open reading frame 1a/b and nucleocapsid protein or envelope protein E)	A
Results	
The results should be reported as positive or negative	A

Table 4 Guidance statements and references: quality control

Guidance statement(s)	Evidence grade
If cell lysate or proteinase K is added to the specimen collection tube, the expiration date and storage conditions should meet the criteria	D
Specimens should be transported to the hospital within 2–4 h to prevent degradation of the RNA	A
The specimens should be processed promptly	D
Set a reagent control, positive control, negative and a positive quality control	A
Place in an ice bath for 3–5 min or at room temperature for >10 min after heating or centrifuging for decreasing the risk of aerosol	D
The cautious use of 75% ethyl alcohol is recommended	D

Table 5 Guidance statements and references: biosafety management and decontamination

Guidance statement(s)	Evidence grade
Sample processing should be carried out by at least two or more individuals	D
Level three protection is recommended. If necessary, one can wear a waterproof apron or waterproof isolation clothing	A
All work regarding specimen treatment should be carried out in a biological safety cabinet with an efflux function	D
Individuals involved in specimen collection must pass the Department of Hospital Infection Management or Superior Management biosafety training	D
The waste generated during testing should be immediately transferred outside of the working area through the waste passage. Three-layer medical waste packaging bags are recommended	A
Terminal disinfection is carried out using a hydrogen peroxide disinfector or other methods	A
Protective clothing, shoe covers, gloves, and masks are sterilized with a 75% ethanol solution and collected in three layers of medical waste packaging bags	A
In order to minimize the possibility of residual nucleic acid contamination, decontamination should be performed as follows: medical waste should be treated with 0.55–1% sodium hypochlorite; 0.55–1% sodium hypochlorite is used to spray or wipe for the disinfection treatment of biosafety cabinets, pipettes, work surfaces, and other supplies; floor disinfection should be done at least once a week; the amplification product should be packed tightly in a disposable medical garbage bag and transferred to the amplification product disposal area through the waste passage. The amplification products may also be treated through immersion in a disinfectant containing 0.55–1% sodium hypochlorite (>1 h treatment is recommended)	A
The operator should dispose of waste promptly and this should be recorded. The waste should not be removed from the laboratory without permission. Medical waste should be treated in accordance with the Administrative Measures on Medical Wastes in Medical and Health Institutions	A

for diagnosed patients can be used to monitor therapeutic effects (further research support is required) (13).

Specimen collecting

(I) Nasopharyngeal swab: the nasopharyngeal swab should be collected from patients in the early-onset stage. Tilt the patient's head slightly backward. The distance between the tip of the nose and the ear lobe is precisely measured with a swab and marked with a finger. Insert the swab to the measured distance. Leave the swab in the nose for 15–30 s, gently rotating 3–5 times then immediately place it in the sample collection tube filled with 2 mL lysate (supplied in the nucleic acid extraction kit) or a cell preservation solution containing the RNase inhibitor (14).

(II) Oropharyngeal swab: the oropharyngeal swab should

be collected from patients in the early-onset stage. It is recommended that a sterile flock swab be used for sampling by wiping the back wall of the pharynx with moderate force. During the process, touching the tongue should be avoided. The swab should be placed into the same collection tube as the Nasopharyngeal swab.

(III) Sputum: deep cough sputum should be collected in a disposable sterile screw-cap sampling cup containing 2 mL of proteinase K (1 g/L) (15,16), closing the container upon collection. The test should be conducted within 30 min if possible. If the specimen needs to be transported over a long distance, proteinase K should not be added in advance.

(IV) Bronchoalveolar lavage fluid (BALF): in the case of severe patients or patients with rapidly progressing pneumonia, the clinician should aseptically collect ≥5 mL BALF into a 50-mL sterile container.

(V) Feces: for patients in the early-onset stage with gastrointestinal symptoms such as diarrhea, 3–5 g (soybean size) stool samples are collected in screw-capped specimen collection tubes containing 2 mL saline (RNase inhibitors added if possible).

(VI) Blood: blood could be collected from patients within 7 days of onset or those considered to have viremia. Usually, 2–4 mL blood samples are collected using vacuum blood vessels containing an ethylenediaminetetraacetic acid (EDTA) anticoagulant.

To increase the accuracy of detection both, one nasopharyngeal swab and one oropharyngeal swab should be both collected into a single collection tube at the same time (17).

Specimen transportation

Specimens should be sent to a qualified SARS-CoV-2 nucleic acid testing laboratory approved by the health administrative organization (18).

