Received: 4 September 2021 | Revised: 9 November 2021 | Accepted: 18 November 2021

DOI: 10.1002/jcla.24152

REVIEW ARTICLE

WILEY

Coronavirus Disease 2019 (COVID-19): Emerging detection technologies and auxiliary analysis

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Funding information
This work was supported by grants from the Qinglan Project of Jiangsu Province of China, Jiangsu Provincial Natural Science Foundation (BK20170252), and Projects for Jiangsu Provincial Young Medical Talents (QNRC2016780)

Abstract

The ongoing COVID-19 pandemic constitutes a new challenge for public health. Prevention and control of infection have become urgent and serious issues. To meet the clinical demand for higher accuracy of COVID-19 detection, the development of fast and efficient methods represents an important step. The most common methods of COVID-19 diagnosis, relying on real-time fluorescent quantitative PCR(RT-qPCR), computed tomography, and new-generation sequencing technologies, have a series of advantages, especially for early diagnosis and screening. In addition, joint efforts of researchers all over the world have led to the development of other rapid detection methods with high sensitivity, ease of use, cost-effectiveness, or allowing multiplex analysis based on technologies such as dPCR, ELISA, fluorescence immunochromatography assay, and the microfluidic detection chip method. The main goal of this review was to provide a critical discussion on the development and application of these different analytical methods, which based on etiology, serology, and molecular biology, as well as to compare their respective advantages and disadvantages. In addition to these methods, hematology and biochemistry, as well as auxiliary analysis based on pathological anatomy, ultrasonography, and cytokine detection, will help understand COVID-19 pathogenesis. Together, these technologies may promote and open new windows to unravel issues surrounding symptomatic and asymptomatic COVID-19 infections and improve clinical strategies toward reducing mortality.

KEYWORDS
auxiliary analysis, comparison, COVID-19, emerging technologies, SARS-CoV-2

1 | INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by SARS-CoV-2 (also named 2019-nCoV by the World Health Organization (WHO), began in late 2019 and has spread quickly around the globe.[1,2]

At present, the source of infection is mainly patients developing pneumonia upon SARS-CoV-2 infection and asymptomatic persons, and the main mode of transmission is respiratory droplets (Figure 1). The virus can also possibly spread by long-term exposure to high concentrations of aerosols.[3] A person can be

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infected by direct contact of mucous membranes with hands that previously touched a surface contaminated with SARS-CoV-2.[4] It should be noted that SARS-CoV-2 can be transmitted through the oral-fecal route.[5] In addition, the existence of SARS-CoV-2-infected asymptomatic persons adds complexity, uncertainty, difficulties, and challenges to epidemic prevention and control because of their invisibility and lack of clinical symptoms. The monitoring, tracking, isolation, and treatment of asymptomatic infected persons are crucial.[6]

Dry cough, fever, shortness of breath, and respiratory distress[7] are the main manifestations of SARS-CoV-2 infection. In severe cases, patients progress to acute respiratory distress syndrome (ARDS).[8] A previous study[9] has shown that elderly people or individuals with underlying diseases are likely to progress to severe and critically severe pneumonia once infected with SARS-CoV-2. Without timely treatment, they can easily develop acute respiratory distress syndrome, which causes respiratory failure. Therefore, early detection, intervention, and treatment of patients with COVID-19 are very important. At present, the diagnosis of COVID-19 includes methods based on etiology, serology, and chest imaging. Real-time fluorescent quantitative PCR (RT-qPCR) and DNA sequencing are the gold standard for detection of this pathogen. Immunoassays such as colloidal gold immunochromatography and enzyme-linked immunosorbent assay (ELISA) are used to detect serum antibodies against SARS-CoV-2. X-ray and computed tomography (CT) are used for imaging detection. In addition, other detection methods based on nucleic acid analysis, such as multiplex PCR and nucleic acid microfluidic detection chip, are being rapidly developed and applied (Figure 1).

Rapid detection of COVID-19 is of great significance for epidemic control and clinical diagnosis. In this review, we provide a brief description of the rapid detection and analytical methods for COVID-19 and evaluate their advantages and disadvantages with regards to sensitivity, specificity, and ease of operation (Table 1). Furthermore, we briefly summarize some of the auxiliary analytical methods that are of great significance for the study of this disease.

2 | COLLECTION OF CLINICAL SAMPLES

For safety purposes, personnel performing sampling must use eye protection goggles, gloves, a full-sleeved gown, an N95 respirator, higher-level respirator, or a face shield.[10,11] The quality and time of sample collection substantially affect the test results. Therefore, professional training should be provided to the sampling personnel in order to reduce the number of false-negative results. Two types of samples are usually collected, depending on the nature of the probed molecules.

2.1 | Clinical samples from the respiratory tract

For virus antigen and RNA nucleic acid detection, samples must be collected from the upper respiratory tract, including a pharyngeal swab, a nasopharyngeal swab, nasopharyngeal extract, sputum, respiratory tract extract, bronchial lavage fluid, and alveolar lavage fluid. In cases of ocular infection, conjunctival swab specimens are collected. Stool samples that may also contain SARS-CoV-2 viral particles should be collected. Recently, some studies have used saliva samples, which have a relatively high positive rate, are non-invasive, and produce no aerosol, compared with swabs. These features are particularly advantageous compared with swabs to collect family samples or samples in areas with limited protection resources.[12] Ryan et al. used exhaled breath condensate (EBC) as a non-invasive lower respiratory tract sampling method and showed that the false-negative rate with this test was lower than that with nasopharyngeal swabs.[13] For antigen detection, samples are eluted in viral transport medium (VTM) or suspended in phosphate-buffered saline (PBS).[14] In nucleic acid-based detection, the nucleic acid needs to be prepared automatically when the RNA-containing samples are collected. The procedure of opening the lid and adding normal saline can be avoided by using a pharyngeal swab or nasopharyngeal swab with virus preservation solution.[15] The viral RNA is extracted after sample collection. Currently, RNA extraction methods vary, and researchers usually

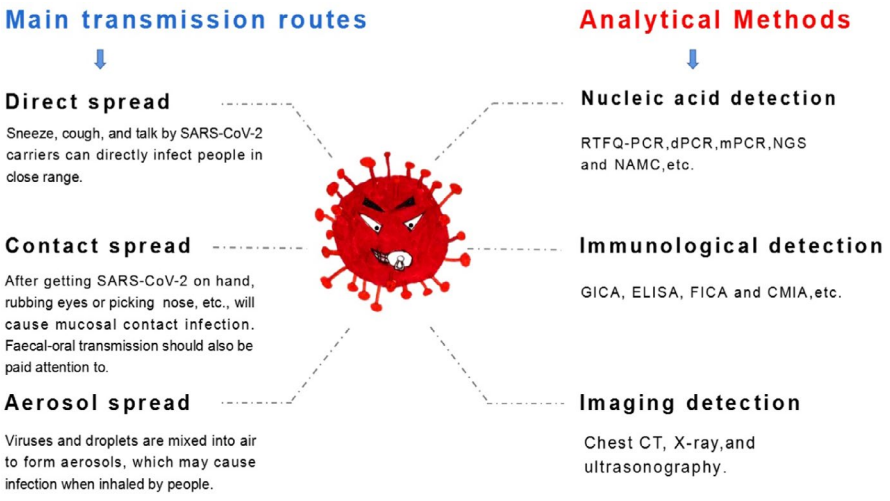

FIGURE 1 Main transmission routes and analytical technologies of SARS-CoV-2

TABLE 1 Comparison of advantages and disadvantages of different analytical technologies for COVID-19

