Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

COVID-19 Diagnostic Methods and Detection Techniques: A Review

Guoguang Rong[a,b], Yuqiao Zheng[a,b], Yin Chen[c], Yanjun Zhang[c], Peixi Zhu[d], and Mohamad Sawan[a,b], [a]School of Engineering, Westlake University, Hangzhou, PR China; [b]Institute of Advanced Study, Westlake Institute for Advanced Study, Hangzhou, PR China; [c]Institute of Microbiology, Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, PR China; [d]College of Pharmaceutical Sciences, Zhejiang University of Technology, Hangzhou, PR China

Abbreviations

COVID-19	Coronavirus disease 2019
CRISPR	Clustered regularly interspaced short palindromic repeats
LAMP	Loop-mediated isothermal amplification
PCR	Polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2

Nomenclature

AI	Artificial intelligence
ASO	Antisense oligonucleotide
AuNP	Gold nanoparticle
C_{dl}	Double layer capacitance
cDNA	Complementary DNA
CE	Counter electrode
COVID-19	Coronavirus disease 2019
CRISPR	Clustered regularly interspaced short palindromic repeats
CT	Computed tomography
DBS	Dried blood spot
DBS	Dried blood spots
DNA	Deoxyribonucleic acid
Eawag	Swiss Federal Institute of Aquatic Science and Technology
EDC	1-Ethyl-3-(3-dimethyl-aminopropyl) carbodiimide
ELISA	Enzyme-linked immunosorbent assay
EPFL	Swiss Federal Institute of Technology in Lausanne
Exo-IQ	Exonuclease probes with internally linked quencher
FET	Field-effect transistor
HDA	Helicase-dependent amplification
IgG	Immunoglobulin G
IgM	Immunoglobulin M
LAMP	Loop-mediated isothermal amplification

LFIA	Lateral flow immunoassay
LOD	Limit of detection
LSPR	Localized surface plasmon resonance
MFCC	Mel-frequency Cepstral Coefficients
mL	Milliliter
NA	Not available
NHS	N-Hydroxysuccinimide
NPV	Negative predicted value
ORF	Open reading frame
ORF1ab	Open reading frame 1ab
OSN-qRT-PCR	One-step single-tube nested quantitative real-time PCR
PCR	Polymerase chain reaction
PPV	Positive predicted value
R_{ct}	Charge transfer resistance
RdRP	RNA-dependent RNA polymerase
rGO	Reduced-graphene-oxide
RNA	Ribonucleic acid
RPA	Recombinase polymerase amplification
rqRT-PCR	Real-time quantitative reverse transcription-PCR
rRT-PCR	Real-time RT-PCR
R_s	Electrolyte resistance
RT-MCDA	Reverse transcription multiple cross displacement amplification
RT-PCR	Reverse transcription PCR
SARS-CoV	Severe acute respiratory syndrome
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SESR	Surface enhanced Raman scattering
SHERLOCK	Specific high-sensitivity enzymatic reporter unlocking
STOP	SHERLOCK testing in one pot
WE	Working electrode
WHO	Word Health Organization
μL	Microliter

Introduction

For more than a year, coronavirus disease 2019 (COVID-19) has been spreading rapidly in many countries, attracting global attention because the infection can be fatal (Zhou et al., 2020). The coronavirus belongs to the coronaviridae family (Zhang et al., 2020b). Genome sequencing of the new coronavirus isolated from patients' bronchoalveolar lavage fluids revealed 96% and 80% similarities with the genomes of the bat coronavirus RaTG13 and severe acute respiratory syndrome (SARS-CoV) virus, respectively (Lu et al., 2020). This coronavirus is known as SARS-CoV-2. By July 15, 2021, there were 188,128,952 confirmed cases and 4,059,339 deaths of COVID-19 globally according to Word Health Organization (WHO). Due to many non-detected asymptomatic cases, the real number of COVID-19 infections could be larger than estimated. Moreover, asymptomatic patients are as contagious as symptomatic patients, and it is estimated that each infected individual may infect about three other individuals (Liu et al., 2020b). Thus, developing rapid and accurate techniques for SARS-CoV-2 detection is crucial to prevent the virus from spreading further.

Owing to the development of molecular biology technologies, molecular diagnostic methods have developed rapidly. Among these, polymerase chain reaction (PCR)-based assays are regarded as the gold standard for virus detection because of their high sensitivity and specificity (Shen et al., 2020). However, PCR-based detection has many limitations such as the requirement of high purity sample, expensive laboratory equipment, training of specialists, and long reaction time (Corman et al., 2020b). Other molecular diagnostic methods such as clustered regularly interspaced short palindromic repeats (CRISPR) and gene sequencing have the same drawbacks. Therefore, researchers sought to develop alternative diagnosis methods such as protein testing and computed tomography. Each method has its own advantages but also some limitations. This manuscript aims at summarizing the current methods for COVID-19 diagnosis to assist researchers in developing rapid and effective techniques. Fig. 1D shows the most representative techniques and new techniques reviewed in this paper.

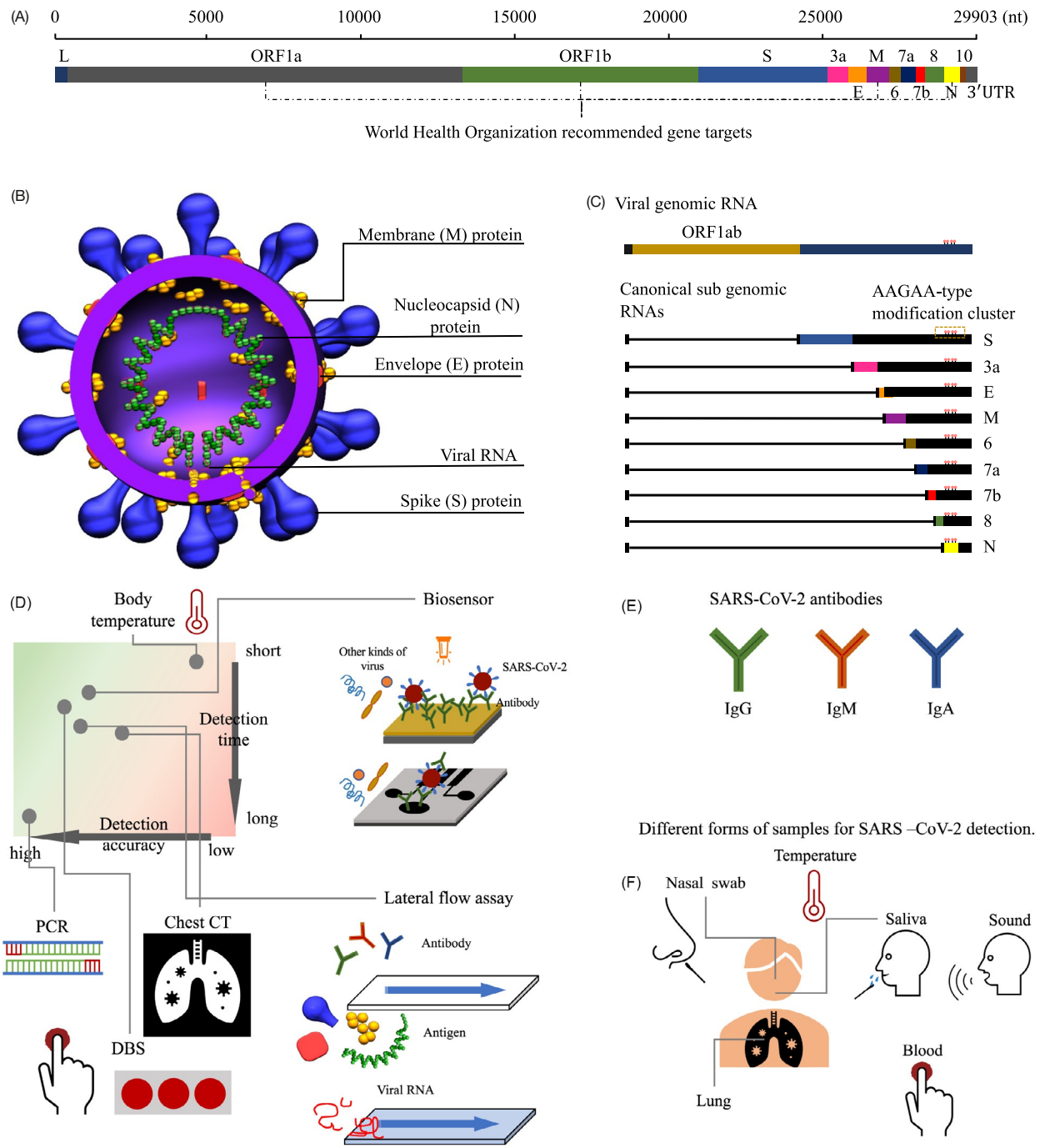

Fig. 1 SARS-CoV-2 viral products and detection techniques: (A) genome organization; the size of the full-length genomic RNA is 29,903 nt; (b) viral structure and antigens; (c) canonical sub genomic mRNAs; (d) typical detection techniques reviewed in this paper and their detection performance; (e) SARS-CoV-2 antibodies; (f) different sample sources for viral detection. ORF, open reading frame; RNA, ribonucleic acid; PCR, polymerase chain reaction; DBS, dried blood spot; CT, computed tomography; IgG, immunoglobulin G; IgM, immunoglobulin M. Partly modified from Kim D, Lee JY, Yang JS, Kim JW, Kim VN et al. (2020) The architecture of SARS-CoV-2 transcriptome. *Cell* 181, 914–921.e10.

Molecular diagnosis

Molecular diagnosis is one of the most important frontiers in contemporary medicine, and its core technology is nucleic acid testing. Due to its capability to identify specific pathogens, nucleic acid testing is suitable for accurate coronavirus diagnosis. At present, nucleic acid detection methods mainly include gene sequencing, CRISPR, and nucleic acid amplification tests such as PCR or isothermal nucleic acid amplification. PCR requires thermal cycling. It is highly sensitive and specific for virus detection. Isothermal

nucleic acid amplification is a quick detection method that can be operated under constant temperature and does not rely on thermocycler operation (Shen et al., 2020). The genome and proteome compositions of the virus were determined by March 24, 2020 despite the host's response to SARS-CoV-2 not being fully understood at that time. (Udugama et al., 2020). Fig. 1A–C shows a schematic representation of SARS-CoV-2. To date, more than 1000 sequences of COVID-19 have been made available to the public (Kim et al., 2020).

