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Review

Current methods and prospects of coronavirus detection

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ABSTRACT

SARS-COV-2 is a novel coronavirus discovered in Wuhan in December 30, 2019, and is a family of SARS-COV (severe acute respiratory syndrome coronavirus), that is, coronavirus family. After infection with SARS-COV-2, patients often experience fever, cough, gas prostration, dyspnea and other symptoms, which can lead to severe acute respiratory syndrome (SARS), kidney failure and even death. The SARS-COV-2 virus is particularly infectious and has led to a global infection crisis, with an explosion in the number of infections. Therefore, rapid and accurate detection of the virus plays a vital role. At present, many detection methods are limited in their wide application due to their defects such as high preparation cost, poor stability and complex operation process. Moreover, some methods need to be operated by professional medical staff, which can easily lead to infection. In order to overcome these problems, a Surface molecular imprinting technology (SM-MIT) is proposed for the first time to detect SARS-COV-2 virus. For this SM-MIT method, this review provides detailed detection principles and steps. In addition, this method not only has the advantages of low cost, high stability and good specificity, but also can detect whether it is infected at designated points. Therefore, we think SM-MIT may have great potential in the detection of SARS-COV-2 virus.

1. Introduction

In December 2019, patients with pneumonia of unknown cause appeared in some medical institutions in Wuhan, Hubei Province, China [1–3]. Wuhan continued to carry out surveillance of influenza and related diseases, and 27 cases of viral pneumonia were found, all diagnosed as viral pneumonia/pulmonary infection. Until January 2020, the World Health Organization officially named it 2019-nCOV [4–6]. Subsequently, the International Committee on Virus Taxonomy announced on its official website that the novel Coronavirus had the English name "SARS-COV-2" [7,8]. To date (October 26, 2020), there have been 35, 347,404 confirmed human cases of SARS-COV-2 worldwide, including 1039,406 deaths (https://www.who.int/home) [9]. The symptoms of SARS-COV-2 virus infection are as follows: Asymptomatic period, the virus was detected only in respiratory tract, but no obvious symptoms. Mild patients are also in the incubation period, patients may have symptoms similar to cold, including fever, cough, fear of cold, physical discomfort and so on. Severe patients, patients will soon develop pneumonia, and appear rapid breathing, respiratory failure, multiple organ damage and so on [10–13].

SARS-COV-2 virus belongs to beta-COV lineage B, which is a group of enveloped single stranded RNA viruses. It is characterized by prominent stick like protrusions on the surface of the virus and abnormally large RNA genome. SARS-COV-2 genome encodes four major structural proteins: spike (s) protein, Nucleocapsid (n) protein, membrane (m) protein and envelope (E) protein, which are essential components of virus particles [14,15]. The onset time of SARS-COV-2 patients was different, which was related to the severity and incubation period of infection. The incubation period of general patients is 1–14 days. For patients with mild infection, it may take 1–2 weeks to develop the disease, and for patients with severe infection, the related clinical symptoms may appear in 2–3 days. But after epidemiological investigation, the patients were infected in 3–7 days [16]. In addition, the SARS-COV-2 virus has demonstrated the possibility of human to human transmission [17] and has found that it can be transmitted through airborne droplets and close contact with patients [18–20]. Like all coronaviruses, SARS-COV-2 uses S-glycoprotein to promote its entry into host cells. The protein has two functional domains: one is S1 receptor binding domain (RBD), the other is S2 domain which mediates the fusion of virus and host cell membrane. SARS-COV-2 protein first binds to ACE2 receptor on host cells through S1 receptor binding domain. Then, S1 domain was detached from the surface of the virus, and S2 domain was fused to the host cell membrane.

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Table 1
CT characterization of various viral infections.

Virus	symptom	CT features	Reference
Influenza A (H1N1) virus	Severe headache, fever	Ground-glass opacity and consolidation	[36]
SARS-COV-2 virus	Fever, cough, fatigue	Double lung multiple ground glass images and infiltrating shadows	[37]
Avian Influenza H7N9 virus	Fever, cough, sputum	Ground-glass opacity and consolidation	[38]
Cytomegalovirus	Fever, cough, dyspnea	Ground glass attenuation, bronchial consolidation and thickening	[39]
MERS CoV virus	Fever, cough, dyspnea	Ground glass attenuation, bronchial consolidation and thickening	[40]

This process requires activation of S protein, which is achieved by cleavage of furin and TMPRSS2 at two sites (S1/S2 and S2 ′). Furin cleavage at S1/S2 site can lead to conformational changes of virus S protein, thus exposing RBD or S2 domains. The researchers believe that the TMPRSS2 cleavage of SARS-COV-2 protein can promote the fusion of virus capsid and host cells, thus allowing the virus to enter [21–23]. Due to the rapid growth of the number of people infected with SARS-COV-2 virus, the pressure of detecting SARS-COV-2 is very great [24–26]. Many people die at home because they can't get timely detection. With the development of the epidemic, the global economy will fall into a serious recession. According to the International Monetary Fund, the global economic recession in 2020 will be more severe than the global financial crisis in 2008. Deutsche Bank, on the other hand, said GDP in the US and Europe could fall by 15.0–30.0% in the second quarter of 2020. At present, the epidemic has led to the sudden stagnation of economic activities in most countries, resulting in the contraction of the global supply chain. Therefore, controlling the epidemic has become the responsibility of people all over the world. The first step in controlling the disease is testing for SARS-COV-2. However, a simple, efficient and short-time detection method has become an important task [27–29]. At present, there are many methods to detect SARS-COV-2. For example: Computed tomography scan, Haematological detection, Polymer chain reaction, Loop mediated isothermal amplification, SHERLOCK technology, Microarray-based methods, Enzyme-linked immunosorbent assay, Electrochemical method and Immunoassay technology, etc. [30–32]. These methods have their own advantages and disadvantages. In this review, a Surface molecular imprinting technology (SM-MIT) for the detection of SARS-COV-2 virus was proposed for the first time, and its steps and mechanism were mentioned in this paper.

2. Detection method

2.1. Simple detection

The simplest way to detect SARS-COV-2 infection is to observe the physiological characteristics. Fever, dry cough and fatigue were the main manifestations [33,34]. A few patients were accompanied by nasal congestion, runny nose, sore throat, myalgia and diarrhea. Severe patients had dyspnea and hypoxemia. Severe cases can rapidly progress to acute respiratory distress syndrome, septic shock, metabolic acidosis, coagulation dysfunction and multiple organ failure [35]. However, these symptoms may also be caused by other viruses or causes, so the accuracy of this method is very poor, which is only suitable for the preliminary work of screening a large number of patients.