Method		Test object	Advantages	Disadvantages	Test time[a]	References
Molecular or nucleic acid-based method	RT-qPCR	*RdRP*, *E* and *N* genes	Real-time; Sensitive and accurate; absolute or relative quantitative analysis; solve contamination problem	False positives occur; depend on reaction efficiency, primer and sample contaminants	~1.5 h	[18,21]
	ddPCR	*ORF1ab* and *N* genes	More sensitive and accurate; Absolute quantitative analysis	High cost	>1 h	[29,30]
	mPCR	*S* and *N* genes	Multiple targets are detected simultaneously	Prone to mismatch	~1.5 h	[33,34]
	mNGS	whole-genome sequence	Ability to detect any portion of genome; hypothesis-free, or unbiased, testing	Long time; complex operation; prone to contamination with environmental species; instrument is expensive	>18 h	[35,36,99]
	NTS	*S*, *E*, *M*, *ORF3a*, *ORF6*, *ORF7a*, *ORF8*, *ORF10* and *N* genes	High sensitive; unmarked; virus mutation monitoring is realized	< 200 bp of nucleic acid fragments cannot be detected; time is longer than qPCR; cheaper than NGS	~4–10 h	[41,42]
	NAMC	*Sand N* genes	High sensitivity, accuracy and efficient; high-throughput parallel detection of multiple target genes	Poor repeatability and stability; subjective interpretation	1.5 h	[60]
	LAMP	*N genes*	Small, simple, cheap, portable; can be performed directly on RNA; purification steps not required	Primer design is complex; quantitative detection is difficult	~1 h	[64–66]
	CRISPR-based method	*ORF1ab and N genes*	Rapid, sensitive, and portable; Specific	Stability needs to be improved; long development time; cannot detect quantitatively	30–50 min	[67–71]
	MS	*N*, *ORF1ab/nsp3*, *ORF1ab/nsp10 genes*	Reliable and cost-effective	Expensive testing equipment	~1 day	[73–75]
Serology-based method	GICA	IgM/IgG antibodies	Rapid; simple operation method; intuitive result	Negative during early infection; sensitivity and specificity need to be improved; most can only qualitatively detect; low flux	~15 min	[44]
	ELISA	IgM/IgG/IgA antibodies	Simple operation method; do not need handling of SARS-CoV–2	not well suited to detect acute infections; poor sensitivity; cumbersome steps	<1 h	[47,49]
	FICA	Nucleocapsid protein	Antigen detection can be used as an early diagnostic marker; good sensitivity and specificity	Fluorescence signal is easy to quench	<30 min	[51]
	CMIA	Total antibody (IgM, IgG and IgA)	rapid; high sensitivity and specificity; high degree of automation	Instrument is expensive	~0.5 h	[52–54]

(Continues)

TABLE 1 (Continued)

Method		Test object	Advantages	Disadvantages	Test time[a]	References
Imaging based method	CT	Whether the lungs have white shadow	High detection rate; can monitor the progression and regression of disease; expedite the identification of patients with severe disease	The specificity of CT findings was relatively low; have radiation; cannot tell which virus is infected	<30 min	[100–102]
	X-ray	Whether the lungs show bilateral lower zone consolidation	Compared with CT, X-ray may minimize the cross-infection; can play a role in the initial screening.	Less sensitive than CT	<30 min	[104]
	USG	As auxiliary method for CT	With non-invasive, non-radiation, and repeatable	Many overlaps exist in ultrasound manifestations of different lung diseases	<30 min	[105]

[a]Explanation: There exist differences in test time because of different products, kits, testing instruments, and laboratory.

choose simple and efficient nucleic acid extraction methods, such as a rapid method using proteinase K digestion and magnetic bead separation.[16]

2.2 | Clinical serum samples

Immunological technology for detection of anti-SARS-CoV-2 antibodies is better applied to serum samples collected in both the acute and recovery phases. The first serum sample should be collected as early as possible (preferably within seven days of disease onset), and the second should be collected 3–4 weeks after disease onset. The sample volume is 5 ml, and it is recommended to use a vacuum blood collection vessel without anticoagulant.[17]

3 | ANALYTICAL METHODS

Currently, the analytical methods of COVID-19 mainly include methods based on PCR, methods based on immunological test, the single nucleotide detection methods (sequencing), imaging test, and other methods such as nucleic acid microfluidic detection chip.

3.1 | Methods based on PCR

The basic idea of traditional PCR is similar to the natural replication process of DNA, including denaturation, annealing, and elongation. In the amplification process, single-stranded DNA is used as the template and oligonucleotides are used as the primers. Under the action of DNA polymerase, specific DNA fragments are amplified toward the direction from 5′ to 3′, enabling the target gene to be copied in large quantities[18] (Figure 2). Since SARS-CoV-2 is an RNA virus, before PCR amplification, two or more target RNA regions are simultaneously transcribed into complementary DNA (cDNA) by reverse transcriptase, and then, these cDNA are used as extension templates.

3.1.1 | Real-time Quantitative Reverse Transcription PCR, Real-time RT-qPCR

RT-qPCR is a technology that can amplify nucleic acid and detect nucleic acid products at the same time. The RNA of the virus is first transcribed into complementary DNA (cDNA) by reverse transcriptase. Subsequently, qPCR reaction is performed using cDNA as template. Take TaqMan probe technique as an example, when the probe is completely annealed to the target sequence, the fluorescence signal emitted by the report group is absorbed by the quencher fluorophore. During PCR amplification, the 5′→3′ exonuclease activity of *Taq* enzyme degrades the probe nucleotides, separates the fluorescent report group and the fluorescent quencher group, and generates a fluorescence signal. That is, for each DNA

