

REVIEW

Advances in laboratory detection methods and technology application of SARS-CoV-2

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Abstract

At present, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is raging worldwide, and the coronavirus disease 2019 outbreak caused by SARS-CoV-2 seriously threatens the life and health of all humankind. There is no specific medicine for novel coronavirus yet. So, laboratory diagnoses of novel coronavirus as soon as possible and isolation of the source of infection play a vital role in preventing and controlling the epidemic. Therefore, selecting appropriate detection techniques and methods is particularly important to improve the efficiency of disease diagnosis and treatment and to curb the outbreak of infectious diseases. In this paper, virus nucleic acid, protein, and serum immunology were reviewed to provide a reference for further developing virus detection technology to provide better prevention and treatment strategies and research ideas for clinicians and researchers.

KEYWORDS

antigen, NAAT, SARS-CoV-2, sequencing, serum

1 | INTRODUCTION

Coronavirus disease 2019 (COVID-19) refers to pneumonia caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. It is mainly spread through respiratory droplets and close contact, and people are generally susceptible.^{1,2} According to the "novel coronavirus Diagnosis and Treatment Program (Trial Eighth Revision)" issued by the National Health and Health Commission, the virus is contagious during the incubation period. Since the outbreak of COVID-19 in December 2019, all walks of life around the world have made efforts to cope with it in various aspects (Figure 1). However, at present, COVID-19 still has no specific and effective treatment plan, and a vaccine against the mutant strain is still under development. Early diagnosis and timely isolation are the keys to preventing the further spread of the epidemic. In this paper, the latest

progress in laboratory detection methods of SARS-CoV-2 was reviewed to provide some ideas for better and more accurate identification of SARS-CoV-2.

2 | NUCLEIC ACID DETECTION OF SARS-CoV-2

COVID-19 nucleic acid detection is the most widely used detection method for SARS-CoV-2. Commonly used nucleic acid detection methods include real-time fluorescence quantitative polymerase chain reaction (PCR), loop-mediated isothermal amplification (LAMP) technology, second-generation sequencing (NGS) technology, and so on.^{3,4} Different nucleic acid detection methods have different characteristics and application values.