2.2. Computed tomography scan (CT-scan)

CT-scan has the characteristics of fast scanning time and clear image, which can be used for the examination of various diseases (See Table 1).

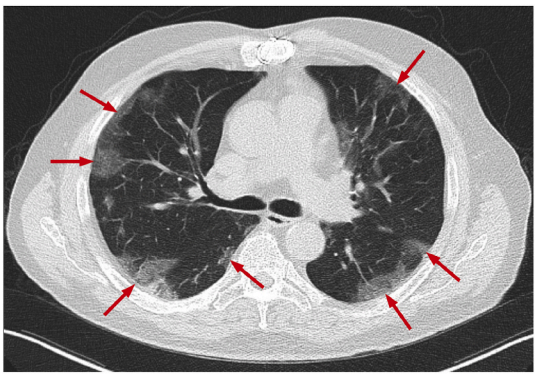

Fig. 1. CT-scan of novel coronavirus pneumonia patients.

Table 2
Serological manifestations of various viral infections.

Virus	Serological features	Reference
Hepatitis B virus	The mean platelet volume, red blood cell distribution width and the ratio of platelet to lymphocyte are increased, while the ratio of lymphocyte to monocyte is decreased.	[50]
SARS-CoV-2 virus	A large number of lymphocytes, $CD4^+$ T cells and $CD8^+$ T cells are lost, on the contrary, inflammatory cytokines and D-dimers are increased.	[2,35]
Schmallenberg virus	The mid-size white blood cell (mid) and red blood cell count (RBC) are decreased, while the average red blood cell hemoglobin is increased.	[51]
Hepatitis E virus	Transaminase and serum bilirubin concentration are significantly increased.	[52]
Ebola virus	Haematological abnormalities were common, including raised haematocrit thrombocytopenia, and granulocytosis.	[53]
Papillomaviruses	The CD8 + T cells of natural killer cells, IFN-γ and IL-17 are increased, on the contrary, the ratio of γδ+ T cells and CD4+/CD8+ is decreased.	[54]

CT-scan can be used to detect suspected and confirmed patients with SARS-COV-2 [41–43]. The CT-scan manifestations of novel coronavirus pneumonia showed multiple small patches and interstitial changes at the early stage. The lung was obviously exuded, and gradually developed into double lung multiple ground glass images and infiltrating shadows (see Fig. 1). Severe cases of pulmonary consolidation and pleural effusion were found [44,45]. CT-can cannot completely determine the presence of a novel Coronavirus, as the CT-scan can only determine the clarity of the chest and lungs, and may also be caused by another virus [46]. It can be seen from Table 1 that the representation phenomena of CT-scan diagram are very similar. Ground glass opacity (GGO) usually refers to the high-density area found in the lung parenchyma during fluoroscopy, which is often manifested as a mass or nodule on chest X-ray or CT. However, GGO and consolidation can appear after infection of various viruses. Therefore, this method is only suitable for the preliminary brush selection in the early stage, and other methods need to be combined to determine whether they are infected.

2.3. Haematological detection

Haematological testing has been applied to various virus screening [47–49] (See Table 2). According to the literature, when the absolute lymphocyte count was less than 0.8×10^9/L or the CD^{4+} and CD^{8+} T cell count significantly decreased, it suggested that the patient might be infected with SARS-COV-2 virus [35]. In addition, the levels of muscle enzymes, liver enzymes, troponin, myoglobin, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were increased in some patients. In severe cases, the lymphocyte count in the blood decreases

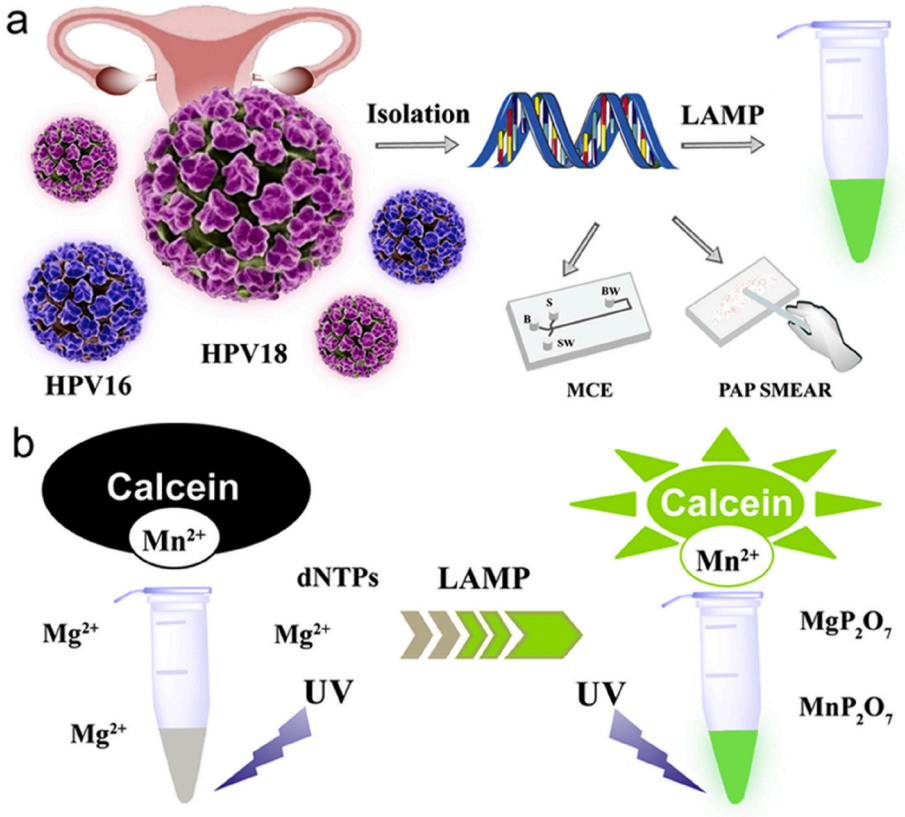

Fig. 2. Working principle of loop-mediated isothermal amplification detection.

gradually. In contrast, inflammatory cytokines and D-dimers were elevated [2]. SARS-COV-2 infection may be considered if prothrombin time is prolonged or aspartate transaminase, creatine kinase, creatinine and lactate dehydrogenase are increased [55,56]. According to reports in the literature, IL1β, IFN-γ, IP10 and MCP1 are expressed at higher levels in SARS-COV-2 patients. In addition, granulocyte colony stimulating factor (GCSF), IP10, MCP1A, MIP1A and TNF-α are more highly expressed in critically ill patients [57]. The changes of these expression factor also can be triggered by various infectious or non-infectious diseases [58]. Therefore, haematological detection is a good method for virus detection. Then, it can be seen from Table 2 that different viruses will have similar changes in blood factors. For example, the number of lymphocytes and inflammatory cytokines increased, CD4 + T cells decreased. Therefore, haematological detection methods need to be further optimized.