PCR-based testing methods

PCR tests use deoxyribonucleic acid (DNA) amplification technology relying on the enzymatic activity of a DNA polymerase that multiplies specifically the desired gene fragment. Coronavirus detection requires a first step of reverse transcription that converts viral ribonucleic acid (RNA) into complementary DNA (cDNA), followed by PCR amplification allowing the quantitative detection of the fluorescent reaction by specific instruments (Adachi et al., 2004; Setianingsih et al., 2019). The two available methods to implement reverse transcription PCR (RT-PCR) are:

(a) the one-step assay, in which a single reaction performs both cDNA synthesis and PCR amplification, minimizing experimental errors. However, the one-step assay is not suitable for testing the same sample multiple times because the RNA serving as template is easy and rapid to degrade.
(b) the two-step assay, which has higher sensitivity compared with the one-step assay, since the reverse transcription occurs separately from the PCR amplification. However, this technique increases the risk of DNA contamination and needs more time (Wong and Medrano, 2005). Corman et al. aligned many severe acute respiratory syndrome coronavirus genome sequences and designed related primers and probes matching conserved sequences located in three different regions: in the RNA-dependent RNA polymerase (RdRP), the Envelope (E), and the nucleocapsid (N) genes. The E gene-based assay was chosen as first-line screening test, while the RdRp gene-based assay is used as confirmatory test, and further confirmation can be done by N gene-based assay. Both the RdRp gene-based and E gene-based assays are highly sensitive. The limit of detection (LOD) is an importance criterion to evaluate the performance of various detection methods. The LOD of the RdRp gene-based assay is 3.9 copies per 25 μL reaction, while that of the E gene-based assay is 3.6 copies per 25 μL reaction. Both tests are highly sensitive compared with N gene-based assay (8.3 copies per 25 μL reaction) (Corman et al., 2020a,b). RT-PCR testing has become a routine coronavirus detection method with high sensitivity, specificity, and a large range of applications (Bernheim et al., 2020; Uhlenhaut et al., 2012).

Currently, real-time quantitative reverse transcription-PCR (rqRT-PCR) is popular for coronavirus detection due to its high specificity, simple quantitative analysis, and higher sensitivity compared with conventional RT-PCR. Many methods have been implemented by researchers to improve real-time RT-PCR tests. The United States' Center for Disease Control and Prevention (CDC) detects SARS-CoV-2 using one-step rqRT-PCR assays. During amplification, the fluorescence quenching probe is cleaved to produce a fluorescent signal in real time (Centers for Disease Control and Prevention, 2020).

Chan et al. designed three real-time RT-PCR (rRT-PCR) assays to detect SARS-CoV-2 RdRp/helicase (Hel), spike (S), and N genes (Chan et al., 2020). The limit of the RdRp/Hel detection (11.2 RNA copy per reaction) was lower than that of the other two assays. Moreover, the RdRp/Hel assay had no cross-reactivity with other coronaviruses, whereas the RdRp assay with probe 2 (RdRp-P2) cross-reacted with SARS-CoV. Wang et al. developed a novel one-step single-tube nested quantitative real-time PCR (OSN-qRT-PCR) assay to detect SARS-CoV-2, which targets the open reading frame 1ab (ORF1ab) and the N genes, and is highly sensitive (1 copy per reaction). In addition, another work presented by Rahman et al. concluded that the reliability of a commercial AusDiagnostic assay (AusDiagnostics Pty Ltd) is not as high as that of in-house real-time PCR using World Health Organization recommended genes, as shown in Fig. 1 (Rahman et al., 2020).

In clinical detection, CDCs around the world typically designed rRT-PCR diagnostic kits in order to reduce the rate of false negative results. False positive reactions of one or more primers and probes with negative template controls indicate sample contamination. Also, individuals diagnosed positive by rRT-PCR may be in the clearance and recovery stage with low viral shedding and not infectious, but isolated unnecessarily (Larremore et al., 2021). False negative results can result from mutations in the SARS-CoV-2 genome affecting the target region of the primers and probes. The resulting mismatches between the primers or probes and the target sequence decrease the assay performance and may generate false negative results. This can be avoided to some extent by using multiple target gene amplifications, but the specificity and sensitivity of the rRT-PCR assay may not be sufficient to definitively solve this problem (Wang et al., 2020b). In addition, internal controls help identify samples that underwent interferences during RNA extraction and/or PCR amplification. Moreover, rRT-PCR assays highly depend on instruments and specifically trained operators, and it can take several hours to obtain results from clinical samples.

Isothermal nucleic acid amplification-based methods

Isothermal nucleic acid amplification techniques are still under development. The reaction involved can be performed at fixed temperature without specialized instruments such as thermal cyclers and has a sensitivity comparable to that of the PCR. Isothermal nucleic acid amplification techniques include recombinase polymerase amplification (RPA), loop-mediated isothermal amplification (LAMP), helicase-dependent amplification (HDA), and many others. They can operate on a tablet-PC-based device (Yoo et al., 2020), and can be used to curb community spread of COVID-19 (James and Alawneh, 2020). LAMP is a one-step nucleic acid

amplification method in which the target DNA can be amplified within an hour. It is based on the PCR technology and is the most popular diagnostic test among the isothermal amplification techniques. The LAMP reaction is highly specific due to the typical use of four to six different primers to bind the six regions of a target DNA. For RNA template, the amplification reaction can be completed in one step by the addition of a reverse transcription, and is then known as RT-LAMP reaction. Some laboratories have developed RT-LAMP tests for COVID-19 diagnosis, and such techniques have already been applied in clinical diagnosis.

Currently, clinical diagnosis for COVID-19 requires special equipment and one or more days before completion. Therefore, many efforts have been made to develop more convenient and faster techniques. Lamb's group proposed an RT-LAMP test able to detect SARS-CoV-2 in simulated patient specimens within 30 min (Lamb et al., 2020). Huang et al. also applied RT-LAMP technology and generated detection results within 30 min (Huang et al., 2020b). They designed four LAMP primer sets targeting three regions in the ORF1ab, S, and N genes of the viral RNA, and obtained a visual detection by using a colorimetric test without the need for expensive or special instruments. The results obtained with this detection method for 16 clinical samples were verified by conventional RT-qPCR. However, this technique may generate carry-over contaminations during the amplification process, resulting in false positives. Zhang's group proposed a technique of RT-LAMP product visualization with pH-sensitive dyes that change color as pH changes (Zhang et al., 2020a). It uses five sets of LAMP primers targeting two regions of the viral genome. However, the sensitivity is low, requiring a few hundred copies per 25 μL reaction. Zhu et al. used a one-step RT-LAMP combined with a nanoparticle-based biosensor assay to rapidly and accurately detect SARS-CoV-2 using simple equipment. The whole detection process requires only about 1 h (Zhu et al., 2020). The detection specificity and sensitivity of this assay were both 100%.

To minimize the false-positive rate, many researchers have designed a series of highly specific primers. Yu's group employed an isothermal LAMP test to amplify a ORF1ab gene fragment using six primers that specifically identify eight different regions of the ORF1ab gene fragment (Craw and Balachandran, 2012). The sensitivity of this assay is similar to that of TaqMan-based qPCR assays. When used to detect synthetic SARS-CoV-2 RNA, the sensitivity was 10 copies/μL (Yu et al., 2020). Yang's group reported a one-step RT-LAMP method that detects three genes (ORF1ab, N, and E genes) for rapid COVID-19 diagnosis (Yang et al., 2020). The specificity of the single-step RT-LAMP was 99%, and the sensitivity was comparable to that of RT-PCR.

Experiments showed that the RT-LAMP tests have different sensitivities for the three genes. N gene detection is highly sensitive, while ORF1ab gene detection brings high specificity, and E gene detection is of medium level compared to the two other genes. However, when one of the three genes was used alone, there was a reduction of specificity and/or sensitivity. Yan's group further optimized the RT-LAMP test. They designed primer sets targeting the ORF1ab (including ORF1ab-212, ORF1ab-4, ORF1ab-33, ORF1ab-22, and ORF1ab-1) and S genes (including S-107, S-123, S-67, S-41, and S-61) of SARS-CoV-2. The sensitivity of primer sets ORF1ab-4 and S-123 was 20 and 200 copies per reaction, respectively. The specificity evaluation showed that only pseudo-SARS-CoV-2 viruses were detected as positive by the RT-LAMP assay with the two primer sets (ORF1ab-4 and S-123). At the same time, the mean detection time (± standard division) of this assay was 26.28 ± 4.48 min, while RT-PCRs take 1–2 h before completion (Yan et al., 2020). In summary, the RT-LAMP-based methods are simple, fast, and accurate for clinical SARS-CoV-2 detection.

Recombinase polymerase amplification (RPA) is another isothermal nucleic acid amplification technique usable for SARS-CoV-2 detection. The advantages of RPA include: (1) it is the fastest nucleic acid amplification technique, requiring only 15 min for completion; (2) it requires moderate heating to only 37–42 °C, which makes the equipment's temperature control module much simpler and cost effective. A suggestion of references using RPA detection include: (1) the use of exonuclease probes with internally linked quencher (Exo-IQ) to rapidly detect SARS-CoV-2 through low-volume real-time single-tube reverse transcription recombinase polymerase amplification (Behrmann et al., 2020); (2) rapid and direct visual detection of SARS-CoV-2 by a reverse transcription recombinase polymerase amplification assay (Lau et al., 2021); (3) single-copy sensitive, field-deployable, and simultaneous dual-gene detection of SARS-CoV-2 RNA by modified RT-RPA (Xia and Chen, 2020).

CRISPR-based tests

CRISPR-based tests detect viral genomes with fewer steps compared to PCR assays and are highly sensitive and specific, offering a promising solution for home testing. This technique provides a new platform for the detection of the virus and bacteria. However, CRISPR-based tests cannot perform in a short time frame if the virus concentration is below 10 nM (Nouri et al., 2021). Thus, most of the CRISPR-based tests use various amplification methods—including PCR, LAMP, and RPA—after viral RNA isolation to enhance their sensitivity (Lee et al., 2017; van Dongen et al., 2020). For SARS-CoV-2 detection, a typical CRISPR-based method includes sample collection, RNA extraction, RNA amplification (like RT-PCR, RT-LAMP and RT-RPA), CRISPR assay (Cas12 and Cas13 effectors), and signal output, such as colorimetry or fluorescence (Kim et al., 2020). To realize onsite applications and avoid using thermal cyclers for their Cas12-based assays, many researchers utilized isothermal amplification methods like RT-LAMP and RT-RPA (Broughton et al., 2020; Ding et al., 2020).