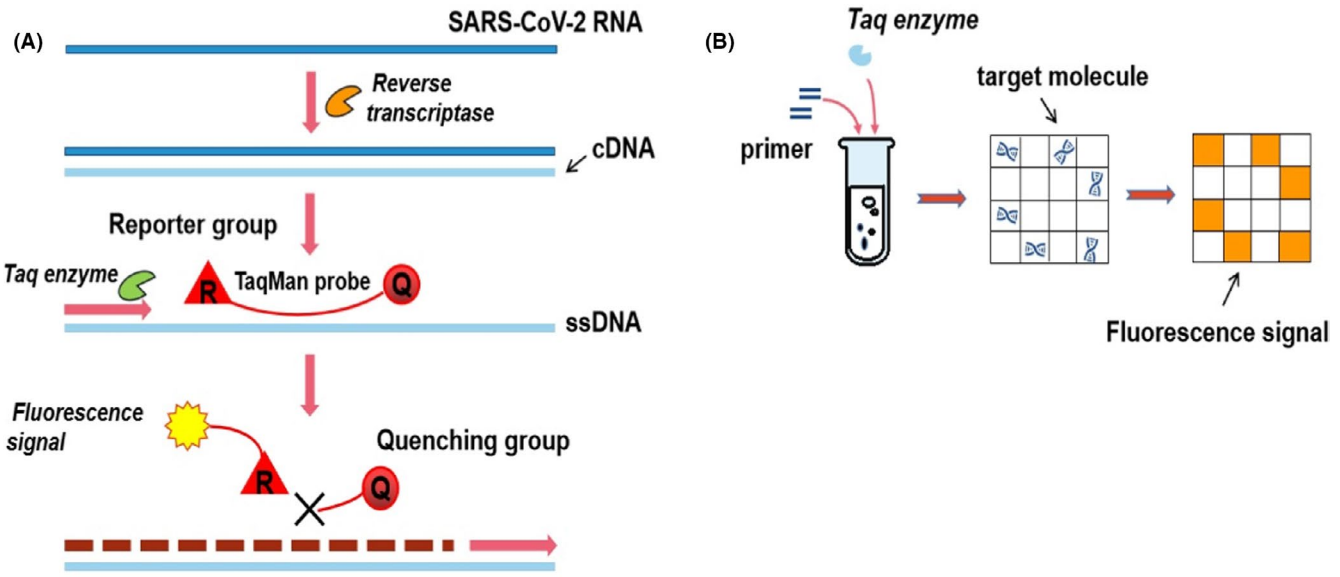

FIGURE 2 Workflow of PCR-based methods for the detection of SARS-CoV-2. (a) RT-qPCR; (b) Droplet digital PCR

strand amplification, a fluorescence molecule is formed. The accumulation of fluorescence signal is completely synchronized with the formation of PCR products[19,20] (Figure 2a). Using this method, three genes loci: *E* gene, *RdRp* gene in Orf1ab fragment, and *N* gene were detected by RT-qPCR.[21] This assay can quickly confirm whether the pathogen is SARS-CoV-2.[22-24] Compared with the traditional PCR methods, this technique has achieved a breakthrough from qualitative to quantitative results, which can measure both relative and absolute gene expression levels, with higher sensitivity, specificity, and accuracy at the same time. The detection process is completely closed, and this can reduce the possibility of sample contamination (Table 1). Meanwhile, the subsequent analysis is not required, so the detection time can be reduced significantly.[25,26]

3.1.2 | Droplet Digital PCR, ddPCR

ddPCR is a new method for the absolute quantification of target DNA or RNA. Before amplification, samples are preprocessed so that the reaction system containing nucleic acid molecules is divided into many partitions which act as an individual PCR microreactor (Figure 2b). After PCR amplification, positive signals are generated in the reaction chamber containing a single template molecule. By detecting each reaction chamber, the number of positive signals can be read out in an absolutely quantitative way. According to the Poisson distribution principle, the initial copy number or concentration of the target molecule can be obtained with the number and proportion of positive droplets.[27] ddPCR is an absolute quantification method for viral load. ddPCR does not require a reference or a standard curve for quantification on the levels of gene expression and viral load. Compared with real-time RT-qPCR, ddPCR is highly tolerate to inhibitors of PCR reaction. ddPCR is capable of analyzing more complex samples. ddPCR is capable of detecting subtle expression change in the tested samples.

A reverse transcription ddPCR was established to detect 194 clinical pharyngeal swab samples of SARS-CoV-2.[28] Compared with RT-qPCR method, the sensitivity of SARS-CoV-2 detection was improved from 28.2% to 87.4% of the fever suspected patients by using ddPCR method. In brief, these studies suggest that ddPCR is a promising tool for overcoming the problem of false-negative SARS-CoV-2 testing. This ddPCR was employed to determine the aerosol RNA concentration. In this assay, 35 aerosol samples of three different types were collected. The assay shows the distribution of the virus which indicate that asymptomatic carriers in the assembled crowds are the potential infection source of SARS-CoV-2.[29] Tao Suo et al. explored the ddPCR to detect SARS-CoV-2 and then compared with RT-qPCR. The results show that ddPCR can reduce the false negatives to achieve its high sensitivity and accuracy.[30]

3.1.3 | Multiplex PCR, mPCR

Multiplex PCR, especially dual fluorescence multiplex-PCR, is a new technique based on traditional PCR. Multiple primers and templates are mixed in one reaction system to amplify different target bands, or multiple primers and a single template DNA are mixed in the same reaction system to amplify different segments of the same template, which is often used for amplification of super-long segments.[31,32] Chenyu Li et al. developed a multiplex-PCR-based method, which can efficiently detected SARS-CoV-2 at low copy numbers and shows higher sensitivity in comparison with RT-qPCR. The assay comprises 172 pairs of specific primers, and positives can be identified directly by electrophoresis.[33]

Theoretically, compared with traditional PCR, multiplex PCR is more efficient, but the sensitivity is reduced. Therefore, the parameters such as primer concentration, annealing temperature, annealing time, and DNA polymerase content of multiple PCR reaction system need to optimize. By replacing primers, the result of the coverage at both regions targeted by the products can successfully be improved, which expected to be used for the detection of lower viral load SARS-CoV-2 samples.[34]

3.2 | Methods based on single nucleotide detection of sequencing

3.2.1 | Metagenomics next-generation sequencing, mNGS

As an unbiased technique that does not need pathogen culture, mNGS, based on next-generation sequencing, is a preferential method of pathogen detection. After high-throughput sequencing of DNA or RNA directly extracted from clinical samples, the sequences are submitted to databases for comparison and biological information analysis.[35,36] A variety of pathogens such as bacteria, fungi, viruses, and parasites can be tested at once. Currently, pathogenic gene sequencing is the most commonly used method in clinical practice.

The mNGS approach could rapidly identify the novel coronavirus, which was the sole pathogen detected in the sample.[37] Ren et al.[38] described the discovery and identification of SARS-CoV-2 by NGS. The results also showed that a previously unknown sequence of a novel coronavirus strain of the *Coronavirus* genus was found in all five tested samples. This new sequence was 79% similar in nucleotides to that of the SARS-CoV virus and was closest to the bat-derived strain (bat-SL-ZC45) but formed a separate evolutionary branch. Currently, mNGS covers a wide range of pathogens and can provide a basis for accurate diagnosis of new pathogens and mixed infections. However, this method has disadvantages such as complex operation, a relatively long turnaround time, lack of standardization, and under time and cost control, it yields insufficient sequencing depth for some samples. Therefore, it is often used in combination with RT-qPCR, which has complementary advantages.

