Showcasing research from Professor Wen's laboratory, Department of Physics, The Hong Kong University of Science and Technology, Hong Kong, China.

Point-of-care testing detection methods for COVID-19

Professor Wen's group from The Hong Kong University of Science and Technology used a thin film silicon-based micro-heater real-time PCR system together with silicon-glass microfluidic chips, reduced the detection time of SARS-CoV-2 nucleic acid to less than 40 min. The portable and rapid SARS-CoV-2 nucleic acid detection system can reach a temperature rising rate up to 30 °C/s, and was able to complete the detection with a sensitivity of up to 500 copies/mL. This article is jointly completed by Professors Jinbo Wu and Bingpu Zhou from Shanghai University and University of Macau respectively.

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Point-of-care testing detection methods for COVID-19

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COVID-19 is an acute respiratory disease caused by SARS-CoV-2, which has high transmissibility. People infected with SARS-CoV-2 can develop symptoms including cough, fever, pneumonia and other complications, which in severe cases could lead to death. In addition, a proportion of people infected with SARS-CoV-2 may be asymptomatic. At present, the primary diagnostic method for COVID-19 is reverse transcription-polymerase chain reaction (RT-PCR), which tests patient samples including nasopharyngeal swabs, sputum and other lower respiratory tract secretions. Other detection methods, e.g., isothermal nucleic acid amplification, CRISPR, immunochromatography, enzyme-linked immunosorbent assay (ELISA) and electrochemical sensors are also in use. As the current testing methods are mostly performed at central hospitals and third-party testing centres, the testing systems used mostly employ large, high-throughput, automated equipment. Given the current situation of the epidemic, point-of-care testing (POCT) is advantageous in terms of its ease of use, greater approachability on the user’s end, more timely detection, and comparable accuracy and sensitivity, which could reduce the testing load on central hospitals. POCT is thus conducive to daily epidemic control and achieving early detection and treatment. This paper summarises the latest research advances in POCT-based SARS-CoV-2 detection methods, compares three categories of commercially available products, i.e., nucleic acid tests, immunoassays and novel sensors, and proposes the expectations for the development of POCT-based SARS-CoV-2 detection including greater accessibility, higher sensitivity and lower costs.

1. Introduction

At the end of December 2019, several patients with flu-like symptoms were admitted to hospitals in Wuhan, China. To identify the infectious pathogen, patient samples were subjected to metagenomic RNA sequencing. The results showed that the pathogen was a novel coronavirus. On January 30, 2020, the World Health Organization (WHO) proclaimed that the pneumonia caused by the newly discovered coronavirus was “2019-nCoV acute respiratory disease”. On January 31, 2020, the WHO declared the novel coronavirus outbreak a public health emergency of international concern (PHEIC). On February 11, 2020, the Coronavirus Study Group (CSG) of the International Committee on Taxonomy of Viruses named this virus “Severe Acute Respiratory Syndrome Coronavirus 2 [SARS-CoV-2]” because of its genetic similarity to the severe acute respiratory syndrome coronavirus (SARS-CoV); the 2 viruses belong to the same clade. The disease caused by SARS-CoV-2 was named coronavirus disease 2019 (COVID-19). Subsequently, the epidemic escalated into a global COVID-19 pandemic. The clinical manifestations of COVID-19 range from mild symptoms, such as cough, fever and dyspnea, to life-threatening syndromes, such as pneumonia and acute respiratory distress syndrome, and even death.1,2 As of the time of writing this article, there were more than 100 million confirmed cases, 2,182,179 deaths. The high infection rate and mortality rate for COVID-19 have caused substantial losses in terms of life, quality of life and the global economy.

Chinese researchers found that SARS-CoV-2 originated from bats, but the intermediate host has not yet been determined.3 The SARS-CoV-2 genome shares approximately 89% sequence homology with bat SARS-like-CoVZXC21 and approximately 82% sequence homology with human SARS-CoV.4

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SARS-CoV-2 enters specific cells through protein–protein interactions; i.e., the S glycoprotein of SARS-CoV-2 binds to angiotensin-converting enzyme 2 (ACE2) on the surfaces of some cell types. Through processing by TMPRSS2 protease, attached virus enters the cells. The S protein consists of 2 functional subunits: S1 and S2. The S1 subunit is binds to host cell receptors, while the S2 subunit fuses the viral membrane and cell membrane. The structural proteins of SARS-CoV-2, i.e., the envelope (E), membrane (M), helicase (Hel) and nucleocapsid (N) proteins, are shown in Fig. 1. Viral replication requires other auxiliary genes, including open reading frame 1a (ORF1a), ORF1b, RNA-dependent RNA polymerase (RdRp), and hemagglutinin esterase (HE). The M protein is the most abundant protein on viral particles. The E protein is the smallest major structural protein of SARS-CoV-2. It is involved in the assembly, release and pathogenesis of the virus. The N protein is the main structural protein of SARS-CoV-2. It is responsible for the transcription and replication of viral RNA, the packaging of the enveloped genome into viral particles, and interactions with the cell cycle of host cells. The N protein is the most abundant phosphorylated protein produced and released during viral infection. It has high immunogenicity and can be detected in serum or urine within the first two weeks of infection. Release of the N protein by the virus peaks approximately ten days after infection.

The main transmission routes of SARS-CoV-2 are contact transmission and droplet transmission. For example, droplets from an infected individual are deposited on the surfaces of objects; if an uninfected individual touches the surface of a contaminated object and then touches his/her mucous membranes of the oral cavity, nasal cavity and eyes, infection may occur. In addition, direct inhalation of the droplets generated by infected individuals when they cough/sneeze/talk or breathe by individuals at close range can also lead to infection. Therefore, the early diagnosis of suspected cases is an important task in managing infected individuals and controlling the spread of pathogens. The WHO emphasizes that early identification and isolation are essential for limiting human-to-human transmission. Therefore, it is necessary to conduct effective large-scale screening using sensitive and high-throughput detection methods; this screening approach allows the identification, isolation and tracking of individuals without obvious symptoms, facilitates a reduction in secondary infections between close contacts and healthcare workers, and leads to improvements in local control. In addition, such screening provides information for regional disease control efforts and prevents further spread of infections.

At present, the methods for the detection of COVID-19 mainly include nucleic acid testing and serological testing. These methods are suitable for the in vitro diagnosis of patients with suspected SARS-CoV-2 infection. Nucleic acid testing includes gene sequencing, reverse transcription-polymerase chain reaction (RT-PCR) and other methods. In the early stage of the novel coronavirus outbreak, the genome sequence of SARS-CoV-2 virus was obtained through gene sequencing, providing an important basis for subsequent testing. RT-PCR is considered the gold standard for detecting COVID-19, especially in the early stage of viral infection. Through targeting the unique RNA sequence of SARS-CoV-2, the genetic material of the pathogen can be directly detected by RT-PCR. However, the currently available quantitative RT-PCR (RT-qPCR) tests vary considerably in sensitivity, stability and detection time. Moreover, the test costs are rather high. These tests are mainly suitable for large and centralized diagnostic laboratories. In addition, the time...
required for sample transportation far exceeds the time spent on testing, further delaying diagnosis. Serological testing involves the use of blood samples to detect viruses. The two main antibodies tested in the blood are immunoglobulin G (IgG) and immunoglobulin M (IgM). Currently, serological testing for COVID-19 mainly involves the direct measurement of antigens and the indirect detection of antibodies in serum. Enzyme-linked immunosorbent assay (ELISA) is also a sensitive method for detecting COVID-19. Imaging also plays an important role in the diagnosis and treatment of COVID-19, especially in the early stages of the disease.

Due to the rapid development of COVID-19 and the limited laboratory-based molecular detection capacity, a rapid point-of-care testing (POCT) method is urgently needed to facilitate COVID-19 diagnosis outside of the laboratory setting. POCT methods, such as some rapid tests for an infectious disease diagnosis, provide results within minutes of the test being administered, allowing for rapid decisions about patient care. These devices are low cost and easy to use, providing sufficient time for the implementation of necessary preventive or therapeutic measures. So POCT devices can also extend testing to communities and populations that cannot readily access care. As quoted the POC test guideline from the United States’ Centers for Disease Control and Prevention (CDC), POC tests are used to diagnose COVID-19 in various settings, such as physician offices, urgent care facilities, pharmacies, school health clinics, long-term care facilities, nursing homes, as well as some temporary locations, such as drive-through sites managed by local organizations. In our review article, the POC test, especially for COVID-19 test, mainly refer to a test scenario where a professional could execute a fast and frequent test, as the above mentioned.

As countries deal with the COVID-19 pandemic in varying ways, one area of agreement is the need to test for the COVID-19 virus in as many people as possible. As reported in the UK media, access to COVID-19 (viral) testing has been limited for some sections of the population including healthcare professionals and careers. There have also been long delays in getting the results back to the person being tested. A higher frequency of test, a higher possibility of finding an infectious people. The most merit of POCT is that it could realize a fast and high frequency test. We could find out the infectious people immediately rather than delivering the sample to a central test lab and waiting a long time for a test result.

POCT doesn’t mean a lack of accuracy. Usually, a POCT machine has the same sensitivity and specificity as the one used in central lab. Especially, some POCT instruments are equipped with automated detection modules by which sample-in and answer-out test procedures could be realized. This not only decrease the operation complexity, but also increase the test sensitivity through an automatic process.

Qin et al. summarized microfluidic technology for the POCT diagnosis of infectious diseases and introduced a fully integrated POCT platform that included sample preparation, on-chip nucleic acid analysis, immune analysis, and system integration. Jiang et al. systematically introduced research progress on microfluidic platforms in the context of basic biological research, clinical applications, environmental monitoring and food safety, focusing on microfluidics-based nucleic acid analysis and protein analysis. In addition, Jiang et al. summarized the integration of microfluidics, nanotechnology, 3D printing and other advanced technologies to provide new platforms and tools for biological analysis. A review article published by Qin et al. focused on the “lab in/on an X” or “LionX” experimental platform and discussed nucleic acid testing on an integrated system that included sample pretreatment, nucleic acid extraction, amplification and signal detection. The review also highlighted promising diagnostic platforms and technologies that were expected to provide better solutions for the diagnosis of COVID-19 and other diseases. On the bases of the aforementioned articles, herein, we review existing POCT methods, as well as POCT methods under development, for detecting COVID-19; these methods include nucleic acid tests, serological immunoassay tests, microfluidic platforms and portable instruments used for the rapid standard diagnosis of COVID-19. We also summarize and highlight various fast-detection technologies and portable instruments for the detection of the novel coronavirus.

2. POCT based on nucleic acids

To detect SARS-CoV-2 nucleic acid, the first step is to collect biological fluids from where the virus is located. Fluids are usually collected using nasopharyngeal swabs and oropharyngeal swabs. The collected fluids are subjected to a series of filtration and isolation steps to obtain the virus. Microfluidic technology allows the miniaturization and integration of nucleic acid purification and large-scale PCR amplification, improving efficiency. After RNA extraction, reverse transcription is used to generate the complementary DNA (cDNA) of the viral RNA. Subsequently, specific regions of the cDNA are amplified, in the presence of DNA probes, by PCR, thereby realizing the real-time detection of the amplification process.