2.4. Polymerase chain reaction (PCR) methods

PCR is an enzyme method with high sensitivity and strong sequence specificity [59–64]. At present, it has become a detection technology of SARS-COV-2 virus [65,66]. However, this method is time-consuming and expensive, so it has great limitations in clinical application.

Real time reverse transcription polymerase chain reaction (RT-PCR), as a specific and simple quantitative detection method [67,68], has been widely used in the detection of various viruses [69–72]. For example, Nunes et al. used RT-qPCR to detect four Brazilian Amazon hantaviruses with a detection limit of 0.9 copies/μL. The specificity and sensitivity of RT qPCR were 100% and 97.63%, respectively [73]. Zhang et al. developed a real-time RT-PCR method to detect the Seneca Valley virus with a detection limit of 7.0 copies/μL. The specificity and sensitivity of RT-qPCR were 98.08% and 100.0%, respectively [74]. In addition, RT-PCR method has been used by many researchers to test SARS-COV-2 virus and has been widely used in clinical trials [75–77]. For example, Li et al. developed a novel qRT-PCR method to detect SARS-COV-2 virus with a detection limit of 365.0 copies/mL. The specificity and sensitivity of RT-qPCR were 100.00% and 97.62%, respectively [78]. Wang et al. detected SARS-COV-2 virus by combining serological total antibody and RT-PCR. The detection limit of RT-PCR was 2.0 copies/μL, and the sensitivity and specificity were 98.6% and 98.7% respectively [79]. Corman et al. developed a novel real-time RT-PCR method to detect SARS-COV-2 virus with 95% specificity and 100% sensitivity. In addition, the limit of detection for E gene and RdRp gene was 3.9 and 3.6 copy RNA per reaction, respectively [80]. At present, all kinds of optimized RT-PCR detection methods are the mainstream of SARS-COV-2 virus detection, which lays a solid foundation for the detection of infection or not. If the virus material is insufficient or the operation is wrong, the RT-PCR test may be false negative. Therefore, the method has strict requirements on material quantity and operation [81]. As a result, this method increases the risk of health care workers contacting suspected infected persons. In addition, when RT-PCR method is used, the temperature setting range of the thermal cycling device of the detection equipment is 50–95 °C, so the temperature requirement is very high. These shortcomings limit its clinical application to a certain extent [60].

2.5. Loop-mediated isothermal amplification (LAMP) methods

LAMP is a novel and efficient isothermal nucleic acid (DNAs and RNAs) amplification method [82–85], showing high sensitivity and specificity [86–88]. LAMP analysis method is often used in clinical detection of coronavirus [89,90]. As shown in Fig. 2, Fan et al. used LAMP method to detect high-risk HPV16 and HPV18 viruses, and the mechanism was as follows: As shown in Fig. 2a, the DNA of the virus was obtained by centrifugation. Then, THE HPV16 and HPV18 viruses were detected by LAMP and PCR-MCE (Polymerase Chain reaction-microchip electrophoresis system), and the accuracy was higher than that of conventional cytology. They were both used for visual detection of HPV16

and HPV18 by Papanicolaou (PAP) staining. As can be seen from Fig. 2b, the virus DNA was obtained by centrifugation. Calcein then acts as a fluorescent indicator and is premixed with manganese ions in the lamp reaction mixture and then irradiated with 365.0 nm ultraviolet light. The binding of manganese ions and pyrophosphate ions results in the release of calcein. Free calcein combines with magnesium ions, resulting in the reaction system turning light yellow and emitting green fluorescence under 365.0 nm UV radiation. These are to determine the presence of HPV in the samples [91]. In addition, Guenther et al. used LAMP method to detect feline Coronavirus virus with a detection limit of 5.0 copies/μL. The specificity and sensitivity of LAMP were 97.3% and 58.8%, respectively [92]. Thai et al. used LAMP method to detect severe acute respiratory syndrome coronavirus with a detection limit of 0.01 copies/mL. The specificity and sensitivity of LAMP were 87.0% and 100.0%, respectively [93]. Pyrc et al. developed a LAMP detection method for human coronavirus nl63 with a detection limit of 1.0 copies/μL. The specificity and sensitivity of LAMP were 100.0% and 100.0%, respectively [94]. Kitagawa et al. developed a LAMP method to detect SARS-COV-2 virus with a detection limit of 1.0×10^1 copies/μL. The specificity and sensitivity of LAMP were 97.6% and 100.0%, respectively [95]. Herein, a number of LAMP-based coronavirus detection methods have been developed and applied in clinical diagnosis.

Reverse transcription loop mediated isothermal amplification (RT-LAMP) is a new method for detecting miRNA. This method has the advantages of low background noise, fast, simple and reliable. We believe LAMP is a promising method for virus detection [96]. According to the literature reports, many researchers use lamp method to detect SARS-COV-2 virus [97,98]. For example, Dao et al. used RT-LAMP method to detect SARS-COV-2 virus with a detection limit of 100.0 copies/μL. The specificity and sensitivity of RT-LAMP were 99.7% and 97.5%, respectively [99]. Klein et al. used RT-LAMP method to detect SARS-COV-2 virus with a detection limit of 10.0 copies/reaction. The specificity and sensitivity of RT-LAMP were 100.0% and 100.0%, respectively [100]. Yan et al. used RT-LAMP method to detect SARS-COV-2 virus with 100% specificity and 100% sensitivity. The lower limit of detection was less than 2×10^1 RNA copies/reaction [101]. Ben-Assa et al. used RT-LAMP method to detect SARS-COV-2 virus with a detection limit of 12.0 copies/reaction. The specificity and sensitivity of RT-LAMP were 100.0% and 100%, respectively [102]. Zhu et al. devised a multiplex reverse transcription loop-mediated isothermal amplification (mRT-LAMP) method to detect SARS-COV-2 virus with a detection limit of 12.0 copies/reaction. The specificity and sensitivity of mRT-LAMP were 100.0% and 100.0%, respectively [103]. Current RT-LAMP detection methods are used by WHO and nursing sites around the world to detect SARS-COV-2 virus, and a large number of optimized RT-LAMP methods have been proposed and even combined with other methods. However, this method also requires a high temperature (52–94 °C) and all virus isolation procedures and RNA extraction are performed in a biosafety level III facility. Therefore, the high cost, complex operation, high detection environment and high equipment requirements of this method limit the clinical application of this method, which requires further optimization [86].

