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Review

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Evolution of the newest diagnostic methods for COVID-19: a Chinese perspective

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Abstract: Coronavirus disease 2019 (COVID-19) has continued to spread globally since late 2019, representing a formidable challenge to the world's healthcare systems, wreaking havoc, and spreading rapidly through human contact. With fever, fatigue, and a persistent dry cough being the hallmark symptoms, this disease threatened to destabilize the delicate balance of our global community. Rapid and accurate diagnosis of COVID-19 is a prerequisite for understanding the number of confirmed cases in the world or a region, and an important factor in epidemic assessment and the development of control measures. It also plays a crucial role in ensuring that patients receive the appropriate medical treatment, leading to optimal patient care. Reverse transcription-polymerase chain reaction (RT-PCR) technology is currently the most mature method for detecting viral nucleic acids, but it has many drawbacks. Meanwhile, a variety of COVID-19 detection methods, including molecular biological diagnostic, immunodiagnostic, imaging, and artificial intelligence methods have been developed and applied in clinical practice to meet diverse scenarios and needs. These methods can help clinicians diagnose and treat COVID-19 patients. This review describes the variety of such methods used in China, providing an important reference in the field of the clinical diagnosis of COVID-19.

Key words: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2); Coronavirus disease 2019 (COVID-19); Diagnosis; Polymerase chain reaction (PCR); Immunoassay; Radiography

1 Introduction

The outbreak of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has posed a significant threat to global public health systems (Cheng and Shan, 2020). SARS-CoV-2 has wreaked havoc on global balance due to its worldwide transmission primarily through person-to-person contact, with fever, fatigue, and a dry cough being the most common symptoms (Wen et al., 2020; Bai and Tao, 2021; Islam et al., 2023c).

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According to Our World in Data (https://ourworldindata.org/explorers/coronavirus-data-explorer), as of February 20, 2023, more than 686 million people have been affected and 6.74 million people have died from coronavirus disease 2019 (COVID-19) infections. Since the outbreak of COVID-19, the SARS-CoV-2 virus has mutated and various variants such as Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), Delta (B.1.6, B.1.6.2), and Omicron (B.1.1.529) have been identified as variants of interest and concern (Sakib et al., 2021; Islam et al., 2022d, 2023c; Soto et al., 2023). Recently, XBB.1.5, a subvariant of the recombinant mutant XBB, has rapidly become the dominant SARS-CoV-2 strain in the USA and has now been detected in mainland China (Yue et al., 2023). Like XBB.1, XBB.1.5 can evade neutralization by plasma and serum from vaccinated or convalescent individuals and monoclonal antibodies (mAbs) (Cao et al., 2023; Kurhade et al., 2023).

However, some patients have not exhibited obvious clinical symptoms of infection in the early stages. These asymptomatic infections account for about 40% to 45% of SARS-CoV-2 infections, and have an even stronger and more persistent ability to transmit the virus (Al-Tawfiq, 2020; Oran and Topol, 2020), so early detection, isolation, and treatment are critical for reducing morbidity and mortality. This ongoing global pandemic is causing researchers to consider new approaches to detect, diagnose, and treat COVID-19.

Recently, wastewater-based epidemiological monitoring (WBEM) has become an efficient and feasible surveillance protocol in a pandemic such as COVID-19. WBEM can be combined with clinical samples for use in early warning of outbreaks, tracking the current trend, detection of genetic diversity and symptomatic individuals, deploying mass vaccination including booster doses, as well as detecting an up-surge in new SARS-CoV-2 variants (Jakariya et al., 2022; Islam et al., 2023a, 2023c). The salient findings of such studies indicate that monitoring the genetic markers of SARS-CoV-2 in wastewater can identify COVID-19 cases, which reduces the burden on the public health system during pandemics. In addition, during the COVID-19 pandemic, monkeypox virus (MPXV) and Langya virus (LayV) are other zoonoses have re-emerged, which can spread dramatically either between humans or from animals to humans by bite/scratch, close contact, or by eating undercooked meat from infected animals. These re-emerging viral diseases have spread swiftly in several countries and constitute an ongoing global public health emergency. Many studies have shown that polymerase chain reaction (PCR) using a specific primer could be a gold standard method for diagnosis of zoonotic diseases, while other diagnostic methods should be investigated further (Chakraborty et al., 2022; Chandran et al., 2022; Islam et al., 2022c, 2023b).

To detect SARS-CoV-2 quickly and accurately, the current mainstream method is real-time reverse transcription-PCR (RT-PCR) (Huang et al., 2020). However, because the test is influenced by the sample materials, handling method, and transport, the sensitivity of the test results fluctuates. There is still an urgent need to improve the sensitivity and specificity of detection, so more accurate and diversified genetic tests are emerging. Serological detection is a good supplement to alleviate the deficiencies of PCR for monitoring the level of antibodies after infection and vaccination. Meanwhile, we should try alternatives to clinical tests for COVID-19, such as combining WBEM and clinical trials further. Imaging examination can also provide the basis for clinical condition monitoring. The goal of this study was to introduce various detection and diagnostic methods at the molecular, immunological, and digital levels, and analyze their utilization in the detection and diagnosis of COVID-19. We focus on the evolution in diagnostic approaches in China, from the beginning of the outbreak to the current stage. We also aimed to better understand the feasibility and acceptability of a variety of diagnostic methods during the pandemic, and address how this understanding has aided the development of more suitable applications for different scenarios. Fig. 1 shows schematics of common detection techniques that will be discussed in detail (Islam et al., 2022a, 2022b, 2022e).

2 Clinical manifestation

Fever, chills, cough, shortness of breath, dyspnea, expectoration, headache, nausea, vomiting, muscle pain, joint pain, weakness, fatigue, and other symptoms are common in COVID-19 patients (Yang et al., 2020). Fever, cough, and fatigue are the three most common in patients, with fever being the most serious. About 90% of patients exhibit more than one clinical symptom. Following COVID-19 infection, patients show hematologic signs of leukopenia, lymphocytopenia, and decreased platelet counts, as well as decreased cluster of differentiation 4-positive ($CD4^+$) and $CD8^+$ T lymphocyte subsets in peripheral blood, eosinophil reduction in most patients, decreased hemoglobin solubility to blood cells, and abnormal coagulation indicators (Shen et al., 2020). In addition to cough and fever caused by lung inflammation, COVID-19 patients exhibit clinical symptoms in the gastrointestinal tract, liver, nerves, kidneys, and eyes.