3.2.2 | Nanopore target sequencing, NTS

By translocating nucleotides through nano-scale pores, NTS can quickly discriminate single nucleotides of target DNA strands due to the ion-current blockades[39] (Figure 3). Solid-state nanopore and protein-pore channels have been studied extensively for NTS applications.[40] Recently, NTS was used for simultaneous detection of respiratory viruses, including SARS-CoV-2,[41] within 6–10 h. Sixty-one nucleic acid samples were tested with qPCR kits and NTS. The NTS method has been confirmed to have the capacity of higher sensitivity and accuracy, as well as monitoring mutated nucleic acid sequences.[42] Phylogenetic analyses demonstrated that SARS-CoV-2 is the closest relative of the bat SARS-related coronaviruses found in Chinese horseshoe bats.

3.3 | Methods based on immunological test

3.3.1 | Colloidal gold immunochromatography assay, GICA

In recent years, GICA has become a fast-developing solid-phase marker immunoassay technique (Figure 4b). It is a new immunolabeling technique using colloidal gold as a tracer marker for antigens and antibodies. Some physical properties of colloidal gold enable a wide range of applications in immunology, histology, pathology, and cell biology. Recently, this technology was extensively used for SARS-CoV-2 detection. A rapid and simple method to simultaneously detect IgG and IgM antibodies against the SARS-CoV-2 virus was developed, which can discriminate COVID-19 patients from healthy individuals in 15 min and can identify patients at different infection stages.[43] Tao Peng et al. have developed a detection method for quantification of IgG and IgM against SARS-CoV-2.[44]

The operation of this technique is simple, and no special equipment is required. The operators do not need any special training. This technique eliminates the competition between non-targeted and targeted amplification products, thereby achieving high specificity. The results can be directly observed by the naked eyes within 15 min. This greatly shortens the test time and allows for rapid diagnosis of suspected patients and on-site screening of people who have had close contact with these patients. Because colloidal gold strips can be stored for a long time at room temperature, this technique is especially applicable to general basic units and point-of-care testing (POCT). However, because colloidal gold particles do not have luminescent properties, these tests rely only on color changes read by the naked eyes, which limits their use for high-sensitivity detection. Therefore, the diagnosis and treatment cannot solely rely on this type of test and should be complemented by clinical history and other laboratory tests.

3.3.2 | Enzyme-Linked Immunosorbent Assay, ELISA

ELISA relies on specific antigen/antibody interaction and enzyme-linked amplification of the reaction signal (Figure 4d). The enzyme conjugated with the antibody or antigen retains its activity, while the immunological reactivity of either the antibody or the antigen is preserved. Thus, the enzyme-labeled antigen or antibody can combine with its respective cognate antigen or antibody. Upon addition of a colorless enzyme substrate, catalysis produces chemical reactions such as hydrolysis, oxidation, or reduction that form colored products that can be qualitatively estimated with the naked eyes or quantitatively measured with a spectrometer.[45] This colored signal

is proportional to the level of antibodies or antigens in the sample.[46] ELISAs are critical tools to define previous exposure and determine seroprevalence in a population. These assays, using plasma/serum, have proven to be specific and sensitive for the screening of individuals who have undergone seroconversion upon SARS-CoV-2 exposure as early as three days after disease onset.[47] With this method, the total Igs, IgGs, and IgMs against SARS-CoV-2 in plasma samples can be detected, constituting a significant sensitivity improvement for the diagnosis of COVID-19 patients.[48]

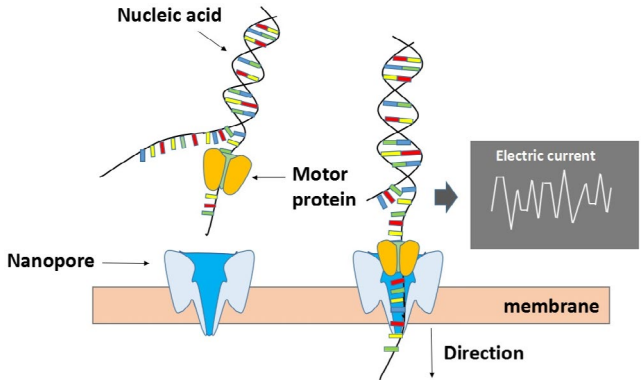

FIGURE 3 Schematic representation of method based on NTS for the detection of SARS-CoV-2

ELISA has the advantages of rapid, sensitive, simple, and easy to standardize. However, difference exists between different in-house and commercial ELISAs. It is demonstrated that in-house ELISAs show higher specificity.[49] In addition, this method relies heavily on antibodies. Moreover, the primary antibody in the test has to be labeled with enzymes, but not every antibody is suitable for labeling, which may limit its application.

3.3.3 | Fluorescence immunochromatographic assay, FICA

FICA is a new detection technique performed on a membrane support and based on specific antibody/antigen recognition. This new immunoassay not only retains the advantage of allowing rapid detection, such as the common colloidal gold strips usable on the spot, but also adds the high sensitivity of the fluorescent detection technology to improve the detection performance of immunochromatography.[50] This method has been tested for the detection of the nucleocapsid protein of SARS-CoV-2 within 10 min. To evaluate this approach, nasopharyngeal swab samples and urine from 239 participants were tested in parallel with a nucleic acid-based test as a reference standard.[51] The results showed that this method provides a rapid, simple, and accurate assay for the diagnosis of COVID-19 (Figure 4a).

