INTRODUCTION

The outbreak of coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2, also known as 2019 novel coronavirus) is a Public Health Emergency of International Concern globally (Association, 2020; Li, Yang, & Ren, 2020; Paraskevis et al., 2020). The World Health Organization (WHO) increased the assessment of COVID-19 as a pandemic at a global level on 11 March 2020. Globally, as of 1 May 2020, more than 3,181,642 confirmed cases and 224,301 deaths caused by SARS-CoV-2 infection were reported in more than 210 countries, territories or areas (https://covid19.who.int/). Therefore, due to the rapid spread of the diseases and the increasing number of patients, timely and accurate detection of SARS-CoV-2 is urgently needed.

Up until now, numerous groups have published methods for detecting the virus. However, the sensitivity and specificity of these methods are quite different. Besides, the false-negative and false-positive rates are relatively high due to possible errors in the sampling and testing process (Li, Yi, et al., 2020; Wang, 2020; Zhang & Li, 2020). In this short review, we summarize the types, characteristics and shortcomings of various detection methods, as well as perspectives for the SARS-CoV-2 diagnosis.

NUCLEOTIDE ACID-BASED METHODS

2.1 | Quantitative real-time RT-PCR

The most widely used methods are nucleotide acid-based detection technology. As recommended by WHO, quantitative real-time RT-PCR (RT-qPCR) is one of the commonly used techniques for virus detection, which has high sensitivity, rapid detection, and other desirable characteristics.

In the past two months, many scientific teams and companies have successively developed methods to detect SARS-CoV-2 (Chan et al., 2020; Chu et al., 2020; To et al., 2020), but different methods have different detection efficiency and some produce more false negatives (Table 1) (Wang, 2020; Zhang & Li, 2020). Therefore, improving the detection efficiency is one of the most important tasks at present. A one-step RT-qPCR targeting ORF1b or nucleocapsid (N) gene of SARS-CoV-2 can detect 10 copies/reaction of plasmid DNA or \(2 \times 10^{-4} \text{ TCID}_{50}/\text{reaction of RNA extracted from virus cultures (Chu et al., 2020). However, this method was designed to react with SARS-CoV-2 and its closely related viruses, such as SARS coronavirus (Chu et al., 2020), which may lead to false-positive reactions for SARS-CoV-2 identification. Furthermore, Chan and colleagues developed a novel RT-qPCR assay targeting the
RNA-dependent RNA polymerase (RdRp)/helicase (Hel) of SARS-CoV-2 and found that the limit of detection (LOD) of the assay was 1.8 TCID_{50}/ml with genomic RNA and 11.2 RNA copies/reaction with in vitro RNA transcripts, which has higher analytical sensitivity than the widely used RdRp-P2 assay in European laboratories (Chan et al., 2020). Notably, the COVID-19-RdRp/Hel assay did not cross-react with other human-origin coronaviruses and respiratory pathogens (Chan et al., 2020), which can be used to differentiate SARS-CoV-2 and other respiratory pathogens. Using RT-qPCR, Lin, Xiang, et al., (2020) and Yang, Yang, et al., (2020) compared detection efficiency of RT-qPCR on throat swabs, nasal swabs and sputum specimens, and found that the positive rates of sputum specimens, nasal swabs and throat swabs were 74.4%–88.9%, 53.6%–73.3% and 44.2%, respectively. This suggests that samples collected from the lower respiratory tract increase the accuracy of diagnosis.

### 2.2 Reverse transcription loop-mediated isothermal amplification

Another promising detection method based on nucleotide acid is the reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Table 1) (Lamb, Bartolone, Ward, & Chancellor, 2020; Lucia, reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Lamb, Bartolone, Ward, & Chancellor, 2020; Lucia, 2020). Another promising detection method based on nucleotide acid is the reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Table 1) (Lamb, Bartolone, Ward, & Chancellor, 2020; Lucia, 2020). Another promising detection method based on nucleotide acid is the reverse transcription loop-mediated isothermal amplification (RT-LAMP) (Table 1) (Lamb, Bartolone, Ward, & Chancellor, 2020; Lucia, 2020).

