



Research progress in laboratory detection of SARS-CoV-2

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Abstract

Background Nucleic acid testing is a reliable method for diagnosing viral infection in clinical samples. However, when the number of cases is huge and there are individual differences in the virus itself, the probability of false-negative results increases. With the advancement in research on the new coronavirus, new detection technologies that use serum-specific antibodies as detection targets have been developed. These detection technologies have high efficiency and shorter turnaround time, which ultimately shortens the time required for diagnosis. This article summarizes the methods that have been reported to date for the detection of the new coronavirus and discusses their principles and technical characteristics.

Aims Compare the advantages and disadvantages of various SARS-CoV-2 detection methods and analyze their principles.

Methods Searched reports on SARS-CoV-2 detection methods published so far, extracted the data and analyzed them. Use the primer blast function of NCBI to analyze the primers used in qRT-PCR detection.

Results The detection sensitivity was the highest when nucleocapsid protein gene was used as the target, reaching 96.6%. The detection efficiency of the remaining targets ranged from 66.7% to 96.0%. Various new detection methods, like Serum specific antibody detection, can speed up the test time. However, due to the complexity of the method and higher testing requirements, it seems that it cannot be used as a complete replacement for qRT-PCR testing.

Conclusions With the advancement of technology and the improvement of methods, the detection methods of SARS-CoV-2 have become more mature. These advances provided great help to the detection of SARS-CoV-2.

Keywords Laboratory detection · Nucleic acid detection · SARS-CoV-2 · Serological detection

Introduction

Since the end of last year, pneumonia has been the primary clinical manifestation in patients infected with a novel coronavirus on a global scale. The novel coronavirus, identified as SARS-CoV-2, causes coronavirus disease 2019 (COVID-19). The characteristic clinical signs of COVID-19 are high fever,

shortness of breath, and dyspnea. However, few patients experience diarrhea and other gastrointestinal symptoms as the first or only manifestation. People of all ages are generally susceptible to the virus, which has an incubation period of 1–14 days or mostly 3–7 days. Middle-aged and older men with diabetes, hypertension, and other underlying disorders have been found to be more susceptible to infection with SARS-CoV-2 [1]. At present, COVID-19 has been incorporated as a class B infectious diseases according to the Law of the People's Republic of China regarding the Prevention and Control of Infectious Diseases. It has been controlled and has been treated as a class A infectious disease. Detection of viral nucleic acid based on quantitative real-time polymerase chain reaction (qRT-PCR) is important for the diagnosis of new coronavirus infection according to the “Diagnosis and Treatment Plan of Novel Coronavirus Pneumonia (seventh trial edition)” [2].

However, many factors, such as sample quality, amplified genes, and the skill level of the testing personnel, affect the accuracy of nucleic acid detection. In the present clinical studies, there have been many cases of false-negatives. Besides sample quality, the quality of the qRT-PCR kit used for nucleic acid

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detection can also affect the detection results [3, 4], such as the genes selected by the primers and the quality of the primers themselves.

Moreover, the use of novel coronavirus-specific IgM and IgG antibodies in the diagnosis and treatment of COVID-19 has been proposed. IgG antibody changes from negative to positive, or the recovery period is 4 times or more higher than the acute period can be one of the basis for case diagnosis. With the development of new technologies for the detection of SARS-CoV-2, antibody detection methods have been used as first-line virus detection methods because of their accuracy and speed. The purpose of this article is to summarize the new methods reported to date for the detection of SARS-CoV-2 and to discuss their principles and technical characteristics.

SARS-CoV-2 laboratory detection methods

qRT-PCR

Viral nucleic acid detection involves exponential amplification of a virus-specific characteristic target sequence using polymerase chain reaction (PCR). Each amplified DNA sequence can bind to a pre-added fluorescent-labeled molecule to produce a fluorescence signal. Greater the amount of target gene amplification, stronger is the accumulated fluorescence signal. If the fluorescence signal reaches the fluorescence threshold, the sample is considered positive. In contrast, specimens that do not contain viruses are negative.

At the beginning of the epidemic, the only criterion for the diagnosis of SARS-CoV-2 was nucleic acid detection. Numerous nucleic acid detection kits have been developed and put into clinical use after completion of SARS-CoV-2 genome-wide nucleic acid sequencing [5]. Compared with those of next generation sequencing (NGS), qRT-PCR has advantages, such as fast detection speed and compatibility with investigation of large sample sizes. Most of the kits target the coding region of SARS-CoV-2-specific genes, among which ORF1ab, N, and E are common. The efficacy of a qRT-PCR assay for respiratory tract samples [6] (pharyngeal swab), tracheal aspirate, bronchoalveolar lavage fluid, and other clinical samples was tested. The detection rate for early-stage samples is high, and the detection efficiency for different types of samples is different [7]. The frequency of use of oropharyngeal swabs was higher in the early stage of the epidemic. However, after collecting 1070 clinical samples for analysis, Wang et al. [8] found that the efficiency of detection of SARS-CoV-2 in oropharyngeal swabs (32%) was much lower than that in nasopharyngeal swab samples (62%). The highest detection efficiency (92%) was obtained with alveolar lavage fluid, but it is not widely used because of the complexity of the procedure involved in sample collection and due to the requirement of high-end equipment. qRT-PCR has improved the clinical detection efficiency of SARS-CoV-2, with

the continuous progression of the epidemic, and the number of samples to be tested has increased sharply; therefore, it would be challenging to meet the testing demands with the available number of first-line kits. Simultaneously, processes, such as the collection, transportation, preservation, and laboratory testing of such a large number of samples have led to an increase in the workload. It is difficult to guarantee quality control. In order to meet the clinical needs, various nucleic acid detection kits have been put into clinical application. As a result, the latest clinical guidelines no longer recommend nucleic acid testing as the only diagnostic criteria for COVID-19, and CT and other imaging findings combined with clinical manifestations have been added to the diagnostic criteria.