Currently, nucleic acid testing for SARS-CoV-2 mainly includes RT-PCR, loop-mediated isothermal amplification (LAMP), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated proteins (Cas)-based testing. This section introduces the latest research results from scientific research teams and commercialized POCT instruments.

2.1 RT-PCR-based tests

PCR is a temperature-controlled biochemical reaction catalyzed by enzymes. PCR instruments are manufactured based on polymerase and are actually thermocyclers that control 3 thermal cycles (namely, denaturation at 95 °C, renaturation at 55 °C and extension at 72 °C). Each PCR cycle exponentially increases the number of DNA fragments, thus producing a large number of specific gene fragments in a certain period of time. In a conventional desktop thermocycler, automated reactions can be conducted in EP tubes, and temperature differences of 20 °C can be attained within 10 s. DNA chips can
attain temperature differences of 20 °C in less than 1 s because of miniaturization, a smaller heat capacity and higher thermal conductivity, greatly improving efficiency.\textsuperscript{17} For example, Wu et al. created a PCR device for rapid DNA amplification and detection.\textsuperscript{18} As shown in Fig. 2, this device consists of an interchangeable PCR chamber, a temperature control system, and an optical detection system. The temperature control system can achieve high-precision temperature control with effective heating/cooling rates. The PCR chip is independent of the temperature control system and the optical detection system. There are other microfluidic rapid-heating systems based on infrared heating,\textsuperscript{19} air heating\textsuperscript{20} and microwave heating.\textsuperscript{21} These noncontact systems have higher heating speeds (65 °C s\textsuperscript{-1}), minimal over- or undershoot (±0.1 °C) and high temperature accuracy (±0.1 °C).\textsuperscript{22}

Currently, RT-PCR is the gold standard for COVID-19 diagnosis. Because RT-PCR can potentially detect a single RNA molecule in one reaction, it is very accurate and sensitive for the detection of viral genomes. There are many molecular targets in the sense-strand RNA genome of SARS-CoV-2 that can be used for PCR detection. The standardized scheme customized by the WHO includes sample collection, sample transportation, sample testing and test result analysis. Sample collection mainly refers to collecting human nasopharyngeal swab samples, oropharyngeal swab samples, sputum samples and saliva samples. Nasopharyngeal swabs and oropharyngeal swabs are used to collect upper respiratory tract specimens. The collection of these specimens requires medical staff to be in close contact with patients, which has certain risks. The “false negative” rate of traditional oropharyngeal swab sampling affects the diagnosis results, Chen et al. used microneedle (MN) patches to design swabs to improve the quality and quantity of virus collection. The antibody (Ab) against viral spike protein was incorporated into the patch to confer viral capture potential with MNs activity. The designed Mn/AB swab can improve the sampling efficiency and reduce the “false negative”.\textsuperscript{23} Sputum, which can be obtained noninvasively, serves as a lower respiratory tract specimen. Lin et al. diagnosed patients with COVID-19 based on RT-PCR analysis of sputum specimens.\textsuperscript{24} However, in a series of COVID-19 cases involving 41 patients, only 28% of the patients produced sputum suitable for diagnosis.\textsuperscript{25} RT-PCR results from the WHO’s Hong Kong laboratory showed that throat swabs obtained from patients tested positive for COVID-19.\textsuperscript{26} A study has shown that saliva has high concordance with nasopharyngeal specimens regarding the detection of coronavirus; the specificity was 90%.\textsuperscript{27} Moreover, sampling for saliva tests is easy and can be completed by patients themselves, thus reducing the risk of infection for medical staff. Taking the S gene of SARS-CoV-2 as the target, Kelvin KW To et al. collected saliva from patients with confirmed disease and performed a 1-step RT-qPCR test using an RT-PCR kit.\textsuperscript{28} COVID-19 was detected in 11 of the 12 patients.

RT-PCR primers have been designed to target different regions of the SARS-CoV-2 genome. Chu et al. developed two 1-step RT-PCR methods to detect the ORF1b and N regions of the viral genome.\textsuperscript{29} To avoid the genetic diversity of coronaviruses, primers and probes were designed to react with the novel coronavirus. This method achieved the detection of SARS-CoV-2. The highly conserved RdRp gene and variable S gene were introduced as the targets in another RT-PCR detection study.\textsuperscript{30} In the RT-PCR-based test developed by the German company Tib-Molbiol, the E gene of the SARS-CoV-2 genome was used for first-line screening, while the RdRp gene was used for confirmatory testing.\textsuperscript{31} This test was highly specific for SARS-CoV-2 and had no cross-reactivity with other coronaviruses. Chan et al. developed three RT-PCR detection methods that targeted the RdRp/HeI, S and N regions of the SARS-CoV-2 genome and compared their performance.\textsuperscript{32} Among these methods, the COVID-19-RdRp/HeI method had high sensitivity and specificity.

Shen et al. have developed an integrated microfluidic system containing a microfluidic chip with a hemagglutinin (HA)/neuraminidase (NA) array.\textsuperscript{33} This system allows automatic virus purification, virus lysis, one-step RT-PCR and fluorescent signal detection. Magnetic microbeads are used for virus extraction and isolation, while pneumatically driven microvalves are used for on-chip liquid control. On this microfluidic platform, up to 12 subtypes of influenza A virus (InfA) can be automatically and simultaneously detected in less than 100 min, with an analysis speed significantly faster than other methods. The structure of the microfluidic chip is shown in Fig. 3. The chip is 84 mm in length, 59 mm in width, and 10 mm in depth. It consists of one thick-film polydimethylsiloxane (PDMS) air control layer, one thin-film PDMS liquid channel layer, and a glass substrate (G-Tech Optoelectronics) to seal the microfluidic chip. The process for InfA detection is as follows. First, polysaccharide-modified magnetic microbeads, a virus-containing sample, RT-PCR reagents, and wash buffer are loaded into the corresponding
weeks. The possible reason is the difference of viral load, that false negatives have occurred in subjects for up to 2 weeks in the early stages of infection. Some reports have claimed that RT-PCR testing sometimes fails to detect SARS-CoV-2 RNA, indicating a positive result after 2 reaction cycles. Finally, RT-PCR signals are detected using an optical detection module.

RT-qPCR has certain limitations. For example, sample purity must be high, and trained professionals are required. In addition, RT-PCR testing sometimes fails to detect SARS-CoV-2 in the early stages of infection. Some reports have claimed that false negatives have occurred in subjects for up to 2 weeks. The possible reason is the difference of viral load, and the amount of virus in various patients is different. Different course of disease can also lead to differences. It is also possible that the sampling site is not suitable, the specimen containing virus are not taken or the detection operation is not standardized. These shortcomings limit the application of RT-PCR testing under many circumstances.

2.2 LAMP-based tests

Although RT-PCR is the gold standard for the molecular diagnosis of COVID, it has disadvantages; it is a time-consuming and laborious method. Isothermal amplification technologies (IATs), such as LAMP, recombinase polymerase amplification (RPA), rolling circle amplification (RCA), exponential amplification reaction (EXPAR), and strand displacement amplification (SDA), allow the amplification of nucleic acids at a constant temperature. IATs are able to overcome the shortcomings of PCR-based amplification, such as high denaturation temperatures and the cumbersome cycling of samples among different temperatures during amplification. IATs have been introduced into microfluidics for nucleic acid detection. Currently, LAMP and RPA have achieved sensitivities that no other IAT can achieve when detecting low-copy-number nucleic acids.

LAMP is a nucleic acid amplification technology that does not require thermal cycling, and it has the ability to probe multiple targets in a single reaction. LAMP synthesizes target DNA under constant temperature (60 to 65 °C) using specifically designed primers and DNA polymerase. This DNA polymerase has strand displacement activity, which improves the sensitivity and specificity of the results. LAMP can recognize 8 distinct target sites on a target sequence using 6 primers, generating 10⁹ copies of a target sequence. The final products are stem-loop DNAs with multiple inverted repeats of the target. The structure of the final products has a “cauliflower”-like appearance.

Some research reports have shown the advantages of LAMP over RT-PCR in terms of sensitivity, accuracy and specificity for SARS-CoV-2 detection. The results reported by Park et al. have shown that LAMP is specific for the detection of SARS-CoV-2 and has no cross-reactivity with other respiratory pathogens such as human coronavirus strains HCoV-OC43 and HCoV-229E. Zhang et al. identified SARS-CoV-2 RNA from purified RNA or lysed cells from patients employing visual and colorimetric detection. Using 130 samples, Yang et al. were able to directly compare RT-PCR with RT-LAMP. It was found that LAMP provided the same clinical diagnosis as RT-PCR and displayed similar sensitivity. Moreover, LAMP was faster, and the results were easier to read. Other research groups reported similar results. LAMP amplification has high sensitivity and specificity when used with samples from patients and improves the accuracy of multigene target amplification.

Some research results are based on a visible colorimetric response or visual readout. In terms of the visual readout for RT-LAMP, Baek et al. employed an assay combining RT-LAMP and a phenol red colorimetric method, which had a fast detection time of 30 min. The RT-LAMP detection primers were designed to target the nucleocapsid (N) protein gene of SARS-CoV-2. The resulting RNA limit of detection was 10⁵, i.e., close to that for qRT-PCR. A color change in phenol red from pink (pH 8.8) to yellow (pH < 8.0) indicates that amplification had occurred. There was no cross-reactivity with related coronaviruses, human influenza viruses and other viruses that cause respiratory diseases. Lu et al. tested a LAMP-based detection method that targeted the RdRp gene. The method was able to detect a colorimetric change after the addition of SARS-CoV-2 RNA, indicating a positive result after...
40 min of amplification. Moreover, the sensitivity of the method reached 5 copies per microliter. Yin et al. described a LAMP-based method, iLACO, for the rapid detection of SARS-CoV-2, producing results consistent with those for RT-PCR. A fragment of ORF1ab was selected as the target region, and the RT-LAMP primers were designed accordingly. The reaction mixture was incubated at 65 °C for 20 min, resulting in a color change from pink to light yellow. The fluorescence signal generated by iLACO was examined under ultraviolet and blue light. A positive signal was visible to the naked eye with a colorimetric pH indicator or under blue light with GeneFinder dye. It was found through serial dilution of the synthesized ORF1ab primers that iLACO could detect as few as 10 copies of the ORF1ab gene and that the detection process could be completed in 20–40 min. Therefore, iLACO is accurate and easy to implement. C. Yan et al. developed an RT-LAMP assay for the visual detection of SARS-CoV-2. The authors correctly identified 58/58 positive and 72/72 negative patients; the results were confirmed by parallel RT-PCR testing. The RT-LAMP analysis of RNA extracts from patient samples only required incubation at 63 °C for 60 min. Abbott ID Now® produced a kit that can detect SARS-CoV-2 within 5 min. This kit has obtained emergency use authorization (EUA) certification from the United States Food and Drug Administration (FDA). It uses NEAR for the qualitative detection of SARS-CoV-2 RNA and only requires a small portable touch screen device, namely, ID Now. It employs an RdRp gene-based molecular detection method, and the test samples can be nasopharyngeal swabs, oropharyngeal swabs or throat swabs. The kit contains 24 tests as well as negative and positive controls, and its application is not restricted by location.