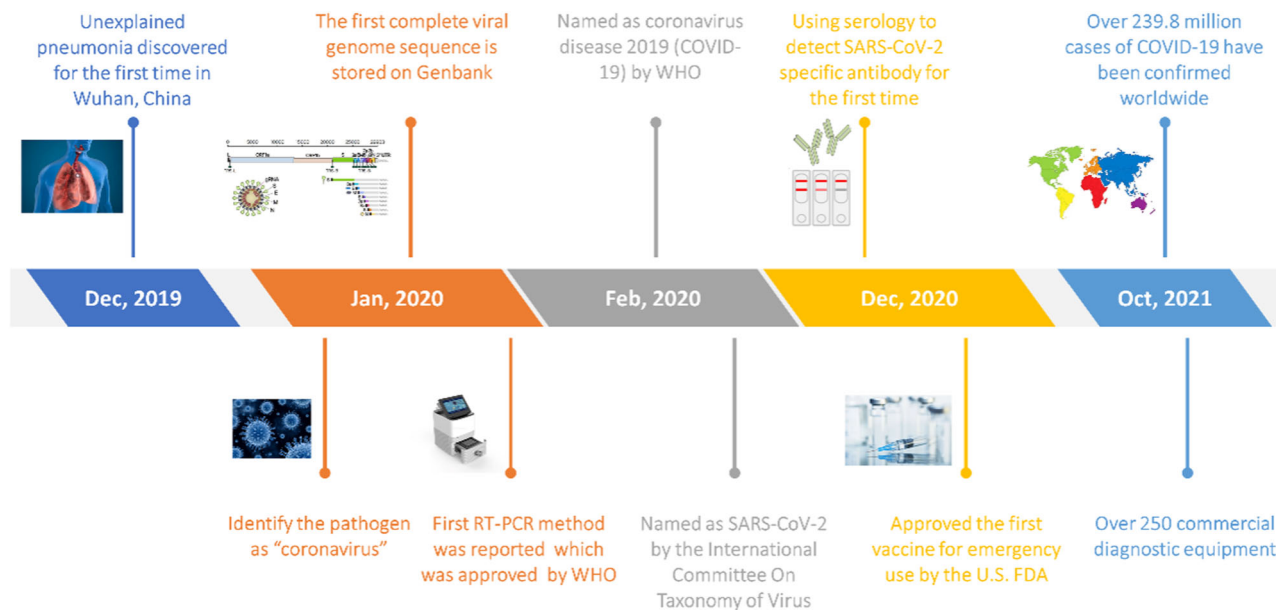


FIGURE 1 Timeline of communication and development in novel coronavirus. COVID-19, coronavirus disease 2019; RT-PCR, reverse transcription-polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization

2.1 | Real-time reverse transcription-PCR

The reverse transcription-PCR (RT-PCR) process of SARS-CoV-2 includes specimen collection, specimen transportation to the laboratory, specimen lysis, virus RNA extraction and purification, RT-PCR amplification, detection, and analysis.⁵ The samples were lysed before RT-PCR amplification, and nucleic acids were extracted to remove potential inhibitors that might hinder target amplification. Both lysis/extraction and RT-PCR amplification can be performed by manually processing the instrument or automated operation. The detection rate of RT-PCR is different in patients with different specimens of COVID-19. As shown in Table 1, the detection rate of bronchoalveolar lavage fluid, sputum, rectal swabs, and nasopharyngeal swabs was higher by RT-PCR. However, the detection rate of the virus in blood and urine samples is meager.

The RT-PCR of SARS-CoV-2 has the characteristics of high sensitivity, strong specificity, rapidity and accuracy, and mature technology, which is widely used in the screening of SARS-CoV-2. However, it is necessary to strictly control the quality control of sample collection, detection, result interpretation, and so forth. In addition, to avoid false positive- or false-negative results, misdiagnosis or missed diagnosis may occur.

2.2 | Isothermal amplification technology

Except for RT-PCR, NAAT research has been launched to develop portable and rapid diagnostic tests for SARS-CoV-2. Isothermal amplification (IAT) replaces the high-temperature melting step in PCR with special enzymes. As it can be carried out under constant temperature, it does not need expensive equipment such as a

thermal cycler. The principle of IATs is thermal denaturation or enzymatic denaturation of nucleic acids, followed by the nucleic acid amplification reaction.¹² Isothermal NAAT technology includes transcription-mediated amplification (TMA), nick enzyme-assisted reaction (NEAR), LAMP, reverse transcription-recombinase polymerase amplification (RPA), and repeating CRISPR-Cas-related systems with short palindromes at regular intervals. The following sections describe examples of IAT and its current and potential applications.

2.2.1 | Reverse transcription-recombinase polymerase amplification

The reaction mechanism of RT-RPA is relatively simple, but the reaction components are relatively complex. Unlike RT-PCR, RT-RPA does not need complex instruments such as thermal cyclers, thus simplifying the detection process. The ease of use of this isothermal technique makes RT-RPA an attractive candidate for molecular testing. RT-RPA technology has been applied to the detection of other RNA viruses, such as the Ebola virus.¹³ However, to date, the data used to detect SARS-CoV-2 are not perfect.

Kim et al. used modified RT-RPA to detect SARS-CoV-2 and achieved a sensitivity of approximately four copies/reaction in a 10-minute reaction using lateral flow immunoassay (LFIA) readings. Their RT-RPA correctly identified 18 artificial samples produced by adding heat-inactivated virus to flocked nasopharyngeal swabs or saliva.¹⁴ Xia and Chen described another improved single-tube RT-RPA version introduced by GenDx, called reverse transcriptase recombinase amplification, and the whole packaging procedure of exponential amplification from RNA.¹⁵

TABLE 1 Real-time fluorescence quantitative PCR for the detection of various specimen types from confirmed patients with COVID-19