The sensitivity and accuracy of nucleic acid detection are influenced by many factors. In addition to subjective factors, such as sample collection, transportation, and the testing personnel's skill and ability, the nature and quality of the test kit can also have a significant impact on the accuracy of the results. Guo et al. [9] selected six nucleic acid detection kits for parallel detection of SARS-CoV-2 using samples from the same patient. The detection rate of only two kits reached 100%. The detection rate of the remaining four kits ranged from 50 to 83.3%. This also further indicates that the current clinical application of nucleic acid detection kits is uneven.

We summarized the primers used in the tests and performed a basic property analysis [10–14] (Table 1) that included primer length, GC%, T_m value, amplification efficiency, and sensitivity of primers (Table 2). The length range of the specific primers collected is 19–30 nt, mainly distributed in 19–22 nt. The difference between the forward and reverse primers lengths is generally not more than 3, and the T_m range is mostly in 52.27–60.93 °C. The difference in T_m between the forward and reverse primers does not exceed 3.68 °C. The G/C range is 31.82–57.89%. The difference in GC% between primer pairs does not exceed 20.81%. Among the genes amplified by the primers summarized in this paper (verified by Primer Blast), 3 were nucleocapsid-coding genes, 2 were E protein-coding genes, 2 were ORF1ab genes, and 1 coded for the S protein. In total, eight primer groups were selected, and N gene was the target gene of the majority of primer pairs (Fig. 1).

Hamid Reza Mollaeid et al. selected five genomic regions of the coronavirus SARS-2 virus, including nucleocapsid (N), envelope (E), RNA-dependent RNA polymerase (RDRP), ORF1Ab, and spike (S), for primer design. In this study, they evaluated the ability of conventional RT-PCR to detect SARS-CoV-2 RNA in five sets of primers. The results showed that the sensitivity of RT-PCR targeting RDRP, N, E, S, and ORF1Ab gene were 95.7%, 96.6%, 66.7%, 85.7%, and 96.0%, and the specificity were 88.9%, 100.0%, 66.7%, 50.0%, and 100.0%, respectively. Significant differences in analytical sensitivity were observed between primer groups studied in RT-PCR kits. The sensitivity, specificity, and positive predictive value of ORF1Ab, N and RDRP primers were higher than those of other primers.

Table 1 Primer sequences

ID	Primer (5'–3')	Product (bp)
Primer 1	F: TAATCAGACAAGGAACTGATTA R: CGAAGGTGTGACTTCCATG	110
Primer 2	F: AGAAGATTGGTTAGATGATGATAGT R: TTCCATCTCTAATTGAGGTTGAACC	118
Primer 3	F: ACTTCTTTTCTTGCTTTTCGTGGT R: GCAGCAGTACGCACACAATC	82
Primer 4	F: ACAGGTACGTTAATAGTTAATAGCGT R: ATATTGCAGCAGTACGCACACA	113
Primer 5	F: CACATTGGCACCCGCAATC R: GAGGAACGAGAAGAGGCTTG	128
Primer 6	F: CCCTGTGGGTTTTACTTAA R: ACGATTGTGCATCAGCTGA	119
Primer 7	F: CCTACTAAATTAATGATCTCTGCTTTACT R: CAAGCTATAACGCAGCCTGTA	158
Primer 8	F: GGGGAACCTTCTCCTGCTAGAAT R: CAGACATTTTGCTCTCAAGCTG	99

F forward primer, *R* reverse primer

Using ORF1ab, N and RdRp primers for RT-PCR analysis can quickly, sensitively, and specifically detect COVID-19 in patients with pneumonia [15].

Serum-specific antibody detection

In the human body, different types of specific antibodies are produced successively after infection with a virus. Initially, IgM antibodies are produced, which quickly reach a peak that is maintained for a short time. In the middle and late stages of the disease, IgG antibodies are produced, which can be maintained in the body for a long time. Meanwhile, some viruses, especially those that infect mucosal epithelial cells (e.g., SARS-CoV-2) can also induce mucosal IgA or secretory IgA production. As most of the IgM, IgG, and IgA molecules are present in serum, antibody-based detection is also called serological detection. Available evidence suggests that an antibody-based detection method can be used for the detection of SARS-CoV-2.

Table 2 Properties of primers

ID	T _m (F/R, °C)	GC (F/R, %)	Length (F/R, bp)
Primer 1	52.27/55.95	31.82/52.63	22/19
Primer 2	55.36/58.82	32.00/40.00	25/25
Primer 3	59.84/ 60.18	37.50/55.00	24/20
Primer 4	58.29/60.93	34.62/45.45	26/22
Primer 5	60.15/58.00	57.89/55.00	19/20
Primer 6	55.70/57.46	42.86/47.37	21/19
Primer 7	58.15/58.18	30.00/47.62	30/21
Primer 8	59.23/58.18	50.00/45.45	22/22