The most common and critical febrile symptoms of COVID-19 patients can be used as crude screening criteria in the general population. Furthermore, the sudden loss of smell and taste has been linked to the possibility of COVID-19 infection, providing a smoking gun for COVID-19. Early symptoms, such as eye symptoms, coughing, and fatigue, may provide a preliminary clinical diagnosis. Patients with certain organ or system injuries, such as gastrointestinal tract and

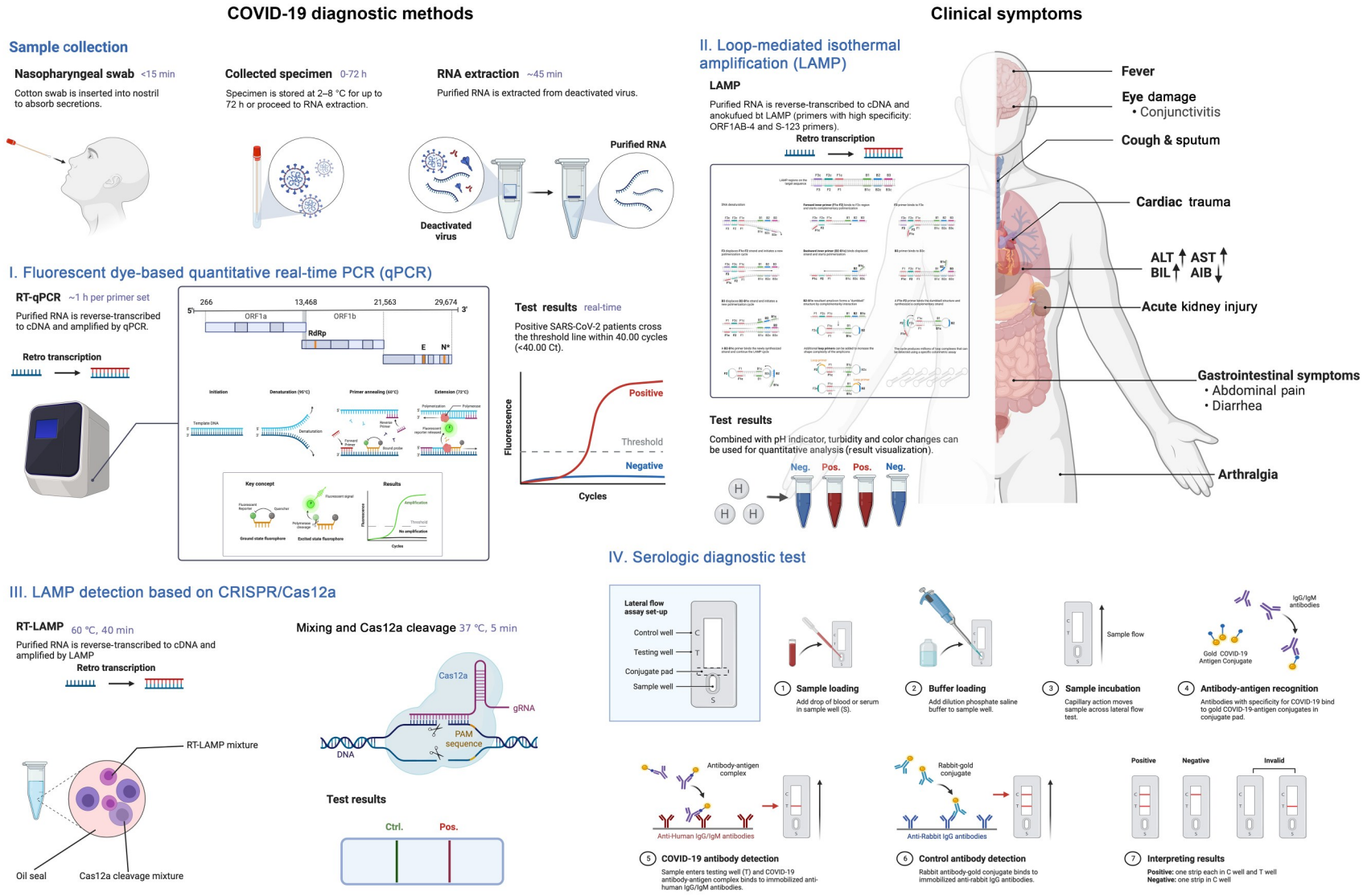

Fig. 1 Schematics of common COVID-19 detection techniques. COVID-19: coronavirus disease 2019; RT-qPCR: reverse transcription-quantitative real-time polymerase chain reaction; cDNA: complementary DNA; ORF: open reading frames; RT-LAMP: reverse transcription-loop-mediated isothermal amplification; CRISPR: clustered regularly interspaced short palindromic repeats; Cas12a: CRISPR-associated (Cas) protein 12a system; gRNA: guide RNA; PAM: protospacer adjacent motif; Neg.: negative; Pos.: positive; Ctrl.: control; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BIL: bilirubin; ALB: albnmin; Ig: immunoglobulin.

eye injuries, may have a more severe course of the disease, which could guide treatment methods to a certain extent. Hematological manifestations such as lymphocytopenia, leucopenia, and thrombocytopenia may aid clinical detection. Brodin (2021) discovered that children under the age of 18 are less likely to encounter severe symptoms of COVID-19, require hospitalization, or die from the disease than the elderly. A combination of the viral spike (S) protein and angiotensin-converting enzyme 2 on the host cells leads to an imbalance of neurons, astrocytes, and oligodendrocytes, which makes the brain vulnerable to being an attractive target for SARS-CoV-2 infections (Adams et al., 2020; Dhochak et al., 2020; Steinman et al., 2020). Furthermore, this phenomenon can cause neurological and cognitive disorders, such as encephalitis, dementia, and neurodegeneration (Verkhratsky et al., 2020; Conte, 2021; Mahalakshmi et al., 2021; Frank et al., 2022).

In screening high-risk groups in China, lung X-ray imaging is also an important way to determine whether a patient is at risk of transmission and disease severity. However, in the case of mass screening, the labor required for film reading is very large, and places high demands on imaging physicians who need experienced support. To alleviate this problem, researchers try to use computer automatic recognition to replace human resources. Among the automated detection tools provided by Alqahtani et al. (2021), a new method was able to detect and determine the degree of COVID-19 lung infection with 91% accuracy compared to the opinions of three experienced radiologists, and also effectively determine the severity of the disease. Rajpal et al. (2021) used computing and deep learning techniques to conduct 10-fold cross-validation and found that the overall classification accuracy of the model in identifying COVID-19 lung infections could reach 97.4%. For the first through tenth editions of the Chinese Health Commission's Guidelines on COVID-19 Diagnosis and Treatment, please refer to Table 1.