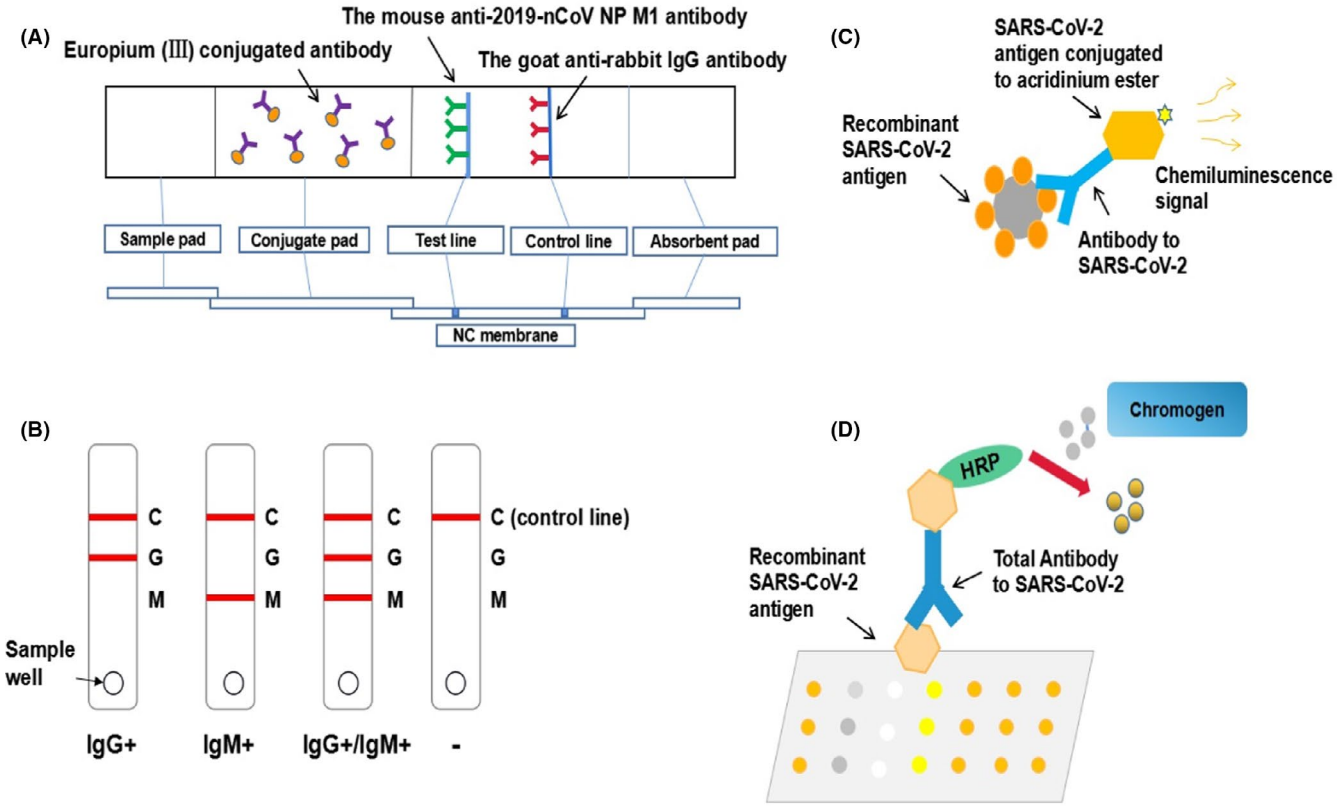

FIGURE 4 Schematic representation of methods based on immunological test for the detection of SARS-CoV-2. (a) Fluorescence immunochromatographic assay (FICA); (b) Colloidal gold immunochromatography assay (GICA); (c) Magnetic particle chemiluminescence immunochromatography assay (CMIA); (d) Enzyme-linked immunosorbent assay (ELISA)

3.3.4 | Chemiluminescence microparticle immunoassay, CMIA

CMIA is an effective detection method based on the specificity of the immune response that heightens the accuracy of COVID-19 diagnosis.[52] CMIA was used to evaluate the immune response in COVID-19 patients co-infected with HIV-1 or HCV by testing plasma IgM and total Igs specific for SARS-CoV-2.[53] The results showed that HIV-1-induced immune dysfunction can influence early SARS-CoV-2 clearance (Figure 4c). The serology of total Igs, IgG, and IgM after SARS-CoV-2 infection was studied with GICA, FICA, and ELISA methods.[54] The antibody level increased rapidly from 6 days postonset, which correlated with the decrease in the viral load. The antibodies showed the highest sensitivity for patients at the early stage of illness.

3.4 | Methods based on imaging tests: chest CT scans and X-rays

Chest CT scans and X-rays are important imaging methods for the preliminary diagnosis of chest diseases.[55] Though they are not specific methods for diagnosis of COVID-19, they played an important role in the screening of suspected patients infected with SARS-CoV-2 in the early phase of COVID-19 pandemic when the diagnostic kits for SARS-CoV-2 infection are not available. CT scans and X-rays can clearly show the lung morphology and lesions of the mediastinum and pleura. The diagnosis of chest diseases by X-rays has certain limitations. It is difficult to find diseases with small lesions, which need further examination by CT. CT can detect occult lesions that cannot be found by chest X-rays.

Imaging examinations of 99 patients were conducted to diagnose SARS-CoV-2 infection[56] (Figure 5a,b). The chest CT scans of all patients showed obvious abnormalities, indicative of pneumonia symptoms. Ninety-eight percent of the cases showed bilateral lung involvement. The typical presentation of patients transferred to the intensive care unit (ICU) is the presence of bilateral multiple lobules and subsegment merging. For patients in non-intensive care units, scans usually show bilateral ground-glass opacity and subsegmental consolidation areas. Recently, 81 patients with COVID-19 who had undergone serial chest CT scans were retrospectively enrolled.[57] The results showed that COVID-19 pneumonia was characterized by chest CT abnormalities. In asymptomatic patients, rapid evolution from local unilateral to diffuse bilateral ground-glass opacity could even be observed, and complications developed within 1–3 weeks.

An automated COVID-19 detection system based on artificial intelligence has been developed, which uses indications from CT images to train the new powered deep learning model U-Net architecture. The proposed algorithm achieved high level of sensitivity and specificity, with an overall accuracy of 94.10%. The U-Net architecture used for chest CT image analysis was found to be effective. Therefore, it can be added to the list of primary tools available for screening of COVID-19 patients by clinicians.[58]

3.5 | Other methods

3.5.1 | Nucleic acid microfluidic detection chip, NAMC

The method based on microfluidic chips can be used for nucleic acid detection. The combination of microfluidic and sensor technologies can integrate the steps of biological sample preparation, analyte labeling, signal amplification, and detection on a miniaturized platform. In this way, the corresponding chemical reaction can be automated with a limited number of reagents with high precision.[59]

Recently, researchers of Capital Biotechnology of China developed an isothermal amplification microfluidic chip detection method to simultaneously detect several respiratory viruses, including the influenza virus, respiratory syncytial virus, and SARS-CoV-2, with CE-IVD certified.[60] This method employs isothermal amplification technology at a constant reaction temperature of 41°C for real-time fluorescence detection. This technology allows for simultaneous multiple target gene detection with a rapid (~1.5 h), highly sensitive (15–25 copies/reaction of SARS-CoV-2), and highly accurate and efficient test.

This method can solve the limitation of nucleic acid detection in common equipment and can realize real-time detection. Microfluidic chips are characterized by miniaturized equipment, integration, low sample and reagent consumption, high throughput, real-time detection, fast analysis speed, and high sensitivity.[61] However, the repeatability and stability of this method still need to be optimized.