Some research groups have combined RPA and LAMP into a two-stage amplification protocol termed RAMP. Song et al. used bioinformatic approaches to design primers specific for the ORF1ab gene in SARS-CoV-2 RNA. The study reported a single-stage COVID-19-LAMP in a closed tube and a two-stage isothermal amplification method (COVID-19-Penn-RAMP) in a closed tube. COVID-19-Penn-RAMP includes 2 isothermal amplification processes (Fig. 4): RPA reaction at 38 °C on the tube cap and a LAMP reaction at 63 °C in the tube. During the detection process, a swab is first eluted in water, and the water is then heated to above 65 °C. After being added to the tubes, the samples are subjected to LAMP single-stage amplification or COVID-19-Penn-RAMP two-stage amplification. The amplification process is monitored in real-time with the fluorescent dye Leuco crystal violet (LCV). The performance of COVID-19-Penn-RAMP is superior to those of COVID-19-PCR and COVID-19-LAMP. The sensitivity of COVID-19-Penn-RAMP is 10 times higher than that of LAMP when using purified samples and 100 times that of LAMP when using minimally prepared samples. The RAMP method displays high specificity, similar to LAMP, enhanced sensitivity resulting from two-stage amplification, and a higher tolerance to inhibitors.

There are also other tools that have application potential for the diagnosis of COVID-19. Yang et al. have described an RNA-based POC device for COVID-19 that combines paper-based POC diagnostic equipment and LAMP assay technology. This device can be integrated with a smartphone application to provide a fast, sensitive, and easy-to-use COVID-19 diagnostic tool (Fig. 5). People can collect nasal swab samples by themselves; perform LAMP analysis; and observe the visible colorimetric test results, record the data and share them with doctors or professionals via the internet. Song et al. developed a microfluidic platform for the detection of ZIKV. The process involves saliva sample collection and lysis, nucleic acid extraction via the isolation membrane of a microfluidic cassette, RT-LAMP amplification and colorimetric DNA detection. Although the operation process can be completed in the same microfluidic chip system, reagents and wash buffers still need to be added manually.

Sun et al. intended to use equine respiratory infection diseases as the model system for corresponding human diseases such as COVID-19 (Fig. 6). Namely, the specific nucleic acid sequences of 5 equine pathogens are LAMP-amplified on a microfluidic chip (reaction volume of 1 μL), and the reaction results are detected using a smartphone. The LAMP reaction takes less than 30 min, and the entire detection process can be completed...
within 1 hour using inexpensive and portable equipment. Silicon microfluidic chips have multiple advantages. These chips display high stability, have no autofluorescence and can be manufactured efficiently. The structure of a microfluidic chips is shown in Fig. 6. The chips are 25 mm × 15 mm × 0.5 mm containing 10 parallel flow channels. The channels are 10 mm in length, 500 μm in width and 200 μm in depth. The volume of each channel is 1 μL, and all channels share the same inlet. The inlet of each chip is an entrance chamber with a diameter of 4 mm, from which the contents are spread to 10 parallel assay channels. There are 2 square markers at the opposite ends of each chip that are used for position alignment during fabrication and automated image recognition during measurement. It has been reported that bare silicon absorbs polymerase, thus inhibiting nucleic acid amplification. Therefore, the surface of the chip is thermally oxidized to grow a 200 nm layer of SiO2. The experimental procedure is as follows: first, the control groups and the targeting primers are deposited into clean chip channels. Once the reagents in the microfluidic chip channels dry, the channels are covered with a transparent double-sided adhesive. Subsequently, LAMP reaction mix is injected from the inlet, and the chip is sealed with cover glass. The chip is heated to 65 °C to drive the LAMP enzymatic amplification reaction and then inserted into a scaffold for endpoint fluorescence imaging. Fu Sun and colleagues planned to use this chip for rapid POC detection of SARS-CoV-2. The fluorescent endpoint images of the LAMP reaction are collected using a mobile phone camera. Data collection can be integrated with telemedicine platforms, which would allow sharing the detection results. In addition, Sun et al. envisaged a detection instrument that could be clipped onto a smartphone. The instrument would have a mechanical adaptor that aligns with the rear-facing camera of several popular mobile phone models.

Wang et al. developed a LAMP-integrated microfluidic chip system to detect multiple respiratory viruses (Fig. 7). The entire detection process can be completed within 1 h. This system is able to specifically recognize influenza A virus subtypes (H1N1, H3N2, H5N1 and H7N9), influenza B virus and human adenoviruses with high specificity and high sensitivity. The microchannels of the chip are approximately 500 μm in width and 200 μm in depth. The chip contains 8 independent reaction microchambers (2 × 1 × 1 mm), with a corresponding reaction volume of approximately 2 μL. The chip system is closed, which reduces aerosol contamination and reagent evaporation during LAMP. The entire detection process includes sample collection, nucleic acid extraction, sample loading, real-time detection, and signal output. The specific process is as follows. First, a throat swab sample is collected from an individual. Nucleic acids are then quickly extracted from the sample using magnetic nanoparticles. After extraction, the nucleic acids are added to the microchambers of the microfluidic chip. The extracted nucleic acid sample reagents are diverted into the 8 microchambers along different channels, which are then covered with film to ensure that LAMP is carried out within a sealed chip. The real-time colorimetric detector provides real-time amplification curves based on color changes in the chip microchambers. Using this

![Fig. 6](image-url)  
**Fig. 6** Ten channel microfluidic chip for multiplexed LAMP detection. (a) The control and the target primer are put into the channel of the chip, which is then covered with double-sided adhesive tape; (b) LAMP reaction mixture is injected at the inlet, and the chip is sealed; (c) the chip is heated to 65 °C for the LAMP reaction, and then the chip is inserted into a scaffold for fluorescence imaging. Adapted with permission from ref. 52. Copyright: 2020, the authors.

![Fig. 7](image-url)  
**Fig. 7** Closed microfluidic chip system for the detection of a variety of respiratory viruses. (a) It consists of 8 independent reaction microchambers (2 × 1 × 1 mm) with a reaction volume of approximately 2 μL. The extracted nucleic acid sample reagents are transferred to 8 microcavities along different channels and covered with a thin film to ensure that the LAMP reaction occurs in a sealed chip; (b) real-time detection instrument; (c) real-time amplification curves. Adapted with permission from ref. 53. Copyright: 2018, the authors.
microfluidic chip, COVID-19 can be detected rapidly through the use of LAMP primers for SARS-CoV-2.

Compared with traditional technology, LAMP provides sensitive, accurate and specific results in a short time. It provides a potential platform for the POC detection of COVID-19 in the community. LAMP reduces the time required for transferring samples to a centralized laboratory and for standard RT-PCR testing. In addition, LAMP does not need to be performed in central laboratories by skilled scientific research and medical personnel, which means that front-line medical workers can perform POC testing for COVID-19 upon demand. Moreover, the isothermal platform is low cost and does not require expensive reagents or instruments, reducing the cost of COVID-19 testing.

2.3 Tests based on the CRISPR/Cas system

The CRISPR/Cas system is the immune system of bacteria, fighting against foreign DNA or RNA invasion. Bacteria recognize target DNA/RNA and cleave the invading foreign nucleic acids through CRISPR RNA (crRNA) and Cas proteins. CRISPR is a powerful gene editing technology that can be used to trim, cut, replace or add to the DNA sequences of organisms. Therefore, CRISPR/Cas is referred to as “molecular scissors”.54 In recent years, it has been shown that CRISPR and its related proteins, mainly Cas12a and Cas13, can be used to detect specific nucleic acids in samples. Cas12a and Cas13 bind to RNA or DNA targets, respectively, specified by guide RNA (gRNA) sequences, they can combine with the fluorophore quencher DNA probe to produce signal amplification. The predicted sequence of COVID-19 was detected by Cas12a and its related proteins, mainly Cas12a and Cas13, and then the virus was confirmed by cleavage of reporter molecule.55,56 Recently, multiple detection methods combining various isothermal amplification techniques (such as LAMP and RPA) and CRISPR have developed into diagnostic tools for the rapid detection of SARS-CoV-2 RNA.

Cas12 protein specifically cuts double-stranded DNA. In addition, Cas12a has accessory activities. Once crRNA specifically binds to a target sequence, Cas12a not only cleaves the target sequence but also cuts any single-stranded DNA (ssDNA) in the system.57 Huang et al. have described a rapid and accurate fluorescence diagnostic method based on RT-PCR/CRISPR-Cas12a. The CRISPR-FDS methods, includes 3 steps, the RNA extraction, target amplification, and fluorescent signal detection (Fig. 8). Detection by this method requires that a sample contains at least 2 copies of the target RNA sequence; no detectable target signal is produced if the DNA target amplified by qPCR has less than 5 copies. This assay has a sample-to-answer time of approximately 50 min, and the reagents and equipment are easy to obtain. Therefore, this method has the potential for use in POCT if the results are analyzed with a portable fluorescence reader.58 Wang et al. have developed a CRISPR/Cas12a-based assay with a naked-eye readout. This method displays high sensitivity and specificity and is capable of detecting as few as 10 copies of a viral gene within 45 min. This detection system provides an ssDNA reporter labeled with a quenched green fluorescence molecule that is cleaved by Cas12a protein in the presence of SARS-CoV-2 nucleic acids. As the result, a green fluorescence signal visible to the naked eye is produced.59 Among the applied SARS-CoV-2 detection systems, the “All-In-One Dual CRISPR-Cas12a” (AIOD-CRISPR) system designed by Ding et al.60 can detect as low as about 5 copies of an RNA target with an incubation time of 40 min. Charles Y. Chiu and colleagues (University of California) have developed a sensitive, rapid, and portable SARS-CoV-2 detection assay based on CRISPR-Cas12 technology. This assay can be run in approximately 30–40 min (ref. 61) and can be used to detect SARS-CoV-2 in respiratory swab RNA extracts. It uses the E gene, N gene and the human RNase P gene as a control and consists of an RT-LAMP reaction at 62 °C for 20–30 min and a Cas12 detection reaction at 37 °C for 10 min. The results are visualized on a lateral flow strip (Fig. 9).