3 SARS-CoV-2 virus isolation

The isolation of the SARS-CoV-2 virus by cell culture contributed to diagnostic precision during the COVID-19 pandemic. This method involves the cultivation of cells in a controlled environment to support the replication and observation of the virus (da Silva et al., 2020; Harcourt et al., 2020; Manenti et al., 2020; Shi P et al., 2020). The procedure provides a critical tool for investigating the behavior of the virus and its interactions with host cells, leading to a deeper understanding of its biology and pathogenesis (Shi R et al., 2020). Furthermore, the method has been instrumental in the rapid development of diagnostic tests and treatments during the COVID-19 pandemic, and continues to play a key role in ongoing efforts to control and prevent the spread of this devastating virus (Guo et al., 2021).

Despite its impressive precision, the method is not without challenges. The potential for contamination is always present, requiring strict sterilization procedures and a meticulous approach to every step of the process (Sandle, 2013; Rutala and Weber, 2017). The cultivation of cells and viruses in the laboratory can be a delicate balancing act, requiring precise conditions, rigorous controls, and constant monitoring (Mackay, 2004; Greenwood et al., 2012; Sirois, 2014). Therefore, more standardized virus isolation procedures will help promote repeatability and accuracy.

4 Gene detection

Current COVID-19 gene detection uses mainly sequencing techniques and PCR (Zhu et al., 2020). SARS-CoV-2 is a virus whose genetic material is RNA. The virus's signature sequence can be detected using direct sequencing or indirect complementary DNA (cDNA) sequencing by reverse transcription. In addition, PCR, based on the principle of complementary base pairing, can directly detect the presence of SARS-CoV-2 viral nucleic acid sequences in infected patients.

4.1 Gene sequencing

Next-generation sequencing (NGS) can not only provide microbiological detection and organism typing for clinical samples from patients, but also be used in epidemiology, lineage tracing, susceptibility prediction, virulence factor determination, and drug-resistance testing (Gu et al., 2019). NGS operates by generating millions or billions of small DNA fragments from a sample and sequencing those fragments simultaneously in parallel (Metzker, 2010). The fragments are then reassembled into a complete genome

Table 1 Content and update of the Chinese Health Commission's Guidelines on COVID-19 Diagnosis and Treatment (from the first to the tenth edition)

Edition	Suspected cases	Confirmed cases	Changes
The first edition 2020-01-15	A suspect case had been to Wuhan or had direct or indirect contact in relevant markets in Wuhan (South China Seafood Market) within two weeks prior to the onset of the disease and had typical clinical manifestations and no significant improvement or progressive aggravation after 3 d of treatment with standard antibiotics.	Based on the suspected cases, the sputum, throat swab, and other respiratory tract specimens are sequenced by the whole genome of the virus, and the results are highly homologous to SARS-CoV-2.	The scope of epidemiological history only focused on the Wuhan region and did not include other regions.
The second edition 2020-01-22	A suspect case has any of the epidemiological history[a] plus all three clinical manifestations[c].	Suspect cases with one of the following etiological evidence: 1. Real-time fluorescent RT-PCR indicates positive for SARS-CoV-2 nucleic acid of respiratory tract specimens or blood samples. 2. Viral gene sequence is highly homologous to SARS-CoV-2.	The definition of observation cases was updated as suspected cases.
The third edition 2020-01-23	A suspect case needs to be identified in combination with epidemiological history[a] and clinical manifestations[c] (the first and third clinical manifestations of the second and third editions are required to be met).	Suspect cases with one of the aforementioned etiological evidence.	The definitions of mild cases and asymptomatic cases were added. There was no imaging sign of pneumonia in mild cases, but the respiratory tract specimens positive for the etiology of SARS-CoV-2.
The fourth edition 2020-01-27	A suspect case needs to be identified in combination with epidemiological history[a] and clinical manifestations[c] (the contents of epidemiological history[a] and clinical manifestations[c] are updated).		The scope of epidemiological history was extended to Wuhan and its surrounding areas, or other communities with reported cases. The criteria for suspicious cases were changed to meet any two clinical manifestations.
The fifth edition 2020-02-05	A suspect case has any of the epidemiological history[a] plus any two clinical manifestations[c] or all three clinical manifestations[c] if there is no clear epidemiological history.		Added the diagnostic classification, clinically diagnosed cases, for Hubei Province (a suspected case with the imaging characteristics of pneumonia becomes a confirmed case when the PCR test for SARS-CoV-2 is positive).

To be continued

Table 1 (continued)

Edition	Suspected cases	Confirmed cases	Changes
The sixth edition 2020-02-19		Suspect cases with one of the following etiological or serological evidence: 1. RT-PCR indicates positive for SARS-CoV-2. 2. Viral gene sequence is highly homologous to SARS-CoV-2. 3. Specific IgM and IgG are detectable in serum and IgG reaches a titration of at least a 4-fold increase during convalescence compared with the acute phase.	The positive detection of specific IgM and IgG in serum was added as the optional criteria.
The seventh edition 2020-03-04	1. A suspect case has any of the epidemiological history[a] plus any two clinical manifestations[c] or all three clinical manifestations[c] if there is no clear epidemiological history. 2. A suspect case has any two clinical manifestations and is positive for SARS-CoV-2 specific IgG antibodies if there is no clear epidemiological history.	Suspect cases with one of the aforementioned etiological or serological evidence.	None
The eighth edition 2020-08-19	1. A suspect case has any of the epidemiological history[b] plus any two clinical manifestations[c] or all three clinical manifestations[c] if there is no clear epidemiological history. 2. A suspect case has any two clinical manifestations[c] and is positive for SARS-CoV-2 specific IgM antibodies if there is no clear epidemiological history[b]. (People who have recently received the COVID-19 vaccine do not take these conditions as reference indicators.)	Suspect cases with one of the following etiological or serological evidence: 1. Tests indicate positive for SARS-CoV-2 nucleic acid. 2. Patients who have not received COVID-19 vaccines are positive for SARS-CoV-2 specific IgM and IgG antibodies.	As the vaccination rate increases, attention should be paid to the exclusion of false antibody positives due to vaccination.