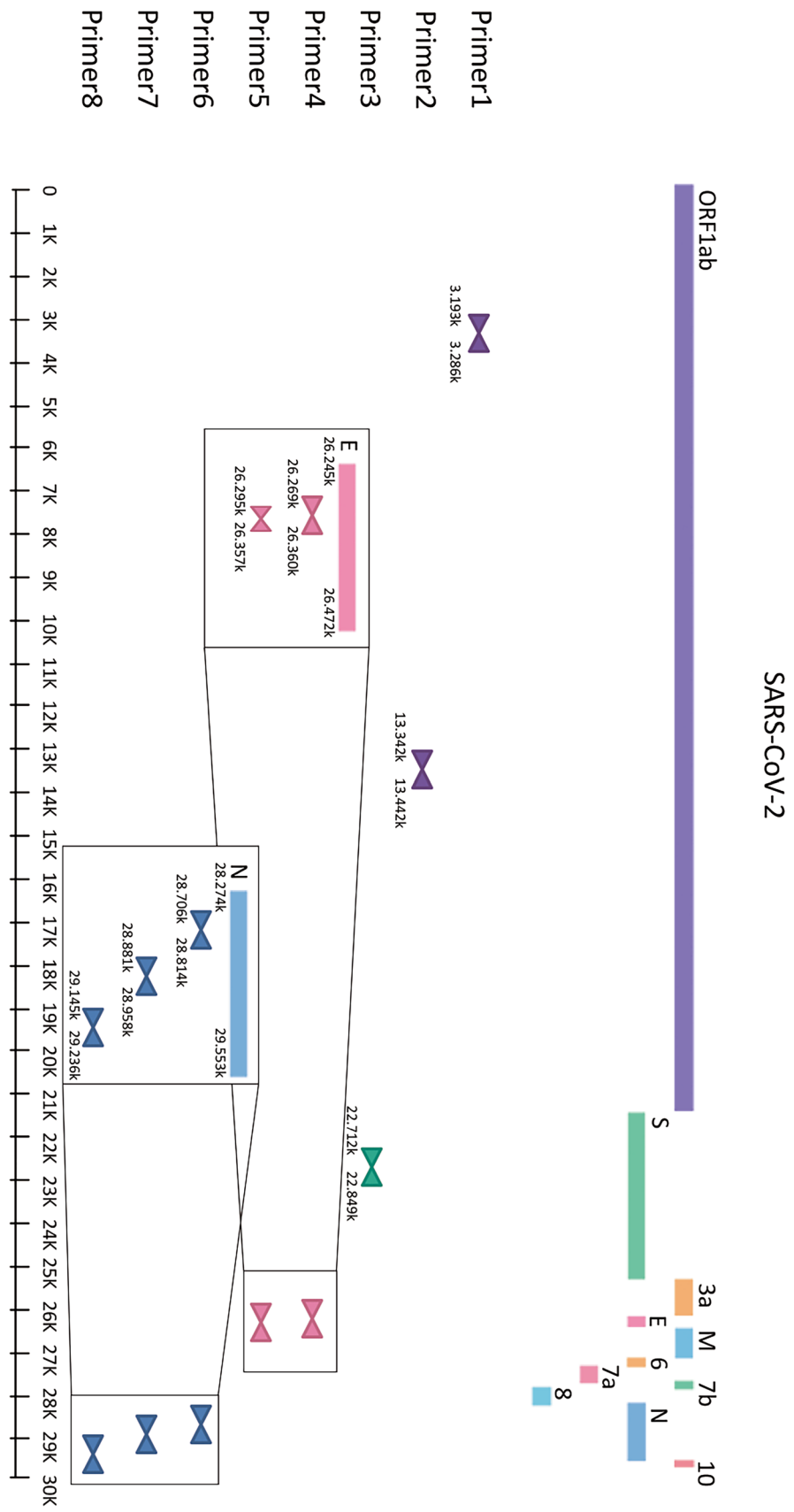
F forward primer, *R* reverse primer

An antibody-based detection method used for the detection of MERS [16] also showed high specificity and sensitivity for the detection of SARS-CoV [17]. Compared with nucleic acid-based detection, antibody-based detection (IgM and IgG) has the advantages of low threshold, convenient sample collection, high sensitivity, timely diagnosis, and wide application range. Nowadays, many antibody detection reagents are available for detection of SARS-CoV-2, and according to the type of antibody, they can be divided into total antibody detection reagent, IgM antibody detection reagent, and IgG antibody detection reagent. Various methods such as colloidal gold method [18, 19], enzyme-linked immunosorbent assay (ELISA) [20], and chemiluminescence [21] are used for antibody detection.

Colloidal gold method

In this method, colloidal gold particles coated with anti-SARS-CoV-2-IgM/IgG and immobilized on nitrocellulose membranes are used for capturing SARS-CoV-2 in human serum based on the principle of lateral immunochromatography. An antigen-antibody complex is formed by the colloidal gold-labeled mouse anti-human IgM/IgG antibody, which, through the mobile phase, accumulates at the detection line that appears as a red colored line [22]. Using the colloidal gold method, Zhang Jian [18] identified 105 clinical samples among 304 clinical samples to be SARS-CoV-2 nucleic acid-positive. The sensitivity of the colloidal gold assay for detection of SARS-CoV-2 IgM and IgG antibodies was 76.2% (80/105) and 86.6% (91/105), respectively. The overall IgM/IgG antibody-positive coincidence rate was 96.1% (101/105). Nucleic acid and antibody were all negative in 73 patients. Among the 126 other clinically diagnosed patients, IgM positivity rate was 69.2% (87/126), IgG-positive

Fig. 1 The distribution of target gene. In the target genes that were primers obtained in this paper (verified by Primer BLAST), there were 3 nucleocapsid protein gene, 2 E protein gene, 2 ORF1ab gene, and 1 S gene



rate was 98.3% (125/126), and IgM/IgG overall compliance rate was 100% (126/126) [18]. Huang et al. designed a lateral flow assay based on colloidal gold nanoparticles (AuNP-LF) to realize rapid diagnosis and field detection of IgM antibodies against SARS-CoV-2 virus by indirect immunochromatography. Compared with the original RT-PCR method, AuNP-LF assay has many advantages, including short test time, less sample consumption, simple operation, no need for expensive instruments, independence of professional experience, and lower cost of each test. In addition, as a reliable immunological assay, the proposed AUNP-LF assay has good specificity and reproducibility [23].