Broughton's group utilized real nasopharyngeal swab RNA samples and developed a two-step Cas12a-based lateral flow assay for SARS-CoV-2 detection. The process requires 30–40 min and the LOD is 10 copies/μL. The assay targets the N and E genes, and provides a positive prediction rate of 95% and a negative prediction rate of 100% (Broughton et al., 2020). Specific high-sensitivity enzymatic reporter unlocking (SHERLOCK) proposed by Sherlock Biosciences (Boston, US) also uses a two-step process for SARS-CoV-2 detection. Sherlock Biosciences and Binx Health (Trowbridge, United Kingdom) proposed a joint product that combines Sherlock's CRISPR test with Binx's analyzer. In this work, Sherlock's assay utilizes a guide RNA that promotes the docking of the Cas13 enzyme on SARS-CoV-2 RNA. Then, the Cas13 enzyme cleaves a reporter sequence connected to an electrochemical

label. A measurable current is generated when the label is oxidized at an electrode (Cormac, 2020). Abudayyeh et al. presented a simple test to detect SARS-CoV-2, known as SHERLOCK testing in one pot (STOP). STOP is a simplified detection method combining virus RNA extraction, isothermal amplification, and CRISPR-based detection. Ding's group utilized a Cas12a-based detection that targets the N gene region after RT-RPA pre-amplification. This experiment consists of a 40-min one-step reaction, and the LOD is 10 copies/μL, representing a typical LOD for Cas12a-based detection (Ding et al., 2020). Fozouni et al. utilized real nasal swab RNA samples and developed a Cas13a-based assay to directly detect SARS-CoV-2 without the need for an amplification step. This assay reaches a sensitivity close to 100 copies/μL in less than 30 min. By combining CRISPR RNAs (crRNAs) that target SARS-CoV-2 RNA with mobile phone-based quantification, the detection becomes more sensitive and specific (Fozouni, 2020). Besides Cas12 and Cas13 proteins, other CRISPR effectors like Cas9 have been studied and applied for the detection of other pathogens (Wang et al., 2020c).

The main limitation of the CRISPR technology stems in that the target sequences of the Cas12 and Cas13 proteins are restricted due to constraints imposed by the protospacer adjacent motifs or protospacer-flanking sites. At the same time, multiplexed detection, allowing for more than one target per reaction, can create interferences between targets and other analytes, and potentially lead to cross-reactivities (Li et al., 2019).

Gene sequencing

Whole genome sequencing is a complex technology requiring specialized equipment to identify complete genome sequences in a specimen. It is a potent tool for the discovery of new diseases (Reuter et al., 2015). For example, gene sequencing is utilized for the identification of coronaviruses isolated from patients infected by SARS-CoV-2 (Ren et al., 2020). Generally, the viral RNA of SARS-CoV-2 is extracted from clinical samples and are then submitted to a multiplexed amplicon sequencing process. Previous research has shown that instead of covering the whole genome of a coronavirus, which is challenging, to analyze clinical samples, researchers can focus on important elements of the genomic sequence (Maurier et al., 2019). Gene sequencing helped researchers discover that SARS-CoV-2S protein was similar to the S protein of a pangolin coronavirus, suggesting the possibility to monitor evolutionary changes of the coronaviruses by sequencing (Lam et al., 2020). This tracking capability can help improve the detection methods and manage coronavirus variants (Fang et al., 2020).

Although nucleic acid amplification-based tests currently represent the gold standard to diagnose COVID-19, they are of limited value to identify major modalities of transmission and localize clusters of infection. Meredith and colleagues developed a combined approach integrating full-genome sequencing of SARS-CoV-2 virus and epidemiological data to keep track of infectious events and track the spreading of the virus (Meredith et al., 2020). They used third-generation sequencing technology based on nanopores. The technology can help decision-makers to adopt appropriate social distancing and other curbing measures in real time to fight COVID-19 more efficiently.

In general, gene sequencing is a complex tool with indispensable equipment and personnel training requirements. Thus, alternative detection methods are still needed to realize on-site tests of individuals.

Antigen/antibody testing

To curb the spreading of the virus, rough and ready surveillance approaches based on frequent, large-scale, and rapid testing would be more effective than nucleic acid amplification-based testing limited to suspected cases (Larremore et al., 2021). Therefore, the development a reliable onsite detection method is very urgent. SARS-CoV-2 can be diagnosed by detecting viral antigens (Fig. 1B) directly, or by detecting antibodies (Fig. 1E) produced by the host immune system in response to SARS-CoV-2 infection. However, SARS-CoV-2 antibodies may cross-react with other coronaviruses, which represents a challenge for the development of accurate serological tests. Lv et al. found cross-reactivity between SARS-CoV-2 and SARS-CoV antibody responses. That is, anti-SARS-CoV antibodies can be detected in plasma samples from SARS-CoV-2 patients (Lv et al., 2020).

Antibody testing

Recently, serologic laboratory diagnostic tests are developed to detect anti-SARS-CoV-2 antibodies in blood and tissue specimens. These tests include enzyme-linked immunosorbent assay (ELISA) and immunochromatography assay. Zhang's group utilized ELISA to detect immunoglobulins M and G (IgM and IgG) from serum samples (Zhang et al., 2020c). In their experiments, the SARS-CoV-2 Rp3 nucleocapsid protein served as viral antigen. This protein is 92% identical to the viral antigens of other SARS-related viruses. Zhang and colleagues found that the level of anti-Rp3 antibodies increases after SARS-CoV-2 infection. Moreover, this method can detect antibodies in blood, respiratory track, and fecal specimens (Zhang et al., 2020c). Liu's group also designed two ELISA kits based on SARS-CoV-2 spike protein and nucleocapsid protein to detect IgG and IgM antibodies against these proteins (Liu et al., 2020a). The positive detection rates of the two ELISAs (spike protein-based and nucleocapsid protein-based) were 82.2% for IgM and 80.4% for IgG. In addition, the spike protein-based ELISA was more sensitive to detect anti-viral IgM than the nucleocapsid protein-based ELISA. The positive rate of IgG and IgM detection increases with the number of days after infection. The positive rate of IgG and IgM increased obviously after 10 days after disease onset, but the positive rate of IgM decreased after 35 days post-disease onset. Meanwhile, the positive rate obtained with the spike protein- and nucleocapsid

protein-based IgM and IgG ELISAs was below 60% in the first 10 days post-disease onset. This indicates that ELISAs could be used as supplementary tests to the nucleic acid-based tests for detecting anti-viral antibodies with a high sensitivity in serum samples from patients, after 10 days post-disease onset.

Immunochromatography is another rapid detection method to diagnose COVID-19, which was used to detect antibodies in the blood. The presence of anti-viral IgM means a short time infection, while anti-viral IgG indicate that the infection is long-standing. Therefore, the rapid detection of anti-viral IgM and IgG antibodies is beneficial for controlling the disease. It can help differentiate individuals at early stage of infection, with low viral load, and who should be isolated to prevent viral spreading, from individuals who are in recovery, with detectable virus or viral RNA. This second group is below the infectious threshold, and therefore need not to be isolated so as to avoid unnecessary economic burden (Larremore et al., 2021). Li's team developed a rapid and simple anti-SARS-CoV-2 IgM/IgG antibody test, as shown in Fig. 2, that can detect both IgM and IgG antibodies at the same time and at different infection stages (Li et al., 2020c). The test strip contains three detection bands, including a control band (C line), an IgG band (G line), and an IgM band (M line). The C line turns red/purple immediately after the specimen reaches the end of the test strip, regardless of whether it is positive or negative. The M and G lines turning red/purple indicate the existence of anti-SARS-CoV-2 IgM and IgG antibodies, respectively. The sensitivity of this test is 88.66% (352 positives from 397 COVID-19 patients' blood samples), and the specificity is 90.63% (12 positives from 128 non-COVID-19 patients' blood samples). The detection sensitivity of the combined IgM/IgG antibody test is higher than that of the individual IgM or IgG antibody tests. However, whether the assay has some cross-reactivity with anti-flu virus antibodies or antibodies recognizing other coronaviruses has not been studied. Choe et al. assessed a rapid COVID-19 detection immunochromatography-based IgG/IgM assay and made comparisons between diagnostic results obtained with this assay with those obtained by RT-PCR assays (Choe et al., 2020). The sensitivity was 92.9% (65/70 of RT-PCR positive group) and the specificity was 96.2% (76/79 of RT-PCR negative group). The diagnostic assay did not cross-react with other flu viruses.

However, immunochromatography-based antibody assays can only identify COVID-19 patients who were recently infected but cannot confirm the presence of the virus in patients. Moreover, these diagnostic methods can lead to false-negative results, if (1) the antibody concentration is below the detection limit; (2) the individuals develop antibodies with different specificities. Finally, 2 weeks after onset, IgM antibodies decrease and finally vanish. Thus far, the effectiveness of immunochromatography-based antibody assays has not been sufficiently verified. To improve the diagnostic sensitivity and specificity of this method it is advisable to further optimize IgM/IgG combined antibody assays against SARS-CoV-2.