To be continued

Table 1 (continued)

Edition	Suspected cases	Confirmed cases	Changes
The ninth edition 2021-03-14	1. A suspect case has any of the epidemiological history[b] plus any two clinical manifestations or all three clinical manifestations if there is no clear epidemiological history. 2. A suspect case has any two clinical manifestations[c] and is positive for SARS-CoV-2 specific IgM antibodies if there is no clear epidemiological history. (People who have recently received the COVID-19 vaccine do not take these conditions as reference indicators.)	Suspect cases with one of the following etiological or serological evidence: 1. Tests indicate positive for SARS-CoV-2 nucleic acid. 2. Patients who have not received COVID-19 vaccines are positive for SARS-CoV-2 specific IgM and IgG antibodies. 3. Tests indicate positive for SARS-CoV-2 antigen.	On the basis of nucleic acid testing, antigen testing is added as a supplement to further improve the ability of early case detection.
The tenth edition 2023-01-05	Cancellation of suspected cases	Clinical manifestations plus microbiological examination (satisfying one is enough) Microbiological examination: 1. Positive for SARS-CoV-2 nucleic acid testing; 2. Positive for SARS-CoV-2 antigen testing; 3. Positive for SARS-CoV-2 isolation and culture; 4. SARS-CoV-2-specific IgG antibody level increased to at least 4-fold level in the acute phase during recovery.	The requirement of mandatory nucleic acid testing has been revoked, and any one of the microbiological tests that are conducted will suffice.

[a] Epidemiological history: 1. History of travel to or residence in Wuhan and its surrounding areas, or in other communities where cases have been reported within 14 d prior to the onset of the disease; 2. In contact with novel coronavirus-infected people (with positive results for the nucleic acid test) within 14 d prior to the onset of the disease; 3. In contact with patients who have a fever or respiratory symptoms from Wuhan and its surrounding areas, or from communities where confirmed cases have been reported within 14 d before the onset of the disease; 4. Clustered cases (two or more cases with fever and/or respiratory symptoms in a small area such as families, offices, and schools within two weeks). [b] Epidemiological history: 1. A history of travel or residence in the reported community within the 14 d prior to onset of illness; 2. History of contact with a novel coronavirus infection within 14 d prior to onset of illness; 3. Patients with fever or respiratory symptoms who had been in contact with communities with their own case reports within 14 d prior to onset of illness; 4. Cluster onset (more than two cases of fever and/or respiratory symptoms within 14 d in a small area such as home, office, and school class). [c] Clinical manifestations: 1. Fever and/or respiratory symptoms; 2. The aforementioned imaging characteristics of COVID-19; 3. Normal or decreased WBC count, normal or decreased lymphocyte count in the early stage of onset. SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; RT-PCR: reverse transcription-polymerase chain reaction; Ig: immunoglobulin; COVID-19: coronavirus disease 2019; WBC: white blood cell.

using advanced computational methods (Quail et al., 2012). In the early stages of the epidemic, in the absence of relevant information such as the SARS-CoV-2 gene sequence and primers, NGS played an important role in pathogen detection. The Chinese Center for Disease Control and Prevention isolated and sequenced the SARS-CoV-2 virus and investigated the etiology of the disease using NGS (Chan et al., 2020; Li et al., 2020; Nie et al., 2020; Zhu et al., 2020). The virus's complete genomic information was subsequently submitted to the World Health Organization (WHO) and stored in Global Initiative on Sharing All Influenza Data (GISAID) (Shu and McCauley, 2017; Tan et al., 2020), aiding the fight against COVID-19.

NGS detection aided the development of specific RT-PCR (Corman et al., 2020; Motayo et al., 2021). Its results can be used to monitor variation among SARS-CoV-2 strains and provide a basis for judging a change in virulence and transmission of a mutant strain (Bal et al., 2020; Holland et al., 2020). For example, in the new outbreak in Guangdong Province, China, in May 2021, the genetic sequencing results of infected patients were found to be highly homologous, and all were Delta variants. Furthermore, NGS can help with the development of antiviral strategies (Dai et al., 2020) and vaccine candidates (Kames et al., 2020), and determining the effectiveness of interventions (Grubaugh et al., 2019; Oude Munnink et al., 2020) for SARS-CoV-2.

4.2 PCR

As a technique for amplifying nucleic acid in vitro with high sensitivity and specificity for early diagnosis, PCR is regarded as the "gold standard" for detecting the presence of viruses. In addition to traditional RT-PCR methods, new techniques are being developed such as isothermal and thermostatic amplification chips, which can achieve higher operational convenience and a shorter detection time. Table 2 summarizes and compares several nucleic-based molecular biological detection methods, serving as a resource for increasingly complex detection occasions and requirements.

4.2.1 RT-PCR

RT-PCR technology converts the virus's RNA genome into cDNA, using RNA-dependent DNA polymerase (reverse transcriptase). The DNA copy of the virus genetic material increases exponentially after repeated cyclic amplification, making detection easier. This cDNA is then used as a template for the PCR, where the DNA is amplified millions of times over, allowing for highly sensitive detection (Lanciotti et al., 1992). The beauty of RT-PCR lies in its ability to detect RNA molecules rapidly and accurately, even at very low concentrations (Iscove et al., 2002). The design of virus-specific primers and probes accelerated following the release of the SARS-CoV-2 genome sequence (Yuan et al., 2021). As reported by Pumford et al. (2020), the process begins with the design of specific primers, which serve as the foundation for the amplification of the viral RNA present in a sample. These primers are then combined with probes, often labeled with fluorescent dyes, to enable the detection of the amplified products and to quantify the amount of virus present. The virus's RNA sequence serves as a highly specific RT-PCR marker, distinguishing it from other pathogens. The design of primers and probes for specific gene detection has greatly improved the technology's sensitivity. Considering the positive rates of various specimens and the degree of acceptance by the subjects, the following are recommended:

(1) Nasopharyngeal swabs should be taken as a priority for screening of asymptomatically infected persons and the general population.

(2) For patients with acute COVID-19, respiratory tract samples should be taken.

(3) At a later stage, additional fecal samples can be collected for monitoring recovery from the disease.

(4) False negatives can be effectively avoided by combining samples from different sources.

In conclusion, RT-PCR is regarded as the best diagnostic option for a wide range of surveillance strategies. There are many available RT-PCR kits based on one-step amplification, which ensures the standardized process and low systematic error (Smyrlaki et al., 2020; Wang CJ et al., 2022). This method, however, has a certain false-negative rate due to non-standard sampling procedures or low viral loads (Sun and Guan, 2020). In the laboratory testing technology guide of the COVID-19 Diagnosis and Treatment Guideline (the fifth edition), the National Health Commission clearly states that a negative PCR test result cannot exclude SARS-CoV-2 infection, and that it is necessary to exclude the factors that may produce a false negative.