Author of article	Types of research	Specimen type	Positive number	Total specimens	Positive rate
Wang et al. ⁶	Cross-sectional study	Bronchoalveolar lavage fluid	14	15	93.3%
		Bronchoscopic brush biopsy	6	13	46.2%
		Phlegm	75	104	72.1%
		Nasal swab	5	8	62.5%
		Swallow swab	126	398	31.7%
		Night soil	44	153	28.8%
		Blood	3	307	1.0%
		Urine	0	72	0.0%
Chen et al. ⁷	Retrospective study	Swallow swab	65	167	38.9%
		Sputum	155	206	75.2%
		Night soil	17	64	26.6%
Xu et al. ⁸	Prospective research	Swallow swab	22	49	44.9%
		Rectal swab	43	49	87.8%
Chan et al. ⁹	Case report	Nasopharyngeal swab	4	5	80.0%
		Swallow swab	2	3	66.7%
		Sputum	2	2	100.0%
		Serum	1	3	33.3%
		Blood plasma	0	4	0.0%
		Urine	0	5	0.0%
		Night soil	0	4	0.0%
Lo et al. ¹⁰	Perspective study	Nasopharyngeal swab	57	84	67.9%
		Sputum	1	1	100.0%
		Urine	0	49	0.0%
		Night soil	46	79	58.2%
Mishra et al. ¹¹	Retrospective study	Saliva	58	250	23.2%
		Urine	8	318	2.5%
		Night soil	396	779	50.8%
		Blood	7	21	33.3%
Summary	-	Bronchoalveolar lavage fluid	14	15	93.3%
		Bronchoscopic brush biopsy	6	13	46.2%
		Sputum	233	313	74.4%
		Nasal swab	5	8	62.5%
		Swallow swab	215	617	34.8%
		Night soil	503	1079	46.6%
		Blood	10	328	3.0%
		Urine	8	444	1.8%
		Nasopharyngeal swab	61	89	68.5%
		Rectal swab	43	49	87.8%
		Serum	1	3	33.3%
		Blood plasma	0	4	0.0%
		Saliva	58	250	23.2%

Abbreviations: COVID-19, coronavirus disease 2019; PCR, polymerase chain reaction.

2.2.2 | Transcription-mediated amplification

TMA is an IAT that amplifies RNA from an RNA template.^{16,17} This technology has been applied to high-throughput analyzers for SARS-CoV-2 diagnosis.^{18–20} In this paper, through the search and collation of relevant literature, some research on the diagnosis of SARS-CoV-2 by TMA technology is also analyzed and experimented with. Details are shown in Table 2.

2.2.3 | Nick enzyme-assisted reaction

The NEAR amplification reaction occurs under isothermal conditions and can be divided into duplex formation and products. The mechanism of NEAR amplification of nucleic acids is relatively complex, but the detection process is rapid and straightforward, and the processing time is as low as 5–15 min. Compared with LDT or commercial NAAT, many recent studies have shown that the consistency of specificity/negative percentage in detecting SARS-CoV-2 by NEAR technology is close to 100%. However, the sensitivity/positive percentage consistency is relatively poor, ranging from 48% to 70%. Other studies have reported a high specificity/NPA (~100%) and sensitivity/PPA value higher than 90%.²⁴

2.2.4 | Reverse transcription-loop-mediated isothermal amplification

RT-LAMP is a new isothermal nucleic acid amplification technology attracting attention.²⁵ Similar to RT-PCR, RT-LAMP first transcribes target RNA into cDNA by reverse transcriptase and then carries out an amplification reaction. As this technology can use different primers to amplify multiple target sequences in the same reaction exponentially, it has high detection efficiency, strong specificity,

and sensitivity. Meanwhile, expensive reagents or instruments are not needed, which is helpful to reduce the detection cost of COVID-19 nucleic acid and improve detection efficiency. A commercial kit for real-time SARS-CoV-2 RT-LAMP was developed and compared with RT-PCR. The results of the commercial RT-LAMP kit were consistent with those of RT-PCR, and the clinical sensitivity was 76.3%.^{26,27} Due to its simplicity, RT-LAMP technology is also used to develop rapid POCT products. With this technology, the results can be obtained within 30 min.