The RT-LAMP result can be evaluated using real-time turbidimeter, electrophoresis or fluorescent, which is faster and more convenient for clinical diagnosis of SARS-CoV-2. Moreover, to provide a rapid, highly sensitive molecular test for point-of-care, such as home, clinic and points of entry, a two-stage isothermal amplification (COVID-19 Penn-RAMP) based on recombinase polymerase amplification (RPA) at 38°C and LAMP at 63°C was developed by Mohamed and colleagues (Mohamed et al., 2020). Both processes of the COVID-19 Penn-RAMP can be performed in a closed tube followed by either fluorescence or colorimetric detection, with 10-fold more sensitivity than COVID-19 LAMP and COVID-19 RT-qPCR (Mohamed et al., 2020). Recent studies showed that an RT-LAMP targeting the N gene of SARS-CoV-2 can specifically detect viral RNAs of SARS-CoV-2 but has no cross-reactivity with related coronaviruses, such as HCoV-229E, HCoV-NL63, HCoV-OC43 and MERS-CoV, as well as human infectious influenza viruses (type B, H1N1pdm, H3N2, H5N1, H5N6, H5N8 and H7N9), and other respiratory disease-causing viruses (RSVA, RSVB, ADV, PIV, MPV and HRV) (Baek et al., 2020). These results demonstrate that the RT-LAMP method has a wider application market for SARS-CoV-2 diagnosis due to its relatively simple operation and low technical requirements for operators.

### 2.3 CRISPR-based methods

Clustered regularly interspaced short palindromic repeats (CRISPR)-based diagnostic platforms have also been developed for point-of-care nucleic acid detection, such as SHERLOCK or DETECTR (Table 1) (Broughton et al., 2020; Kostyusheva et al., 2020; Lucia et al., 2020; Metsky et al., 2020; Zhang et al., 2020; Abudayyeh, & Gootenberg, 2020).

The CRISPR-based nucleic acid detection platforms combine recombinase polymerase amplification with CRISPR-Cas enzymology for specific recognition of targeted DNA or RNA sequences (Kellner, Koob, Gootenberg, Abudayyeh, & Zhang, 2019; Myhrvold et al., 2018). It can sensitively detect as low as 10 copies/µL for synthetic RNA or in vitro viral RNA transcripts. The results of the CRISPR-based methods can be analysed by fluorescent or lateral flow strip in <1 hr with a setup time of less than 15 min (Kellner et al., 2019). It can be used in areas at greatest risk of transmitting SARS-CoV-2 infection, including airports, emergency departments and local community hospitals, particularly in low-resource countries (Broughton et al., 2020; Zhang, Abudayyeh et al., 2020). Moreover, Broughton and colleagues compared the workflow of the DETECTR, SHERLOCK and the RT-qPCR recommended by CDC/WHO for SARS-CoV-2 detection and found that the LOD of these methods is 70–300 copies/µL, 10–100 copies/µL or 3.16–10 copies/µL input sample, respectively (Broughton et al., 2020). The assays can be finished in 30, 60 and 120 min, respectively (Broughton et al., 2020). These results suggest that RT-qPCR is more sensitive than the CRISPR-based assay, while the latter is more convenient and timesaving than RT-qPCR. However, due to the lack of clinical detection samples, the sensitivity and specificity of the CRISPR-based method needs further verification in the clinic.