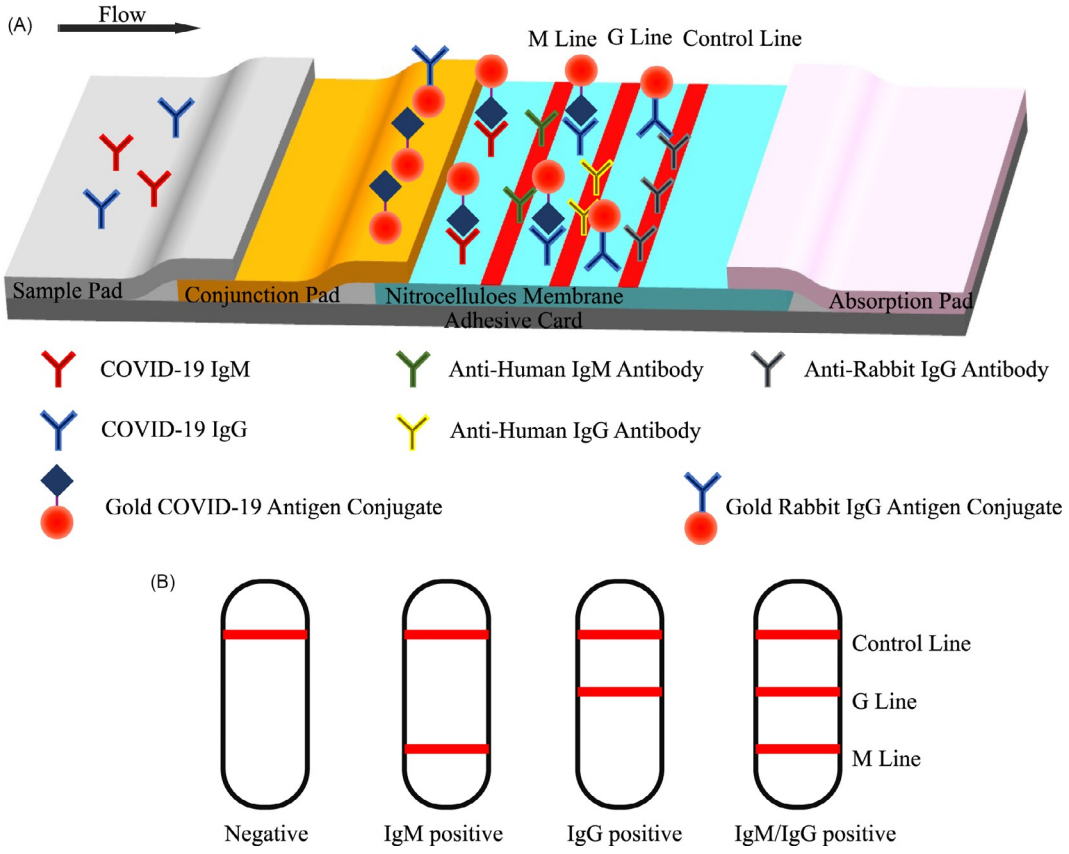

Fig. 2 Diagram showing rapid combined detection of anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulin M/immunoglobulin G (IgM/IgG) antibodies: (A) detection device, (B) images of various test results. Adapted from Li ZT, Yi YX, Luo XM, Xiong N, Liu Y, et al. 2020c. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *Journal of Medical Virology* 92: 1518–1524.

Dried blood spot (DBS) has long been used in serologic diagnosis in many medical fields (Amendola et al., 2021). Some researchers developed serum assays able to detect anti-SARS-CoV-2S1 protein antibodies contained in dried blood samples (Karp et al., 2020). This test can be operated by any individual given that it requires only dried blood samples pricked from fingers. The authors evaluated the sensitivity and specificity of this method by testing 31 patients infected by SARS-CoV-2 and 80 healthy volunteers. The result indicated that the technique can perfectly predict the positive and negative cases and both the sensitivity and specificity reached 100%. Another work by McDade's group also detected anti-SARS-CoV-2 IgG antibodies using blood samples from fingers (McDade et al., 2020). Their assay was done against DBS and the sensitivity reached 97%, by testing 29 positives out of 30 confirmed COVID-19 patients. Morley et al. concluded that although further optimizations are required for DBS, it still has high sensitivity and specificity against anti-SARS-COV-2 S protein antibodies relatively to serum specimens (Morley et al., 2020). Table 1 shows some DBS assays for COVID-19 diagnosis. Improving detection throughput has also been studied. Gaugler et al. proposed an automatic DBS processing and extracting technique in detecting human SARS-CoV-2 IgG antibody (Gaugler et al., 2021). This work largely reduces detection cost and can support at-home testing. It is worth mentioning that the quality of DBS detection can be strongly affected by storage temperature. High temperature and sunlight can lead to bad extraction yield, as shown in Fig. 3 (Zava and Zava, 2021).

In general, DBS tests are inexpensive compared to PCR and other methods and can be conveniently operated by individuals at home. According to Table 1, various researchers have validated the sensitivity and specificity of DBS. Thus, these tests are considered promising for large-scale COVID-19 screening in the population.

Antigen testing

Besides detecting antibodies produced in response to SARS-CoV-2 infection for diagnosis purposes, direct detection of SARS-CoV-2 antigens to confirm the presence of the virus is also an option. Antigens are molecules that are specifically recognized and bound by antigen receptors on the surface of T/B lymphocytes, can trigger antibody production and induce cell-mediated immune responses. The E, N, and S proteins of the SARS-CoV-2 virus have been used as antigens, the S and N protein being the two main antigen targets. The S protein locates on the virus surface and is necessary for virus' entry into human cells. Various forms of the S protein are used as antigens, including full-length (S1 + S2) and partial proteins (receptor binding domain (RBD) or S1 domain) (Ernst et al., 2021). The N protein shares ~90% sequence identity with the N protein of SARS, ~49% with that of MERS-CoV, and 20–36% with those of other coronaviruses including HCoV-OC43, 229E, NL63, and HKU1. The S protein has only 75% homology with SARS S protein, and only 28–33% of its amino acid sequence is identical to those of other human coronaviruses. The RBD on the C-terminus portion of S1 of SARS-Cov-2 shares 74% homology with the RBD of SARS, and 19–21% of identical amino acids with those of HKU-1, NL63, OC43, and 229E. Thus, the selection of the target protein can affect the sensitivity and specificity of detection (Ernst et al., 2021). Wilson et al. proposed a pan-human coronavirus proteome-wide programmable phage display assay (ReScan) that can recapitulate the antigen specificities specifically enriched in serum IgGs of COVID-19 patients (Zamecnik et al., 2020). This study identified epitopes from the N, S and ORF3a proteins by ReScan, which is a multiple diagnostic pipeline, pans human sera for

Table 1 Comparison of some DBSs used for COVID-19 diagnosis.

Sample amount	*PPV/NPV*	*Sensitivity/Specificity*	*References*
52	DBS: 21 positives, 28 negatives, 3 inconclusive Serum test: 23 positives, 28 negatives, 1 inconclusive		Amendola et al. (2021)
111	NA	100%/100%	Karp et al. (2020)
87	100%/96.88%	98.11%/100%	Morley et al. (2020)
13	NA	86%/100%	Gaugler et al. (2021)
177	IgG S1: 100%/99.3% IgG nucleocapsid: 100%/98%	IgG S1: 90.9%/100% IgG nucleocapsid: 86.4%/95.2%	Zava and Zava (2021)

Ref., reference; DBSs, dried blood spots; PPV, positive predicted value; NPV, negative predicted value; NA, not available; IgG, immunoglobulin G.

Amendola A, Bianchi S, Gori M, Barcellini L, Colzani D, et al. (2021) Dried blood spot as an alternative to plasma/serum for SARS-CoV-2 IgG detection, an opportunity to be sized to facilitate COVID-19 surveillance among schoolchildren. *Pediatric Infectious Disease Journal* 40: E46–E47.

Karp DG, Danh K, Espinoza NF, Seftel D, Robinson PV, et al. (2020) A serological assay to detect SARS-CoV-2 antibodies in at-home collected finger-prick dried blood spots. *Scientific Reports* 10.

Morley GL, Taylor S, Jossi S, Perez-Toledo M, Faustini SE, et al. (2020) Sensitive detection of SARS-CoV-2-specific antibodies in dried blood spot samples. *Emerging Infectious Diseases* 26: 2970–2973.

Gaugler S, Sottas PE, Blum K and Luginbuhl M (2021) Fully automated dried blood spot sample handling and extraction for serological testing of SARS-CoV-2 antibodies. *Drug Testing and Analysis* 13: 223–226.

Zava TT and Zava DT (2021) Validation of dried blood spot sample modifications to two commercially available COVID-19 IgG antibody immunoassays. *Bioanalysis* 13: 13–28.

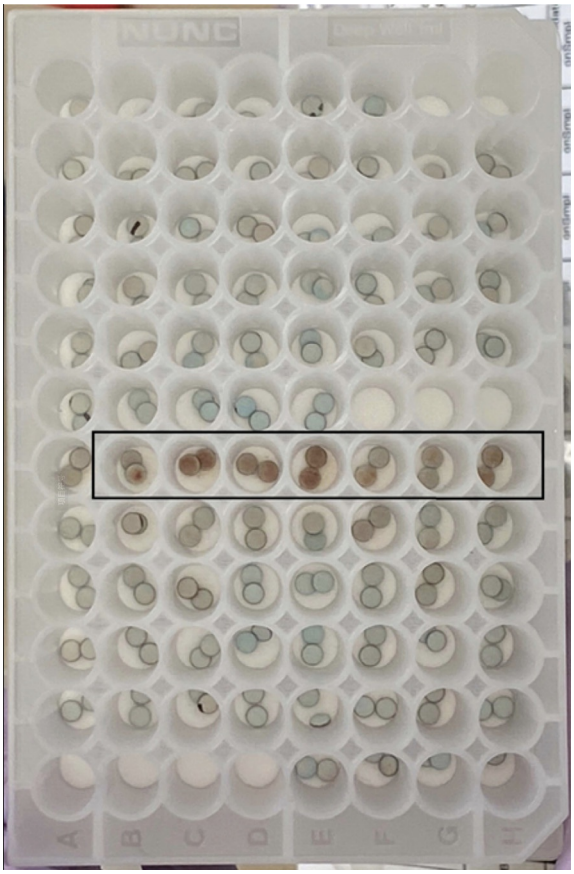

Fig. 3 Extracted DBSs in the fritted filter block. Samples were stored under 55 °C for 14 days in order to test the stability and precision of the assay. The marked part illustrates samples that were only partially extracted. Excerpted from Zava TT and Zava DT (2021) Validation of dried blood spot sample modifications to two commercially available COVID-19 IgG antibody immunoassays. *Bioanalysis* 13: 13–28.