Compared with RT-PCR, when tested on 21 nasal swabs, the sensitivity was 80%, and the specificity was 73%, which was relatively low.²⁸ The authors of this study believed that although the sensitivity and specificity are poor, the analysis is still valuable in some clinical applications. Other studies using RT-LAMP show that compared with LDT based on RT-PCR, the performance of common sample types using RT-LAMP is different.²⁹ Generally, the range of reaction copies is consistent with some LDT and commercial RT-PCR results. Due to the high sensitivity of LAMP technology, aerosol pollution easily occurs during the experiment, which leads to false positives. In addition, this method has relatively high requirements for primer design, which is developed late, and the technology is not mature enough. There was no difference between LAMP operation, personal protection, laboratory requirements, and RT-PCR. However, the amplification time of LAMP is greatly reduced, so the exposure time of operators is reduced.

2.2.5 | CRISPR-Cas technology

Gene-editing technology can modify and edit genes or transcripts in organisms at fixed points and achieve the function of detecting pathogens by identifying specific sequences in test samples. The CRISPR-Cas system represents this technology. CRISPR and its Cas protein are derived from the photonuclear defense system against foreign nucleic acids.³⁰

TABLE 2 Performance verification of transcription-mediated amplification technology in the diagnosis of SARS-CoV-2

Author of article	Research method	Specimen type	Number of specimens	Positive coincidence rate	Negative coincidence rate
Pham et al. ²¹	Hologic Panther Fusion	Nasopharyngeal swab	140	100%	98.7%
Schneider et al. ²²	Hologic Aptima	Upper respiratory tract specimen	103	95.7%	–
Smith et al. ¹⁹	Hologic Panther Fusion	Nasopharyngeal swab	150	98.7%	100%
	Hologic Aptima	Nasopharyngeal swab	150	94.7%	100%
	BioFire Defense	Nasopharyngeal swab	150	98.7%	100%
Cordak et al. ²³	Hologic Aptima	Upper respiratory tract specimen	157	99.4%	99%
Summary	Hologic Panther Fusion	Nasopharyngeal swab	290	99.3	99.3
	Hologic Aptima	Upper respiratory tract specimen	260	95.8	–
		Nasopharyngeal swab	150	94.7	100%
	BioFire Defense	Nasopharyngeal swab	150	98.7%	100%

Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

When activated, the Cas protein can show local DNase or RNase activity, which leads to local cleavage of target DNA or RNA (*cis*-cleavage) and incidental damage to adjacent single-stranded DNA or RNA (*trans*-cleavage). By cutting RNA or DNA from a band, the detection result is visually visible, and the purpose of diagnosis is achieved.

It has become an active research direction to apply CRISPR–Cas technology to SARS-CoV-2, but its analytical performance needs further verification. Studies have shown that CRISPR-based detection can identify SARS-CoV-2 at a low viral load.³¹ In summary, the data show the difference in detection performance based on CRISPR, and the sensitivity ranges from 80% to 100%. Although further verification is needed to fully understand the advantages of CRISPR–Cas technology, it has great potential in applying POCT equipment or a high-throughput test platform. In addition, if CRISPR–CAS technology is combined with recombinant polymerase amplification technology, it can amplify a large number of trace nucleic acids in samples at a constant temperature and has the advantages of simple operation, high sensitivity, and fast detection speed, which may compensate for the shortcomings of other existing technologies.