### 3 ANTIBODY-BASED METHODS

Although nucleotide acid-based methods are the recommended methods by many groups and WHO, some experts have recently reported that the sensitivity of nucleic acid detection in SARS-CoV-2 is low, even as low as 42.10% (Cai et al., 2020; Jia et al., 2020; Li, Yi, et al., 2020; Wang, 2020; Zhang & Li, 2020). In some cases of nucleotide acid-based detection, a positive result will appear after repeated negative tests. Nasopharyngeal or throat swabs are negative many times, but finally, positive results are detected in sputum specimens or respiratory lavage fluid samples (Li, Yi, et al., 2020; Wang, 2020; Zhang & Li, 2020). Moreover, there are several limitations in nucleotide acid-based detections, such as long turnaround time, complex operation, expensive equipment and trained technicians (Cai et al., 2020; Li, Yi, et al., 2020). Therefore, many experts suggest using specific antibody detection as a supplement for nucleic acid detection,
| Test                        | Target gene/protein          | Sensitivity (limit of detection, LOD) | Clinical specimens         | Cross-reactivity | Positive rate | Sample source in the literature                                                                 | Output                           | References            |
|-----------------------------|------------------------------|---------------------------------------|----------------------------|------------------|---------------|------------------------------------------------------------------------------------------------|----------------------------------|-----------------------|
| COVID-19-RdRp/Hel assay     | RNA-dependent RNA polymerase (RdRp)/helicase (Hel) | 1.8 TCID<sub>50</sub>/ml genomic RNA from culture lysate, 11.2 RNA copies/reaction (in vitro viral RNA transcripts) | 10<sup>-5</sup> fold dilution of RNA, 3.21 × 10<sup>6</sup> RNA copies/ml | NA               | 119/273 (43.6%) | Nasopharyngeal aspirates/swabs and/or throat swabs, saliva specimens, sputum specimens, plasma specimens, faeces or rectal swabs | Real-Time PCR System           | Chan et al., (2020)   |
| RdRp-P2 assay               | RNA-dependent RNA polymerase (RdRp) | 1.8 × 10<sup>5</sup> TCID<sub>50</sub>/ml RNA from culture lysate, 3.6 RNA copies/reaction (in vitro viral RNA transcripts) | 10<sup>-4</sup> fold dilution of RNA, 3.21 × 10<sup>6</sup> RNA copies/ml | React with SARS-CoV | 77/273 (28.2%) | Nasopharyngeal aspirates/swabs and/or throat swabs, saliva specimens, sputum specimens, plasma specimens, faeces or rectal swabs (partial efficiency) | Real-Time PCR System           | Chan et al., (2020)   |
| RT-qPCR                     | S gene                       | NA                                    | NA                         | NA               | 91.7%         | Saliva                                                                 | Real-Time PCR System              | To et al., (2020)       |
| RT-qPCR                     | ORF1b and N gene             | 10 copies/reaction for plasmid, 2 × 10<sup>-4</sup>-2000 TCID<sub>50</sub>/reaction for RNA extracted from virus cultures | NA                         | NA               | NA            | NA                                                                 | Real-Time PCR System              | Chu et al., (2020)      |
| RT-LAMP                     | ORF1ab, N and E gene         | 4.8 copies/μl for synthetic RNA       | 1.000 RNA copies/ml (5 copies) | Specific         | 99%-100% agreement with the commercial RT-qPCR | Throat and nasal swabs                                                                 | Real-time turbidimeter, electrophoresis or Fluorescent | Yang, Dang, et al. (2020), Zhao, Odiwuor, et al. (2020) |
| RT-LAMP, iLACO              | ORF1ab gene                  | 10 copies of synthesized RNA          | 2 μl sample loading (RNA concentration, 0.2–47 ng/μl) | NA               | 97.6% (42/43) | Respiratory samples                                                                 | Real-time turbidimeter, electrophoresis or Fluorescent | Yang, Dang, et al. (2020), Zhao, Odiwuor, et al. (2020) |
| RT-LAMP                     | Genomic RNA                  | 1.02 fg genomic RNA                   | NA                         | Specific         | NA            | Simulated patient samples were generated by spiking serum, urine, saliva, oropharyngeal swabs, and nasopharyngeal swabs with a portion of the COVID-19 nucleic acid | Real-time turbidimeter, electrophoresis or Fluorescent | Lamb et al., (2020)    |
| Closed-tube Penn-RAMP (COVID-19 Penn-RAMP) | ORF1ab | 7 copies of Synthesized DNA, 10 times higher than LAMP and RT-PCR. | NA                         | Specific         | NA            | Nasal swabs                                                                 | Real-time turbidimeter, electrophoresis or Fluorescent | Mohamed et al., (2020)  |
| Test | Target gene/protein | Sensitivity (limit of detection, LOD) | clinical specimens | Cross-reactivity | Positive rate | Sample source in the literature | Output | References |
|------|---------------------|--------------------------------------|--------------------|-----------------|---------------|--------------------------------|--------|------------|
| CRISPR-based methods | S, N, E, RNase P, or ORF1ab genes | DNA plasmids/RNA transcripts/RNA from culture lysate | 10 copies/μl for synthetic RNA/in vitro viral RNA transcripts | NA | NA | Synthetic RNA/in vitro viral RNA transcripts | Fluorescent or lateral flow strip | Metsky et al., (2020), Lucia et al., (2020), Zhang, Abudayyeh, et al. (2020), Broughton et al., (2020) |
| COVID IgM/IgG antibodies kit | IgM or/and IgG | NA | NA | NA | Single detection: IgM, 60.61%-79.17%; IgG, 45.45%-66.67%. Combination of IgM and IgG, 72.73%-87.50%. | Blood | Fluorescence intensity | Jia et al., (2020) |
| Novel coronavirus IgG/IgM antibody ELISA | IgM or/and IgG | NA | NA | NA | Single detection: IgM, 44.4% (28/63), IgG, 82.54% (52/63). Combination of IgM and IgG, 55/63 (87.3%). | Serum samples | Chromogenic reaction (OD450), GICA strip | Xiang, Yan, et al. (2020) |
| Novel coronavirus IgG/IgM antibody GICA kits | IgM or/and IgG | NA | NA | NA | Single detection: IgM, 57.1% (52/91), IgG, 81.3% (74/91). Combination of IgM and IgG, 75/91 (82.4%). | Plasma samples | Chromogenic reaction (OD450), GICA strip | Xiang, Yan, et al. (2020) |
| Rapid IgM-IgG Combined Antibody Test strip | IgM and IgG | NA | NA | NA | 88.66%-94.83% | Fingerstick blood, serum, and plasma of venous blood | Lateral flow immunoassay, GICA strip | Li, Yi, et al. (2020) |
| Peptide-based Magnetic Chemiluminescence Enzyme Immunoassay | IgG or IgM, a synthetic peptide derived from S protein | NA | Specific | IgG, 71.4% (197/276)-97.5% (78/80); IgM, 57.2% (158/276)-87.5% (21/24). Combination of IgM and IgG, 81.52% (225/276) | Serum samples | Luminescent immunoassay | Cai et al., (2020), Lin, Liu, et al. (2020), Xiang, Wang, et al. (2020), Xiang, Yan, et al. (2020) |