Similarly, CRISPR diagnosis is suitable for RNA molecular diagnosis. In 2016, Zhang et al. found that Cas13a would cleave any nearby RNA after cleaving the target RNA sequence.62 Such phenomenon is known as “collateral cleavage”. Doudna et al. employed the Cas13a protein for RNA diagnosis.57 However, the development of CRISPR-based RNA diagnostic technology has been limited due to low sensitivity. In 2017, Zhang et al. optimized the CRISPR/Cas13a diagnostic platform. The SHERLOCK (specific high-sensitivity enzymatic reporter unLOCKing) system

Fig. 8 A CRISPR-based fluorescence diagnosis system for COVID-19 (COVID-19 CRISPR-FDS). (a) Schematic diagram of a CRISPR-FDS assay for the detection of SARS-CoV-2 RNA; (b) SARS-CoV-2 genome map of COVID-19 CRISPR-FDS target sequences. Adapted with permission from ref. 58. Copyright: 2020, the authors.
enhances diagnostic sensitivity by 1 million-fold and is capable of detecting ZIKV or Dengue virus at as low as 1 copy per microliter. This method is able to consistently detect synthesized SARS-CoV-2 RNA within a range of 10–100 copies per microliter of input and allows a visual readout of the detection results via a lateral flow strip. In addition, the detection process can be completed within 40 to 57 min after the RNA extraction step.

In February 2020, Zhang et al. employed the CRISPR/Cas13-based SHERLOCK technique to detect the novel coronavirus and successfully detected the virus within the range of 10–100 copies per microliter of input. This method is simple to implement. It only requires purified nucleic acid samples and can be completed in 1 h. Moreover, this method is able to quickly and accurately detect SARS-CoV-2 and exhibits high sensitivity.

2.4 Commercial nucleic acids POCT products

Nucleic acid detection method was the gold standard for the diagnosis of COVID-19 at the beginning, and it currently has the most products (Table 1). Some of the products are “all in one” and can achieve fully automated “sample-in answer-out” detections, while others mainly focus on the detection process. The “all in one” products’ operations are simpler, while the others are more flexible and affordable.

Cepheid used a special reaction tube in the Smart Cycler system, integrated and fast real-time fluorescence PCR detection is obtained. Based on that, Cepheid developed a pre-processing cartridge that uses the rotation and movement of a central plunger to achieve connectivity and liquid movement between different chambers on the cartridge and integrated nucleic acid extraction and nucleic acid amplification detection on one instrument—GeneXpert. In addition, this test can quickly detect the currently prevalent SARS-CoV-2 within 30 min, with reduced sample preparation duration down to 1 min.

Wei Zhen’s group evaluated 3 POCT nucleic acid detection systems using 108 symptomatic patients’ nasopharyngeal swabs and found that the Cepheid Xpert Xpress SARS-CoV-2 Assay had a positive agreement of 98.3% and a limit of detection of 100 copies per mL, the Abbott ID NOW COVID-19 Assay had a positive agreement of 87.7% and a limit of detection of 20,000 copies per mL, and the GenMark ePlex SARS-CoV-2 Test had a positive agreement of 91.4% and a limit of detection of 1000 copies per mL. In terms of detection time, ID NOW was much faster (17 min) when compared with Cepheid (46 min) and ePlex (approximately 1.5 h).

Bosch Healthcare Solutions announced the development of a fully automated PCR assay that integrated sample
<table>
<thead>
<tr>
<th>No.</th>
<th>Device</th>
<th>Company</th>
<th>Detection technology</th>
<th>Sample type</th>
<th>Detection duration</th>
<th>All-in-one?</th>
<th>Regulatory</th>
<th>Detection vessel</th>
<th>Positive/ Negative agreement</th>
<th>Number of samples per test</th>
<th>Limit of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ID NOW COVID-1994,95</td>
<td>Abbott Diagnostics Scarborough, Inc.</td>
<td>NEAR</td>
<td>NS/OS/NPS</td>
<td>5–13 min</td>
<td>Yes</td>
<td>FDA EUA</td>
<td>Cartridge</td>
<td>93.3%/98.4%</td>
<td>1</td>
<td>125 genome equivalents per mL</td>
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<tr>
<td>2</td>
<td>VitaPCR Platform96</td>
<td>A. Menarini Diagnostics s.r.l.</td>
<td>Real-time PCR</td>
<td>NPS/OS</td>
<td>20 min</td>
<td>No</td>
<td>CE</td>
<td>Tube</td>
<td>100%/100%</td>
<td>1</td>
<td>2730 copies per mL</td>
</tr>
<tr>
<td>3</td>
<td>BioFire Respiratory Panel 2.1 (RP2.1/2.1-EZ)97,98</td>
<td>BioFire Diagnostics, LLC</td>
<td>Nested multiplex PCR</td>
<td>NS</td>
<td>45 min</td>
<td>Yes</td>
<td>FDA EUA, CE</td>
<td>Cartridge</td>
<td>97.1%/99.3%</td>
<td>1–12*</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Biomeme SARS-CoV-2 Real-Time RT-PCR Test99</td>
<td>Biomeme, Inc.</td>
<td>Real-time PCR</td>
<td>NPS/NS/OS/ NPW-/NA/ aspirate</td>
<td>1 h</td>
<td>No</td>
<td>FDA EUA</td>
<td>Tube</td>
<td>97.46%/98.51%</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Vivalytic VR1 Multiplex Test72,100</td>
<td>Bosch Healthcare Solutions GmbH</td>
<td>Multiplex PCR, μArray-detection</td>
<td>NS/OS</td>
<td>39 min</td>
<td>Yes</td>
<td>CE</td>
<td>Cartridge</td>
<td>98%/100%</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>Xpert Xpress SARS-CoV-2 Test101,102</td>
<td>Cepheid</td>
<td>Real-time PCR</td>
<td>NPS/NW/ aspirate</td>
<td>45 min</td>
<td>Yes</td>
<td>FDA EUA, CE</td>
<td>Cartridge</td>
<td>100%/100%</td>
<td>1–80*</td>
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<tr>
<td>7</td>
<td>ePlex Respiratory Pathogen Panel 290,103</td>
<td>GenMark Diagnostics, Inc.</td>
<td>eSensor technology</td>
<td>NPS</td>
<td>2 h</td>
<td>Yes</td>
<td>FDA EUA, CE</td>
<td>Cartridge</td>
<td>99.02%/98.41%</td>
<td>1–24*</td>
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<tr>
<td>8</td>
<td>iC-COVID19 Assay104,105</td>
<td>iCUBATE</td>
<td>ARM-PCR</td>
<td>NPS/NS/MNS</td>
<td>6 h</td>
<td>Yes</td>
<td>—</td>
<td>Cartridge</td>
<td>—</td>
<td>1</td>
<td></td>
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<tr>
<td>9</td>
<td>Automatic Integrated Gene Detection System106,107</td>
<td>Lifereal Biotechnology Co., Ltd.</td>
<td>Real-time PCR</td>
<td>OS/ALF/ sputum-/stool</td>
<td>1.5 h</td>
<td>Yes</td>
<td>CE</td>
<td>Cartridge</td>
<td>97.62%/100%</td>
<td>1–4*</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Lucira COVID-19 All-In-One Test kit108,109</td>
<td>Lucira Health, Inc.</td>
<td>LAMP</td>
<td>NS</td>
<td>30 min</td>
<td>Yes</td>
<td>FDA EUA</td>
<td>Tube</td>
<td>94%/98%</td>
<td>1</td>
<td>900 copies per mL</td>
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<tr>
<td>No.</td>
<td>Device</td>
<td>Company</td>
<td>Detection technology</td>
<td>Sample type</td>
<td>Detection duration</td>
<td>All-in-one?</td>
<td>Regulatory</td>
<td>Detection vessel</td>
<td>Positive/ Negative agreement</td>
<td>Number of samples per test</td>
<td>Limit of detection</td>
</tr>
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<tr>
<td>11</td>
<td>Microchip RT-PCR COVID-19 Detection System</td>
<td>Lumex Instruments</td>
<td>Real-time PCR</td>
<td>NS/OS/LRTA/BLF/NPW/NA/aspirate/sputum</td>
<td>50 min</td>
<td>No</td>
<td>RUO</td>
<td>Microfluidic Chip</td>
<td>—</td>
<td>7</td>
<td>9000 copies per mL</td>
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<tr>
<td>12</td>
<td>ARIES SARS-CoV-2 Assay</td>
<td>Lumex Corporation</td>
<td>Real-time PCR</td>
<td>NPS</td>
<td>2 h</td>
<td>Yes</td>
<td>FDA EUA</td>
<td>Cartridge</td>
<td>100%/100%</td>
<td>1–12*</td>
<td>1500 copies per mL</td>
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<tr>
<td>14</td>
<td>LamPORE assay</td>
<td>Oxford Nanopore Technologies</td>
<td>Nanopore sequencing combined with LAMP</td>
<td>NS/NPS/Os</td>
<td>2 h</td>
<td>Yes</td>
<td>CE</td>
<td>Flow cell</td>
<td>99.1%/99.6%</td>
<td>24–480</td>
<td>7000–10 000 genome copies per mL of extracted RNA</td>
</tr>
<tr>
<td>15</td>
<td>QIAsat-Dx</td>
<td>QIAGEN GmbH</td>
<td>Real-time PCR</td>
<td>NPS/OS/ALF/Sputum/serum/whole blood/stool</td>
<td>1 h</td>
<td>Yes</td>
<td>FDA EUA, TGA, CE</td>
<td>Cartridge</td>
<td>100%/93%</td>
<td>1–4*</td>
<td>500 copies per mL</td>
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<tr>
<td>16</td>
<td>iPonatic</td>
<td>Sansure BioTech Inc.</td>
<td>Real-time PCR</td>
<td>NPS/OS/BLF</td>
<td>15–45 min</td>
<td>Yes</td>
<td>CE, NMPA</td>
<td>Tube</td>
<td>98.68%/99.35%</td>
<td>1</td>
<td>6750 copies per mL VTM</td>
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<tr>
<td>17</td>
<td>Sherlock CRISPR SARS-CoV-2 Kit</td>
<td>Sherlock BioSciences, Inc.</td>
<td>RT-LAMP and CRISPR</td>
<td>NS/NPS/OS/NPW/ aspirate/NA/BLF</td>
<td>1 h</td>
<td>No</td>
<td>FDA EUA</td>
<td>HybriDetect Strips</td>
<td>97%/100%</td>
<td>Observe results with naked eyes, unlimited</td>
<td></td>
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<tr>
<td>18</td>
<td>PCR Nucleic Acid Analyzer</td>
<td>Shineway Technology Corporation</td>
<td>Real-time PCR</td>
<td>NS/OS/sputum</td>
<td>50 min</td>
<td>No</td>
<td>CE</td>
<td>Microfluidic Chip</td>
<td>99.5%/100%</td>
<td>3</td>
<td>500 copies per mL</td>
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<tr>
<td>19</td>
<td>Automatic CPA Nucleic Acid Analyzer</td>
<td>Ustar Biotechnologies (Hangzhou) Ltd.</td>
<td>CPA</td>
<td>OS/sputum</td>
<td>55 min</td>
<td>Yes</td>
<td>TGA, NMPA</td>
<td>Tube</td>
<td>98%/95.4%</td>
<td>1–2</td>
<td>—</td>
</tr>
<tr>
<td>20</td>
<td>WizDx F-150 Real-Time PCR System</td>
<td>Wizbiosolutions Inc.</td>
<td>Ultra-fast real-time PCR</td>
<td>NPS/OS/sputum</td>
<td>40 min after RNA extraction</td>
<td>No</td>
<td>CE, MFDS</td>
<td>Microfluidic Chip</td>
<td>—</td>
<td>10</td>
<td>19.7 copies per rxn (E gene) 23.4 copies per rxn (RdRP gene)</td>
</tr>
</tbody>
</table>