The above IAT techniques have advantages and disadvantages in experimental operation and effect, among which RT-RPA does not need complex instruments such as thermal cyclers. The detection process is simplified. At present, the data used for detecting SARS-CoV-2 by this method are not perfect. Compared with RT-PCR, TMA technology has similar sensitivity and detection coincidence rate and relatively high accuracy. Its clinical performance is worth looking forward to. The specificity of the NEAR technique for SARS-CoV-2 detection is close to 100%, but the sensitivity is relatively poor, which needs further optimization. RT-LAMP technology does not need expensive instruments and is easy to operate to develop rapid POCT products. However, aerosol pollution and false positives easily occur when the cap is opened in the experimental process, and it has high requirements for primer design. Technology is not yet mature; CRISPR–Cas technology has high efficiency and low cost, but the CRISPR/Cas system has occasional off-target effects. Experimental personnel should wear protective articles for biosafety level 3 laboratories and complete nucleic acid extraction in biosafety cabinets with biosafety level 2 laboratories. Subsequent fluorescence detection was carried out on the CRISPR platform without opening the cover again. It can effectively ensure the safety of operators.

2.3 | Genome sequencing-SARS-CoV-2 NGS

Gene sequencing technology has been successfully applied to identify unknown viruses, conducive to our rapid response to the outbreak of new infectious diseases. Scientists in China isolated SARS-CoV-2 from patients at the early stage of the outbreak using the metagenomics technology of NGS sequenced it and provided the sequence to the World Health Organization on December 12, 2020. It was conducive to the global response to the epidemic. For the 30 kb SARS-CoV-2 genome, NGS can quickly obtain high-quality sequences directly from clinical specimens, and strategies such as

amplification enrichment or bait capture are used to facilitate the sequencing of the virus targets.³² NGS involves synthetic sequencing, ligation sequencing, and ion semiconductor sequencing, each with its advantages and limitations.^{33,34}

According to FDA EUA guidelines, a single commercial kit for atypical pneumonia (Illumina, Inc.) has been approved as a clinical diagnostic test in COVID-19, which is based on synthetic sequencing. However, there are no data to describe its performance, advantages, or limitations compared with standard detection methods such as RT-PCR. In addition, only a limited number of studies have explored the purpose of NGS for SARS-CoV-2 detection.³²

For example, using the NGS protocol developed by the laboratory, Bhojar et al. compared the results of repeated treatment of 752 clinical specimens by NGS and real-time fluorescence quantitative RT-PCR on single flow cells.³⁵ The coincidence rate of the two methods was very high, and the positive rate of NGS diagnosis increased by 5.7% (6 cases were negative by PCR, and 21 cases were uncertain by PCR). This study demonstrated the feasibility of processing 1536 samples in a total of 17 h (11 h for sequencing and 6 h for analysis).³⁵ In another study, a low-cost NGS method proved to be highly sensitive to SARS-CoV-2, and its sensitivity was equal to or higher than that of some RT-PCR methods. However, only 10 samples (five positive samples and five negative samples) were tested in this study. If the flux is increased to their proposed workflow of 192 samples within 8 h, it is unclear whether high sensitivity will still occur. Compared with limited samples (31 positives and 33 negatives), the coincidence rate between NGS and RT-PCR was 100%.³⁶ Generally, some data support the potential of NGS as a diagnostic tool for SARS-CoV-2, but further analysis is needed to understand its advantages and limitations.

In summary, NGS is conducive to the initial identification and in-depth study of SARS-CoV-2, and its accuracy is very high. However, NGS requires more equipment, takes more time and costs, and is unsuitable for rapid clinical screening.

2.4 | Detection of SARS-CoV-2 antigen

Antigen detection methods, such as NAAT, detect active replication viruses in the early stage of SARS-CoV-2 infection. Antigen detection is mainly based on two antigens in SARS-CoV-2 detection, namely, the S protein and N protein. High-throughput antigen-based detection can be performed on semiautomatic or automated instruments using enzyme immunoassay technology. SARS-CoV-2 AG-RDT is usually advertised as POCT equipment and used for rapid testing and instant management of patients in doctor's offices or clinics. Examples approved in laboratories or POCT environments in the United States or Canada include relying on colorimetric LFIA with visual readings, antigen detection using fluorescence-based LFIA, microfluidic immunofluorescence analysis chromatographic digital immunoassay, and so forth.