SARS-CoV-2 antigens. This technique is a good candidate for enhancing diagnosis specificity. Benjamin et al. proposed a half-strip lateral flow assay that detects SARS-CoV-2 nucleocapsid N protein (Grant et al., 2020). When tested on a buffer containing a commercial SARS-CoV-2 nucleocapsid N protein as target, the LOD of this half-strip lateral flow assay was 0.65 ng/mL. Kyosei et al. presented an antigen-based detection technique for SARS-CoV-2 that combined thio-nicotinamide adenine dinucleotide cycling and a sandwich ELISA (Kyosei et al., 2020). For spike protein detection, the LOD was 2.3×10^{-18} M/assay in the range of 63–500 pg/mL, which equals to a level of 10^{-20} mol per assay—supposing that each virus has ~25 spike proteins on its surface—when detecting SARS-CoV-2. Another work proposed by Dan Liu et al. presented a nanozyme chemiluminescence paper-based test that can rapidly detect SARS-CoV-2 (Liu et al., 2021a). This test delivers detection results within 16 min, and the LOD for the detection of the spike protein and pseudo virus are of 0.1 ng/mL and 360 $TCID_{50}$/mL, respectively.

Antigen tests have the advantage that they can accommodate virus mutants. Since mutated viruses still bind human cells through ACE2-binding protein, by employing ACE2 as receptor to engage with the spike proteins of SARS-CoV-2, antigen tests can capture S-D614G mutants with higher affinity (about twice that of the wild-type S-614D protein) (Zhou et al., 2021). In addition, trimeric ACE2 can be engineered to bind coronavirus S proteins with affinities several tens or even hundreds times higher (Guo et al., 2021). This represents a big advantage compared with nucleic acid amplification-based tests, which can be impaired by mutations occurring naturally in the RNA virus variants.

To summarize "Antigen/Antibody testing" section, the key to antigen/antibody recognition-based diagnosis is to obtain highly sensitive and specific antigens and antibodies for detection. Antigen-based detection requires a process of antibody preparation that is tedious and time-consuming. It is crucial to choose the right sampling time window, due to the facts that both the viral load and infectiousness are influential (He et al., 2020), and that appearance and disappearance of antibodies in the human body (Zhang et al., 2021) are dynamic. If the properties and status of antigen/antibody-based diagnosis is taken into consideration, currently it cannot be used as a highly accurate diagnosis tool for COVID-19. Through a combination of multiple detection methods, including nucleic acid-based and antigen/antibody recognition-based tests, the detection time window will be shortened, and the positive detection rate will increase. IgG/IgM antibody detection plays a vital role in the auxiliary diagnosis of SARS-CoV-2 infection by providing more information about the infection stage.

Computed tomography (CT)

Chest CT detection is a significant method to diagnose patients suspected of having a COVID-19 infection. It is a non-invasive detection method based on multiple X-ray detections performed on patient chest at different angles to generate cross-sectional images. Doctors can then analyze these images to find abnormal features and render a diagnosis (Lee et al., 2020). Many studies have concluded that most COVID-19 patients have typical radiologic features in chest CT images, such as multifocal plaque consolidation and ground glass opacity (Chung et al., 2020; Li et al., 2020b). Further, researchers found that the imaging characteristics of COVID-19 is determined by the infection stage. Pan's group performed chest CT detections on 21 COVID-19 patients and established that the lung infection reaches a peak about 10 days after the onset of symptoms. They divided lung CT characteristics into four stages based on chest CT scans from 0 to 26 days after the beginning of the infection: the first corresponds to the ground glass opacity stage (0–4 days); the second is the increased stage, characterized by a crazy-paving pattern (5–8 days); the third is the consolidation stage (9–13 days); the last stage corresponds to the gradual resolution of consolidation and absence of crazy-paving pattern ($\geq$14 days) (Pan et al., 2020). Bernheim et al. found that 56% of the patients cannot be diagnosed by CT scans at early stages of symptom onset (Bernheim et al., 2020). With time, consolidation, linear opacity and other pathological features became more obvious on the CT scans. This observation is largely consistent with the findings by Pan et al. Therefore, patients who test negative by RT-PCR but whose CT scans are positive should be isolated for further assessment and to prevent wide-spreading of the infection.

Some studies have also reported that some patients tested positive by RT-PCR show initially normal X-rays or chest CT scans (Bernheim et al., 2020; Ai et al., 2020). There are two explanations for this:

(a) Patients may not have typical radiological abnormalities during early onset of the symptoms, and it is easy to miss some lesions due to the low resolution of chest X-rays, but the false negative rate of chest CT-based diagnosis is relatively low.
(b) In some patients, the lung may not be the target organ of the SARS-CoV-2 infection, which may infect multiple organs such as the liver, kidney, and heart (Huang et al., 2020a). Thus, when patients show severe clinical symptoms but normal chest CT scans, they need to be further checked by more comprehensive examinations. In addition, further research is urgently needed to provide theoretical support for the diagnosis of COVID-19 patients by chest CT.

Currently, RT-PCR is used to diagnose COVID-19 and CT scans are used for screening. Each detection method has its own drawbacks. For RT-PCR-based diagnosis, the availability of RT-PCR kits cannot meet the demand, and the diagnostic efficacy depends on the presence of SARS-CoV-2 in the collected samples. For CT-based diagnosis, the equipment is expensive and requires operation by professional doctors. In order to reduce the workload and improve diagnosis accuracy, artificial intelligence (AI) has also been used to analyze CT images for COVID-19 diagnosis (Wang et al., 2021), preferably in automated fashion (Ozturk et al., 2020). Importantly, CT scan alone cannot specifically diagnose COVID-19. Thus, it is often complemented with a PCR-test. A combined approach provides more accurate results (Ai et al., 2020).

Biosensor-based detection

Sensitive and "Naked-eye" techniques of SARS-CoV-2 detection that do not rely on advanced instruments and specialized trained personnel are desirable to speed up the diagnosis process. Therefore, researchers are studying different types of biosensors to make the detection more convenient and suitable for at-home test. These biosensors include optical, electrical, and electrochemical sensors targeting different virus gene, nucleic acid or antibody (Kim et al., 2021; Huang et al., 2021; Ke et al., 2021; Liu et al., 2021b; Eissa and Zourob, 2021; Zhao et al., 2021; Mojsoska et al., 2021; Alafeef et al., 2020; Li et al., 2020a; Funari et al., 2020; Wang et al., 2020a).

Parikshit et al. reported a gold nanoparticle (AuNPs)-based colorimetric test for COVID-19 diagnosis. This technique can give output in 10 min from positive isolated RNA specimens covered with suitable N-gene specific thiol-modified antisense oligonucleotides (ASOs). This method detected SARS-CoV-2 RNA sequences selectively and with a LOD of 0.18 ng/μL (Moitra et al., 2020). The specificity of this biosensor was verified using MERS-CoV virus RNA, which produced no obvious change in absorbance. Seo's group assessed clinical specimens using a graphene field-effect transistor-based biosensor with anti-SARS-CoV-2 spike antibodies (Seo et al., 2020). Their biosensor had a LOD of 242 copies/mL on clinical samples and shows specificity for SARS-CoV-2 antigen over MERS-CoV antigen. Ali et al. reported a biosensing platform created by three-dimensional (3D) nanoprinting that can detect SARS-COV-2 antibodies in a few seconds (Ali et al., 2021). Electrodes were fabricated by 3D nanoprinting and the 3D structure allowed sensors to be loaded with a large amount of antigen. The detection principle of this biosensor is illustrated in Fig. 4. It has a LOD of 2.8 $\times$ 10–15 M towards spike S1 protein while anti-RBD antibodies provide a LOD of 1.6 $\times$ 10–15 M. Yousefi et al. proposed a reagent-free sensor-modified electrode chip that detects SARS-CoV-2 viral particles in specimens within 5 min. This technique is based on an electrode-tethered sensor that uses antibodies to bind the analyte displayed on a negatively charged DNA linker. The sensor can detect SARS-CoV-2 in unprocessed patient's saliva in 5 min, and the LOD is 4 $\times$ 103 virus particles/mL (Yousefi et al., 2020). Molecularly imprinted polymer-based biosensor, as shown in Fig. 5, is also popular in molecular detection. Raziq et al. developed a molecularly imprinted polymer-based biosensor for SARS-CoV-2 nucleoprotein detection, which performs at a LOD of 15 fM (Raziq et al., 2021). Table 2 shows recent developments in SARS-CoV-2 detection using different types of biosensors. These types of biosensors do not rely on any sophisticated instruments, and thus are easy for on-site use in high-risk environments.

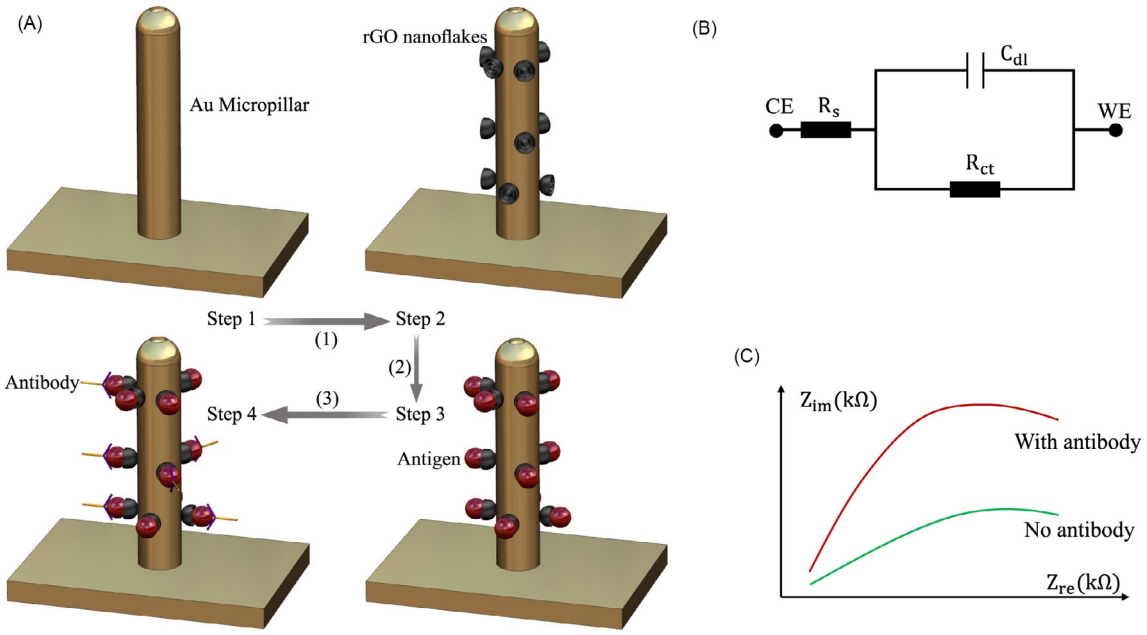

Fig. 4 Principle of the electrochemical biochip for COVID-19 detection; (A) functionalization of the micropillar electrode and biosensor. This technique includes four steps and three processes: (1) coating of the electrodes by carboxylate rGO sheets; (2) use of EDC-NHS to couple viral antigens with rGO; (3) selective attachment of antibodies to antigens; (B) electrical circuit of the 3D-printed COVID-19 detection chip; (C) circuit response of antibody binding to antigens. rGO, reduced-graphene-oxide; EDC, 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide; NHS, N-Hydroxysuccinimide; CE, counter electrode; WE, working electrode; R_s, electrolyte resistance; R_{ct}, charge transfer resistance; C_{dl}, double layer capacitance. Adapted form Ali MA, Hu CS, Jahan S, Yuan B, Saleh MS, et al. (2021) Sensing of COVID-19 antibodies in seconds via aerosol jet Nanoprinted reduced-graphene-oxide-coated 3D electrodes. *Advanced Materials* 33(7): e2006647.