Enzyme-linked immunosorbent assay

The principle of ELISA involves immobilization of the SARS-CoV-2 antigen to the polystyrene carrier, followed by addition of calibrator or diluted sample, and use of capture ELISA for capture of the virus IgM/IgG antibody in the sample. Virus IgM/IgG antibodies in the sample bind to antigens immobilized on the vector to form antigen-antibody complexes. After washing, the enzyme-labeled anti-IgM/IgG antibody is added as the second diagnostic antibody for indirect detection of the IgM/IgG antibodies. Finally, the enzyme-substrate is added to the medium for enzymatic reaction, which leads to the formation of a colored product, whose concentration is then determined [24]. The efficiency of ELISA for the detection of a virus depends to some extent on the virus domain corresponding to its target serum antibody. When the more conserved nucleocapsid protein [25] of SARS-CoV-2 was used as a target, the probability of cross-reaction with other coronaviruses associated with diseases increased, and therefore, the specificity of detection decreased [26]. The virus spiny protein (SP protein), as the most diverse protein, has the greatest potential to be used as an efficient ELISA target [27]. The results showed that the sensitivity for detection of SARS-CoV-2 IgM and IgG was 70.1% and 87.7%, and the specificity was 89.2% and 83.9%, respectively [20]. This indicates that ELISA can be used as a SARS-CoV-2 detection method and has higher reliability than nucleic acid detection. In addition, the assay can be used as a complementary test for RT-PCR, especially 10 days after the onset of the disease, and it was used for serum samples of patients with COVID-19 [28].

The main advantages of ELISA (especially for the diagnosis of COVID-19) are high sensitivity, unified detection for a wide range of patients, fast speed, and simple operation. However, when direct antibody screening is used to improve the simplicity and rapidity of analysis, false-positive results may be recorded. This is usually related to interfering factors, for example, the discovery of moderate to high levels of rheumatoid factor can lead to false-positive IgM results [29]. On the other hand, indirect antigen or antibody screening may be affected by nonspecific immobilization [30].

Chemiluminescence

The chemiluminescence method is based on the principle of high-tech detection of magnetic bead-labeled SARS-CoV-2 recombinant protein. The samples, magnetic particles coated with the recombinant antigen, and the reaction mixture were mixed, and the antigen was bound to the recombinant protein labeled with the magnetic beads. Acridine ester-labeled SARS-CoV-2 recombinant antigen was added after washing to form a “magnetic bead-SARS-CoV-2 antibody bound SARS-CoV-2 recombinant antigen-labeled acridine ester” complex. The pre-excitation and excitation solutions were added after another washing step, and then, the resulting chemiluminescence signal was detected. The results were expressed in terms of relative light units (RLU). The luminescence is positively correlated with the concentration of SARS-CoV-2 antibodies present in the sample [31]. Li Quan reported that the plasma samples of 25 COVID-19 patients were positive for IgM and IgG when analyzed using nucleic acid detection and chemiluminescence assay, respectively [21]. Of these, 24 samples were positive with a sensitivity of 96%. All the control samples of 60 normal subjects were negative, and the specificity reached 100%. Moreover, 17 patients with negative nucleic acid test but presenting bilateral, multifocal, and peripheral ground glass opacities on chest CT scan were suspected to have COVID-19. Two IgM and IgG were positive by chemiluminescence antibody at the same time, and two IgM and IgG were positive, respectively. The results suggest that chemiluminescence can serve as an effective supplement for nucleic acid-based detection.

Due to the potential for automation, high specificity, low interference levels, high stability of the conjugate and reagent, reduced incubation time, compatibility with immunoassay regimens, and wide dynamic range, CLIA is extremely attractive [32]. The validity of CLIA analysis is often verified by comparing with ELISA results. However, the large-scale application of CLIA is limited due to the limited test panel, high cost, and non-linear response to analyte concentration due to complex chemical reactions [30].

Other new COVID-19 detection methods

CRISPR-based detection

Clusters of regular interval short palindromes (CRISPR)-mediated gene detection techniques have emerged in recent years [33]. CRISPR/Cas9-based clinical diagnosis has become a hot spot in the field of virology. CRISPR/Cas is a powerful analytical tool for modifying genomes and gene function. Cas13, the latest discovery of a RNA-targeting enzyme in CRISPR-Cas, has inspired the next generation of biosensors with higher specificity, sensitivity, and rapid nucleic acid detection capabilities [34]. Cas13 is an RNA