Although EUA has approved some AG-RDTs and high specificity has been observed in antigen-based detection methods, the sensitivity of AG-RDTs is low (Table 3). Seo et al. studied a biosensor based on a field-effect transistor, which can detect SARS-CoV-2 with

a concentration of 2.42×10^2 copies/ml in clinical specimens in approximately 3 min without sample pretreatment.³⁹ Antigen detection is suitable for early detection and has the characteristics of rapid detection without expensive equipment and laboratories, and the false positive rate of detection results is also low.

2.5 | Serological immunological methods for the detection of SARS-CoV-2

SARS-CoV-2 infection can stimulate the body to produce specific antibodies. This means that the laboratory can detect SARS-CoV-2-specific

TABLE 3 Performance verification of table antigen detection technology in SARS-CoV-2 diagnosis

Research method	Sample number or sample size	Average sensitivity	Average specificity	References
Antigen detection	328	57.6	99.5%	37
Antigen detection	148	30.2	–	38
Antigen detection	1.8×10^5 copies/ml	100%	–	39
	9.4×10^3 copies/ml	70.6%		
	4.9×10^2 copies/ml	46.9%		
Antigen detection	138	50%	100%	40

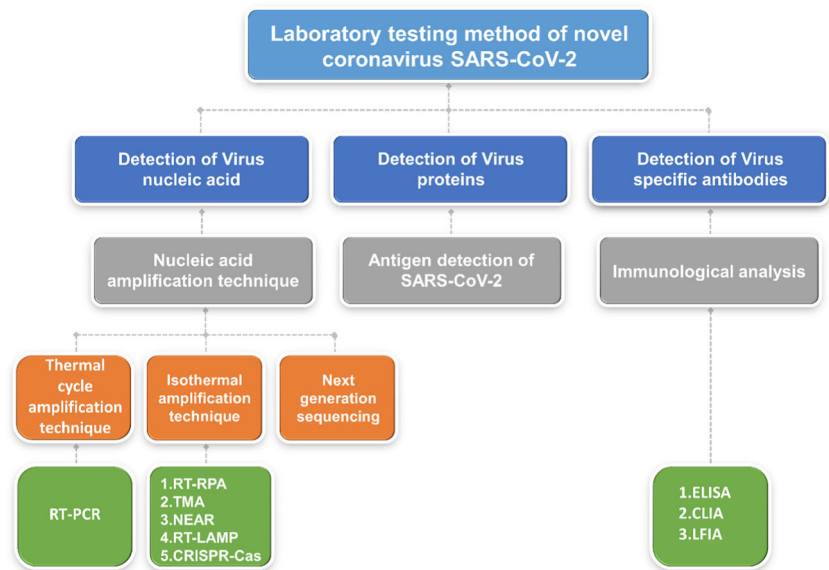
Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

TABLE 4 Performance verification of serological detection technology in SARS-CoV-2 diagnosis

Research method	Detection object	Onset time	Average sensitivity	Average specificity	References
LFIA	IgM	Week 1	25.3%	–	42
		Week 2	51.8%		
		Week 3	69.9%		
	IgG	Week 1	13.4%		
		Week 2	50.1%		
		Week 3	79.7%		
ELISA	IgM	Week 1	26.7		
		Week 2	57.6%		
		Week 3	78.4%		
	IgG	Week 1	23.7%		
		Week 2	65.3%		
		Week 3	82.1%		
CLIA	IgM	Week 1	50.3%		
		Week 2	74.3%		
		Week 3	90.6%		
	IgG	Week 1	53.2%		
		Week 2	85.4%		
		Week 3	98.9%		
LFIA	IgM	–	61.8%	96.6%	39
	IgG		64.9%	97.6%	
ELISA	IgM		81.7%	99.7%	
	IgG		80.6%	98.9%	
CLIA	IgM		84.3%	96.6%	
	IgG		93.5%	97.8%	

Abbreviations: CLIA, chemiluminescence; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IgM, immunoglobulin M; LFIA, lateral flow immunoassay; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