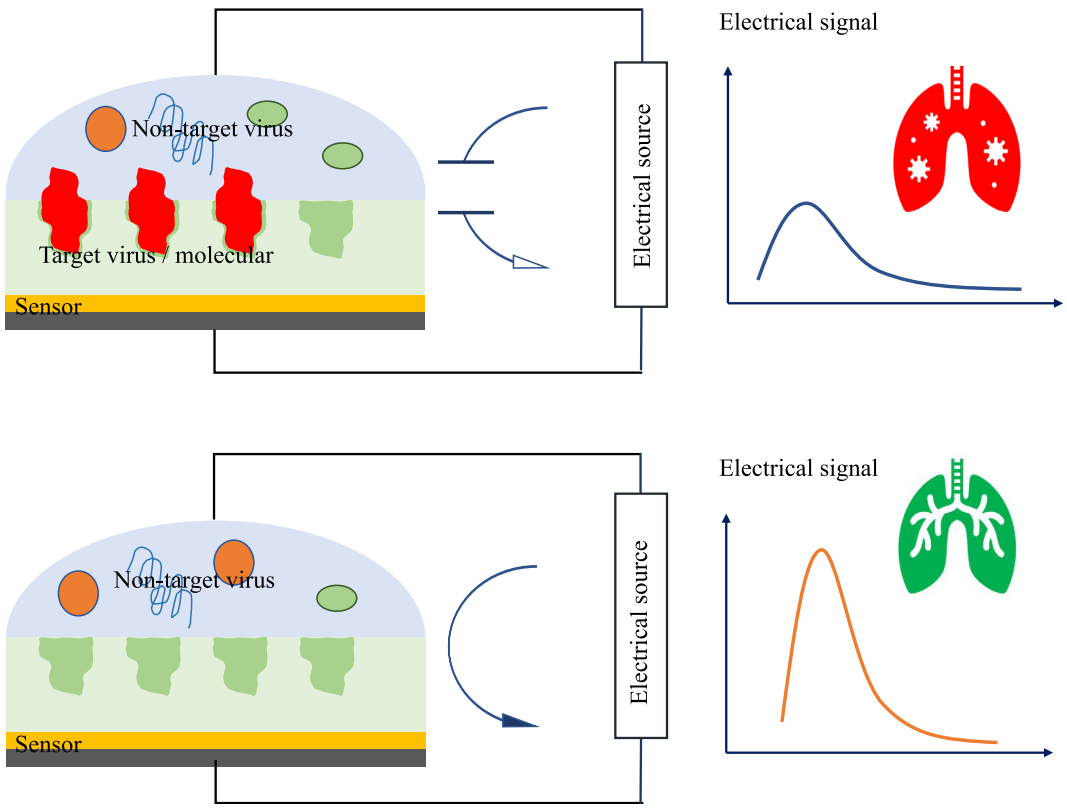

Fig. 5 An example of molecularly imprinted polymer-based biosensor.

Table 2 Representative biosensors for detection of SARS-CoV-2.

Biosensor type	*Biomarker*	*LOD (or sensitivity)*	*Detection time*	*References*
LFIA	N protein	Antigen protein: 2 ng/reaction Cultured virus: 2.5×10^4 pfu/reaction	20 min	Kim et al. (2021)
CRISPR Cas12a	N gene	10 copies/μL	NA	Huang et al. (2021)
Graphene FET	RdRp gene	∼0.1 fg/mL	∼10 min	Ke et al. (2021)
	IgM/IgG antibodies	∼1 fg/mL	∼5 min	
SERS	IgM/IgG antibodies	1 pg/mL	25 min (suitable)	Liu et al. (2021b)
Electrochemical immunosensor	N protein	0.8 pg/mL	NA	Eissa and Zourob (2021)
Electrochemical biosensor	ORF1ab gene	200 copies/mL	NA	Zhao et al. (2021)
Electrochemical biosensor	S protein	20 μg/mL	45 min	Mojsoska et al. (2021)
Electrochemical biosensor	N gene	6.9 copies/μL	<5 min	Alafeef et al. (2020)
RT-MCDA	ORF1ab and N gene	5 copies/reaction	60 min	Li et al. (2020a)
LSPR	S protein	0.08 ng/mL	30 min	Funari et al. (2020)
LFIA	IgM/IgG antibodies	$1{:}10^6$ dilution from 1 μL serum	15 min	Wang et al. (2020a)

Note that all the biomarkers in the table are for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). LOD, limit of detection; Ref., reference; min, minute(s); LFIA, lateral flow immunoassay; CRISPR, Clustered Regularly Interspaced Short Palindromic Repeats; FET, field-effect transistor; IgG, immunoglobulin G; IgM, immunoglobulin M; SESR, surface enhanced Raman resonance; RT-MCDA, reverse transcription multiple cross displacement amplification; LSPR, localized surface plasmon resonance.

Kim HY, Lee JH, Kim MJ, Park SC, Choi M, et al. (2021) Development of a SARS-CoV-2-specific biosensor for antigen detection using scFv-Fc fusion proteins. *Biosensors & Bioelectronics*, 175.

Huang D, Shi ZW, Qian JJ, Bi K, Fang MJ, et al. (2021) A CRISPR-Cas12a-derived biosensor enabling portable personal glucose meter readout for quantitative detection of SARS-CoV-2. *Biotechnology and Bioengineering*, 10.

Ke GJ, Su DK, Li Y, Zhao Y, Wang HG, et al. (2021) An accurate, high-speed, portable bifunctional electrical detector for COVID-19. *Science China-Materials* 64: 739–747.

Liu HF, Dai EH, Xiao R, Zhou ZH, Zhang ML, et al. (2021b) Development of a SERS-based lateral flow immunoassay for rapid and ultra-sensitive detection of anti-SARS-CoV-2 IgM/IgG in clinical samples. *Sensors and Actuators B-Chemical* 329: 10.

Eissa S and Zourob M (2021) Development of a low-cost cotton-tipped electrochemical immunosensor for the detection of SARS-CoV-2. *Analytical Chemistry* 93: 1826–1833.

Zhao H, Liu F, Xie W, Zhou TC, OuYang J, et al. (2021) Ultrasensitive supersandwich-type electrochemical sensor for SARS-CoV-2 from the infected COVID-19 patients using a smartphone. *Sensors and Actuators B-Chemical* 327: 9.

Mojsoska B, Larsen S, Olsen DA, Madsen JS, Brandslund I, et al. (2021) Rapid SARS-CoV-2 detection using electrochemical immunosensor. *Sensors* 21: 11.

Alafeef M, Dighe K, Moitra P and Pan D (2020) Rapid, ultrasensitive, and quantitative detection of SARS-CoV-2 using antisense oligonucleotides directed electrochemical biosensor chip. *Acs Nano* 14: 17028–17045.

Li SJ, Jiang WJ, Huang JF, Liu Y, Ren LJ, et al. (2020a) Highly sensitive and specific diagnosis of COVID-19 by reverse transcription multiple cross-displacement amplification-labelled nanoparticles biosensor. *European Respiratory Journal* 56.

Funari R, Chu KY and Shen AQ (2020) Detection of antibodies against SARS-CoV-2 spike protein by gold nanospikes in an opto-microfluidic chip. *Biosensors & Bioelectronics* 169: 8.

Wang CW, Yang XS, Gu B, Liu HF, Zhou ZH, et al. (2020a) Sensitive and simultaneous detection of SARS-CoV-2-specific IgM/IgG using lateral flow immunoassay based on dual-mode quantum dot nanobeads. *Analytical Chemistry* 92: 15542–15549.

Newest diagnosis methods

At present, researchers around the world are sparing no effort to study the SARS-CoV-2 from many different perspectives in order to design more effective diagnosis and treatment methods. One of these methods relies on DBS, as mentioned above, which has become widely used around the world. Researchers at the Swiss Federal Institute of Technology in Lausanne (EPFL) and Swiss Federal Institute of Aquatic Science and Technology (Eawag) have proposed a technique that detects SARS-CoV-2 in wastewater (Sandy Evangelista, 2020). By analyzing wastewater samples, the virus can be detected before clinical diagnosis. This research paves the way for the development of early warning systems. In addition, there are many emerging diagnostic technologies for SARS-CoV-2 detection. Coughing sounds analysis by AI is another new direction, as shown in Fig. 1F. A work by Laguarta et al. allows pre-screening of COVID-19 patients from cough recordings by simply using an acoustic biomarker feature extractor. The general structure of this diagnosis method is shown in Fig. 6 (Laguarta et al., 2020). This method achieves 98.5% sensitivity and 94.2% specificity for officially confirmed patients, and 100% sensitivity and 83.2% specificity for asymptomatic patients. On other aspects, AI-assisted Internet of Things (Adly et al., 2020) and big data monitoring and surveillance systems (Pham et al., 2020) can help limit the spread of COVID-19.

Although these new techniques may seem attractive, more experimental validation is needed, and further research should be done to improve their practicality before they can be released for clinical applications.