3.5.2 | Sensitive splint-based one-step isothermal RNA Detection, SENSR

A SENSR technology for rapid detection of SARS-CoV-2 was recently developed. This method was shown to be a sensitive and effective assay for the detection of SARS-CoV-2, as well as five other pathogens, including influenza A viruses and Middle East respiratory syndrome-related coronaviruses, with a detection limit of 10^{-16} mol/m^3.[62]

3.5.3 | Loop-mediated isothermal amplification, LAMP

LAMP employs a DNA polymerase, and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA. The cycling reaction proceeds with the accumulation of 109 copies of the target in less than one hour.[63]

Improved and upgraded LAMP can detect SARS-CoV-2 RNA at significantly low levels, corresponding to a more higher sensitivity compared with common RT-PCR methods.[64] Based on conventional LAMP, researchers developed the capture and improved loop-mediated isothermal amplification (Cap-iLAMP) method, which combines a hybridization capture-based RNA extraction of gargle

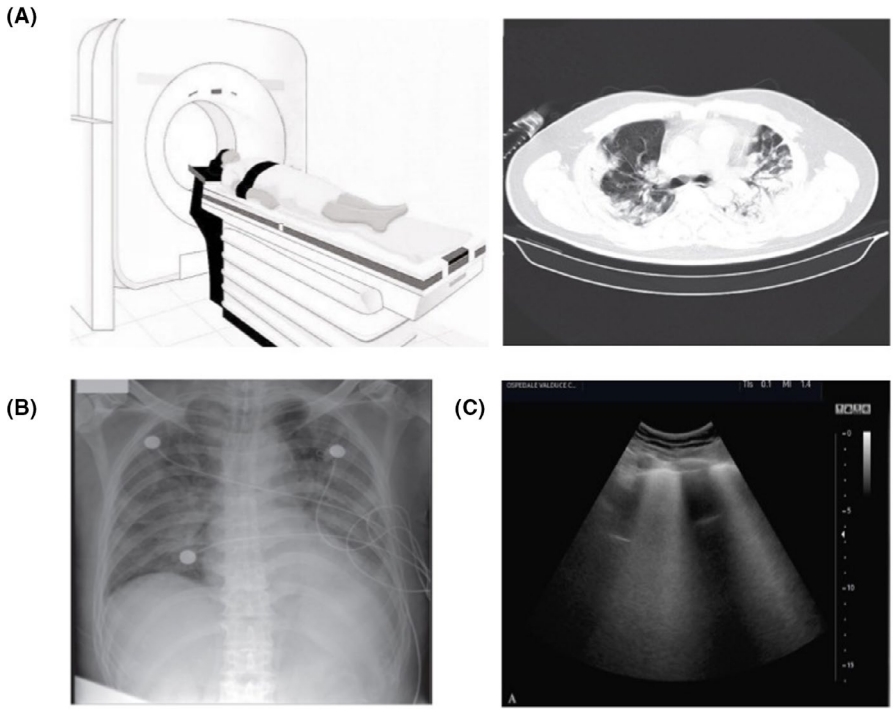

FIGURE 5 Methods based on CT, X-ray, and ultrasonography for the detection of COVID-19. (a) CT detection result; (b) X-ray detection result; (c) Ultrasonography detection result

lavage samples with an improved colorimetric RT-LAMP assay and smartphone-based color scoring. Cap-iLAMP enables the detection of SARS-CoV-2-positive samples in less than one hour.[65,66] Compared with RT-PCR, this method amplifies DNA with high specificity, efficiency, and rapidity, without the need for expensive instruments, under isothermal conditions. These characteristics are a huge advantage for POCT.

3.5.4 | CRISPR-based RNA Detection

The fastest way to test for the presence of a coronavirus may be by using the CRISPR genome editor, better known for adding or deleting DNA in cells.[67] Researchers have recently adapted accurate CRISPR-Cas12-based lateral flow assay for SARS-CoV-2 detection.[68] This method uses RT combined with LAMP assays, followed by Cas12 detection of predefined coronavirus sequences and confirmation of virus detection. With this method, the nucleoprotein and envelope genes can be detected with a performance comparable to that of the SARS-CoV-2 RT-qPCR method developed in the United States by the Centers for Disease Control and Prevention (CDC). This new assay has been reported to have a limit of detection of 10 copies/μL input RNA and a detection time of 30 min, which can compete with the CDC's standard RT-qPCR. Specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) is a platform based on CRISPR-Cas13 systems. It combines reverse transcription and recombinase polymerase amplification (RT-RPA) with Cas13a. SHERLOCK can detect both DNA and RNA viruses with single-base discrimination sensitivity.[69] CRISPR-based diagnostic platforms provide rapid, sensitive, and specific tests for SARS-CoV-2 detection. CRISPR-Cas-based diagnosis tests can also detect the viral genome at early stages of the incubation period. However, this technique is more qualitative than quantitative.[70,71]

3.5.5 | PfAgo-based detection of SARS-CoV-2, PAND

PfAgo is a prokaryotic argonaute protein (pAgo) from *Pyrococcus furiosus*. As a nucleic acid-guided endonuclease, PfAgo preferentially cleaves DNA substrates under the guidance of short 5′-phosphorylated single-stranded DNA without the presence of a protospacer-adjacent motif (PAM). Briefly, SARS-CoV-2 PAND starts with an RT-PCR step that amplifies conserved regions in the viral genome. Next, PfAgo, guide DNAs, and molecular beacons in appropriate buffer are added to the PCR product and are incubated at 95°C for 20–30 min. Finally, the fluorescence signal is measured. This method alleviates the shortage caused by the saturation of expensive real-time PCR instruments by shortening the reaction time to only 3–5 min per batch. It is a rapid, sensitive, and accurate method for nucleic acid detection. A study showed its suitability for large-scale genotyping of SARS-CoV-2 variants.[72]

3.5.6 | Mass spectrometry, MS

MS is a method of detection based on the movements of ions activated by electric and magnetic fields according to their mass charge ratio. It is an important technique in medical laboratory used in a multitude of applications for diagnosis.[73]

A study used MS to detect viral nucleocapsid genes (N, ORF1ab/nsp3, and ORF1ab/nsp10 genes) as signature of SARS-CoV-2. The

results obtained by MS and RT-qPCR were concordant. However, MS detection takes more time than RT-qPCR.[74] In another study, Cazares et al. established a targeted MS assay for the detection of the SARS-CoV-2 spike protein and nucleoprotein in vitro derived mucus. MS-based methods for viral antigen detection may deliver higher throughput and could complement RT-qPCR as a diagnostic tool.[75]

3.5.7 | Detection based on biosensors

Biosensors are inexpensive, sensitive, rapid, miniaturized, and portable platforms. In recent years, biosensors developed rapidly, and today, they provide more approaches for the detection of SARS-CoV-2.[76]

An article reported the development of a field-effect transistor (FET)-based biosensing device for SARS-CoV-2 detection in clinical samples. The sensor was produced by coating the graphene sheets of an FET with an antibody specific for the SARS-CoV-2 spike protein.[77] Surface plasmon resonance (SPR) is an optical technique that can measure the refractive index changes in the vicinity of thin metal layers in response to biomolecular interactions.[78] Wang et al. improved SPR to build a spectrum-based SPR imaging sensing system with fast wavelength scanning capability. They configurated the system to perform SPR high-throughput detection of the SARS-CoV-2 spike protein.[79] An MIP-based electrochemical sensor has also been developed for detection of the SARS-CoV-2 nucleoprotein (ncovNP).[80] Gao et al. developed a rapid and sensitive triple-mode detection of the SARS-CoV-2 virus-specific genes. The triple-mode signals of the sensor were verified with one another to increase the accuracy of the experimental results.[81]

4 | AUXILIARY ANALYSIS

4.1 | Pathological anatomical analysis

The findings of pathological anatomy can help in understanding the pathogenesis of COVID-19 and assist doctors to devise a timely therapeutic strategy. *The Lancet* published a pathological anatomy case report of a patient who died of COVID-19.[82] The results showed bilateral diffuse alveolar damage with cellular fibromyxoid exudates. Among other clinical features, the right lung showed evident desquamation of pneumocytes indicating ARDS. Viral cytopathic-like changes were identified in the intra-alveolar spaces.