FIGURE 2 Methods and techniques for detecting or identifying severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in novel coronavirus. CLIA, chemiluminescence; ELISA, enzyme-linked immunosorbent assay; LFIA, lateral flow immunoassay; NEAR, nick enzyme-assisted reaction; RT-LAMP, reverse transcription-loop-mediated isothermal amplification; RT-PCR, reverse transcription-polymerase chain reaction; RT-RPA, reverse transcription-recombinase polymerase amplification; TMA, transcription-mediated amplification



antibodies in blood sources such as serum, plasma, or whole blood based on the immunological principle of antigen-antibody specific binding.⁴¹ Given that the typical time of detecting the SARS-CoV-2 immune response is approximately 1–2 weeks, serological detection has a limited diagnostic effect on SARS-CoV-2 in the acute stage of disease, but it may be valuable once the immune response occurs in time. Commonly used methods include enzyme-linked immunosorbent assay, chemiluminescence (CLIA), and LFIA. According to a meta-systematic evaluation and meta-analysis published in the past, we summarized the sensitivity and specificity of the three methods as follows (Table 4). The sensitivity of the three methods is related to the duration of onset, and the sensitivity of the three methods in the third week of onset is higher than that in the first 2 weeks. The CLIA method is the most sensitive of the three serological detection methods, and the specificity is not much different.

The diagnosis of SARS-CoV-2 is mainly based on nucleic acid detection and gene sequencing. However, nucleic acid detection will be affected by many factors, such as the infection cycle of patients, specimen types, specimen collection methods, specimen preservation, and transportation conditions, and the performance of detection kits, and false positives or false negatives may occur. In addition, gene sequencing has high requirements for equipment, a long time, and high cost. It is not suitable for the rapid screening of large clinical samples. Serum immunology technology can be used to supplement molecular biology technology to increase the accuracy and reliability of SARS-CoV-2 detection. For example, when the SARS-CoV-2 test is false negative or the infection time is long, it can be combined with SARS-CoV-2 specific antibody (immunoglobulin M/immunoglobulin G [IgM/IgG]) detection results in serum to determine whether it has been infected with SARS-CoV-2 recently or in the past. This method is helpful for the diagnosis of nucleic acid-negative but clinically suspected cases. According to the “novel coronavirus Diagnosis and Treatment Plan (Seventh Edition),” the detection of specific antibodies in serology has been included in the diagnosis basis of SARS-CoV-2 infection.

Serum immunological detection has the characteristics of high efficiency and high specificity and can be used as a powerful auxiliary diagnostic method. However, the detection of antibodies will be affected by some interfering substances in blood samples, such as rheumatoid factor, antinuclear antibody, nonspecific IgM, and so forth, making false-positive results. Therefore, it is necessary to detect IgM and IgG simultaneously and improve the accuracy through multiple dynamic monitoring.

3 | CONCLUSIONS

Under the background of the current epidemic situation in COVID-19, it is still the most important task to develop diagnostic reagents and methods with high sensitivity, high specificity, low cost, easy operation, and short time. Figure 2 is the mode diagram of the detection technology summarized in this study. Real-time fluorescence quantitative PCR has become the gold standard for SARS-CoV-2 detection because of its high sensitivity and strong specificity. However, it is easy to have false negatives due to various factors. How to solve the problem of false negatives is worth considering. LAMP and CRISPR/Cas have apparent advantages in nucleic acid quantification and sensitivity. Sequencing technology is reliable, but it needs expensive instruments and high-tech personnel, so it is not widely used at the grassroots level. Antibody detection assists the detection of SARS-CoV-2 to some extent. In addition, facing the problem of virus traceability, countries worldwide need to work together to develop new detection methods and technologies that are more efficient, more accurate, small, and cheap and provide genuinely effective prevention and control measures for all humankind to control the COVID-19 epidemic.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Qing Ye had the idea for and designed the study and took responsibility for the integrity of the data and the accuracy of the data analysis. Hanyan Meng and Huihui Liu contributed to the writing of the report. Xiucui Zhang and Hanyan Meng contributed to the critical revision of the report. Xiucui Zhang and Hanyan Meng contributed to the statistical analysis. All authors contributed to data acquisition, data analysis, or data interpretation and reviewed and approved the final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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