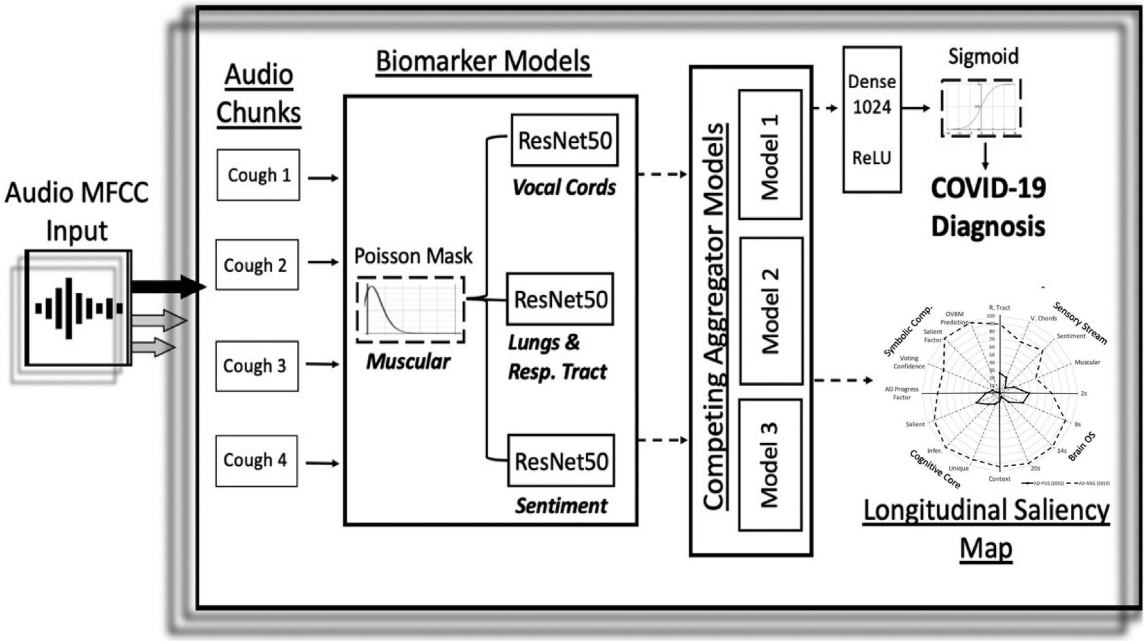

Fig. 6 Schematic architecture of COVID-19 diagnosis by cough recording. MFCC: Mel-Frequency Cepstral Coefficients. Excerpted from Laguarta J, Hueto F and Subirana B (2020) COVID-19 artificial intelligence diagnosis using only cough recordings. *IEEE Open Journal of Engineering in Medicine and Biology* 1: 275–281.

Discussions and conclusion

A variety of COVID-19 diagnosis methods have been developed by researchers. In general, these approaches are based on techniques including molecular tests that detect viral genomic material, antigen and antibody-based tests that detect proteins, CT examination that directly checks patient's lung, and other rapid tests like biosensors. The molecular tests have various formats. PCR tests that detect different regions of the SARS-CoV-2 genome have become the dominant method for controlling COVID-19. They are of high accuracy and stability and are generally applied in hospitals and medical institutions. However, they require centralized labs, trained personnel, and thermocycling machines for rounds of nucleic acid amplification. These methods are not suitable for large-scale applications. During outbreaks, PCR tests can lead to test shortages and delays in receiving the results, which greatly reduce the efficiency of disease prevention. Techniques such as LAMP are also molecular tests, and are less time consuming than PCR tests. They were proven comparable to PCR in terms of high sensitivity and accuracy. Antigen and antibody-based detection are established techniques and present obvious advantages for the reduction of detection time. They can be deployed in high-risk applications, such as clinics, supermarkets, public transportations, and educational institutions, and operated by personnel with little or no specialized training. The sensitivity of rapid antigen-based tests is about one order of magnitude lower than that of the PCR-based tests (1000 copies/mL for PCR compared with 10,000 copies/mL for LFA antigen test) (Cormac, 2020). Given delay between the onset of infection and detectable antibody responses, antibody-based tests are of little value for early capture of active infections in individuals. Therefore, antigen-based tests are more suitable for large-scale detection and discovery of latent infections, while antibody-based tests can be applied to patients who already had obvious symptoms. It is worth mentioning that a number of researchers are studying the use of dried blood spots for COVID-19 detection. This technique is inexpensive and can be operated by individuals in-home with high convenience. Therefore, this method has great potential to help realize large-scale detection. CT have been used as an auxiliary method to nucleic acid-based tests for COVID-19 diagnosis in hospitals. Emerging detection methods like biosensors are thought to realize large-scale detection in all kinds of public areas. Other detection methods like microfluidic devices and smartphone surveillance systems are also under research to prevent COVID-19 from further spreading.

It should be pointed out that epidemic prevention, control, and clinical diagnosis require different systems. At present, epidemic prevention and control rely on disease diagnosis. However, epidemic prevention and control do not need highly sensitive and accurate test, as required in clinical diagnosis, but rather rapid, frequent, and large-scale screening of the population. A recent study by Professor Mina of the Harvard University has shown that with rapid and large-scale screening methods, even if the LOD is 100–1000 time worse than RT-PCR and there is a 10% false negative rate, as long as the population coverage can reach 50–75% and the screening can be performed periodically with a period of 1–7 days, the epidemic could be quickly contained within a few weeks without using vaccine (Larremore et al., 2021). Therefore, epidemic prevention and control require large-scale population screening and rapid results. The effectiveness of this strategy has been proven by the world's first population scale screening in Slovakia (Mahase, 2020). In this regard, nucleic acid amplification-based detection methods are actually neither practical nor convenient.

Rather, rapid detection, such as those relying on antigen detection or isothermal nucleic acid amplification, is needed. Serological antibody testing methods are suitable for judging whether infected individuals are at early stage of infection or in recovery, so as to avoid unnecessary isolation. In summary, there is no COVID-19 test that can fit all scenarios (Mina and Andersen, 2021). Different testing scenarios require different testing technologies with varying sensitivity, specificity, speed, frequency, coverage, cost, and ease of operation (Weissleder et al., 2020). Technologies under development and new products should consider all of these factors. An integrated approach combining various methods could be an effective way to control the COVID-19 pandemic.

References

Adachi D, Johnson G, Draker R, Ayers M, Mazzulli T, et al. (2004) Comprehensive detection and identification of human coronaviruses, including the SARS-associated coronavirus, with a single RT-PCR assay. *Journal of Virological Methods* 122: 29–36.

Adly AS, Adly AS, and Adly MS (2020) Approaches based on artificial intelligence and the internet of intelligent things to prevent the spread of COVID-19: Scoping review. *Journal of Medical Internet Research* 22: e19104.

Ai T, Yang ZL, Hou HY, Zhan CN, Chen C, et al. (2020) Correlation of chest CT and RT-PCR testing for coronavirus disease 2019 (COVID-19) in China: A report of 1014 cases. *Radiology* 296: E32–E40.

Alafeef M, Dighe K, Moitra P, and Pan D (2020) Rapid, ultrasensitive, and quantitative detection of SARS-CoV-2 using antisense oligonucleotides directed electrochemical biosensor chip. *ACS Nano* 14: 17028–17045.

Ali MA, Hu CS, Jahan S, Yuan B, Saleh MS, et al. (2021) Sensing of COVID-19 antibodies in seconds via aerosol jet Nanoprinted reduced-graphene-oxide-coated 3D electrodes. *Advanced Materials* 33(7), e2006647.

Amendola A, Bianchi S, Gori M, Barcellini L, Colzani D, et al. (2021) Dried blood spot as an alternative to plasma/serum for SARS-CoV-2 IgG detection, an opportunity to be sized to facilitate COVID-19 surveillance among schoolchildren. *Pediatric Infectious Disease Journal* 40: E46–E47.

Behrmann O, Bachmann I, Spiegel M, Schramm M, Abd El Wahed A, et al. (2020) Rapid detection of SARS-CoV-2 by low volume real-time single tube reverse transcription recombinase polymerase amplification using an exo probe with an internally linked quencher (Exo-IQ). *Clinical Chemistry* 66: 1047–1054.

Bernheim A, Mei XY, Huang MQ, Yang Y, Fayad ZA, et al. (2020) Chest CT findings in coronavirus disease 2019 (COVID-19): Relationship to duration of infection. *Radiology* 295: 685–691.

Broughton JP, Deng XD, Yu GX, Fasching CL, Servellita V, et al. (2020) CRISPR-Cas12-based detection of SARS-CoV-2. *Nature Biotechnology* 38: 870–U54.

Centers for Disease Control and Prevention (2020) *Research Use Only Real-Time RT-PCR Protocol for Identification of 2019-nCoV*.

Chan JFW, Yip CCY, To KKW, Tang THC, Wong SCY, et al. (2020) Improved molecular diagnosis of COVID-19 by the novel, highly sensitive and specific COVID-19-RdRp/Hel real-time reverse transcription-PCR assay validated in vitro and with clinical specimens. *Journal of Clinical Microbiology* 58: e00310-2.

Choe JY, Kim JW, Kwon HH, Hong HL, Jung CY, et al. (2020) Diagnostic performance of immunochromatography assay for rapid detection of IgM and IgG in coronavirus disease 2019. *Journal of Medical Virology* 92: 2567–2572.

Chung M, Bernheim A, Mei XY, Zhang N, Huang MQ, et al. (2020) CT imaging features of 2019 novel coronavirus (2019-nCoV). *Radiology* 295: 202–207.

Cormac S (2020) Coronavirus testing finally gathers speed. *Nature Biotechnology* https://www.nature.com/articles/d41587-020-00021-z.

Corman V, Bleicker T, Brünink S, and Zambon M (2020a) *Diagnostic Detection of Wuhan Coronavirus 2019 by Real-Time RT-PCR*. WHO.

Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, et al. (2020b) Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveillance* 25: 23–30.

Craw P and Balachandran W (2012) Isothermal nucleic acid amplification technologies for point-of-care diagnostics: A critical review. *Lab on a Chip* 12: 2469–2486.

Ding X, Yin K, Li ZY, Lalla RV, Ballesteros E, et al. (2020) Ultrasensitive and visual detection of SARS-CoV-2 using all-in-one dual CRISPR-Cas12a assay. *Nature Communications* 11: 4711.

Eissa S and Zourob M (2021) Development of a low-cost cotton-tipped electrochemical immunosensor for the detection of SARS-CoV-2. *Analytical Chemistry* 93: 1826–1833.

Ernst E, Wolfe P, Stahura C, and Edwards KA (2021) Technical considerations to development of serological tests for SARS-CoV-2. *Talanta* 224: 121883.

Fang B, Liu L, Yu X, Li X, Ye G, et al. (2020) *Genome-Wide Data Inferring the Evolution and Population Demography of the Novel Pneumonia Coronavirus (SARS-CoV-2)*.