Table 2 Comparison and evaluation of major molecular biological detection methods

Method	Principle	Time	Advantages	Disadvantages	Evaluation	Samples	References
RT-PCR	Reverse transcriptase converts the viral RNA genome to cDNA. The DNA copy of the virus's genetic material grows exponentially after repeated cycles of amplification, making it easy to detect.	2 h	1. Low cost; 2. Good specificity; 3. High sensitivity; 4. Can be used for early diagnosis.	1. It takes a long time and the operation is complicated; 2. False-negative is easy to occur due to the influence of multiple factors; 3. It has high personnel and laboratory equipment requirements, making it difficult to popularize in primary medical institutions.	It is the most widely used and one of the "gold standards" for diagnosis. It can be used in a variety of situations, including case diagnosis, condition analysis, and population screening.	Blood, tissue, and cell lines	Lanciotti et al., 1992; Gill et al., 2009; Grubaugh et al., 2019; Kames et al., 2020; Oude Munnink et al., 2020; Tsang et al., 2021
Isothermal amplification technique	For each region of the target gene, 4–6 pairs of specific primers were designed. The nucleic acid was then amplified by incubating them with reverse transcriptase and DNA polymerase with strong chain replacement activity and high-temperature tolerance for 20 to 60 min at a constant temperature.	15–60 min	1. High sensitivity; 2. Quick and portable; 3. Results visualization.	The possibility of non-specific binding of primers is high, resulting in false positives.	Used to monitor a large number of exposed individuals and to facilitate screening in hospitals and the public sector.	Saliva, urine, and sputum	Liu et al., 2016; Li et al., 2018; Gao W et al., 2019; Pumford et al., 2020; Smyrlaki et al., 2020; Sun and Guan, 2020; Gao YP et al., 2022

To be continued

Table 2 (continued)

Method	Principle	Time	Advantages	Disadvantages	Evaluation	Samples	References
Isothermal amplification chip method	Combining isothermal amplification technology with microfluidic chip technology.	50 min	1. Simultaneous detection of multiple pathogens from the same sample; 2. Strong integration ability and a high degree of automation; 3. Detection time is so short that suitable for real-time detection.	Chip design, material selection, processing, packaging, and storage are all challenging tasks.	Multiple indicators can be combined to effectively distinguish influenza patients from COVID-19 patients and achieve an accurate patient diagnosis.	Saliva, urine, and sputum	Gill et al., 2009; Pumford et al., 2020; Smyrlaki et al., 2020; Sun and Guan, 2020; Tsang et al., 2021; Wang et al., 2021; Gao YP et al., 2022
LAMP detection based on CRISPR/Cas12a	The target sequence was amplified. To activate Cas12a, RNA was directed to recognize the target sequence and interact with the Cas12a-gRNA RNP complex. As a result, the target sequence is specifically cut and produces fluorescence.	40 min	1. Reduced operational complexity and the possibility of cross-contamination; 2. Strong system stability; 3. Low requirements on operating environment, and can be used for field testing.	The development began relatively late, and further research is required to ensure detection accuracy.	This method is in good agreement with qPCR and provides a simple and reliable field diagnosis method suitable for community testing.	Bacterial cultures, environmental samples, and clinical specimens	Li YY et al., 2017; Hu et al., 2020; Yan et al., 2020; Zhang et al., 2020; Li F et al., 2021

RT-PCR: reverse transcription-polymerase chain reaction; cDNA: complementary DNA; COVID-19: coronavirus disease 2019; LAMP: loop-mediated isothermal amplification; CRISPR: clustered regularly interspaced short palindromic repeats; Cas12a: CRISPR-associated protein 12a system; gRNA: guide RNA; RNP: RNA polymerase; qPCR: quantitative PCR.

4.2.2 Isothermal amplification technique

Isothermal amplification is a new and simple nucleic acid amplification technology. In contrast to RT-PCR, the reaction process is kept at a constant temperature, eliminating the need for temperature cycling, and rapid nucleic acid amplification can be accomplished by adding active enzymes and specific primers. By leveraging the use of heat-stable enzymes, such as recombinase polymerase amplification (RPA) and ligase chain reaction (LCR), this method has shown remarkable efficiency and specificity in detecting and quantifying low-abundance target sequences (Li et al., 2017; Gao YP et al., 2022). The requirements of isothermal amplification for specialized instruments are greatly simplified and such instruments may even not be needed, and the reaction time is greatly reduced, allowing the technique to better meet the needs of rapid and simple detection. Currently, the most widely used isothermal amplification technique is loop-mediated isothermal amplification (LAMP), whose main advantage is the ability to visualize the results. It can be used for quantitative analysis based on turbidity and color changes. Because LAMP performs the amplification reaction at a lower temperature, the possibility of nonspecific binding of primers increases, which makes false positives more likely. Fortunately, detection specificity can be improved by combining fluorescent probes and designing appropriate primers. Yan et al. (2020) designed five groups of primers based on the reverse transcription-LAMP (RT-LAMP) method, among which the open reading frames 1ab (ORF1ab)-4 and S-123 primers amplified the gene in the shortest time. Hu et al. (2020) confirmed that RT-LAMP had a higher sensitivity and specificity in the diagnosis of SARS-CoV-2 infection than RT-qPCR. Therefore, the RT-LAMP detection and LAMP sequencing can broaden the range of available test methods and supplement RT-PCR-based single and combination tests with faster, simpler, and potentially less expensive test methods.

The isothermal amplification chip method, combining isothermal amplification technology with microfluidic chip technology, can be used to detect multiple pathogens in the same sample. It is highly integrated with a high degree of automation, short detection time, and low detection limit (up to 100 copies/mL).

4.2.3 LAMP detection based on CRISPR/Cas12a

Clustered regularly interspaced short palindromic repeats (CRISPR) technology is a nucleic acid testing tool with a high degree of specificity. The CRISPR/CRISPR-associated protein 12a system (Cas12a)-based LAMP combines RT-LAMP with CRISPR/Cas12a, and is a groundbreaking approach to disease diagnosis. An RT-LAMP reaction is carried out at 65 ℃ to amplify the SARS-CoV-2-specific nucleic acid sequence, and detection is then performed at 37 ℃ using CRISPR/Cas12a technology. This system works using a guide RNA (gRNA) to direct the Cas12a enzyme to a target DNA sequence, where it cleaves the DNA. In the LAMP detection method, the target DNA is amplified in an isothermal reaction and the resulting amplicons are then detected using specific fluorescent dyes. The entire amplification and detection process is completed within 40 min (Chen et al., 2020). This method is highly specific and sensitive, avoiding cross-reactivity with related coronaviruses. Pang et al. (2020) developed a single-tube method for detecting SARS-CoV-2 in patients, combining the benefits of RT-LAMP isothermal exponential amplification and the sequence recognition specificity of the CRISPR/Cas12a system, which further simplifies operation and reduces contamination at a constant temperature. Wang et al. (2021) proposed the "opvCRISPR" detection system, which combines RT-LAMP amplification and CRISPR cutting. This system has a high specificity, a sensitivity similar to that of RT-PCR, and short detection time, and requires less equipment. Thus, it compensates for the shortcomings of RT-PCR, which is not suitable for large-scale diagnosis, requires expensive equipment, and has a long reaction time. The opvCRISPR method has enormous potential for SARS-CoV-2 detection in next-generation point-of-care molecular diagnostics (Wang et al., 2021).