guided RNase that can produce multiple cleavages in non-specific target single stranded RNA (ss-RNA). To make it specific, CRISPR RNA (crRNA) is needed, and the resulting Cas13-crRNA complex has higher target specificity and can be directed to the target RNA sequence [35]. Therefore, RNA sensing of cas13 with non-specific endonuclease activity is used as a detection strategy, in which the enzyme modified with RNA guide sequence is activated after binding to a specific target. Therefore, the RNA sensing of Cas13 with non-specific endonuclease activity was used as a detection strategy. Enzymes modified with RNA-guided sequences are activated after binding to a specific target. When the activated enzyme interacts with the fluorescence quenching agent, a fluorescence signal is generated, indicating the presence of RNA or DNA with high sensitivity and selectivity as high as fmol L⁻¹ [36]. Studies have confirmed that this technique can be used for the clinical detection of human papillomavirus, Zika virus, and dengue virus [35, 37], and this method exhibits advantages with respect to sensitivity, specificity, rapidity, and simplicity. Wang et al. [38] reported that CRISPR/Cas9 detection technology could be used for the rapid detection of SARS-CoV-2. This method can detect 10 viral loci in samples within 45 min. The results obtained using CRISPR/Cas9 were 100% consistent with those obtained via nucleic acid detection. To facilitate on-site diagnosis, Wang and their team have also developed Cas12 protein, SARS-CoV-2-specific CRISPR and single-stranded (ss) DNA reporter genes, and green fluorescence protein-labeled ssDNA. In the sample that contained SARS-CoV-2, the reporter gene was degraded by Cas12, and the resulting color (480 nm) could be seen with the naked eye.

One of the biggest challenges in applying CRISPR-based strategies is the significant complexity of the technology. It requires a team of experts and extraordinary reagents. Other difficulties include lack of standardization of tests, sequence limitations, and limited literature in the CRISPR trial. Moreover, an additional step of DNA amplification is usually required to lower LOD. Because of these characteristics, the CRISPR technique is less economically viable than RT-PCR and LAMP [39].

Digital PCR

Digital PCR technology (dPCR) was developed by Vogelstein in 1993 [40]. It is a quantitative and precise detection method based on traditional nucleic acid detection. dPCR verifies the expression of target genes in samples by adding samples to hundreds of detection units and detecting amplification information for each set of samples. In contradiction to the methodology used in traditional qRT-PCR, no standard curve is required for analyzing the results of dPCR. The detection sensitivity of high abundance target can be improved by eliminating the influence of local signals on detection results [40]. At

present, dPCR technology is widely used in early diagnosis, colony counting, and phage research [41]. Clinical evidence suggests that dPCR-based nucleic acid detection has higher detection efficiency. Furthermore, the use of dPCR for complex samples such as feces can reduce the influence of other factors on the detection results. In all, Liu et al. tested samples from 77 SARS-CoV-2 patients via dPCR and compared the results with the results of qRT-PCR. Of the 77 patients, 26 were found to be positive via digital PCR [42]. The results showed that dPCR had lower detection limits and higher detection performance than qRT-PCR, as it could detect the virus even in samples with low SARS-CoV-2 viral load [43]. dPCR can reduce the number of false-negatives and can serve as a powerful tool complementary to qRT-PCR.

However, the limitations and shortcomings of dPCR as a clinical diagnostic tool are evident; it is complex to operate and skilled technicians are required for its operation. Because of the large number of samples and the need for more sophisticated experimental equipment, it is difficult to employ dPCR at community or township level medical institutions. Furthermore, dPCR also results in false-positives. In a study by Kiselina [44], three of the negative control groups tested positive for HIV-1 RNA when tested by dRNA. When large a number of samples have to be transported or used simultaneously, cross-contamination is a possibility. Poor detection design and other non-specific targets can also affect the accuracy of results.

RT-LAMP

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is a one-step nucleic acid amplification method that combines loop-mediated isothermal amplification (LAMP) with reverse transcription [22]. RT-LAMP serves as a new means of rapid virus detection. LAMP reactions are usually performed at a constant temperature, generally using 4 or 6 primers that bind to six regions in the target DNA. LAMP is based on the principle of strand displacement DNA synthesis by DNA polymerase. As a result, the target sequence is continuously extended throughout the reaction, and finally, some DNA molecules with different stem-ring structures are produced. WarmStart RTx Reverse Transcriptase (UK New England Biolabs, NEB) makes it possible to combine reverse transcription with LAMP. WarmStart RTx significantly reduces the time required for reverse transcription and LAMP [45], and positive amplification can be evaluated based on visual changes in color (orange to yellow) [46]. Therefore, WarmStart RTx could be used for the rapid detection of SARS-CoV-2.

Because RT-LAMP relies on the use of different primers and shows the best results only at high temperatures, its application is limited and challenging under normal

conditions [47]. Another challenge for scale-up RT-LAMP analysis is the need for genetic primers for nucleic acid amplification reactions, which are expensive and the reagents available are not cheap and cannot be kept for long.

qRT-PCR plus serum antibody detection

Novel coronavirus (SARS-CoV-2) nucleic acid test is the gold standard, but due to the high false-negative nucleic acid test, antibody test will be a beneficial complement to nucleic acid test. Zhao et al. tested the serum of 173 patients diagnosed with nucleic acid by using the total antibody (AB), IgM antibody, and IgG antibody reagent of Wantai Biocolon in Shenzhen Third Hospital. The results showed that the sensitivity of RNA alone was only 67.1%, while the sensitivity of combined serum total antibody detection was as high as 99.4%, which was 32.3% higher than that of nucleic acid detection alone. The sensitivity of the combined detection of RNA and antibody at different stages of the disease was analyzed. The results showed that, even in the early stage (1 to 7 days after treatment), the sensitivity of the combined test was 78.7%, which was 12.0% higher than that of the RNA alone test (66.7%). It is worth mentioning that the nucleic acid detection rate was only 45.5% in the 15–39 days after onset, but combined with total antibody detection, 100% detection can be achieved. It is suggested that PCR combined with antibody detection can improve the detection rate of new crown in different infection stages [48].