Notes: 1. Sort by first letter of company name. 2. All the carriers (cartridges, microfluidic chips and tubes) are for single use. ‘*’ means one carrier can detect one sample, but the machine has the ability to test more carriers at one time. 3. ‘#’ means this product is authorized for prescription home use. 4. List of abbreviations: (1) NEAR, nicking endonuclease amplification reaction; ARM-PCR, amplicon-rescued multiplex PCR; CPA, cross priming amplification; LAMP, loop-mediated isothermal amplification. (2) FDA EUA, U.S. Food & Drug Administration Emergency Use Authorizations; CE, Conformite Europeenne; NMPA, National Medical Products Administration; TGA, Therapeutic Goods Administration; MFDS, Ministry of Food and Drug Safety; RUO, research use only. (3) NS, nasal swabs; TS, throat swabs; NPS, nasopharyngeal swabs; OS, oropharyngeal swabs; MNS, mid-turbinate nasal swabs; NPW, nasopharyngeal wash; NW, nasal wash; ALF, alveolar lavage fluid; BLF, bronchoalveolar lavage; LRTA, lower respiratory tract aspirates; NA, nasal aspirate. https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/vitro-diagnostics-euas/; Web: http://app1.nmpa.gov.cn/data_nmpa/facel/dir.html#maodian2; Web: https://www.finddx.org/covid-19/pipeline/.
preparation, DNA amplification, detection (ion probe array), and analysis in a single cartridge.72 A cotton swab (nasopharyngeal or oropharyngeal) is inserted into the cartridge and instrumentally analyzed. The results are delivered within 2.5 h. This test platform, named Vivalytic VRI, allows the selection of different test samples and analytic methods. A cartridge for COVID-19 detection only is also released and it only takes 39 min to get a result. However, the diagnostic kit can only be used in specific analytical equipment, and many medical institutions do not yet have those devices.

The FilmArray system invented by BioFire Defense uses a flexible bag instead of a cartridge to achieve POCT molecular diagnosis.73,74 Different reagents are preinstalled in different parts of the bag. Liquid flow is controlled by squeezing the reagent bag with the instrument, and magnetic bead nucleic acid extraction and nested PCR with melting curve analysis are used to detect viruses. The system can detect more than 20 pathogens at the same time. Zaneeta Dhesi et al. used the FilmArray system to detect secondary infections in 94 COVID-19 patients in the ICU of 5 UK hospitals.75 They found that the FilmArray system generated more (54% vs. 28%) positive secondary infection results than traditional bacterial culture did. Using the FilmArray system, Yosuke Hirotsu et al. screened 191 patients with cold symptoms and found that 32 patients were infected with at least 1 virus, among which 8 were infected with only SARS-CoV-2.76

Nature Biotechnology reported an on-site rapid molecular diagnostic system (Shineway) for COVID-19 detection (Fig. 10a).77 The system was developed by Wen’s group in Hong Kong University of Science and Technology and input a mass production in Shenzhen Shineway Technology Corporation.78 It used a novel thin film silicon-based micro-heater for rapid heating and a silicon-based microfluidic chip for nucleic acid PCR amplification.18 The heater and Pt thermal sensor were combined together, which improved the speed of heating and cooling while greatly reduced the complexity of the temperature control system. This heater can reach a temperature rising rate up to 30 °C s⁻¹.79 The microfluidic chip was made of silicon and glass, and silicon is a good heat conductor, therefore, the rapid temperature change of the heater can be transmitted to the reaction solution in time and significantly reduce the detection time. The reaction chamber was made on silicon wafer by dry etching, and then a glass wafer was bonded to the silicon wafer. A robust package and sealing technology were developed to prevent water evaporation during a fast PCR thermal cycling. In their previous researches, SRY gene was successfully detected from saliva samples within 25 min.18 And *Escherichia coli* and Salmonella were detected within 25 minutes, and the detection results were consistent with the result on Roche LightCycler 480 machine.79

The Shineway system can achieve the SARS-CoV-2 PCR detection within 40 min,77 which is only half or less of the time of traditional PCR machines. The dimension of the machine is only 280 mm × 240 mm × 162 mm, and it incorporates a touch-screen control system that eliminates the requirement of a computer. The Shineway system is compatible with third party reagents. 42 clinical samples of SARS-CoV-2 were tested and the specificity was 100%. The detection limit of SARS-CoV-2 on this machine was around 500 copies per mL. Shenzhen Shineway Technology also proposed a complete solution for POCT SARS-CoV-2 detection, the portable PCR machine, a preprocessor and all other accessories were all integrated into a suitcase, and can be taken to detection site easily. This system has been used in inspection vehicles and public places on-site inspection.

Wizbio Solutions invented a WizDx F-150 ultrafast real-time PCR system (Fig. 10b)80,81 this system can be used with a PCR chip and COVID-19 CrystalMix to detect SARS-CoV-2. The PCR chip is made of a transparent polymer, and the inlets are sealed with paste; 10 samples can be tested together on 1 chip. The limit of detection is 19.7 copies per rxn. WizDx F-150 can control temperature at a rate of 8 °C s⁻¹.

ID NOW COVID-19 system from Abbott Diagnostics, Inc. can achieve SARS-CoV-2 detection in less than 13 minutes.48 The system uses an isothermal nucleic acid amplification technology called “NEAR” (nicking endonuclease amplification reaction). There is a sample receiver to release nucleic acids from the swabs, a test base to do the detection and a transfer cartridge to remove nucleic acids from sample receiver to the test base. Harrington et al. did a comparison of Abbott ID Now and Abbott m2000 methods for the detection of SARS-CoV-2, and they found that the negative agreement was 99% while positive agreement was 75%.82

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**Fig. 10** Commercial POCT nucleic acid detection products on SARS-CoV-2. (a) Shineway’s portable fast microchip based PCR Analyser (ref. 77); (b) WizDx F-150 ultra-fast real-time PCR system. Adapted with permission from ref. 81. Copyright: 2020, the websites.
Sequencing technology is another way to detect SARS-CoV-2 nucleic acids. In the early stage of the COVID-19 outbreak, genome sequencing was used to obtain the genome sequence information of the virus, which provided information for the subsequent PCR detection of SARS-CoV-2. Limited by long detection times and high detection costs, sequencing technology has not been applied to SARS-CoV-2 detection on a large scale. However, genome sequencing still plays an important role in detecting gene mutations, researching and analyzing the evolution of SARS-CoV-2, researching its pathogenic mechanism and so on.

Ming Wang et al. used Oxford Nanopore MinION/GridION to detect SARS-CoV-2 and other respiratory viruses by nanopore sequencing. When a nucleic acid passes through the nanopore protein, an electrical signal can be recorded in real-time. In the tests, 22 of 61 suspected COVID-19 samples were diagnosed as positive by nanopore sequencing, while negative or inconclusive results were obtained by RT-qPCR. However, it took 6–10 h for each sequencing test. The results showed that when samples cannot be accurately diagnosed, sequencing can be a complementary approach to RT-qPCR.

Recently, Oxford Nanopore Technologies developed a LamPore assay. LamPore combines LAMP with nanopore sequencing and greatly shortens the detection time. The assay takes approximately 2 h to test 1–96 samples in a single flow cell. The ePlex SARS-CoV-2 Test system from GenMark Diagnostics also uses a cartridge to achieve nucleic acid extraction and detection. Different from Cepheid’s real-time PCR method, ePlex uses an electrowetting method (EWOD) to manipulate droplets moving in a planar electrode array. Competitive DNA hybridization and electrochemical detection are used in the ePlex system. Katharine Uhtrieg et al. used clinically negative samples spiked with known concentrations of SARS-CoV-2 RNA as evaluation samples and obtained a limit of detection of 600 copies per mL. In addition, tests for both positive and negative samples showed 100% repeatability. A comparative experiment conducted by Wei Zhen et al. showed that among 104 clinical samples, ePlex showed a 96% positive coincidence rate and a 100% negative coincidence rate, and the detection sensitivity of ePlex was 1000 copies per mL.

3. POCT based on immunoassay

According to the classification of detection targets, SARS-CoV-2 immunoassays mainly include antigen detection and antibody detection. Theoretically, SARS-CoV-2 antigen can be detected once an individual is infected. However, in reality, the sensitivity of antigen detection is lower than that of RT-PCR method and is better to be used a few days after symptom onset. Different from antigen detection, antibody detection can only be achieved after the body produces an immune response. There is a window period from when the virus enters the human body to the time the immune response produces antibodies. During the immune response, detection tools are prone to produce “false negative” results.

After the window period, as the antibody concentration in the patient’s body rises, the accuracy of the immune detection method gradually increases. Antibody screening mainly involves IgM and IgG detection. When SARS-CoV-2 invades the human body, IgM antibody will appear in about 1 week, and then IgG antibody will appear in about 1–3 weeks or even longer; the concentration will be much higher and duration much longer than those for IgM. Immunoassays can help clinicians determine different stages of viral infection in patients and are effective complementary methods to SARS-CoV-2 nucleic acid detection.

Based on different detection techniques, immunoassays mainly can be divided into colloidal gold/immunofluorescence chromatography (lateral flow immunoassay, LFA), enzyme linked immunosorbent assay (ELISA) and chemiluminescence. Most LFA methods produce test results within 10–15 min. They have the advantages of simple operation, low price, fast detection, etc. ELISA and chemiluminescence are more sensitive and specific than LFA, but they require complex and long protocols as well as large machines. However, recently, some studies make POCT via ELISA and chemiluminescence possible, and other methods such as SERS and digital immunoassay are also expected to be used in the detection of SARS-CoV-2.

3.1 Lateral flow assay-based tests

Benjamin D. Grant et al. used commercially available reagents and developed a half-strip lateral flow assay method for POCT SARS-CoV-2 antigen detection (Fig. 11a). The assay has a limit of detection of 0.65 ng mL−1 recombinant antigen. Tian Wen and Chao Huang et al. optimized the pH, antigen concentration and several other parameters for the lateral flow immunoassay strip and obtained specific and stable detection results for IgG and IgM antibodies against SARS-CoV-2 (Fig. 11b). The sensitivity and specificity of IgM detection were 100% and 93.3%, using RT-PCR results as a comparison. Zhenhua Chen et al. used lanthanide-doped...
polystyrene nanoparticles (LNPs) as a fluorescent reporter and detected anti-SARS-CoV-2 IgG in clinical samples. They observed good consistency with RT-PCR results (Fig. 11c).138

The use of LFA method to detect COVID-19 has been widely commercialized, and it has low requirements for the detection environment and instruments, and can realize a on-the-go inspection and expand the application scenarios. But because the samples are directly tested without processing, some interfering substances may prone to lead to false positive results. To reduce the false positive rates, optimizing the processing of the strip materials to enhance the filtering ability of interfering substances, and adjusting reagent formulations to enhance the anti-interference ability are necessary.