5 Immunodiagnosis

Serological tests are quick, simple, sensitive, accurate, and stable, making them ideal for large-scale testing and tracing previously infected patients (Dheda et al., 2013; Mercer and Salit, 2021). There are numerous serological detection methods for SARS-CoV-2, including direct detection and indirect detection. Direct detection involves detecting SARS-CoV-2 RNA sequences or landmark proteins like the S protein (Mohammadi et al., 2022). Antibody detection, which looks for SARS-CoV-2-specific antibodies in the body, is an example of indirect detection. Immunoglobulin

G (IgG), IgM, or both antibodies were used to test for COVID-19 (la Marca et al., 2020; Safiabadi Tali et al., 2021). The National Health Commission included the SARS-CoV-2 serum antibody test as an auxiliary detection method in the Protocol on Prevention and Control of COVID-19 (Edition 6) (http://en.nhc.gov.cn/2020-03/29/c_78468.htm). Regarding the definition of specific IgM and IgG positivity, IgM or IgG changes from negative to positive, or IgM or IgG has a titer of at least 4-fold increase during convalescence compared with the acute phase. The rapid and sensitive immunological test method is being promoted as an auxiliary test method for the rapid diagnosis of SARS-CoV-2 across the country. It can reduce the false-negative rate when performed in conjunction with a PCR test, track the progression of the disease, and reduce the risk of exposure of medical personnel due to the ease of sampling (Chau et al., 2020; Kucirka et al., 2020).

5.1 Antigen detection

Antigen detection has high accuracy and a fast reporting speed (usually about half an hour). It can also provide results at an earlier stage of infection (Santiago, 2020; Yüce et al., 2021). Rapid antigen testing can identify SARS-CoV-2 positive individuals by detecting the SARS-CoV-2 nucleocapsid protein or S protein in swabs taken from the upper respiratory tract of suspected infected subjects (Taleghani and Taghipour, 2021). A lateral flow test employs the immunochromatographic principle: the test sample is moved across the matrix to bind antigens to antibodies through capillary action, allowing these complexes to be detected and visualized (Singh et al., 2015; Koczula and Gallotta, 2016).

Antigen detection is not only important for diagnosing infected patients, but also can reduce the reliance on the PCR assay to some extent (Singh et al., 2015). The sensitivity of rapid point-of-care antigen detection for SARS-CoV-2 infection within 7 d of symptom onset ranged from 77.3% (duration 1 to 33 d) to 100% in a low-prevalence setting (Chen et al., 2021; Muhi et al., 2021). Corman et al. (2021) and Hirotsu et al. (2021a, 2021b) recently comprehensively evaluated PCR-based quantitative antigen tests and found that the Roche and Lumipulse antigen tests showed high concordance up to 9 d after symptom onset using RT-PCR as a reference. The Lumipulse® antigen assay has a sensitivity and specificity of over 90% in RT-PCR positive samples, demonstrating that the antigen detection system can be safely used for screening in the general population (Gili et al., 2021). When it comes to detecting recombinant proteins or viruses, the RapiGEN test outperforms all others.

Krüttgen et al. (2021) researched the sensitivity and specificity of the SARS-CoV-2 Rapid Antigen Test (Roche) using swabs from patients previously tested by SARS-CoV-2 PCR and concluded that the sensitivity and specificity of the antigen assay are inferior to those of a PCR assay. A study found that immunochromatographic AMP rapid antigen test was highly sensitive at low cycle threshold (C_T) values when rapid RT-PCR tests were not feasible, making it a valuable tool for front-line testing (Leixner et al., 2021). All these studies suggest that antigen tests are somewhat feasible, but may be limited by viral load.

The sensitivity and specificity of currently approved antigenic assays are not as good as those of PCR assays, but antigenic assays may provide an alternative that is quick and easy to perform (García-Fiñana and Buchan, 2021). Their accuracy is heavily reliant on a high viral load (Menchinelli et al., 2021), which means that positive results are more likely in the early stages of the disease. This also explains why the LUMIPULSE G600II automated immunoassay analyzer (FujireBio) had a sensitivity of only 55% when applied to long-term follow-up samples from a small number of patients (Hirotsu et al., 2020). According to the WHO interim guidelines, negative predictive value (NPV) is more reliable in populations with low infection rates, whereas a higher positive predictive value (PPV) can reduce the time burden and cost of procalcitonin (PCT) in populations with high infection rates. Therefore, we believe that attention should be paid to how antigen detection and PCR tests should be allocated in the diagnosis of COVID-19 patients with different infection ratios, infection tolerance, and detection conditions in different regions (Mina et al., 2020; Fitzpatrick et al., 2021). When the viral load is reduced to a concentration unlikely to be detectable by antigen-rapid diagnostic testing (Ag-RDT) in patients going to hospital more than seven days after symptom onset, the best option is to confirm COVID-19 using a combination of molecular and antibody tests (Peeling et al., 2021).

More notably, recent studies have shown that some antigen tests are also good at identifying mutated viruses. Soni et al. (2022) compared the sensitivity

of Ag-RDT to the Delta and Omicron variants and found that the sensitivity was highly consistent. Although certain mutated sites affected the accuracy of some antigen tests, other tests were unaffected when comparing results for antigen-negative samples identified by Bourassa et al. (2021) using different tests. Therefore, careful screening and cross-use of different antigen detection methods could reduce the probability of missed diagnosis.

The development of antigen detection deserves further attention as its accuracy still lags a little behind the gold standard. Whether a detection method is worthy of research and large-scale implementation depends not only on the accuracy and sensitivity of the method. A test method for COVID-19 is always intended to be used on patients or the public, and its operability on a large scale must also be considered. The operational difficulty of antigen detection is very low and the result interpretation is relatively simple. These advantages are very important in large-scale deployment. Therefore, further development of antigen detection methods is desirable.

5.2 Antibody detection

Antibody detection is now widely used in clinical detection and is included in diagnostic evidence along with RT-PCR (Li et al., 2020). However, antibody detection cannot replace PCR detection and viral gene sequencing as the gold standard for confirming SARS-CoV-2 infection (Lv et al., 2020). On the one hand, serological detection can only indirectly prove the existence of the virus (Peto, 2020). On the other hand, at the stage of large-scale vaccination, it is unclear whether the positive antibody is the result of SARS-CoV-2 infection or vaccination (Yue et al., 2022). This rapid, simple, and highly sensitive method can improve the sensitivity of the diagnosis of COVID-19 and represents an appropriate supplement to a PCR test (Wu et al., 2022).