2.6. SHERLOCK technique

SHERLOCK technique is a detection method which combines isothermal amplification with CRISPR-mediated detection [104–107], and the technology reduces reliance on equipment. At present, this method is often used for virus detection [108–110]. Moreover, the SHERLOCK technique can be used to detect SARS-COV-2 virus and has clinical applications [111]. For example, Joung et al. used SHERLOCK technique to detect SARS-COV-2 virus with a detection limit of 100.0 copies/reaction. The specificity and sensitivity of SHERLOCK technique were 100.0% and 100.0%, respectively [112]. Broughton et al. used CRISPR-based lateral flow assay to detect SARS-COV-2 virus with 95.0% positive predictive agreement and 100.0% negative predictive agreement. The detection limit of each reaction was 1.0 copy per μl reaction [113]. Ail et al. used CRISPR-based method to detect SARS-COV-2 virus with a detection limit of 10.0 copies/reaction. The specificity and sensitivity of SHERLOCK technique were 100.0% and 100.0%, respectively [114]. This detection method not only has good sensitivity and specificity, but also does not require complex instruments. However, this method requires DNA extraction and amplification, and the operation of the instrument is complex, and patients cannot self-detect, which increases the risk of contact and infection of medical staff [107].

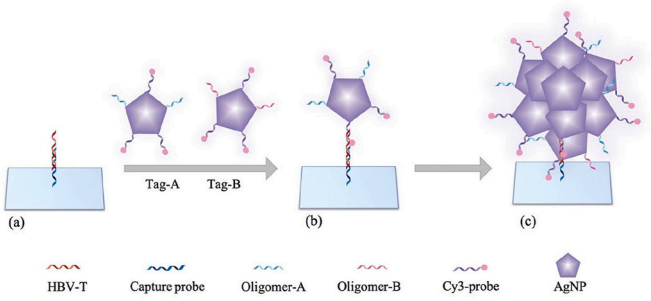

Fig. 3. Working principle of Enzyme-free fluorescence microarray detection.

2.7. Microarray-based methods

Microarray is a rapid, high-throughput detection method [115–117], which can reverse transcription of viral RNA and produce a specific probe labeled cDNA. The cDNA was then loaded into each well and hybridized with solid-phase oligonucleotides on the Microarray, which is then washed to remove the free DNAs. Finally, viral RNA is detected by a detection-specific probe, and Microarray has been widely used in the detection of virus [118–122]. As shown in Fig. 3, Jin et al. used Enzyme-free fluorescence microarray to detect hepatitis B virus DNA, and the mechanism was as follows: Fig. 3 shows the formation mechanism of AgNP aggregates for target detection on DNA microarrays. Two kinds of nucleic acid-modified AgNP probes, Tag-A and Tag-B were obtained separately by the attachments of recognition probes (Cy3-probe) and hybrid probes (Oligomer-A and Oligomer-B) to AgNPs through Ag–S bonds [123]. In addition, Lung et al. used Multiplex RT-PCR and Automated Microarray for Detection of rinderpest virus (RPV) with good specificity and sensitivity. The limit of detection of the microarray assay was as low as 1.0 $TCID_{50}$/ml for RPV [124]. Guo et al. used 6 a single nucleotide polymorphism DNA microarray method to detect SARS virus with 100% accuracy, and this method has able to detect 24 SNPs and determine the type of a given strain [125]. De Souza Luna et al. used nonfluorescent low-density microarray method to detect severe acute respiratory syndrome virus with a detection limit of 100.0 copies/reaction. The specificity and sensitivity of nonfluorescent low-density microarray were 100.0% and 100.0%, respectively [126]. Hardick et al. used a spotted array Mobile Analysis Platform (MAP) method to detect respiratory syncytial virus with a detection limit of 30.0 copies/reaction. The specificity and sensitivity of MAP were 100.0% and 100.0%, respectively [127]. In addition, we think this method may be of great significance for the rapid detection of SARS-COV-2. However, this method also requires DNA extraction and amplification, and the operation of the instrument is more complex, and this method not only operates at high temperature, but also takes a long time. These reasons will limit its future clinical application [118].

2.8. Enzyme linked immunosorbent assay (ELISA) method

At present, ELISA is usually used to detect viruses in clinical practice [128–132]. ELISA is an analytical method based on the specific binding between antigen and antibody [133–136]. For example, Li et al. used a

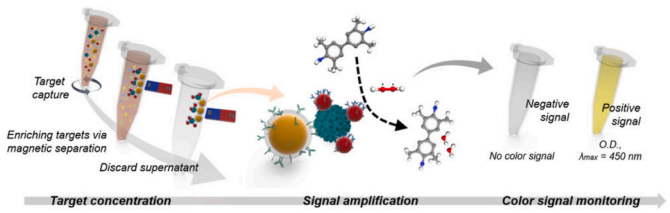

Fig. 4. Working principle of ELISA colorimetric detection.