Abbreviations: CLIA, Chemiluminescence-immunoassay; CRISPR, Clustered regularly interspaced short palindromic repeats; E, Envelope protein; ELISA, Enzyme-linked immunosorbent assay; fg, Femtogram; GICA, Colloidal gold immunochromatographic assay; LAMP, Loop-mediated isothermal amplification; N, nucleocapsid phosphoprotein; NA, not applicable, or not analysed in the literature; RT, Reverse transcription; S, spike protein.
because the antibody-based methods are relatively cheap, easy to operate and have low technical requirements (Table 1).

To date, enzyme-linked immunosorbent assay (ELISA), colloidal gold immunochromatographic assay (GICA) and chemiluminescence-immunoassay (CLIA) are the most commonly used methods for detection of SARS-CoV-2. As reported, antibody-based methods targeting IgG and IgM induced by the recombinant N and S proteins of SARS-CoV-2 are consistent with the results obtained by nucleic acid-based assay (Cai et al., ; Jia et al., 2020; Li, Yi, et al., 2020; Lin, Liu, et al., 2020; Xiang, Yan, et al., 2020; Zhong et al., 2020). Furthermore, the receptor-binding domain (RBD) of the viral S protein showed a better antigenicity than that of the viral N protein for the diagnosis of SARA-CoV-2 infection (Ma et al., 2020). Moreover, a recent report showed that IgA level in patient serum is positively correlated with the severity of the COVID-19 (Ma et al., 2020), indicating that serum IgA can also be used as a biological marker for the COVID-19 identification.