The main methods of antibody detection include chemiluminescent immunoassay (CLIA), enzyme-linked immunosorbent assay (ELISA), and lateral flow immunoassay (LFIA) (Nicol et al., 2020). Montesinos et al. (2020) evaluated CLIA, ELISA, and LFIA, and pointed out that they were accurate and equivalent in detecting SARS-CoV-2 antibodies. This confirmed their suitability for clinical use and for formulating an epidemiological strategy for the COVID-19 pandemic (Montesinos et al., 2020; Yadav et al., 2021). Antibody detection is mainly for IgM and IgG antibodies. Li et al. (2020) used a lateral immunoassay to simultaneously detect IgM and IgG antibodies against SARS-CoV-2 in human blood within 15 min. They found that this antibody detection method has high sensitivity and specificity, and that IgM-IgG combined detection has better practicability and sensitivity than IgM or IgG detection alone (Li et al., 2020).

The change of antibody titer in serum provides a very important reference value for the judgment of the course of infection and prognosis. According to Wu et al. (2020), the presence of antibodies in COVID-19 patients was <40% within one week after onset and rapidly increased to 100.0% (antibody), 94.3% (IgM), and 79.8% (IgG) by Day 15. In contrast, RNA detection decreased from 66.7% (58/87) in samples collected prior to Day 7 to 45.5% (25/55) during Days 15–39 (Wu et al., 2020). The dynamic monitoring of antibodies can improve understanding of the rules of antibody production and play an important role in follow-up investigations of the COVID-19 epidemic, the development of antibody drugs and vaccines, and clinical diagnosis and treatment. Moreover, it can be more effective in preventing and controlling COVID-19 (Qiu et al., 2020; Wang XN et al., 2020). If the SARS-CoV-2 antibody detection is combined with the PCR test to interpret the results of the "nucleic acid+antibody" test, it will be effective for improving the detection rate of diseases, monitoring diseases and controlling the epidemic situation, and achieving "early detection, early diagnosis, and early treatment." Table 3 summarizes the different possible results and diagnostic significance of coordinated nucleic acid and antibody testing. Table 4 summarizes the current methods commonly used for antibody detection.

The COVID-19 Diagnosis and Treatment Guideline (the seventh edition) adds a serological test method and states explicitly that specific antibody testing, PCR testing, and viral gene sequencing should be taken together as diagnostic evidence. In this case, the serological test results can not only be used to monitor the immune level, but also be a further supplement to the PCR test result. Compared to PCR, serological detection has advantages like having a simple operation, simple sample collection, and low exposure risk.

Table 3 Co-detection results of nucleic acids and antibodies of SARS-CoV-2

Result	IgM	IgG	Diagnostic significance
Positive (+)	+	+	The virus is active, but the body has a certain immunity.
	+	−	In the early stage of infection, IgG is not present or have low.
	−	+	Be in the middle or late stage of infection or have recurrent infection.
	−	−	In the window period of infection, no antibody has been detected yet.
Negative (−)	+	+	Be in recovery, but still have a certain number of antibodies.
	+	−	Most likely in the infection period, nucleic acid should be retested.
	−	+	Have recovered from a previous infection.
	−	−	There was no infection and no protective antibodies.

The data are referenced from the studies by Kubina and Dziedzic (2020), Fang et al. (2021), Yan et al. (2021), and Dou et al. (2022). SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; Ig: immunoglobulin.

Table 4 Characteristics and comparison of the main antibody detection methods

Method	Sensitivity (%)	Specificity (%)	Time	Advantages	Disadvantages
LFIA	71.4–88.7	90.6–98.4	15–20 min	1. Small and portable; 2. Quick and sensitive at a low cost; 3. Fingertip blood can be used for testing.	1. Quantitative analysis is unavailable; 2. It is easy to be contaminated by other factors and lead to false positives.
ELISA	75.6–100.0	85.7–100.0	1–5 h	1. High specificity and sensitivity; 2. Low difficulty in carrier standardization; 3. Low requirements for equipment.	1. Time-consuming and complicated; 2. Only semi-quantitative detection.
CLIA	96.0–98.0	89.4–100.0	1–2 h	1. High sensitivity and time-saving; 2. No enzymes; 3. Capable of quantitative analysis and automated high-throughput detection.	1. Special equipment is required; 2. High cost.
Protein microarray				1. Photosensitive dye marking; 2. Less specimen consumption but high sensitivity; 3. High throughput and parallel analysis.	1. High requirements for equipment; 2. Possibility of false positives.

LFIA: lateral flow immunoassay; ELISA: enzyme-linked immunosorbent assay; CLIA: chemiluminescent immunoassay.

Additionally, monitoring of SARS-CoV-2 antibodies using dried blood spots (DBSs) is a cutting-edge innovation in the fight against the COVID pandemic. This test can be operated by any individual given that it requires only dried blood samples pricked from fingers. In a study using this method to evaluate sensitivity and specificity based on 31 SARS-CoV-2 infected patients and 80 healthy volunteers, Karp et al. (2020) and Beyerl et al. (2021) stated that the technique perfectly distinguished positive and negative cases, and both the sensitivity and specificity reached almost 100%. This method allows for a simple, non-invasive, and cost-effective way to measure the presence of antibodies in an individual's blood, and also may be a convenient protocol for school age children or the disabled (Karp et al., 2020; Beyerl et al., 2021). By using DBS, researchers can easily collect and store blood samples, eliminating the need for complicated laboratory procedures (Beyerl et al., 2021; Miesse et al., 2022). In other studies by McDade et al. (2020) and Morley et al. (2020), DBS analysis was proven to be a highly reliable and accurate method for detecting SARS-CoV-2 antibodies. Gaugler et al. (2021) improved detection throughput using an automatic DBS processing and extracting technique. This technique, which greatly reduces detection cost and supports at-home testing, also demonstrates the potential of DBS for widespread sero-surveillance and tracking the evolution of the pandemic (Zava and Zava, 2021). Though there have been few studies involving DBS

analysis in China, especially in special populations such as school age children, it has great potential for use in the post-pandemic era.

Note that regardless of the method, serological test results provide only circumstantial evidence, which cannot be used as confirmed diagnostic evidence.