sandwich ELISA method to detect Chinese sacbrood virus (CSBV) with a detection limit of 3.675×10^4 copies/μL. The method is specific to CSBV and has no cross reactivity with other bee viruses [137]. Tan et al. used an indirect ELISA based on glycoprotein B gene method to detect Feline herpesvirus type 1 virus. The indirect ELISA, characterized by high sensitivity showed no cross-reaction with two types of feline virus, had detection limit at 1:2000 dilution [138]. Li et al. used IDVET ELISA method to detect Akabane virus with a detection limit of 1:64 dilution. The specificity and sensitivity of IDVET ELISA were 82.31% and 93.46%, respectively [139]. Wang et al. used GP5 Protein-based ELISA method to detect Porcine reproductive and respiratory syndrome virus with a detection limit of 1:800 dilution. The specificity and sensitivity of GP5 Protein-based ELISA were 100.0% and 80.39%, respectively [140]. Qi et al. used an antigen-capture enzyme-linked immunosorbent assay (AC-ELISA) to detect equine arteritis virus with a detection limit of 36.0 PFU/mL. The specificity and sensitivity of AC-ELISA were 100.0% and 100.0%, respectively [141]. In, addition, specific antibodies (such as angiotensin-converting enzyme 2, ACE2) were immobilized and then bound to the spinous glycoprotein (S protein, viral surface spinous protein) on the surface of SARS-COV-2 to form a solid phase complex. Then the complex was combined with the enzyme labeled antibody. After adding the substrate of enzyme reaction, the substrate was catalyzed into colored products. The color reaction is analyzed according to the depth of color reaction [142]. As shown in Fig. 4, Oh et al. used Magnetic Nanozyme-Linked Immunosorbent Assay to detect Influenza A Virus, and the mechanism was as follows: Firstly, the nucleic acid was enriched by magnetic separation. Then, MagNB-Abs, which is a specific combination of magnetic nanoparticles (MagNB) and Influenza A virus antibody (Abs), acts as capture probe to recognize target viral nucleic acid through specific antigen antibody interaction. The AuNZs, which is represent the enzyme like activity of gold nanoenzyme, acts as signal amplifier to form sandwich structure with magnbs complex. After the immune response, the sandwich like structures were collected by magnetic force. AuNZs on sandwich like immune complexes act as catalysts to promote the oxidation of TMB by H_2O_2, thus producing colorimetric signals. Meanwhile, the absence of virus cannot assemble the immunocomplex, leading to the lack of oxidation reaction of TMB for colorimetric signaling. The concentration of influenza virus directly related to the color change of TMB can be detected by the catalytic activity of AuNZs with H_2O_2 as electron acceptor [143]. The method is accurate and effective, but it has some defects such as high cost of antibody preparation, poor stability and complex operation process, which limits its wide application [144,145];

2.9. electrochemical method

In recent years, electrochemical biosensor has become a reliable analytical equipment [146–148], which can detect various viruses that threaten human health [149–151]. Electrochemical biosensor is a kind of equipment which has both electrochemical sensing and biosensor [152,153]. It can convert biochemical information, and has the advantages of simple instrument, high sensitivity, high cost-effectiveness and miniaturization [154]. The working principle of electrochemical biosensor is the enzyme catalyzed reaction between biomolecules and target analytes, which generates electrons and affects the electrical properties of the solution [155]. In addition, electrochemical biosensors have different kinds of sensors due to different biological elements [156, 157]. In recent years, electrochemical biosensors have achieved great success in the field of pathogen detection due to its unique performance. For example, it can be seen from Fig. 5 that various viruses (Influenza, Zika, HIV, HEP-B, COVID-19) can be detected by electrochemical biosensor. The method monitors the activity of living cells or enzymes by measuring interactions between analytes and biological receptors with electrochemical biosensors. The strength of each virus's electrochemical signal is different, so that the presence of such a virus can be accurately detected [146]. In addition, Zhou et al. used a novel Electrochemiluminescence Immunosensor (P-RGO@Au@Ru–SiO_2) to detect HIV-1 p24 Antigen with a detection limit of 1.0×10^{-9} mg/mL. The specificity and sensitivity of Electrochemiluminescence Immunosensor were 100.0% and 100.0%, respectively [158]. Kor et al. used an electrochemical sensor, which is based on electropolymerized molecularly imprinted polymer, to detect furosemide with good specificity and sensitivity. The detection limit of electrochemical sensor was 7.0×10^{-8} mol/L [159]. Heo et al. developed a new electrochemical method to

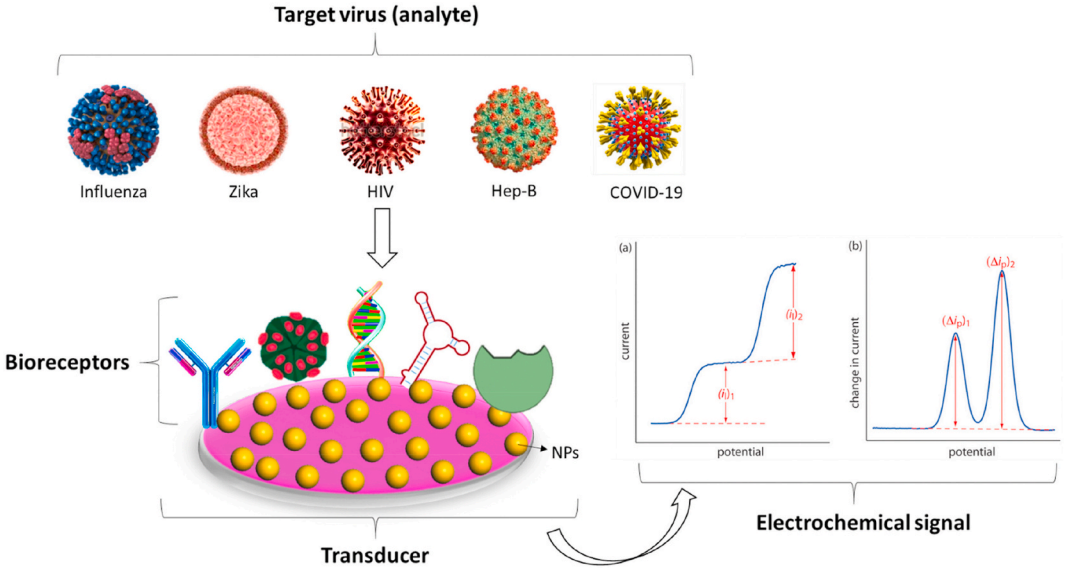

Fig. 5. Potential electrochemical biosensor platforms for the detection of various pathogenic viruses including COVID-19.

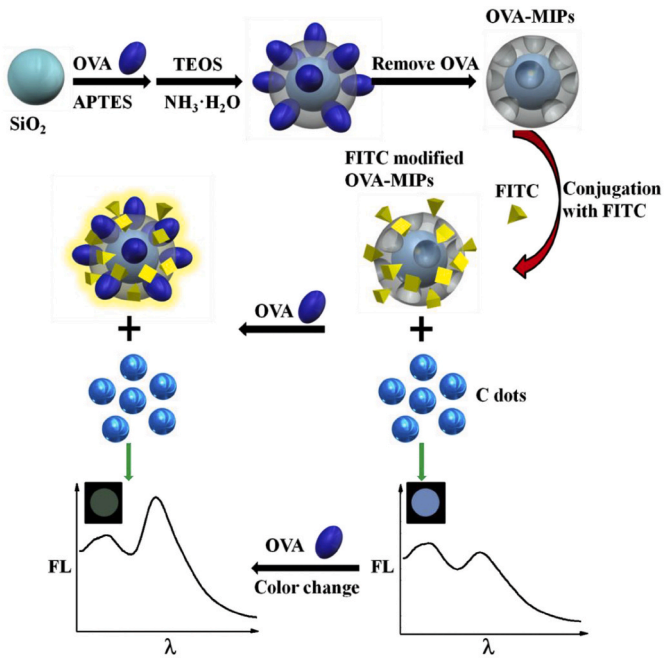

Fig. 6. Construction of molecularly imprinted double fluorescence emission system.