Transport should be done in a three-layer packaging system: inner container, along with middle, and outer packaging. They should be water-proof, breakage-proof, leak-proof, and be both resistant to high pressure along with high and low temperatures. Relevant biohazard labels, warnings, and prompts should be displayed on the transport containers and packaging materials. The leak-proof inner container is to be packaged with a biohazard symbol pasted on it and placed in the middle container. Infectious materials identification is placed on the outer packaging. A sufficient amount of absorbent material should be placed between the inner and middle containers. The middle container should be secured in a hard outer container, and gel ice packs should be placed between the middle and outer containers (19,20).

If a specimen needs to be transported over a long distance, a "Qualified Transportation Certificate" should be processed in accordance with the "Management Regulations on the Transport of Highly Pathogenic Microorganisms (Poison) Species or Samples Infecting Humans" (21). SARS-CoV-2 specimens belong to category A, with the UN identification number UN2814. If transportation is by air, the packaging should also comply with the PI602 classification and packaging requirements of the International Civil Aviation Organization (ICAO) document Doc9284-AN/905 "Technical Rules for the Safe Transport of Dangerous materials by Air" (19,22).

Two individuals should be sent to accompany the transportation of the specimens (23). If conditions permit, shipping containers should be equipped with a specimen transfer monitoring device.

Specimen handover

Both the specimen delivery personnel and the receiving personnel should sign during the specimen handed over. Before receiving specimens, the outer packaging of the specimen transfer container is to be checked for damage, and the specimens should be stored in a designated refrigerator (24). If the specimens cannot be tested immediately, they may be stored at 4 ℃ for a short period (the total duration from the collection time should not be >24 h) or at −70 ℃ for a prolonged period (17). Samples that have been refrigerated over 4 hours at 4 degrees may still be viable; yet, the rate of decay has not been documented. Further research is needed to study the rate of sample decay.

Nucleic acid isolation and amplification

Virus inactivation

It is recommended that a water bath be used for virus inactivation. The virus can be inactivated by heating to a temperature of 56 ℃ for 30 min or 60–65 ℃ for 20 min (25,26). In order to prevent specimens from floating, a heavy object may be over-top of them. Specimens are to be agitated gently, once every 10 minutes.

Nucleic acid isolation

In order to ensure the safety of the personnel along with the

purity and efficiency of nucleic acid extraction, automated nucleic acid extraction methods are recommended.

(I) Nasopharyngeal swab and oropharyngeal swab: specimens with cell lysate can be used directly for nucleic acid isolation. If necessary, virus inactivation steps can be added (19).
(II) Sputum: samples are incubated for 15 min at 55 ℃ for homogenization (15). If proteinase K was not added in advance, this step should be performed after virus inactivation.
(III) BALF and feces: samples are individually placed into a sealed bag and should be agitated thoroughly to mix well.
(IV) Blood: plasma is obtained by centrifuging at 1,500 ×*g* for 10 min, and then incubated on ice for 3–5 min or at room temperature for >10 min. Subsequently, the nucleic acid is extracted.

Amplification reagents

The amplification reagent should contain at least two sites of the *SARS-COV-2* gene (open reading frame 1a/b and nucleocapsid protein or envelope protein E) (19).

Results

According to the Laboratory Testing Technical Guide of New Coronavirus Infection Pneumonia (19), the results should be reported as positive or negative and supply interpretation for each result, along with suggestions for the next steps.

(I) Positive results: *ORF1ab* gene and *N* gene are both positive (19).
(II) Negative results: if the result shows no cycle threshold (Ct) value or Ct ≥40 at the two detection sites (refer to the reagent manual for details), it can be reported as negative.
(III) Gray zone results: when the Ct value is between 37 and 40, it is a gray zone result (refer to the reagent manual for details).

Quality control

Specimen

If the cell lysate or proteinase K is added to the specimen collection tube, the expiration date and storage conditions should meet the criteria.

Specimens should be transported and examined as soon as possible after collection, ideally within 2–4 h. The transportation time should not exceed 24 h when transporting at 2–8 ℃. If the transportation time exceeds 24 h, they should be stored and transported at ≤–70 ℃ (17). Sputum, oropharyngeal, and nasopharyngeal swabs are preserved in homogenization agents or cell preserving agents, which might cause degradation of nucleic acids due to prolonged operation.

Control and quality control

(I) The reagent control contains only PCR amplification reagents.
(II) It is recommended that the nucleic acids of the positive samples be used as a positive control.
(III) As Nucleic acid may form an aerosol, for negative quality control, after each test 3–5 tubes containing 2 mL of sterile water should be placed in different positions at different working areas to monitor for airborne contaminants that could affect test results.
(IV) Samples with a lower viral load can be used as a positive quality control after inactivation. When the corresponding quality control materials are provided by the inter-room quality assessment agency, the laboratories should participate in the inter-room quality assessment.