4.2 | Ultrasonographic analysis

Ultrasonography (USG) is now used in auxiliary diagnosis of COVID-19 as well as for its complications (Figure 5c). COVID-19 changes rapidly, but repeated CT examination to monitor the course of the disease is difficult to implement. At the same time, for severe or critically ill patients, in addition to lung disease itself, the assessment of systemic parameters, as for any diseases, is essential for monitoring the condition and for guiding treatment. Ultrasound examination represents a unique advantage for the diagnosis and treatment of COVID-19. In addition, for pregnant patients and children with pneumonia, ultrasound can play an extremely important role as it does not involve radiation.[83] For pregnant women diagnosed with COVID-19, intrauterine monitoring of the fetus should be strengthened toward continuous ultrasound examination to assess fetal structure and growth.

During COVID-19 complications, USG is a sensitive and specific examination method for the diagnosis and identification of venous thrombosis, pleural effusion, pericardial effusion, and abnormal cardiac function. Therefore, USG is a useful supplement to CT imaging diagnosis, providing pulmonary infection specialists with rich imaging information. Besides the screening of early suspected cases or assessment of the systemic condition of critically ill patients, due to its non-invasiveness, radiation-free property, and repeatability, USG can help clinicians to detect critical situations in time and improve the timeliness of diagnosis and treatment.[84,85]

4.3 | Cytokine detection and analysis

Accumulating evidence indicates that patients with severe COVID-19 may develop a cytokine storm syndrome.[86] Once a cytokine storm initiates, the immune system kills normal lung cells in addition to infected cells to eliminate the virus. This dysregulated response severely damages the ventilation function of the lungs. Many studies have reported that cytokine storms can be triggered by SARS-CoV-2 infection. Release of multiple cytokines, including IL-6, causes ARDS and multiple organ failure. The cytokine profile occurring during severe COVID-19 resembles that of secondary hemophagocytic lymphohistiocytosis (sHLH), characterized by increased interleukin (IL)-2 and tumor necrosis factor-α.[5,86] These observations suggest that COVID-19-related mortality is caused by virus-driven hyperinflammation. Cytokines and T-cell subset profiles could be used as a basis to predict the transition from mild to severe disease.[3,87-89] IL-6 is one of the key inflammatory factors that trigger the inflammatory storm in COVID-19 patients.[90] The IL-6 level could be used for early diagnosis of acute infections and may become a biomarker for early warning of cytokine storms[91] to prevent the number of severe patients and reduce the mortality rate.

4.4 | Immunocyte analysis

Research shows that patients with COVID-19 have lower lymphocyte counts, higher leukocyte counts, and neutrophil-lymphocyte ratio (NLR), as well as lower percentages of monocytes, eosinophils, and basophils. T-cell number decreases significantly below normal levels. Both T helper (Th) cells and T suppressor cells are below normal levels in patients with COVID-19, with lower levels of Th cells in the severe group. The percentage of naive CD4+ T cells

increases and that of memory Th cells decreases in severe cases. Patients with COVID-19 also have lower numbers of regulatory T cells, which are more obviously decreased in severe cases. This may be because SARS-CoV-2 infects T lymphocytes through receptor-dependent, S protein-mediated membrane fusion, resulting in lymphocyte apoptosis in lymphoid organs.[92] Severe pulmonary lesions are associated with interstitial mononuclear infiltrates caused by CD4+ and CD8+ T lymphocytes.[82] Neutrophilia contributes to inflammation, cytokine dysregulation, and autoimmune and thrombotic manifestations. Surveillance of NLR and lymphocyte subsets is helpful in the early screening of critical illness, diagnosis, and treatment of COVID-19.[93]

4.5 | Blood coagulation function analysis

COVID-19 is strongly associated with various coagulopathies that may result in either bleeding and thrombocytopenia or hypercoagulation and thrombosis. Thrombotic and bleeding or thrombotic pathologies are significant concomitants to acute respiratory syndrome and lung complications in COVID-19. During disease progression, the levels of von Willebrand factor (vWF), P-selectin, and fibrinogen are high, with normal or slightly increased D-dimer levels. Progression toward vWF and fibrinogen decreases, and high D-dimer levels and even higher P-selectin levels are indicative of poor prognosis.[94-97]

5 | DISCUSSION

COVID-19 is a public health emergency. The most effective way to curb the spread of the epidemic is to identify and isolate infected individuals. To date, various detection kits have been developed successfully, which has fulfilled the urgent clinical needs to the greatest extent. However, there are still unsolved problems, including differences in the positive detection rate of the different kits[98] or in targets (ORF1ab, *E*, and *N* genes), the selection of reagents matching the hardware equipment of laboratories, and the emergence of virus variants.

Nucleic acid assays based on RT-qPCR or NGS[99] and CT were the most important techniques for COVID-19 diagnosis at the beginning of the outbreak. Today, molecular, antigenic, and antibody detection methods are the most commonly used. All these methods have their own advantages. RT-qPCR has high detection sensitivity and a low detection cost and therefore became the gold standard for SARS-CoV-2 detection. In addition to the conventional methods cited above, technologies such as dPCR, multiplex PCR, ELISA microfluidic detection chips, LAMP, and some biosensors are equally important supplementary technologies to fight against COVID-19.

However, there are limitations inherent to nucleic acid-based technologies: (1) False-negative results for positive patients may occur; PCR-based methods are sensitive, but the false-negative rate is detrimental to large-scale testing; correct execution of the analysis is crucial, because negative results do not mean an absence of the virus; (2) the equipment is expensive and not always available in smaller facilities; (3) high-quality clinical samples are important for optimal detection of the virus; (4) the time necessary for completion of the test might be longer than that for serological tests; (5) the CRISPR method may lack specificity because guide RNA might recognize interspersed sequences of the patient's genome, producing false-positive results; (6) a short development time and shortage of specially trained technical staff are issues that need to be considered when using nucleic acid-based technologies; (7) only infected individuals can be detected, whereas previously ill persons cannot be identified, which has little significance for epidemiological research; (8) these tests cannot distinguish between SARS-CoV-2 RNA from infectious virus and SARS-CoV-2 RNA from noninfectious virus.