detect hepatitis B virus (HBV) with good specificity and sensitivity. The detection limit of electrochemical method was 0.14 ng/mL [160]. Layqah et al. used an electrochemical immunosensor to detect MERS-CoV and HCoV virus with good specificity and sensitivity. The detection limits for HCoV and MERS-CoV were as low as 0.4 pg/ml and 1.0 pg/ml, respectively [161]. To sum up, we believe that the electrochemical method will be applied to the detection of new coronavirus in the near future. However, they also face many challenges. For example, high cost, limited equipment and complex operation limit its clinical application [153].

2.10. Immunoassay technology

Immunoassay is a technology which combines specific reaction of antigen and antibody [162–164]. At present, many researchers use this technology to detect new coronavirus [165–168]. For example, Mairesse et al. used Chemiluminescence immunoassay technology to detect SARS-CoV-2 IgM antibodies. Using optimized cut-off, the specificity and sensitivity for IgM was 94.7% and 81.6%, respectively. The detection limit is 2.81 AU/mL for IgM [169]. Kohmer et al. used automated immunoassays technology to detect SARS-CoV-2 IgG antibodies with 100.0% specificity and 77.8% sensitivity [170]. Liu et al. used Chemiluminescence Microparticle Immunoassay to detect SARS-CoV-2 IgM and Ab antibodies. The specificity of IgM and Ab detection were 99.3% and 98.9%, respectively. The sensitivity of IgM and Ab detection were 72.3% and 90.8%, respectively [171]. Montesinos et al. used chemiluminescent immunoassays to detect SARS-CoV-2 IgM and IgG antibodies. The specificity of IgM and IgG detection were 58.7% and 53.2%, respectively. The sensitivity of IgM and IgG detection were 100.0% and 100.0%, respectively. The detection limit is 1.0 AU/mL for IgM and IgG [172]. To sum up, Immunoassay technology is a particularly good method for the detection of specificity and sensitivity, and has been widely used for the detection of various viruses. However, the cost of this method is too high, the operation is complex and time-consuming, which limits its clinical application [165].

2.11. New method

As mentioned above, the existing detection methods still have room for further improvement in the aspects of detection cost, detection time, convenience, and susceptibility of detection personnel [173]. Therefore, how to design a low cost, convenient, rapid, specific and sensitive SARS-COV-2 detection method is one of the important issues that need to be solved urgently at present. Surface molecular imprinting technology (SM-MIT) can be applied to the identification of biological macromolecules (such as proteins and nucleic acids), viruses and cells [174–181]. SM-MIT is a technique for synthesizing polymer networks around template molecules to obtain rich polymer materials that can be used to identify specific target molecules. This technique has good physical and chemical stability, simple preparation method, low cost, and high selectivity in recognition and recombination. Due to these excellent properties, SM-MIT has been widely used in protein specific recognition [182,183]. For example, the basic principle is that the virus specific protein is used as a template. A surface imprinting material was synthesized by using silica gel particles [184], organic polymer microspheres [185] or chitosan [186] as inert carriers, and amino and carboxyl groups as binding sites [187–189]. Then, the template protein was removed to obtain a surface imprinting material with specific and selective recognition of viral protein. For example, as can be seen from Fig. 6, Wang et al. synthesized silica core-shell surface molecularly imprinted microspheres (MIPs) using ovalbumin (OVA) as template, and used fluorescein isothiocyanate (FITC) modified microspheres as fluorescence enhancement signal, and then prepared a ratio nano sensor for the fluorescence determination of ovalbumin (OVA) by mixing blue carbon quantum dots (C dots). The sensor can be observed by naked eyes, and the fluorescence color changes from blue to dark olive green and finally to green. In addition, the detection limit is as low as 15.4 nm [190]. Klangprapan et al. used a quartz crystal microbalance molecularly imprinted polymer material to detect classical swine fever virus with good specificity and sensitivity, and the detection limit was 1.7 μg/mL [191]. Yang et al. prepared a fluorescent molecularly imprinted sensor material based on a metal-organic framework for identifying Japanese encephalitis virus (JEV). It is found through experiments that it has high selectivity and high sensitivity, and its maximum detection limit is 13.0 pmol/L [192]. Luo et al. prepared a magnetic molecularly imprinted polymer resonance light scattering sensor material to identify Japanese encephalitis virus. It is found through experiments that it has high selectivity and high sensitivity, and its maximum detection limit is 1.3 pM [193]. For example, as can be seen from Fig. 7, Zhang et al.

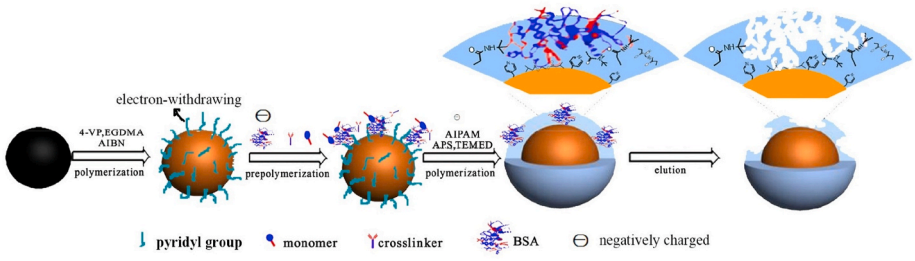

Fig. 7. Schematic diagram of protein separation by core-shell molecularly imprinted polymer microspheres.