Negative and positive quality control materials should be operated in parallel with the specimen testing (27).

Cautious use of 75% ethanol

For reasons of laboratory safety and to inhibit the effects of gene amplification, the cautious use of 75% ethyl alcohol is recommended.

Aerosol formation

In order to reduce the formation of aerosols, procedures should be performed gently during the specimen treatment. Subsequently, specimens can be placed in an ice bath for 3–5 min or at room temperature for >10 min, after heating or centrifuge.

Optimizing work process

Optimized working procedures are beneficial for detection. An expedited work process is conducive to reducing the degradation of nucleic acids. In order to reduce the cross-contamination of nucleic acids in the various sections of the laboratory, it is advisable to carry out SARS-CoV-2 nucleic

acid testing through the division of labor and cooperation.

Biosafety management and decontamination of nucleic acid

Personal

Individuals involved in specimen collection should be trained and certified in biosafety as organized by the Department of Hospital Infection Management or Superior Management. Sample processing should be carried out at least by two or more individuals depending on the specimen numbers (23).

Protective equipment

It is recommended that individuals should use level three protection equipment during the whole process, including work clothes, disposable work hats, double gloves, protective clothes, KN95/N95 masks, or higher-level particulate protective masks or a powered air-purifying respirator, a protective face screen, work shoes or rubber boots and waterproof boot covers. If necessary, one may wear a waterproof apron or waterproof isolation clothing (20).

The work place for specimen collection should be equipped with garbage bins for infectious waste, medicine for emergency incidents, and appropriate ventilation to prevent the spread and infection of pathogenic microorganisms (28).

In order to deal with accidental spillage, the specimen transportation personnel should carry 75% ethanol.

Handling specimens

All work relating to the treatment of specimens should be carried out in a biological safety cabinet with an efflux function. The biosafety cabinet should be equipped with a waste bucket containing a 0.55–1% chlorine disinfectant. If possible, a layer of water-absorbent material should be spread on the operating surface of the biosafety cabinet.

Disinfection

A solution of 75% ethanol is used to spray the inner wall of the nucleic acid extractor and other parts of the equipment that can be treated with it, followed by irradiation with ultraviolet light for 30 min (20). A 75% ethanol or 0.55–1% chlorine-containing disinfectant is used to clean work surfaces, followed by UV irradiation for 30 min to sterilize the surface. The terminal disinfection is carried out using a hydrogen peroxide disinfector or other methods (29).

The frequency of floor disinfection can be determined according to the number of specimens, but at least once a week is recommended. Ground disinfection can be carried out using a 0.55–1% chlorine-containing disinfectant after ultraviolet disinfection.

Waste disposal

Protective equipment including clothing, shoe covers, mask, and gloves are sterilized with 75% ethanol and collected in three layers of medical waste packaging bags. The outer packaging bags should be labelled with "medical waste generation site", department, date, category, and marked as "new coronavirus infection pneumonia" or abbreviated as "new coronavirus" in the special instructions. All items should be treated as normal medical waste after autoclaving. Waste should be immediately transferred outside of the working area through the waste passage. Protective face screens can be treated with 75% ethanol (30).

Decontamination of nucleic acid

In order to minimize the possibility of residual nucleic acid contamination, decontamination can be performed as follows:

(I) Medical waste that has been in contact with a specimen, such as the pipette tips, sample tubes, and small centrifuge Eppendorf (EP) tubes, should be treated with a 0.55–1% sodium hypochlorite (31).

(II) A disinfection treatment of 75% ethanol or 0.55–1% sodium hypochlorite solution is used to spray or wipe the biosafety cabinets, work surfaces, pipettes, and other supplies.

(III) The frequency of floor disinfection can be determined according to the amount of work and specimens, but at least once a week is recommended. Ground disinfection methods are the same as floor disinfection.

(IV) The amplification products should be packed tightly in a disposable medical garbage bags and transferred to the amplification product disposal area through the waste passage. The amplification products can also be treated in a specific room, and the amplification products should be immersed in a disinfectant containing a 0.55–1% Sodium hypochlorite (>1 h treatment is recommended) (31).

Waste disposal management

The operator should dispose of waste promptly and this should be recorded. The waste should not be removed from

the laboratory without permission. Medical waste should be treated in accordance with the Administrative Measures on Medical Wastes in Medical and Health Institutions (32). Those that can be incinerated should be burned promptly. If not, they should be transported to a landfill after disinfection.