Isothermal amplification plus CRISPR technique

Feng et al. demonstrated the possibility of combining isothermal amplification with CRISPR to improve the detection of SARS-CoV-2 [49]. The principle is to extract SARS-CoV-2 RNA from suspected patient samples, and then the purified RNA samples need to be reversely transcribed to form cDNA copies. Therefore, isothermal RT-LAMP and RT-RPA were used for amplification. The resulting cDNA Amplicons are either directly mixed into the Cas12-CRISPR system or firstly transcribed into SSRNA copies. And then it is mixed into the Cas13-CRISPR system [50].

Based on the CRISPR detection technology, the product isothermal amplification sequence generates a signal when the sequence is correct. Because it is non-specific, it has a higher analytical specificity than that used with pH indicators or fluorescent dyes [51]. In contrast, analyses using only Cas12 or Cas13 were less analytical sensitive in detecting viral RNA in the sample. In a short time, exponential amplification was accomplished by isothermal amplification; so, the combination of CRISPR technology helps overcome the limitations of analytical sensitivity. The method can detect 10 copies of RNA/liter of SARS-CoV-2 RNA extract. Amplification and detection can be completed within 30 min of incubation and are recommended for field analysis and POC detection.

Discussion

Nucleic acid detection is a useful method for the diagnosis of viral diseases, and its reliability has been confirmed in the clinical detection of SARS, MERS, and other coronaviruses. During this COVID-19 pandemic, nucleic acid detection is still regarded as the gold standard for the detection of the causative virus (SARS-CoV-2). At present, RT-PCR is still the main nucleic acid detection technology. A large number of databases and software have been developed for designing RT-PCR detection. While designing specific primers for clinical detection, it should be considered that the selected template genes and the nature of the primers themselves would affect the PCR and the accuracy and sensitivity of the final detection reaction. Meanwhile, various factors, such as sample collection and storage, virus infection area, RNA extraction method, and quality of nucleic acid detection kit could affect the detection of SARS-CoV-2 nucleic acids [52], and thus, the positivity rate is only about 50% [53, 54].

Besides nucleic acid detection, serological detection has also become a powerful method for the early diagnosis of COVID-19. Serological detection can help confirm the diagnosis of patients with negative nucleic acid test but with obvious clinical symptoms [54]. Besides, antibody levels can provide information about the progression of infection in patients. In addition, based on the antibody levels, we can predict the severity of the disease in patients with COVID-19 [55–57]. Colloidal gold test can be performed using samples such as blood from the patient's fingertip or venous blood; its operation is convenient, its results can be directly visualized, and generally, it takes approximately 15 min to complete the test [58]. However, the results of IgM/IgG antibody colloidal gold method can only be used as serological evidence and they cannot replace the results of qRT-PCR as etiological evidence. Serological detection may be affected by factors, such as sample hemolysis, fibrin, bacterial contamination, or patient autoantibodies, resulting in a high false-positive rate. The detection window of IgM/IgG antibody is longer than that of qRT-PCR. ELISA is generally sensitive, but the results of ELISA need to be interpreted using a routine enzyme marker, and it is difficult to obtain positive results in the early stage of infection because IgM/IgG antibody detection has a window period. The detection time of ELISA (about 1.5 h) is long, and the number of steps is more. Measures should be taken to avoid infection while performing the assay. The sensitivity of chemiluminescence is also high. An automatic chemiluminescence immune analyzer is used to complete the detection without the involvement of much manual operation. Detection time is generally about half an hour, but the clinical promotion process is also associated with practical problems similar to those observed when using ELISA. With respect to limitations, IgM/IgG antibody detection window is longer, and the false positivity

rate is higher. In the case of an epidemic, logistics and cold chain transportation are more complicated, which may easily lead to the variable performance of reagents [22].

Conclusions

Laboratory testing has become an essential method for diagnosing COVID-19. Most countries and regions use a nucleic acid detection method to diagnose COVID-19, which involves collection of respiratory tract specimens from patients suspected with new coronavirus pneumonia. Based on various studies and experiences regarding many viral diseases, the detection of serum-specific IgM and IgG antibodies has also been employed. Compared with nucleic acid detection, serological detection is convenient, straightforward, and faster. However, serological tests are not well suited for screening asymptomatic individuals or for the detection of low viral loads. At present, serological methods cannot completely replace the status of qRT-PCR in the diagnosis and screening of patients. However, the combined detection of PCR and antibody can improve the detection rate of new crowns at different stages of infection and provide an important basis for the resumption of work and produce. Besides, new genetic techniques such as dPCR and RT-LAMP have also been employed for the clinical detection of SARS-CoV-2. Biosensors are also used as emerging diagnostic strategies for COVID-19, such as paper-based biosensors and plasma-based biosensors. They can be used not only for immune sensing and genetic testing but also for alternative testing principles that do not require special biometrics. Their emergence could improve and extend the diagnostics of COVID-19, which could lead to low-cost large-scale testing methods and/or improved accuracy [59]. We believe that, with progress in research, detection technologies will be further improved.

Declarations

Ethical statement The authors declare that this research does not involve any experiments on animals or humans.

Conflict of interest The authors declare that they have no conflict of interest.