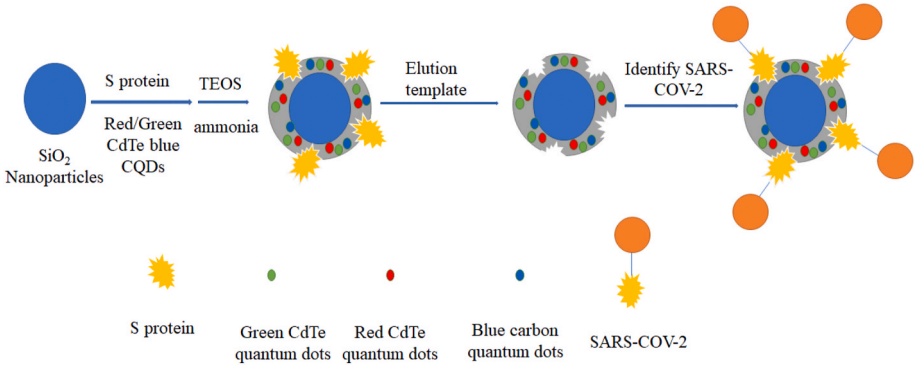

Fig. 8. Synthesis of S-protein molecularly imprinted microspheres with core-shell trichromatic fluorescence SARS-COV-2.

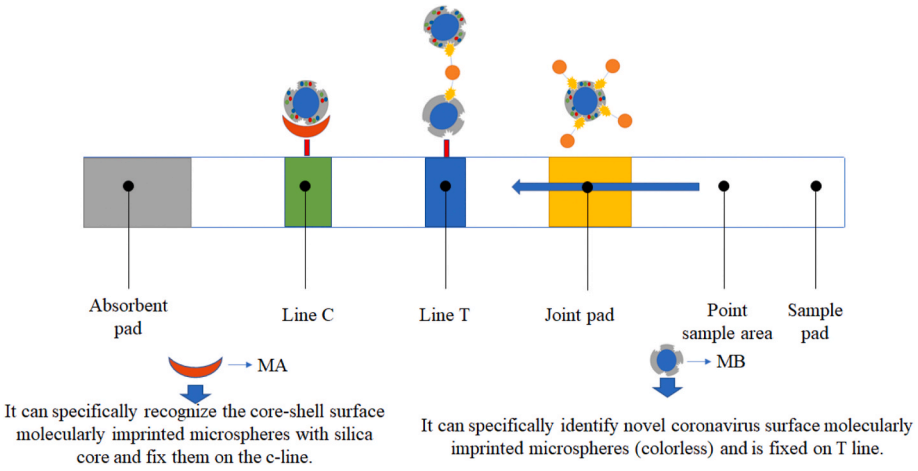

Fig. 9. Construction of three-color fluorescence-like immunotype test paper. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

prepared a core-shell protein surface molecularly imprinted microsphere material (MIMPs), the preparation of MIMPS consists of three steps. Firstly, magnetic beads were prepared by improved solvothermal reaction. Then, 4-Vinyl pyridine (4-VP) was polymerized on the surface of the magnetic beads. Finally, the polymer membrane complementary to the template was obtained. N-isopropylacrylamide (NIPAM) monomer is added as a temperature sensitive component, which allows expansion and contraction with the change of temperature, thus realizing the recognition and release of bowene serum albumin (BSA). Pyridine group is hydrophilic, which is conducive to the dispersion of carrier microspheres in water. In addition, recognition of pyridine group at the bottom of the cavity and recognition of polymer chain are conducive to the interaction between BSA and electrostatic and hydrogen bonds, which is suitable for the selective adsorption of BSA.

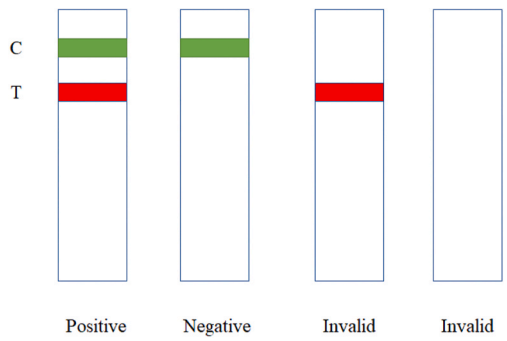

Fig. 10. Schematic diagram of test paper determination.

After the removal of the template protein, a surface molecularly imprinted material was obtained to specifically recognize BSA [194]. To sum up, we think SM-MIT technology provides an important reference value for the research on how to target and recognize the spike glycoprotein (s protein) on the surface of SARS-COV-2. Fluorescence emission detection technology provides a visual and effective way to detect infection. The concentration of the detected substance can be reflected by the fluorescence intensity of the fluorescent emission group, which can be easily captured by the naked eye. In recent years, a series of detection technologies based on single fluorescence emission and double fluorescence emission systems have been developed for the detection of proteins, small molecules and ions [195–197]. In addition, the single fluorescence emission system can identify very few kinds of colors, and the degree of visualization is low [198]. However, the dual fluorescence emission system can not only self-tuning, but also expand the range of color change [199,200]. Therefore, silica core-shell surface molecularly imprinted microspheres [201] embedded with red, green and blue fluorescent quantum dots can be constructed by sol-gel polymerization. Then, the response of the ratio of red, green and blue quantum dots to spike glycoprotein (S-protein) on the surface of SARS-COV-2 was studied (as shown in Fig. 8). With the increase of the concentration of S-protein on the surface of SARS-COV-2, the color change effect of fluorescence color from green, yellow, orange, red, purple to blue can be observed. The surface molecularly imprinted microspheres were mixed with fluorescent quantum dots, and then grafted onto the surface of appropriate chromatographic test paper by grafting, so as to construct the SARS-COV-2 type immune test paper with visualization, specific and selective detection and convenient operation (as shown in Fig. 9). The

Table 3
Pros and Cons of virus Testing Methods.