The sensitivities of IgG and IgM-targeted methods were >71.4% and >57.2%, or even up to 97.5% and 87.5%, respectively (Cai et al.; Jia et al., 2020; Lin, Liu, et al., 2020; Xiang, Wang, et al., 2020; Xiang, Yan, et al., 2020). It was reported that ELISA is superior to lateral flow immunoassay (LFIA) in specific detection and quantification of SARS-CoV-2 IgM and IgG, and is highly sensitive to IgG 10 days after symptoms appear (Adams et al., 2020). Notably, the sensitivities of RBD-specific IgA, IgM and IgG were 98.6%, 96.8% and 96.8%, and the specificities of RBD-specific IgA, IgM and IgG were 98.1%, 92.3% and 99.8%, respectively (Ma et al., 2020). Recently, Lin et al. found that serological CLIA based on the recombinant N protein of SARS-CoV-2 had a higher performance for diagnosis of COVID-19 than that of the commercial ELISA kit, with more reliable sensitivity and specificity of 82.28% and 97.5%, respectively (Lin, Liu, et al., 2020). Therefore, antibody-based methods are also effective methods to detect COVID-19.

To improve the detection efficiency, several groups developed antibody-based methods for simultaneous detection of IgG and IgM (Guo et al., 2020; Jia et al., 2020; Li, Yi, et al., 2020; Ma et al., 2020; Xiang, Yan, et al., 2020) and found that the sensitivity of test detecting IgM and IgG simultaneously was significantly higher than the nucleic acid, IgM or IgG single detection (Jia et al., 2020). The sensitivities of ELISA and GICA for simultaneous detection of IgM and IgG antibodies were 87.3% and 82.4%, respectively, with 90.63%–100% specificity (Li, Yi, et al., 2020; Xiang, Yan, et al., 2020). Besides, the IgA/IgG or IgA/IgM/IgG combination can provide improved diagnostic reliability compared to conventional IgM/IgG combinations (Ma et al., 2020).

Clinically, specific IgA and IgM against SARS-CoV-2 can be detected 7 days after virus infection or 3–4 days after symptoms appear, and specific IgG of the virus appears in 7–10 days after SARS-CoV-2 infection (Adams et al., 2020; Cai et al., ; Guo et al., 2020; Li, Yi, et al., 2020; Xiang, Wang, et al., 2020; Xiang, Yan, et al., 2020; Xiang, Yan, et al., 2020). The median concentrations of IgA and IgM reached a peak of 8.84 and 7.25 μg/ml at 16–20 days after onset, respectively (Adams et al., 2020). IgG titres increased within 3 weeks after symptoms appeared and the median concentration reached a peak of 16.47 μg/ml in 21–25 days after onset and began to decrease at the 8th week, but remained above the detection threshold (Adams et al., 2020). For patients of different stages, the sensitivities of GICA strips targeting viral IgM or IgG antibody were 11.1%, 92.9% and 96.8% for the nucleic acid confirmed cases of the early (1–7 days after onset), middle (8–14 days after onset) and late stage of the COVID-19 (over 15 days), respectively (Pan et al., 2020). These results suggest that antibody detection of SARS-CoV-2 can be performed in the middle to later stages of COVID-19.

Generally, a clinical diagnosis can be finalized in as little as 5–15 min via antibody-based methods (especially by commonly used serological GICA strip), using different types of blood samples, such as fingerstick blood, serum and plasma of venous blood (Cai et al.; Jia et al., 2020; Li, Yi, et al., 2020; Pan et al., 2020; Xiang, Yan, et al., 2020). Therefore, combined with nucleic acid detection, the detection of virus-specific antibody can significantly reduce "false negatives" of SARS-CoV-2 infection at the clinical level.