Discussion

Safety and quality control are two major issues during molecular testing, especially for highly pathogenic microorganism tests, like SARS-CoV-2. In order to protect laboratory staff and the environment, it is necessary to inactivate SARS-CoV-2 through either chemical or physical methods (33). A 75% ethanol solution may also be used for inactivation. A lysis buffer is recommended, and may be used instead of a specimen preservation reagent, however, any changes in test procedure will affect the performance of the test (34). There are no consistent opinions about how virus inactivation will affect test sensitivity. Reasons for this lay in the different inactivation methods and specimen preservation reagents. The consensus within the literature suggests that preventing RNA degradation is the most important (35). If the specimen was collected in a virus preservation liquid, human respiratory epithelial cells will be destroyed and the RNase released, which is a major factor in RNA degradation. There is also a general consensus that inactivation through heating will decrease the sensitivity of the test (35). Other researchers have found the effect of RNA degradation will be decrease obviously when the specimen is added to an RNase inhibitor such as guanidine salt (not published officially). Because the lysis buffer contains guanidine salt, it can be used for virus lysis; at the same time, it will provide a protective effect for the RNA.

In theory, specimens can be directly used for RNA isolation when they are stored in a lysis buffer; however, it is hard to confirm whether the virus is inactivated completely using a lysis buffer because there is no conclusive evidence. For safety specimens should be inactivated by both preserving them in a lysis buffer and through heating. It was reported that the Middle East respiratory virus could be inactivated at 56 or 65 ℃ effectively (26). So taken together, using lysis buffer in specimen collection and inactivation of the virus through heating ought to protect laboratory staff efficiently. Heat the sample for 20 minutes to inactivate it. The virus inactivation time indicates the time required after a specimen reaches the set temperature. Due to different types of sample collection containers, the time to reach the set temperature is also different and should be tested in advance.

Along with virus inactivation, personal protection is very important. During the whole detection process, level three protection is recommended during testing. However, it is not necessary for all laboratory staff. The staff involved specimen collecting, specimen transportation, reagents preparation and amplification can appropriately decrease the protection level. In this way, it is possible to optimize the use of protective clothes and decrease laboratory staff costs.

Nucleic acid contamination should be avoided. The most suitable substance for decontamination is hydrochloric acid. However, it is rarely used because of its associated danger. A 0.55–1% sodium hypochlorite solution can destroy nucleic acid effectively (31), so it is recommended that nucleic acid decontamination be done using this solution. It should also be noted that sodium hypochlorite is corrosive; therefore, a clean water flush and then ventilating is necessary after treatment.

There are many methods for the homogenization of sputum, such as using sodium hydroxide (36), proteinase K, phosphate-buffered saline (PBS) and N-acetyl-L-cysteine and sodium citrate (NALC) (37). The Technical Guidelines for the Prevention and Control of New Coronavirus Infections in Medical Institutions recommends the use of a mixed reagent or proteinase K for homogenizing sputum. A proteinase K is suitable for sputum homogenization, and using 1 g/L proteinase K is convenient and recommended (16).

A comparison of the predicted coding regions of *SARS-COV-2* showed that they possessed a similar genomic organization to *bat-SL-CoVZC45*, *bat-SL-CoVZXC21*, and *SARS-CoV*. At least 12 coding regions were predicted, including 1ab, S, 3, E, M, 7, 8, 9, 10b, N, 13, and 14 (38). Detecting more sites will increase sensitivity, but it is hard to report results, and it will increase the cost of the reagent. According to the Technical Guidelines for the Prevention and Control of New Coronavirus Infections in Medical Institutions, we recommend the amplification reagent contains at least two sites of the *SARS-COV-2* gene (open reading frame 1a/b and nucleocapsid protein or envelope protein E). Meanwhile, an amplification reagent with a large reaction system and large sample loading volume is recommended. In addition, amplification kits with different primer pairs could be used to check the results.

Negative results cannot completely exclude a SARS-CoV-2 infection. Sample quality, specimen type, sample collection timing (whether it is in a period of low viral load), along with specimen storage, transportation, and

processing can affect the test results (39). When the clinical manifestations and other examinations highly suspect a SARS-CoV-2 infection, specimen re-collection or the collection of specimens from other parts of the body and repeating the test is recommended. It is necessary to supply an interpretation for each result; at the same time, suggestions for the next steps are needed.

When the test results are ambiguous, the laboratory can implement the following measures: (I) check whether the whole process has an impact on the sample quality, specimen type, sample collection timing (whether it is in a period of low viral load), along with specimen storage, transportation, and processing. (II) The kits from different manufacturers are utilized for repeating the experiment or using another sensitive method (such as a digital PCR method) to confirm the results further. (III) It is recommended that the clinicians re-collect the specimens for re-testing or utilize different types of samples for testing.

This consensus provides detailed information for the detection of *SARS-CoV-2*; however, with a greater understanding of the virus and more scientific evidence, some of these areas can be improved. Further study is recommended on the development of new methods for virus inactivation.

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Footnote

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