Method	Pros and cons	Reference
Simple	A simple method can be used to determine whether a virus is infected by some symptoms of the body, but the accuracy of this method is poor, and it can only be used for pre-screening in special period.	[34]
CT-scan	CT-scan can determine whether a certain organ is infected with virus to some extent, but there are many kinds of viruses that can infect an organ. Therefore, it needs to combine with other methods to determine. This method is suitable for the examination of respiratory tract infection.	[45]
Haematological	Haematological detection has been used in the screening of various viruses, and the change of blood components can be used to determine whether some viruses are infected. However, the detection time of this method is too long, and its blood components may change the same after different virus infection. Therefore, this method also needs further optimization.	[2,54]
PCR	PCR is a highly sensitive and sequence specific enzyme method. This method is widely used in virus detection and has been widely accepted by researchers. However, the process is time-consuming and requires a variety of biochemical reagents, laboratory level instruments and trained professionals.	[63]
LAMP	LAMP is an efficient isothermal nucleic acid (DNAs and RNAs) amplification method with high sensitivity and specificity, and is widely used in the detection of coronavirus. However, this method also needs high temperature, so it has certain risk. Not only that, all virus isolation procedures and RNA extraction are carried out in a tertiary biosafety facility. Therefore, the cost of this method is high, the operation is complex, and the equipment requirements are high.	[86]
SHERLOCK	SHERLOCK technology is very simple, convenient and low-cost to detect viruses. It can detect gene expression and interaction in human or animal genomes one by one or even in batches, so as to clarify the function and regulatory network of genes. However, this method is easy to miss the target and is relatively difficult to transfect. It has the preference of base recognition, which limits the application scope of gene editing, and leads to different editing efficiency of different gene sites.	[107]
Microarray	Microarray-based method is to detect DNA hybridization signal to realize rapid, parallel and efficient detection or medical diagnosis of biological samples. This method can achieve high-throughput and parallel detection of microorganisms. All the results can be obtained in one experiment, and the operation is simple and fast. But this method requires a large amount of viral nucleic acid. In addition, there are many kinds of this method, so it is difficult to establish a unified quality standard, which limits its clinical application.	[116]
ELISA	At present, ELISA is commonly used in clinical detection of viruses, which is based on the specific binding of antigen and antibody. The procedure of this method is simple, because there is no need to use the second antibody, it can avoid the interaction reaction, high sensitivity and high specificity, and the antigen does not need to be purified in advance, so it can be applied to relatively impure samples, and the data reproducibility is very high. However, this method is not repeatable and easy to be interfered by autoantibodies and heterophilic antibodies. In addition, the first antibody in the test must be labeled with enzyme, but not every antibody is suitable for labeling, and the cost is relatively high.	[130]
Electrochemical	At present, electrochemical biosensors are often used to detect various viruses. It has the advantages of simple instrument, high sensitivity and low cost. However, this method has poor selectivity, small temperature range and short instrument life. Therefore, this method needs further optimization.	[147]
Immunoassay	Immunoassay is a method based on the specific binding of antigen and antibody. This method has the advantages of high specificity, simple and rapid operation, relatively few influencing factors, easy control, good repeatability and easy standardization. However, the results of this method have great influence on the source and affinity of the antibody used, and the sensitivity is relatively low. Therefore, this method needs further optimization.	[167]
SM-MIT	SM-MIT is a technology of synthesizing polymer networks around template molecules to obtain rich polymer materials, which can be used to identify specific target molecules. The technology has good physical and chemical stability, simple preparation method, low cost and high selectivity for recognition and recombination. Moreover, the surface molecularly imprinted materials can be reused. Because of these excellent properties, SM-MIT has been widely used in protein specific recognition.	[187]

core-shell surface molecularly imprinted microspheres with silica core could specifically recognize the S-protein of SARS-COV-2 virus and form a complex with SARS-COV-2 virus after the detection sample was added to the sample area. Then the complex moves forward under the traction of the chromatographic test paper. When it moves to the position of T line, the complex can be captured by MB, and the color of T line can be determined according to the concentration of the tested substance. Some parts of the surface molecularly imprinted microspheres that are not bound to SARS-COV-2 cannot be captured by MB. Continue to move forward with the chromatography test paper. When it moves to the C line position, the surface molecularly imprinted microspheres will be captured by the MA and trapped on the C line. At this time, the C line shows green, indicating that there is no virus infection. The determination of the test results is shown in Fig. 10. If color rendering occurs on both Lines T and C, the result is positive; if only line C produces color rendering but line T does not, the result is negative; if no color rendering occurs on Line C, the test result is invalid and should be tested again. Therefore, this method can effectively shorten the detection time, reduce the cost and reduce the risk of infection of the detection personnel, and the operation is particularly simple (suspected patients can test by themselves). Therefore, early screening and early detection of SARS-COV-2 pneumonia patients can be carried out. It is believed that SM-MIT will become the focus of attention of all researchers and has great potential for application in the detection of SARS-COV-2 virus.

3. Summary and prospect

As SARS-COV-2 virus swept the world, a large number of people were infected and even died. Moreover, with the development of the epidemic, the global economy will fall into a serious recession. Some countries even disintegrated because of the epidemic. The epidemic has seriously affected the normal survival of people all over the world. The first step in controlling the epidemic is to screen suspected patients. At present, the detection methods of SARS-COV-2 virus are changing with each passing day. For the published methods, this paper not only analyzes their advantages and disadvantages (see Table 3), but also discusses their clinical performance (see Table 4). It is found from Tables 3 and 4 that the detection time of Surface molecular imprinting technology (SM-MIT) method is short, the detection limit is low and the accuracy is high. To sum up, this review is the first time to propose SM-MIT for the detection of SARS-COV-2 virus, and the steps and mechanisms of

Table 4
Different detection methods detect different viruses.

Method	Virus species	Accuracy (%)	Detection limit	Detection time	Reference
Simple	/	/	/	/	[33]
CT-scan	/	/	/	/	[41]
Haematological	/	/	/	/	[47]
PCR	SARS-CoV-2	100.0	5.0 copies/reaction	70.0 min	[69]
	Brazilian Amazon hantaviruses	97.6	0.9 copies/μL	92.0 min	[73]
LAMP	Zika virus	100.0	20.0 copies/reaction	60.0 min	[90]
	Feline Coronavirus virus	100.0	5.0 copies/μL	77.0 min	[92]
SHERLOCK	SARS-CoV-2	100.0	100.0 copies/reaction	70.0 min	[112]
	SARS-CoV-2	100.0	10.0 copies/μL	45.0 min	[113]
Microarray	Severe acute respiratory syndrome	100.0	100.0 copies/reaction	45.0 min	[126]
	Respiratory syncytial virus	100.0	30.0 copies/reaction	110.0 min	[127]
ELISA	Chinese sacbrood virus	100.0	3.67×10^4 copies/μL	70.0 min	[137]
	Equine arteritis virus	100.0	36.0 PFU/mL	60.0 min	[141]
Electrochemical	Chikungunya virus	100.0	8.0 ng/mL	90.0 min	[149]
	Hepatitis B virus	100.0	0.14 ng/mL	120.0 min	[152]
Immunoassay	SARS-COV-2	100.0	20.0 copies/μL	48.0 min	[90]
	SARS-COV-2	88.6	20.0 copies/μL	15.0 min	[162]
SM-MIT	Swine fever virus	100.0	1.7 μg/mL	10.0 min	[191]
	Japanese encephalitis virus	100.0	13.0 pmol/L	20.0 min	[192]

this method are described in detail. We believe that the SM-MIT method will become a hot spot for many researchers and has great potential in the detection of SARS-COV-2 virus.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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