References

- Shi HS, Han XY, Fan YQ et al (2020) Clinical features and imaging manifestations of pneumonia infected by new coronavirus (2019-nCoV). *J Clin Radiol (Chinese)* 39(01):8–11
- National Health Commission of the People's Republic of China. Diagnosis and treatment guideline of COVID-19 (version 7.0) <http://www.nhc.gov.cn/xcs/zhengcwj/202003/46c9294a7dfe4cef80dc7f5912eb1989/files/ce3e6945832a438eaae415350a8ce964.pdf>
- Feng Y, Lu YY, Yan JY et al (2005) Fluorescence quantitative RT-PCR technology for rapid detection of SARS virus nucleic acid. *Chinese Virol (Chinses)* 20(03):228–231
- Huiyu Z, Zhenhen Z, Xingbo S et al (2020) Clinical points and experience in nucleic acid testing of SARS-CoV-2. *Int J Lab Med (Chinese)* 41(05):523–526
- Lai CC, Wang CY, Ko WC et al (2020) In vitro diagnostics of coronavirus disease 2019: Technologies and application. *J Microbiol Immunol Infect S1684–1182(20)*:30140–30147
- Gaunt ER, Hardie A, Claas EC et al (2010) Epidemiology and clinical presentations of the four human coronaviruses 229E, HKU1, NL63, and OC43 detected over 3 years using a novel multiplex real-time PCR method. *J Clin Microbiol* 48(8):2940–2947
- LM J, Yiwei T (2020) Laboratory diagnosis of emerging human coronavirus infections - the state of the art. *Emerg Microbes Infect* 9(01):747–756
- Wenling W, Yanli X, Ruqin G et al (2020) Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA* 323(18):1843–1844
- Guo YY, Wang K, Zhang Y et al (2020) Comparison and analysis of the detection performance of six new coronavirus nucleic acid detection reagents. *Chongqing Medicine (Chinese)* 49(15):2435–2439
- Corman VM, Landt O, Kaiser M et al (2020) Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* 25(3):2000045
- Lu R, Zhao X, Li J et al (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. *Lancet* 395(10224):565–574
- Dawei W, Bo H, Chang H et al (2020) Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan. *China JAMA* 323(11):1061–1069
- Huang C, Wang Y, Li X et al (2020) Clinical features of patients infected with 2019 novel coronavirus in Wuhan. *China Lancet* 395(10223):497–506
- Chan JF, Yuan S, Kok KH et al (2020) A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. *Lancet* 395(10223):514–523
- Mollaei HR, Afshar AA, Kalantar-Neyestanaki D et al (2020) Comparison five primer sets from different genome region of COVID-19 for detection of virus infection by conventional RT-PCR. *Iran J Microbiol* 12(3):185–193
- Yixin C, Kwok-Hung C, Congming H et al (2016) A highly specific rapid antigen detection assay for on-site diagnosis of MERS. *J Infect* 73(1):82–84
- Wpc Y, Lsk P, Wbh L et al (2004) Detection of specific antibodies to severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein for serodiagnosis of SARS coronavirus pneumonia. *J Clin Microbiol* 42(5):2306–2309
- Zhang WJ, Lv X, Huang C et al (2020) Clinical evaluation and application of colloidal gold immunochromatography for detection of novel coronavirus IgM/IgG antibodies. *J Virol (Chinese)* 36(03):348–354
- Deng JL, Wang YF, Wang TT et al (2020) Discussion on the clinical value of colloidal gold immunochromatography for detection of SARS-CoV-2 serum antibodies. *Int J Lab (Chinese)* 41(08):964–966+970
- Li DC, Zhang MX, Lin DC et al (2020) Sensitivity and specificity evaluation of a new coronavirus ELISA kit. *J Shenzhen University (Science and Technology Edition) (Chinese)* 37(03):224–226