3.2 ELISA-based tests

Siddhartha Tripathi and Amit Agrawal proposed a microfluidic sandwich ELISA system to detect SARS-CoV-2 antibodies. On the microfluidic chip, a T-shaped microchannel is used to separate plasma from whole blood (Fig. 12a).139–141 The plasma is then used for a SARS-CoV-2 ELISA. Approximately 10 μL of plasma can be isolated from 1 mL of whole human blood in approximately 3 min. Xudong Fan’s group invented a microfluidic ELISA method to quantitatively and sensitively detect SARS-CoV-2. IgG and viral antigen-S protein in serum were used as targets (Fig. 12b).142 The ELISA took 15–20 min to complete. A capillary sensor array with 12 channels was used as an ELISA reactor. An automated system was used to operate the microfluidic chip.143,144 Humanized chimeric SARS-CoV-2 antibodies were used to test the limit of detection, and the LOD was 2 ng mL⁻¹. In addition, commercial human-cell-expressed SARS-CoV-2 S1 protein can be detected at an LOD of 0.4 ng mL⁻¹.

Sthitodhi Ghosh et al. developed a microchannel capillary flow assay (MCFA) platform.145 The platform comprises an MCFA chip, a highly-sensitive optical detector and a smartphone. This platform performs chemiluminescence-based ELISA detection. For malaria, the LOD was 8 ng mL⁻¹. There is a capillary pump on the chip, and the capillary force drives the sample through channels and detection regions; no extra power supply was required. This research result is expected to be used in the detection of COVID-19.

The ELISA method can quantitatively detect SARS-CoV-2, and the result is more accurate than LFA method. At the same time, this method has lower requirements for the detection environment than nucleic acid detection method. However, due to the complicated operation steps, only parts of the operation of ELISA or chemiluminescence were achieved by microfluidic methods in many researches. Few have achieved full-process automated POC ELISA or chemiluminescence. A large number of commercial chemiluminescence products have been introduced to the market. We believe that POC chemiluminescence devices are also on the way to researches and products.

3.3 Other methods

In addition to LFA, ELISA, and chemiluminescence, many researchers have developed other methods to achieve immunoassay of COVID-19.

Jilie Kong’s group developed a point-of-care microfluidic platform to detect SARS-CoV-2 IgG, IgM and antigen (Fig. 13a).146 The diagnostic microchips need to be used in a fluorescence detection analyzer, with results available in 15 min. There is a loading chamber, a waste reservoir and a fluorescence immunoassay fluid channel on the microchip. The fluorescence immunoassay fluid channel has a capture region and a test region. Fluorescent microsphere (FMS)-labeled capture antibodies are printed in the capture region. When sample added into the loading chamber, SARS-CoV-2 biomarkers (IgG/IgM/antigen) specifically bind to the
antibody in the capture region. Then, due to capillary effect, the “antigen–antibody complexes” and FMS flow to and immobilize in the test region. After 10 min, the microchip must be centrifuged for 10 s and placed in a portable fluorescence analyzer to obtain the results. This platform offered a rapid and easy-to-use SARS-CoV-2 detection method.

Benjamin L. Miller’s group reported an arrayed imaging reflectometry (AIR) platform that can detect 9 antibodies of upper respiratory pathogens, including SARS-CoV-2, on 1 chip (Fig. 13b).147 The microfluidic chip has a silicon dioxide substrate with a thickness specific to an array of antigen probes. When antibodies were captured, the light signal can be reflected and detected by using a CCD camera.148 Each chip contains a 15-plex array (including a negative control): 1 target is detected twice, and each antigen is printed in 6 spots. The chip can be placed in 1 well of a 96-well plate. The SARS-CoV-2 detection results have a generally good correlation with those obtained through ELISA. The AIR assay has been commercialized as Adarza Ziva, and the commercial instrument can process 1000 samples in 24 h.149 Weian Zhao’s group developed a 3D-printable portable imaging platform, “TinyArray imager”, to read antigen microarrays for SARS-CoV-2, SARS-1, MERS and 3 other respiratory viruses (Fig. 13c).150 The imaging platform consists of a camera module controlled by Raspberry Pi, 2 LED sources and 2 filters to spectrally select for the emitted fluorescence. The portable imager has a large imaging field of $35 \times 26$ mm. Positive and negative COVID-19 sera was used to evaluate the imager. The TinyArray imager showed good consistency with a commercial imager that is 100× more expensive. These two kinds of image array platforms are suitable for the rapid detection and screening of a variety of antigens or antibodies.

SERS method and digital immunoassay chip are two of sensitive and accurate methods in detecting antibodies and antigens. But they haven’t been used in detecting SARS-CoV-2 yet. C. Yang’s group combined digital microfluidics (DMF) with surface enhanced Raman scattering (SERS) to detect H5N1.151 The DMF platform automatically manipulates reagents to conduct sandwich immunoassays of antigens in samples. SERS is used to detect signals. The DMF-SERS method has an LOD of 74 pg mL$^{-1}$ and a detection time less than 1 h. After modifying the detection kit, the platform, with high sensitivity and automation, can be used for SARS-CoV-2 detection. Ying Mu’s group developed a self-compartmentalization bead-based digital immunoassay chip.152,153 The reagent in the chip is self-driven. Their group also developed a portable smartphone-based device for digital PCR detection.154 These two technologies can be used together to obtain ultrasensitive results for SARS-CoV-2 biomarkers on a digital platform.

3.4 Commercial POCT immunoassay products

On the list of FDA’s “In Vitro Diagnostics EUAs”, about half of the serology/antibody tests products use lateral flow method, and antibody detection products is more than antigen detection products (Table 2).

Guangzhou Wondfo’s SARS-CoV-2 antibody test uses lateral flow method to detect IgM and IgG antibody.155,156 The detection results can be interpreted by naked eyes without any instrument. This is a relatively common method, and it does not rely on detection equipment, so it is particularly suitable for a rapid POCT detection. Jhong-Lin Wu et al. tested four POCT lateral flow immunoassays for diagnosis of COVID-19, they found that the specificity was 100% from the 1st day and after 21 days of symptom onset the sensitivity also became 100%.157

Recently, Abbott’s antigen detection kit get the FDA EUA approval.158 This BinaxNOW COVID-19 Ag Card works with a nasal swab sample, extraction reagent and a credit card-sized reactive card to detect the nucleocapsid protein antigen from SARS-CoV-2.159 Test results are interpreted visually at 15 minutes. Abbott also developed an app on mobile phones, the negative detection result would be shown on the app as a digital health pass.

Hotgen company invented an up-converting phosphor immunochromatographic technology (UPT) and used it on

![Fig. 13](image-url) Other microfluidic immunoassay methods for detecting SARS-CoV-2. (a) Microfluidic platform to detect SARS-CoV-2 IgG, IgM and antigen. Reused with permission from ref. 146. Copyright 2020, the American Chemical Society. (b) Arrayed imaging reflectometry (AIR) platform assay for antibodies to respiratory viruses. Reused with permission from ref. 147. Copyright: 2020, Elsevier. (c) 3D-printable portable imaging platform (TinyArray imager) work flow and fluorescence images acquired with the imager and a commercial imager. Adapted with permission from ref. 150. Copyright: 2020, the Royal Society of Chemistry.
Table 2 Commercial immunoassay POCT products (Since there are too many colloidal gold and immunofluorescence strip products, only some of them are listed in this table)

<table>
<thead>
<tr>
<th>No.</th>
<th>Device</th>
<th>Company</th>
<th>Detection target</th>
<th>Sample type</th>
<th>Detection duration</th>
<th>Results reading</th>
<th>Regulatory</th>
<th>Positive/Negative agreement</th>
<th>Detection technology</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BinaxNOW COVID-19 Ag Card1,159,167</td>
<td>Abbott Diagnostics Scarborough, Inc.</td>
<td>Antigen</td>
<td>NS</td>
<td>15 min</td>
<td>Naked eye</td>
<td>FDA EUA</td>
<td>91.7%/100%</td>
<td>Lateral flow</td>
</tr>
<tr>
<td>2</td>
<td>Novel Coronavirus 2019-nCoV Antibody Test169</td>
<td>Beijing hot view Biotechnology Co., Ltd.</td>
<td>IgM and IgG antibody</td>
<td>Serum/plasma</td>
<td>15 min</td>
<td>Naked eye</td>
<td>NMPA, CE</td>
<td>—</td>
<td>Lateral flow, (up-converting phosphor immunochromatographic)</td>
</tr>
<tr>
<td>3</td>
<td>WANTA SARS-CoV-2 Ab Rapid Test168</td>
<td>Beijing Wantai Biological Pharmacy Enterprise Co., Ltd.</td>
<td>Total antibody</td>
<td>Serum/plasma (dipotassium EDTA, lithium heparin and sodium citrate)/VWB</td>
<td>15 min</td>
<td>Naked eye</td>
<td>FDA EUA, CE, TGA</td>
<td>100%/98.8%</td>
<td>Lateral flow (colloidal gold)</td>
</tr>
<tr>
<td>4</td>
<td>BioCheck SARS-CoV-2 IgG and IgM Combo test169,170</td>
<td>BioCheck, Inc.</td>
<td>IgM and IgG antibody</td>
<td>Serum</td>
<td>30 min</td>
<td>Machine</td>
<td>FDA EUA, CE</td>
<td>99.1%/97.2%</td>
<td>Chemiluminescence</td>
</tr>
<tr>
<td>5</td>
<td>qSARS-CoV-2 IgG/IgM Rapid Test171,172</td>
<td>Cellex Inc.</td>
<td>IgM and IgG antibody</td>
<td>Serum/plasma (EDTA or citrate)/VWB</td>
<td>15-20 min</td>
<td>Naked eye</td>
<td>FDA EUA, CE</td>
<td>93.8%/96%</td>
<td>Lateral flow (colloidal gold)</td>
</tr>
<tr>
<td>6</td>
<td>Ellume COVID-19 Home Test173,174</td>
<td>Ellume Limited</td>
<td>Antigen</td>
<td>NS</td>
<td>15 min</td>
<td>Machine</td>
<td>FDA EUA</td>
<td>95%/97%</td>
<td>Lateral flow (fluorophore)</td>
</tr>
<tr>
<td>7</td>
<td>SARS-CoV-2 Antibody Test175,176</td>
<td>Guangzhou Wondfo Biotech Co Ltd.</td>
<td>IgM and IgG antibody</td>
<td>Whole blood/serum/plasma</td>
<td>15 min</td>
<td>Naked eye</td>
<td>NMPA EUA, CE, TGA</td>
<td>45.2%/81.8%</td>
<td>Lateral flow (colloidal gold)</td>
</tr>
<tr>
<td>8</td>
<td>SARS-CoV-2 IgG/IgM Antibody Rapid Test Kit177</td>
<td>Lumigenex co. LTD</td>
<td>IgM and IgG antibody</td>
<td>Serum/plasma/whole blood</td>
<td>10 min</td>
<td>Machine</td>
<td>—</td>
<td>—</td>
<td>Lateral flow (time resolved fluorescence)</td>
</tr>
<tr>
<td>9</td>
<td>xMAP SARS-CoV-2 Multi-Antigen IgG Assay177,178</td>
<td>Luminex Corporation</td>
<td>IgG antibody</td>
<td>Serum/dipotassium EDTA plasma</td>
<td>Less than 3 h</td>
<td>Machine</td>
<td>FDA EUA, Health Canada</td>
<td>96.3%/99.3%</td>
<td>96 plate (multiplexed microsphere-based assay)</td>
</tr>
<tr>
<td>10</td>
<td>LumiraDx SARS-CoV-2 Ag Test178</td>
<td>LumiraDx UK Ltd.</td>
<td>Antigen</td>
<td>NS</td>
<td>12 min</td>
<td>Machine</td>
<td>FDA EUA, CE</td>
<td>97.6%/96.6%</td>
<td>Microfluidic immunofluorescence assay</td>
</tr>
<tr>
<td>11</td>
<td>Sofia SARS Antigen FIA181</td>
<td>Quidel Corporation</td>
<td>Antigen</td>
<td>NPS/NS</td>
<td>15 min</td>
<td>Machine</td>
<td>FDA EUA, CE</td>
<td>96.7%/100%</td>
<td>Lateral flow immunofluorescent sandwich assay</td>
</tr>
<tr>
<td>12</td>
<td>Accre 6161,179,180</td>
<td>Shenzhen Tisenc Medical Devices Co., Ltd.</td>
<td>IgM and IgG antibody</td>
<td>Serum/plasma</td>
<td>22 min</td>
<td>Machine</td>
<td>—</td>
<td>96.6%—</td>
<td>Chemiluminescence</td>
</tr>
<tr>
<td>13</td>
<td>Diagnostic Kit for IgM/IgG Antibody to Coronavirus (SARS-CoV-2)181</td>
<td>Zuhai Livzon Diagnostics Inc.</td>
<td>IgM and IgG antibody</td>
<td>Serum/plasma/VWB</td>
<td>15 min</td>
<td>Naked eye</td>
<td>NMPA, CE</td>
<td>90.6%/99.2%</td>
<td>Lateral flow (colloidal gold)</td>
</tr>
</tbody>
</table>