6 Imaging diagnosis

In an emergency, laboratory testing techniques may not be appropriate, and RT-PCR may produce a false negative if the viral load is insufficient. According to some studies, computed tomography (CT) scanning has higher sensitivity (88%–98%) and a lower false-negative rate than RT-PCR (Fang et al., 2020). In a study of 1014 patients, the sensitivity of chest CT scans for COVID-19 diagnosis was 97% (Xu et al., 2020). With analysis of serial RT-PCR assays and CT scans, the mean interval between the initial negative to positive RT-PCR results was about 5 d, indicating that CT scans could play a key role in the early detection and treatment of COVID-19. Because of the high sensitivity of imaging detection, the COVID-19 Diagnosis and Treatment Guideline (the fifth edition) added clinically diagnosed cases for Hubei Province, China. Cases with suspected pneumonia based on imaging characteristics but negative PCR test results were included in the scope of confirmed cases for unified isolating diagnosis and treatment.

The presence of ground-glass opacity (GGO) in the peri-pulmonary and subpleural areas is the most notable CT feature of COVID-19 pneumonia (Wang YH et al., 2020). The characteristics change over time, depending on the stage and severity of the pulmonary infection (Kobayashi and Mitsudomi, 2013). The imaging stages and manifestations of COVID-19 are summarized in Table 5.

Pan et al. (2020) investigated changes in the lung over time in patients recovering from COVID-19. They discovered that pulmonary involvement gradually decreased 10 d after symptom onset and then increased, showing mainly imaging signs such as GGO, paving stone signs and consolidation shadows. These symptoms then subsided 14 d after they first appeared. Bernheim et al. (2020) discovered a high frequency (56%) of normal CT results in the early stages of disease (0–2 d), with the most severe pulmonary infection peaking about 10 d after symptom onset. Tsang et al. (2021) studied longitudinal changes and confirmed that isolated GGO was most common after symptom onset, and that a mixed pattern of GGO and irregular linear opacity peaked from 6 to 11 d of the disease. Notably, the main limitation of CT in confirming COVID-19 is its low specificity (25%). Due to imaging features that may overlap, it is difficult to distinguish COVID-19 from other viral pneumonias (Bai et al., 2020). Previous diagnostic data from radiology departments at several hospitals in Hubei Province, China, showed that 30% to 40% of patients with COVID-19 symptoms based on chest CT had negative PCR test results. CT detection has a higher positive rate than nucleic acid detection, which allows medical staff to make more accurate clinical diagnoses. Furthermore, CT detection can help guide clinical decision-making and provide prognostic information.

7 Authors' opinion

Since the start of the pandemic, the requirements for the quality and speed of sample detection have increased. To some extent, this has promoted progress

Table 5 Imaging stages and manifestations of COVID-19

Period	Imaging findings
Early period	In the first week of onset, the thin ground glass density shadow is common. The thickening of the bronchial vascular bundle can be seen, or with local gridded thickening of the interlobular septum.
Exacerbation	The range of GGO shadows increases with increased density. Some are fused into lobules, while others are widely fused into bands or large sheets of dense shadows, with visible and multiple consolidations.
Critical period	Diffuse gridded GGO shadows appeared in both lungs, and a few individuals showed "white lungs." The majority of the lesions consisted of small flake consolidation. Both pleural cavities may have a small effusion.
Absorption	The lesion's scope and density were reduced. The GGO shadow may completely disappear as the exudate is gradually absorbed. Some patients, however, may have residual pulmonary fiber strip shadow.

COVID-19: coronavirus disease 2019; GGO: ground-glass opacity.

in detection technologies in China and increased the level of attention to detection. In addition, rapid and accurate testing has been proven to be a key component of the fight against COVID-19. At the current stage of the pandemic development in China, it is almost impossible for a large-scale epidemic to occur under the strict prevention and control of the government, the united efforts of grassroots organizations, and the meticulous cooperation of the entire nation (Cheng et al., 2023). However, due to the continual evolution of the virus and the emergence of individuals who remain positive after sitting through the formal quarantine period, a small number of sporadic cases cannot be completely avoided. Nationwide testing can be performed occasionally, but requires a lot of labor and materials, which depletes available medical resources. At the moment, it is critical to distinguish between healthy and infectious patients swiftly and accurately. Therefore, the most important current aim is to improve detection efficiency and accuracy while keeping detection methods simple. Further, to minimize the risk of infection and prevent the spread of the virus, it is crucial to adhere to strict biosafety and biosecurity measures in the laboratory. These measures include wearing personal protective equipment, implementing decontamination procedures, and following standard operating procedures (SOPs) for handling the virus.

Based on the detection methods described above, the main diagnosis method continues to rely on a combination of antigen and antibody detection, RT-PCR, and clinical symptoms, each with its own set of advantages and disadvantages. In the case of antigen detection, although the detection time is very short, it has certain viral load requirements, which may mean that the patient is already infectious by the time the virus reaches detectable levels. Although the amplification used in RT-PCR can greatly improve accuracy and the technology has advanced significantly, RT-PCR still cannot match the reporting speed of antigen detection, due to the limits of its concept. Therefore, we believe that the main focus should be on further improving the speed and accuracy of RT-PCR detection. On the other hand, there is also a need to find alternative techniques that can replace RT-PCR as the gold standard for confirming SARS-CoV-2 infection. By raising the sensitivity of antigen detection to a level comparable to that of RT-PCR and considering aspects such as the detection principle, a rapid and accurate detection approach with low environmental requirements may be created.

Recent research has indicated that nanomaterials such as graphene, or related antigen detection technologies, can considerably enhance accuracy. Thus, the viral load requirement can be minimized and the utilization rate of antigen testing can be improved in China. Zhang et al. (2022) used a graphene mixture to boost the sensitivity of interleukin-6 (IL-6) detection. Wang YW et al. (2022) created an electrochemical immunosensor based on staphylococcal protein A (SPA) and reduced graphene oxide-polyethyleneimine-silver nanoparticles-Nafion (rGO-PEI-Ag-Nf) for rapid detection of mAbs. Kuntip et al. (2021) discovered that graphene quantum dots have a high capacity for microRNA (miRNA) adsorption. In other words, graphene shows a higher adsorption capacity for the detection of particular biological macromolecules, which improves the sensitivity of detection and reduces the detection threshold. Gao JW et al. (2022) have developed a field-effect transistor biosensor based on a graphene oxide (GO)/graphene van der Waals heterostructure for selective and ultra-sensitive SARS-CoV-2 protein detection. It can effectively improve the fixed density of SARS-CoV-2 captured antibody, with strong selectivity and high sensitivity, and represents a potential method for rapid and accurate detection of SARS-CoV-2. To some extent, graphene has been shown to improve the sensitivity of biological macromolecule detection. Extending this technique's application to the rapid identification of COVID-19 is a very promising research direction, especially in the application of fast antigen detection.

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Author contributions

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Compliance with ethics guidelines

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HUANG, and Baoqing SUN declare that they have no conflict of interest.

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