21. Li Q, Liu DB, Qiao ZR et al (2020) Value of SARS-Cov-2 IgM / IgG antibody detection in diagnosis of new coronavirus pneumonia. *Int J Lab Med (Chinese)* 41(18):2244–2246
22. Xiao B, Zhou Q, Lei T et al (2020) Application and prospect of SARS-Cov-2 laboratory detection technology. *J Southern Medical University (Chinese)* 40(04):601–605
23. Huang C, Wen T, Shi FJ et al (2020) Rapid detection of IgM antibodies against the SARS-CoV-2 virus via colloidal gold nanoparticle-based lateral-flow assay. *ACS Omega* 5(21):12550–12556
24. Lindström P, Wager O (1978) IgG Autoantibody to human serum albumin studied by the ELISA-technique. *J Scand J Immunol* 7(5):419–425
25. Sz F, Mx J (2004) Antigenic cross-reactivity between the nucleocapsid protein of severe acute respiratory syndrome (SARS) coronavirus and polyclonal antisera of antigenic group I animal coronaviruses: implication for SARS diagnosis. *J Clin Microbiol* 42(5):2352–2352
26. Yixuan W, Yuyi W, Yan C et al (2020) Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (COVID-19) implicate special control measures. *J Med Virol* 92(6):568–576
27. Chen YZ, Qiu F (2020) Detection and treatment of spike protein and new coronavirus. *J Biomed Eng (Chinese)* 37(02):246–250+261
28. Liu W, Liu L, Kou G et al (2020) Evaluation of nucleocapsid and spike protein-based enzyme-linked immunosorbent assays for detecting antibodies against SARS-CoV-2. *J Clin Microbiol* 58(6):e00461-e520
29. Wang Q, Du Q, Guo B et al (2020) A method to prevent SARS-CoV-2 IgM false positives in gold immunochromatography and enzyme-linked immunosorbent assays. *J Clin Microbiol* 58(6):e00375-e420
30. Aydin S (2015) A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. *Peptides* 72:4–15
31. Li XF, Liu GM, Li WL et al (2020) The clinical application value of chemiluminescence detection of new coronavirus antibodies. *J Shandong University (Medical Edition) (Chinese)* 58(5):46–59
32. Cinquanta L, Fontana DE, Bizzaro N (2017) Chemiluminescent immunoassay technology: what does it change in autoantibody detection? *Auto Immun Highlights* 8(1):9
33. Shan LL, Xia HB (2018) Advances in genome editing technology mediated by clusters of regularly spaced short palindromic repeats (CRISPR). *J Cell Mol Immunol (Chinese)* 34(09):856–862
34. Li Y, Li S, Wang J et al (2019) CRISPR/Cas systems towards next-generation biosensing. *Trends Biotechnol* 37(7):730–743
35. Gootenberg JS, Abudayyeh OO, Lee JW et al (2017) Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science* 356(6336):438–442
36. O'Connell MR (2019) Molecular mechanisms of RNA targeting by Cas13-containing type VI CRISPR-Cas systems. *J Mol Biol* 431(1):66–87
37. Chen JS, Ma E, Harrington LB et al (2018) CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science* 360(6387):436–439
38. Wang X, Zhong M, Liu Y et al (2020) Rapid and sensitive detection of COVID-19 using CRISPR/Cas12a-based detection with naked eye readout, CRISPR/Cas12a-NER. *Sci Bull (Beijing)* 65(17):1436–1439
39. van Dongen JE, Berendsen JTW, Steenbergen RDM et al (2020) Point-of-care CRISPR/Cas nucleic acid detection: recent advances, challenges and opportunities. *Biosens Bioelectron* 166:112445
40. Vogelstein B, Kinzler KW (1999) Digital PCR. *Proc Natl Acad Sci U S A* 96(16):9236–9241
41. Li L, Sui ZW, Wang J et al (2012) Research progress of single-molecule DNA quantitative technology based on digital PCR. *Adv Biochem Biophys (Chinese)* 39(10):1017–1023
42. Li ZJ, Liu Zt, Li JY et al (2019) Research progress of digital PCR technology. *Specialty Products Res (Chinese)* 41(01):120–123
43. Xinjin L, Jiangpeng F, Qiuhan Z et al (2020) Analytical comparisons of SARS-COV-2 detection by qRT-PCR and ddPCR with multiple primer/probe sets. *Emerg Microbes Infect* 9(1):120–123
44. Maja K, O PA, Ward DS et al (2014) Comparison of droplet digital PCR and seminested real-time PCR for quantification of cell-associated HIV-1 RNA. *PLoS One* 9(1):e85999
45. Huang WE, Lim B, Hsu CC et al (2020) RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2. *Microb Biotechnol* 13(4):950–961
46. Lamb LE, Bartolone SN, Ward E et al (2020) Rapid detection of novel coronavirus/severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by reverse transcription-loop-mediated isothermal amplification. *PLoS ONE* 15(6):e0234682
47. Zhang Y, Odiwuor N, Xiong J et al (2020) Rapid molecular detection of SARS-CoV-2 (COVID-19) virus RNA using colorimetric LAMP
48. Zhao J, Yuan Q, Wang H et al (2020) Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. *Clin Infect Dis* 71(16):2027–2034
49. Feng W, Newbigging AM, Le C et al (2020) Molecular diagnosis of COVID-19: challenges and research needs. *Anal Chem* 92(15):10196–10209
50. Broughton JP, Deng X, Yu G et al (2020) CRISPR-Cas12-based detection of SARS-CoV-2. *Nat Biotechnol* 38(7):870–874
51. Kellner MJ, Koob JG, Gootenberg JS et al (2019) SHERLOCK: nucleic acid detection with CRISPR nucleases. *Nat Protoc* 14(10):2986–3012
52. Li H, Liu Z, Ge J (2020) Scientific research progress of COVID-19/SARS-CoV-2 in the first five months. *J Cell Mol Med* 24(12):6558–6570
53. Li H, Pan J, Su Y et al (2020) SARS-CoV-2 IgM/IgG antibody detection confirms the infection after three negative nucleic acid detection. *J Cell Mol Med* 24(14):8262–8265
54. Zhao J, Yuan Q, Wang H et al (2020) Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. *Clin Infect Dis* 71(16):2027–2034
55. To KK, Tsang OT, Leung WS et al (2020) Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis* 20(5):565–574
56. Sun B, Feng Y, Mo X et al (2020) Kinetics of SARS-CoV-2 specific IgM and IgG responses in COVID-19 patients. *Emerg Microbes Infect* 9(1):940–948
57. Yongchen Z, Shen H, Wang X et al (2020) Different longitudinal patterns of nucleic acid and serology testing results based on disease severity of COVID-19 patients. *Emerg Microbes Infect* 9(1):833–836
58. Shen B, Zheng Y, Zhang X et al (2020) Clinical evaluation of a rapid colloidal gold immunochromatography assay for SARS-Cov-2 IgM/IgG. *Am J Transl Res* 12(4):1348–1354
59. Mattioli IA, Hassan A, Oliveira ON Jr et al (2020) On the challenges for the diagnosis of SARS-CoV-2 based on a review of current methodologies. *ACS Sens* 5(12):3655–3677