lateral flow strips. The UPT material can absorb long wave light and emit short wave light. This method has an advantage of low background interference and can achieve no fluorescence fading. But the tests results must be read by a instrument. The IgM and IgG antibodies can be tested at the same time in 15 minutes. And a portable machine is used to read the result in 5 s.

At present, Tisenc has developed an IgM and IgG antibody single person chemiluminescence SARS-CoV-2 detection kit. Reagent has been pre-stored in a strip and a desktop machine was used to automatically drive the fluid to accomplish the detection. They have tested 90 samples (including 58 cases of nucleic acid positive samples), the IgM clinical coincidence rate was more than 90%, IgG clinical coincidence rate was more than 95%. It took 22 min to get the result. This is a direction for the development of POCT ELISA and chemiluminescence detection systems in the future.

Sofia SARS Antigen FIA from Quidel Corporation is the first device detecting SARS-CoV-2 antigen that get the FDA EUA approval. It uses immunofluorescence-based lateral flow technology in a sandwich design to detect SARS-CoV-2 antigen in swabs within 15 min for result release. However, this product also need a portable machine to read the results.

Blusense has proposed a BluBox together with ViroTrack cartridge to detect COVID-19 IgA + IgM/IgG antibodies. The cartridge only use one drop of blood and can detect COVID-19 in 10 min. In the cartridge, magnetic nanoparticles were coated with specific antibodies beforehand. Antigens will be trapped and format nanoclusters under a strong magnetic field. The fluorescence of the nanoclusters will be detected in the machine. Maria Engel Moeller et al. have evaluated the BluBox system with ELISA method, in 35 plasma samples from COVID-19 patients, 29 could be detected by BluBox. And for samples collected 14 days after symptom appear, the sensitivity of both BluBox system and ELISA was around 90%, specificity of BluBox system could reach 100%, while ELISA IgA was 95%.

The essence of immunoassay is the specific combination of antigens and antibodies. Commercial products have used lateral flow, chemiluminescence, magnetic nanoparticles capture and other methods to achieve POCT immunoassay of COVID-19. Since immunoassay can provide the information of the patient's disease course, and the accuracy of immunoassays improves, China's National Health Commission has added serological testing method into the diagnosis standard in “Diagnosis and Treatment Protocol (version 7)”.  

4. Biosensor-based identification

Apart from the aforementioned methods, there is still an urgent need for rapid and sensitive SARS-CoV-2 identification techniques as alternative POCT systems. Recently, miniature biosensors have exhibited potential as analytical platforms because of their unique characteristics, such as sensitivity, reliable specificity and rapid diagnosis, etc. A biosensor, typically composed of a functional receptor, transducer, and signal detector/analyzer, can sense the intruded target and directly provide sufficient feedback to the end-user with optical signals, electrical signals, etc. With continuous development in nanoscience and technology, functional materials and intensified structures have reduced the signal-to-noise ratio and sample-to-answer time for devices. Biosensor-based diagnosis is considered an alternative solution for relieving the heavy pressure on PCR-based testing, which has been proved as a promising platform during the pandemic. From this perspective, biosensors may be key components in field of POCT for COVID-19 and lead to wearable devices for facile and rapid transmission from signal collection to detector without sophisticated equipment for data analysis.  

4.1 Plasmonic biosensors

Based on localized surface plasmon resonance (LSPR) effects from artificial nanostructures, plasmonic biosensors have been considered as important and sensitive tools for POCT. Combined with surface functionalization processes, plasmonic nanostructures can therefore enable the fast, real-time and label-free detection of analytes even under ultralow concentrations. Developments in material science have now made it possible to precisely control the morphologies of nanomaterials with tunable plasmonic properties and sensitivities. Alternatively, advanced nanofabrication techniques, e.g., electron-beam lithography, also provide platforms to engineer nanopattern arrays on various substrates as plasmonic sensing devices. Moitra et al. presented a colorimetric assay based on gold nanoparticles (AuNPs) that were customized with caps of thiol-modified antisense oligonucleotides (ASOs). The functional plasmonic carrier thus enables the specific detection of the N gene (nucleocapsid phosphoprotein) from SARS-CoV-2-infected samples through spectral resonance shifts in a fast manner (within 10 min). Interestingly, the authors further incorporated RNaseH to cleave the RNA strand from the RNA–DNA hybrid, resulting in a naked-eye detectable assay in which agglomeration among the AuNPs plays an important role (Fig. 14a). Via functionalization with complementary DNA receptors, Qiu et al., took advantage of the combinational mechanism from plasmonic photothermal effect and LSPR

![Fig. 14](image-url)
sensing. Apart from the well-known LSPR effect, the thermoplasmionic heat can further elevate the in situ nucleic acid hybridization temperature, which enhances the detection ability with a lower detection limit of SARS-CoV-2 sequences (as low as 0.22 pM). The two-dimensional gold nanoislands (AuNIs) can thus serve as a promising solution for the reliable clinical diagnosis of COVID-19 (Fig. 14b).194 Based on previous research, Murugan et al. proposed a plasmonic fiber-optic absorbance biosensor that can be adapted for a 1-step, wash-free monitoring platform for detecting SARS-CoV-2 directly from saliva samples.195 As a conceptual idea, the authors envisaged two different types of biosensors to detect the N protein of SARS-CoV-2 within 15 min that can well satisfy the urgent demand for rapid and low cost diagnosis under the current situation. Along with microfluidic systems, Funari et al. developed an opto-microfluidic chip that uses LSPR to detect SARS-CoV-2 antibodies.196 When antibodies bind to antigens on the chip, the peak shift of resonant wavelength from the gold nanospikes appears for direct monitoring. The results can be obtained in 30 min with an LOD of approximately 0.08 ng mL\(^{-1}\) (~0.5 pM), which allows the quantitative SARS-CoV-2 diagnostics to be performed in an easier, cheaper and faster way.

4.2 Electrochemical biosensors

Electrochemical biosensors are another type of widely-used miniaturized device which attracts much attention thanks to the superiorities such as simplicity, low cost, and ease of miniaturization. An electrochemical biosensor normally relies on a customized electrode acts as the receptor/transducer for real-time, specific, and precise target monitoring. The information from the sensing electrode can be derived to electrical signals, e.g., potentiometric or amperometric, associated with the presence of the analyte of interest.197,198 A potential solution for the diagnosis of COVID-19 was proposed by Tripathy et al. where a miniaturized electrochemical biosensor was fabricated via electrodeposited AuNPs as the transducing element.199 By integrating surface immobilization and smartphone software, the authors claimed that this portable electrochemical analysis system could provide rapid data acquisition, exhibiting the potential as a smart POCT tool to reduce the dependence on bulky instrumentation.

Recently, researchers are also investigating alternative electrochemical solutions that can further improve the detection capability, as well as paving the way to reduce the operation procedure or total cost. By introducing advanced 3D printing method, Md. Ali et al. created a three-dimensional reduced-graphene-oxide electrode and integrated with a microfluidic device as an electrochemical sensor.200 Such 3D electrodes were functionalized with viral antigens to enable sensitive detection of antibodies specific to SARS-CoV-2, with the detection limit down to 2.8 × 10\(^{-15}\) M. Fabiani et al. applied the magnetic beads with the carbon black-based electrode to realize a miniaturized electrochemical sensor for SARS-CoV-2 detection (Fig. 15a).201 With assistance from the magnetic beads, the external magnetic field can provide advantages such as promoting the pre-concentration and eliminating the washing step, while preserving the superiorities such as sensitive and reliable detection, anti-interference from seasonal H1N1 influenza virus, etc. Another two recent studies also introduce paper as the substrate material for electrochemical sensor due to the advantages including low cost, portability, and disposable property. Via printing electrode patterns on paper, Yakoh et al. produced the electrochemical device to capture SARS-CoV-2 antibodies for detection within 30 min (Fig. 15b).202 With the acceptable sensitivity and specificity, the paper-based sensor exhibits as the potential POCT platform especially for the unique cost-effective, portable and disposable behaviors. Alafeef et al. immobilized the sensing probes to a paper-based electrochemical platform, thus yielding a device towards the nucleic acid testing.203 With precise design of the essential sensing materials (gold nanoparticles), the sensor provides a significant improvement in the sensitivity and output signals within 5 min. Interestingly, the device can also quantitatively measure the targets in a linear range from 585.4 copies μL\(^{-1}\) to 5.854 × 10\(^{7}\) copies per μL with a sensitivity of 231 (copies per μL\(^{-1}\)), which reveals the potentials to indicate the progression of SARS-CoV-2 infection after initial infected confirmation.