X-rays and chest CT scans, especially high-resolution CT scans, are better used for the detection of early changes in lungs.[100-102] Combined with medical history and other comprehensive analytic tools, clinicians can make early diagnoses and implement treatment. However, due to infection control issues related to patient transport to CT suites, the inefficacy of CT room decontamination, and lack of CT availability in parts of the world, portable chest radiography will likely be the most commonly utilized mean of identification and follow-up of lung abnormalities.[103] However, imaging examination methods cannot solve all problems. For instance, they cannot identify the infectious agents, contrary to nucleic acid tests that can accurately identify viruses. In addition, some patients do not present typical COVID-19 images, even though they have clinical symptoms or tested positive by nucleic acid tests. Therefore, negative imaging results do not rule out COVID-19. Thus, it is important to feed a dispute opposing nucleic acid-versus CT-based diagnosis as a standard for COVID-19 since both methods have their respective values and advantages and should complement each other to improve the accuracy of diagnosis.

It is worth noting that immunological assays, which use serum to detect antibodies against SARS-CoV-2, are significantly more informative because they provide information on the evolution of the pandemic over time. Moreover, the detection time of serological tests is shorter than that of nucleic acid-based methods. However, serological tests rely heavily on antibodies and they can usually detect antibodies produced by the human body only from three days onwards (for anti-SARS-CoV-2 IgM) after virus infection.[47] Thus, a negative result does not guarantee that the patient is not infected. At the same time, with the vaccination, antibody-dependent detection methods may be affected. Patients who have been in contact with the virus should undergo molecular testing. In addition, due to the cross-reactivity with other antibodies, false positives can also occur with this method; that is, a positive result may be due to a past coronavirus infection. These limitations make immunological assays not that useful at early stages of an infection and for test-and-trace strategies. However, serology tests are an important complement to nucleic acid tests, especially to uncover false-negative results of COVID-19 nucleic acid tests, for pathogenic specific detection, and

for the evaluation of the immune status of patients.[15] Well-validated antibody tests are recommended in public health practice to ameliorate the prevention and control of COVID-19.[54]

In addition to the abovementioned laboratory diagnosis methods, research on the influence of the ABO blood type[104] or on the presence of anti-A antibodies in serum,[105] as well as the discovery of pathological anatomy and immunocyte analysis, may open a new window for unraveling issues related to COVID-19 and help in understanding its pathogenesis toward ameliorating clinical strategies and reducing mortality. USG is also a useful supplement to CT imaging diagnosis, providing pulmonary infection specialists with rich imaging information.[106] Detection of cytokines such as IL-6 can be used to assist the early diagnosis of acute infections to reduce the number of critically ill patients caused by SARS-CoV-2, as well as the mortality rate. Analysis of blood coagulation function can provide information on a patient's physical condition.

According to disease surveillance data, other respiratory infectious diseases, such as the respiratory syncytial virus, influenza virus, and adenovirus, have overlapping epidemic seasons with that of new coronavirus pneumonia. The clinical manifestations and chest images associated with the diseases caused by these viruses are similar to those observed in SARS-CoV-2 infections, rendering their distinction difficult. These respiratory viruses are mixed in the current new coronavirus pneumonia pandemic, which not only interferes with the prevention and control of the pandemic, but also threatens the safety and health of the public.[107,108] Therefore, it is crucial to identify and prevent these respiratory viruses from spreading. Parallel detection of SARS-CoV-2 and the common respiratory viruses will effectively improve the efficiency of disease detection and help medical staff to quickly distinguish healthy individuals from those infected with SARS-CoV-2 or influenza. Achieving accurate diagnosis will lead to precise treatment of patients, in the most convenient, fast, and suitable way. Some cases of false-negative results for SARS-CoV-2 detection in COVID-19 patients co-infected with the influenza A virus have been reported.[109] This indicates that medical staff should also pay particular attention to co-infections with SARS-CoV-2 and other viruses or bacteria in order to prevent misdiagnosis. Combined therapy for non-anti-SARS-CoV-2 co-infection should be further investigated.[110]

Increasing experience in coronavirus infections, development of kits, and related detection technologies provide pivotal guidance for the development of SARS-CoV-2 rapid detection methods. In the future, more technologies will be developed, such as fully automated and integrated nucleic acid detection techniques.[23,111] POCT products with a small size, simple operation, and timely results have been developed rapidly and have great potential. For example, Abbott Laboratories (Abbott, San Diego, CA) recently received emergency use authorization from the Food and Drug Administration (FDA) of the United States for the ID NOW™ testing platform. This represents the fastest available molecular POCT for SARS-CoV-2 detection, giving positive results in 5 min, and offers rapid results for health care workers. The FilmArray® Respiratory EZ Panel uses a molecular syndromic approach to accurately detect and identify a wide range of pathogens, including common coronaviruses.[112] However, parameters of these technologies such as specificity, sensitivity, detection time, detection cost, and if the parallel detection should be evaluated and worthy of attention.

Finally, effective prevention and control of infection in medical institutions are worthy of attention. Independently of the SARS-CoV-2 detection technology used, technicians should pay special attention to negative results for early detection of COVID-19. They should also pay close attention to self-protection and laboratory disinfection and should avoid cross infections. In addition, false-positive, asymptomatic infected persons need further diagnosis and identification. Nucleic acid detection and serum epidemiological investigation based on detection of anti-SARS-CoV-2 IgGs and IgMs can help untangle asymptomatic infections and serologic status among healthy people and provide a scientific basis for the adjustment of control and prevention strategies. Sampling methods and kits suitable for home testing need to be further developed and promoted. Regarding the detection equipment, it would be meaningful to simplify the operation in order to decrease the risk of contamination and to reduce the size of the equipment. Direct detection of viruses in the air and the detection of some environmentally polluted samples also need further follow-up, as they are highly relevant to the safety of people in public areas. Recently, new variants of coronavirus have been identified, and some variants are more infectious and lethal.

This emergence brings greater challenges for the prevention and control of the pandemic. Detection methods to distinguish between these mutants need to be thoroughly studied, and whether the original detection targets are mutated also needs to be carefully examined. Lastly, multisectoral partnerships can minimize social and economic impact.[113] All scientists should work together and endeavor to overcome the pandemic, protect lives and livelihoods, and dedicate more research and development funding for vaccines, medicines, and diagnosis methods, as well as strengthen international scientific cooperation and leverage digital technologies.

ACKNOWLEDGMENT

We thank Accdon (www.accdon.com) for its linguistic assistance during the preparation of this manuscript.

CONFLICTS OF INTEREST

All authors have completed the ICMJE uniform disclosure form. The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

YC conceived of and designed the study. YC, SXH, LYZ, and XW wrote the article. HY and WQL generated the table and figures. All authors reviewed the article and approved its submission for publication.

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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How to cite this article: Chen Y, Huang S, Zhou L, Wang X, Yang H, Li W. Coronavirus Disease 2019 (COVID-19): Emerging detection technologies and auxiliary analysis. *J Clin Lab Anal*. 2022;36:e24152. doi:10.1002/jcla.24152