4 | OPTIMIZATION OF THE SAMPLING METHODS

It is worth noting that the nucleic acid-based methods need to extract nucleic acid in advance, which is a labour-intensive and time-consuming operation and also has risks of affecting nucleic acid extraction, thus misleading the diagnosis (Zhao et al.,). To overcome this problem, a virus RNA extraction method was developed based on poly (amino ester) and carboxyl-coated magnetic nanoparticles, which combines the lysis and binding steps into one step and can be directly incorporated into RT-qPCR or RT-LAMP (Zhao et al., 2020). The simplified method can purify viral RNA from multiple samples in 20 min (Zhao et al., ).

Moreover, to obtain the virus effectively, it is usually necessary to collect respiratory tract samples, such as nasopharyngeal aspirates/swabs or throat swabs. As reported by Wu et al., the positive predictive values of RT-qPCR for SARS-CoV-2 in sputum, nasopharyngeal swab, blood, anal swabs, faeces are 48.68% (148/304), 38.13% (180/472), 3.03% (4/132), 10.00% (12/120) and 9.83% (24/244) (Wu et al., 2020). However, collecting samples from the respiratory tract, especially low respiratory tract, might pose a risk of virus transmission to the healthcare workers. Thus, To and colleagues evaluated saliva viral load and found that live SARS-CoV-2 could be detected in the initial saliva specimens of 11 patients (91.7%), suggesting saliva is also a promising non-invasive specimen for diagnosis and monitoring of SARS-CoV-2 infection (To et al., 2020). Zhang et al. found nucleic acid detection of COVID-19 in faecal specimens was equally accurate to that of pharyngeal swab specimens (Zhang, Wang, & Xue, 2020). Chen and colleagues found SARS-CoV-2 nucleic acid could be detected in the serum of the patients, and serum SARS-CoV-2 viral load (RNAemia) is closely associated with drastically increased interleukin 6 (IL-6) level in critically ill patients (Chen et al., 2020),
indicating RNAemia and IL-6 can be used to predict the poor prognosis for COVID-19 patients. These results are conducive to the selection of specimens and improvement of the accuracy of diagnosis. However, only certified health laboratories with professionals and specialized equipment can carry out relevant experimental operations, especially nucleic acid extraction, which will prolong the diagnosis time and delay the treatment and control of the disease.

5 | CONCLUSION AND PERSPECTIVE

In conclusion, nucleic acid-based methods are sensitive, but prone to false-positive. The sensitivity of the antibody-based method is slightly lower, but the accuracy is higher. Therefore, it is suggested that the two methods should be combined to improve the detection accuracy of COVID-19. Moreover, developing a risk-free sample preparation method for detection is one of the urgent tasks to be solved at present. Also, because a few recovered patients that have been discharged from hospitals have tested positive in nucleic acid tests, it is still necessary to develop new sensitive and specific detection methods for the confirmation of virus-infected persons, carriers and recovered patients. At the same time, the establishment of a differentiation method between SARS-CoV-2 and other respiratory viruses is also urgently needed.

Notably, SARS-CoV-2 is a zoonotic virus, but the host spectrum of the virus is still controversial. As reported, the virus was detected not only in humans, but also in dogs, ferrets, domestic cats, tigers and lions (Gollakner & Capua, 2020; Shi et al., 2020), while a survey by Deng et al. showed that dogs and cats remained serologically negative to SARS-CoV-2 (Deng et al., 2020). However, sequence analysis of angiotensin-converting enzyme 2 (ACE2) receptor between humans and animals indicates a low species barrier for transmission of SARS-CoV-2 to farm animals (Sun et al., 2020), demonstrating the virus might infect other species, especially pets and farm animals, from humans and vice versa. Therefore, it is also necessary to monitor SARS-CoV-2 infection in pets and domestic animals.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

No ethical approval was required as this is a review article with no original research data.

DATA AVAILABILITY STATEMENT

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

ORCID

Lin Zhu Ren https://orcid.org/0000-0002-1092-6435

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