To facilitate the fast result release to end-user and at-home diagnosis, the data transmission is another aspect that should be taken into consideration apart from the electrochemical sensing component. Zhao et al. demonstrated that

Fig. 15 (a) The magnetic beads based assay for COVID-19 detection in untreated saliva. Reused with permission from ref. 201. Copyright: 2021, Elsevier. (b) Schematic diagram showing the detection principle of paper-based electrochemical biosensor for COVID-19 detection. Reused with permission from ref. 202. Copyright: 2021, Elsevier.
the electrochemical sensor can be adapted to a smartphone for SARS-CoV-2 RNA detection, which relieves the heavy dependence on large-scale instrument and laboratory processes. Such ‘plug-and-play’ manner can possibly provide a portable channel for the customers to assess the test result conveniently. A recent study further combined the electrochemical platform with wireless module to enable the ultra-rapid identification of COVID-19 based on mass-producible graphene electrodes. The demonstrated ‘SARS-CoV-2 RapidPlex’ exhibits high sensitivity and low cost detection, providing the multiplexed information on three key aspects of COVID-19 disease (the viral infection, immune response, and disease severity) with potential of at-home diagnosis.

4.3 Other methods

Apart from the aforementioned methodologies based on miniature biosensors for COVID-19 identification, recent efforts

### Table 3 Summary of biosensor-based SARS-CoV-2 detection

<table>
<thead>
<tr>
<th>No.</th>
<th>Detection technology</th>
<th>Material</th>
<th>Detection target</th>
<th>Sample type</th>
<th>Detection duration</th>
<th>Selectivity</th>
<th>Limit of detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Surface plasmon resonance and colorimetric assay</td>
<td>Gold nanoparticles</td>
<td>Nucleic acid</td>
<td>Isolated RNA</td>
<td>10 min</td>
<td>Against MERS-CoV viral RNA</td>
<td>180 ng mL⁻¹</td>
</tr>
<tr>
<td>2</td>
<td>Plasmonics and photothermal effect</td>
<td>Gold nanoislands</td>
<td>Nucleic acid</td>
<td>Synthesized samples</td>
<td>—</td>
<td>Against SARS-CoV</td>
<td>0.22 pM</td>
</tr>
<tr>
<td>3</td>
<td>Opto-microfluidic chip</td>
<td>Gold nanospikes</td>
<td>Antibodies</td>
<td>Diluted human plasma</td>
<td>30 min</td>
<td>Against BSA (bovine serum albumin), IL-6 (interleukin 6), CRP (C-reactive protein), H1N1 influenza and seasonal H1N1 influenza virus</td>
<td>0.08 ng mL⁻¹ (0.5 pM)</td>
</tr>
<tr>
<td>4</td>
<td>3D electrochemical sensor</td>
<td>Reduced-graphene-oxide nanoflakes</td>
<td>Antibodies</td>
<td>—</td>
<td>Within seconds</td>
<td>Against N antibodies, IL-6 (interleukin 6)</td>
<td>2.8 × 10⁹ M⁻¹</td>
</tr>
<tr>
<td>5</td>
<td>Magnetic beads based biosensor</td>
<td>Magnetic beads and carbon black-based electrodes</td>
<td>Spike (S) protein and nucleocapsid (N) protein</td>
<td>Untreated saliva</td>
<td>30 min</td>
<td>Against 2009 H1N1 influenza and seasonal H1N1 influenza virus</td>
<td>19 ng mL⁻¹ (S protein) and 8 ng mL⁻¹ (N protein)</td>
</tr>
<tr>
<td>6</td>
<td>Paper-based electrochemical sensor</td>
<td>Graphene-based materials</td>
<td>Antibodies</td>
<td>Human serum</td>
<td>30 min</td>
<td>—</td>
<td>1 ng mL⁻¹</td>
</tr>
<tr>
<td>7</td>
<td>Paper-based electrochemical sensor</td>
<td>Gold nanoparticles</td>
<td>Nucleic acid</td>
<td>COVID-19 positive patients</td>
<td>&lt;5 min</td>
<td>Against MERS-CoV and SARS-CoV viral RNA</td>
<td>6900 copies per mL</td>
</tr>
<tr>
<td>8</td>
<td>Electrochemical sensor</td>
<td>Gold@Fe₃O₄ nanocomposite</td>
<td>Nucleic acid</td>
<td>Artificial and clinical RNA samples</td>
<td>—</td>
<td>Against SARS-CoV, MERS-CoV, HCoV-OC43</td>
<td>200 copies per mL</td>
</tr>
<tr>
<td>9</td>
<td>Electrochemical sensor</td>
<td>Multi-wall carbon nanotubes</td>
<td>Reactive oxygen species</td>
<td>Fresh sputum</td>
<td>&lt;30 s</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>Nanomaterials-based breath sensor</td>
<td>Gold nanoparticles</td>
<td>Disease-specific biomarkers</td>
<td>Exhaled breath</td>
<td>In seconds</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>11</td>
<td>Field-effect transistor</td>
<td>Graphene sheet</td>
<td>Spike (S) protein</td>
<td>Clinical samples from COVID-19 patients</td>
<td>Real-time electrical response</td>
<td>—</td>
<td>1 fg mL⁻¹</td>
</tr>
</tbody>
</table>
also demonstrate that the detection of other bio-markers or advanced sensing mechanisms can be applied as promising tools. Miripour \textit{et al.} applied an electrochemical sensor with integrated carbon nanotubes for reactive oxygen species (ROS) monitoring, which is based on the fact that an increase in ROS is a side effect of COVID-19.\textsuperscript{206} Within less than 30 s, the electronic device can directly analyze a sputum sample for real-time ROS, with an accuracy of 94\% and a sensitivity of 92\% after calibration (Fig. 16a). In view of the volatile organic compounds (VOCs) emitted by viral agents, Shan \textit{et al.} applied gold nanoparticles as the sensor array to detect the disease-specific biomarkers from exhaled breath.\textsuperscript{207} Through the surface linking of organic ligands, the device enables the reaction with VOCs which finally causes the signal variation from electric resistance. Even such breath analyzer exhibits potential for rapid COVID-19 screening, factors such as environmental humidity or background diseases can also influence the sensitivity and accuracy that should be considered before the system to be commercialized. Seo \textit{et al.} reported a field-effect transistor (FET)-based biosensor for the identification of SARS-CoV-2 in clinical samples, where the sensor was produced by coating graphene sheets with a specific antibody against SARS-CoV-2 spike protein (Fig. 16b).\textsuperscript{208} Sensor performance was evaluated using antigen protein, cultured virus, and nasopharyngeal swab specimens from COVID-19 patients with SARS-CoV-2 spike protein detection capability of 1 fg mL\textsuperscript{-1} in phosphate-buffered saline and 100 fg mL\textsuperscript{-1} in clinical transport medium.

Without the requirement of sample pretreatment or labeling, this method might serve as a suitable platform to realize the rapid and sensitive diagnosis for COVID-19 or other emerging viral diseases (Table 3).

5. Conclusion and perspective

This article provides a summary analysis of the laboratory methods and commercial products for POCT detection of COVID-19. At present, the POCT methods for the detection of SARS-CoV-2 can be divided into three categories: nucleic acid testing, immune testing, and biosensor testing. In terms of nucleic acid testing, the products developed by Cepheid, Biofire and other companies integrate nucleic acid extraction and RT-PCR amplification into a cassette, with automated instrument operation. These products allow fully automated and integrated nucleic acid testing, thus reducing the risk of cross-contamination and the requirements for detection conditions and enhancing the detection efficiency. Due to its fast detection speed and constant temperature, LAMP-based detection methods have been used by many researchers in the study of POCT for novel coronavirus detection. These LAMP-based methods have achieved fast and convenient nucleic acid detection of the novel coronavirus. And these types of isothermal amplification method are expected to be applied to home test. In addition, the CRISPR method uses a chromatographic strip to detect target nucleic acids, and the results can be observed with the naked eye. Therefore, the CRISPR method is convenient to implement. LamPORE launched by Oxford combines the LAMP method with sequencing, which improves the detection efficiency of sequencing and renders the possibility to use sequencing methods for the POCT detection of SARS-CoV-2. In terms of immune testing, there are a large number of studies regarding colloidal gold- and fluorescence-based immunochromatographic assays and many resulting products. Such methods have advantages such as rapid detection and low cost. There are very few products that use ELISA and chemiluminescence due to the complicated equipment required. However, because ELISA and chemiluminescence results are more accurate and reliable than those derived from chromatographic assays, many researchers are now conducting POCT studies. In terms of biosensors, new sensor detection technologies, such as SPR methods and electrochemical methods, have fast detection capability and low detection limits, providing alternative solutions for the detection of the novel coronavirus. However, due to selectivity limitations, sample preprocessing and preparation costs, there is still substantial room for development. Multiple detection methods complement each other, thereby covering multiple stages of COVID-19, such as screening suspected cases, initial infection, disease treatment, and prognosis. However, there is a clear gap between scientific research results and commercial products. Although diverse and novel research results are introduced in this review, products in the market are mainly concentrated in PCR and LFA detection methods. Lack of stability, high costs and patent restrictions may be some of the reasons that hinder the commercialization of scientific research achievements. We also hope that more scientific research results can be assisted by enterprises and transformed into market-oriented competitive products.

To date, this epidemic has been developing for more than one year and without any signs of ending. COVID-19 has high infectivity and has now undergone widespread transmission. Moreover, there are patients with asymptomatic infection. High-sensitivity, high-accuracy, rapid, and cost-effective detection methods are conducive to the early detection of patients in the initial infection stage and patients with asymptomatic infection, rendering it possible to make early diagnoses and screen close contacts. In addition, the northern hemisphere is entering flu season. The number of patients with influenza A, influenza B, and adenovirus infections will increase, which will impose challenges regarding the diagnosis and treatment of COVID-19. Therefore, rapid and high-throughput multiple-screening tests are also very important.

The existing tests for SARS-CoV-2 are mainly performed by large laboratories. Therefore, sample transportation and results reporting take a rather long time. Moreover, the testing of large numbers of samples easily puts pressure on laboratories and increases the risk of cross-contamination. In view of the shortcomings and deficiencies of the existing detection methods, the research and development of high-throughput, multitarget, fast and automated POCT detection systems...
while ensuring high precision and accuracy will reduce the dependence on laboratory conditions, dispense pressure regarding screening and detection, improve detection efficiency, and reduce the risk of cross-infection. This will also be the main research direction for novel coronavirus detection in the field of microfluidics.

In addition, researchers, doctors and manufacturers from all over the world are researching and developing COVID-19 vaccines. At present, the Pfizer-BioNTech COVID-19 vaccine has obtained emergency use authorization from the United States. And China has announced free vaccinations for all people and more than 24 million people have been vaccinated. People who has the COVID-19 vaccine jointly developed by Oxford University and AstraZeneca has also entered a phase III clinical trial. Before a vaccine is widely available, early detection and the effective control of transmission routes are still the most effective ways to deal with the COVID-19 pandemic.

Author contributions
Qi Song, Xindi Sun, Ziyi Dai, Yibo Gao, Xiuxing Gong, Bingpu Zhou, Jinbo Wu and Weijia Wen all contributed to the writing, reviewing and editing of this manuscript.

Conflicts of interest
There are no conflicts of interest to declare.

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