Fozouni P (2020) *COVID-19/SARS-CoV-2 News From Preprints; Direct Detection of SARS-CoV-2 Using CRISPR-Cas13a and a Mobile Phone*. Medical Letter on the CDC & FDA.

Funari R, Chu KY, and Shen AQ (2020) Detection of antibodies against SARS-CoV-2 spike protein by gold nanospikes in an opto-microfluidic chip. *Biosensors & Bioelectronics* 169: 8.

Gaugler S, Sottas PE, Blum K, and Luginbuhl M (2021) Fully automated dried blood spot sample handling and extraction for serological testing of SARS-CoV-2 antibodies. *Drug Testing and Analysis* 13: 223–226.

Grant BD, Anderson CE, Williford JR, Alonzo LF, Glukhova VA, et al. (2020) SARS-CoV-2 coronavirus nucleocapsid antigen-detecting half-strip lateral flow assay toward the development of point of care tests using commercially available reagents. *Analytical Chemistry* 92: 11305–11309.

Guo L, Bi WW, Wang XL, Xu W, Yan RH, et al. (2021) Engineered trimeric ACE2 binds viral spike protein and locks it in "three-up" conformation to potently inhibit SARS-CoV-2 infection. *Cell Research* 31: 98–100.

He X, Lau EHY, Wu P, Deng XL, Wang J, et al. (2020) Temporal dynamics in viral shedding and transmissibility of COVID-19. *Nature Medicine* 26: 672–675.

Huang C, Wang Y, and Li X (2020a) Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China (vol 395, pg 497, 2020). *Lancet* 395: 496.

Huang WE, Lim B, Hsu CC, Xiong D, Wu W, et al. (2020b) RT-LAMP for rapid diagnosis of coronavirus SARS-CoV-2. *Microbial Biotechnology* 13: 950–961.

Huang D, Shi ZW, Qian JJ, Bi K, Fang MJ, et al. (2021) A CRISPR-Cas12a-derived biosensor enabling portable personal glucose meter readout for quantitative detection of SARS-CoV-2. *Biotechnology and Bioengineering* 118(4): 1587–1596.

James AS and Alawneh JI (2020) COVID-19 infection diagnosis: Potential impact of isothermal amplification technology to reduce community transmission of SARS-CoV-2. *Diagnostics* 10: 399.

Karp DG, Danh K, Espinoza NF, Seftel D, Robinson PV, et al. (2020) A serological assay to detect SARS-CoV-2 antibodies in at-home collected finger-prick dried blood spots. *Scientific Reports* 10: 20188.

Ke GJ, Su DK, Li Y, Zhao Y, Wang HG, et al. (2021) An accurate, high-speed, portable bifunctional electrical detector for COVID-19. *Science China Materials* 64: 739–747.

Kim D, Lee JY, Yang JS, Kim JW, Kim VN, et al. (2020) The architecture of SARS-CoV-2 transcriptome. *Cell* 181: 914–921.e10.

Kim HY, Lee JH, Kim MJ, Park SC, Choi M, et al. (2021) Development of a SARS-CoV-2-specific biosensor for antigen detection using scFv-Fc fusion proteins. *Biosensors & Bioelectronics* 175: 112868.

Kyosei Y, Namba M, Yamura S, Takeuchi R, Aoki N, et al. (2020) Proposal of de novo antigen test for COVID-19: Ultrasensitive detection of spike proteins of SARS-CoV-2. *Diagnostics* 10: 594.

Laguarta J, Hueto F, and Subirana B (2020) COVID-19 artificial intelligence diagnosis using only cough recordings. *IEEE Open Journal of Engineering in Medicine and Biology* 1: 275–281.

Lam TTY, Jia N, Zhang YW, Shum MHH, Jiang JF, et al. (2020) Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. *Nature* 583: 282–285.

Lamb LE, Bartolone SN, Ward E, and Chancellor MB (2020) *Chancellor Rapid Detection of Novel Coronavirus (COVID-19) by Reverse Transcription-Loop-Mediated Isothermal Amplification*.

Larremore DB, Wilder B, Lester E, Shehata S, Burke JM, et al. (2021) Test sensitivity is secondary to frequency and turnaround time for COVID-19 screening. *Science Advances* 7: eabd5393.
Lau YL, Ismail IB, Mustapa NIB, Lai MY, Soh TST, et al. (2021) Development of a reverse transcription recombinase polymerase amplification assay for rapid and direct visual detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). *PLoS One* 16: e0245164.
Lee SH, Yu J, Hwang GH, Kim S, Kim HS, et al. (2017) CUT-PCR: CRISPR-mediated, ultrasensitive detection of target DNA using PCR. *Oncogene* 36: 6823–6829.
Lee EYP, Ng M-Y, and Khong P-L (2020) *COVID-19 Pneumonia: What Has CT Taught us Lancet Infectious Disease 2020*.
Li Y, Liu L, and Liu G (2019) CRISPR/Cas multiplexed biosensing: A challenge or an insurmountable obstacle? *Trends in Biotechnology* 37(8): 792–795.
Li SJ, Jiang WJ, Huang JF, Liu Y, Ren LJ, et al. (2020a) Highly sensitive and specific diagnosis of COVID-19 by reverse transcription multiple cross-displacement amplification-labelled nanoparticles biosensor. *European Respiratory Journal* 56: 2002060.
Li XM, Zeng WB, Li X, Chen HN, Shi LP, et al. (2020b) CT imaging changes of corona virus disease 2019(COVID-19): A multi-center study in Southwest China. *Journal of Translational Medicine* 18: 154.
Li ZT, Yi YX, Luo XM, Xiong N, Liu Y, et al. (2020c) Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. *Journal of Medical Virology* 92: 1518–1524.
Liu WB, Liu L, Kou GM, Zheng YQ, Ding YJ, et al. (2020a) Evaluation of nucleocapsid and spike protein-based enzyme-linked immunosorbent assays for detecting antibodies against SARS-CoV-2. *Journal of Clinical Microbiology* 58: e00461-20.
Liu Y, Gayle AA, Wilder-Smith A, and Rocklov J (2020b) The reproductive number of COVID-19 is higher compared to SARS coronavirus. *Journal of Travel Medicine* 27: taaa021.
Liu D, Ju CH, Han C, Shi R, Chen XH, et al. (2021a) Nanozyme chemiluminescence paper test for rapid and sensitive detection of SARS-CoV-2 antigen. *Biosensors & Bioelectronics* 173: 112817.
Liu HF, Dai EH, Xiao R, Zhou ZH, Zhang ML, et al. (2021b) Development of a SERS-based lateral flow immunoassay for rapid and ultra-sensitive detection of anti-SARS-CoV-2 IgM/IgG in clinical samples. *Sensors and Actuators B: Chemical* 329: 10.
Lu RJ, Zhao X, Li J, Niu PH, Yang B, et al. (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: Implications for virus origins and receptor binding. *Lancet* 395: 565–574.
Lv H, Wu NC, Tsang OT-Y, Yuan M, Perera RAPM, et al. (2020) Cross-reactive antibody response between SARS-CoV-2 and SARS-CoV infections. *Cell Reports* 31: 107725.
Mahase E (2020) Covid-19: Mass testing in Slovakia may have helped cut infections. *BMJ [British Medical Journal]* 371: m4761.
Maurier F, Beury D, Flechon L, Varre JS, Touzet H, et al. (2019) A complete protocol for whole-genome sequencing of virus from clinical samples: Application to coronavirus OC43. *Virology* 531: 141–148.
McDade TW, McNally EM, Zelikovich AS, D'Aquila R, Mustanski B, et al. (2020) High seroprevalence for SARS-CoV-2 among household members of essential workers detected using a dried blood spot assay. *PLoS One* 15: e0237833.
Meredith LW, Hamilton WL, Warne B, Houldcroft CJ, Hosmillo M, et al. (2020) Rapid implementation of SARS-CoV-2 sequencing to investigate cases of health-care associated COVID-19: A prospective genomic surveillance study. *Lancet Infectious Diseases* 20: 1263–1272.
Mina MJ and Andersen KG (2021) COVID-19 testing: One size does not fit all. *Science* 371: 126–127.
Moitra P, Alafeef M, Dighe K, Frieman MB, and Pan D (2020) Selective naked-eye detection of SARS-CoV-2 mediated by N gene targeted antisense oligonucleotide capped Plasmonic nanoparticles. *ACS Nano* 14: 7617–7627.
Mojsoska B, Larsen S, Olsen DA, Madsen JS, Brandslund I, et al. (2021) Rapid SARS-CoV-2 detection using electrochemical Immunosensor. *Sensors* 21: 11.
Morley GL, Taylor S, Jossi S, Perez-Toledo M, Faustini SE, et al. (2020) Sensitive detection of SARS-CoV-2-specific antibodies in dried blood spot samples. *Emerging Infectious Diseases* 26: 2970–2973.
Nouri R, Tang Z, and Dong M (2021) CRISPR-based detection of SARS-CoV-2: A review from sample to result. *Biosensors and Bioelectronics* 178: 113012.
Ozturk T, Talo M, Yildirim EA, Baloglu UB, Yildirim O, et al. (2020) Automated detection of COVID-19 cases using deep neural networks with X-ray images. *Computers in Biology and Medicine* 121: 103792.
Pan F, Ye TH, Sun P, Gui S, Liang B, et al. (2020) Time course of lung changes a chest CT during recovery from coronavirus disease 2019 (COVID-19). *Radiology* 295: 715–721.
Pham QV, Nguyen DC, Thien HT, Hwang WJ, and Pathirana PN (2020) Artificial intelligence (AI) and big data for coronavirus (COVID-19) pandemic: A survey on the state-of-the-arts. *IEEE Access* 8: 130820–130839.
Rahman H, Carter I, Basile K, Donovan L, Kumar S, et al. (2020) Interpret with caution: An evaluation of the commercial AusDiagnostics versus in-house developed assays for the detection of SARS-CoV-2 virus. *Journal of Clinical Virology* 127: 104374.
Raziq A, Kidakova A, Boroznjak R, Reut J, Öpik A, et al. (2021) Development of a portable MIP-based electrochemical sensor for detection of SARS-CoV-2 antigen. *Biosensors and Bioelectronics* 178: 113029.
Ren LL, Wang YM, Wu ZQ, Xiang ZC, Guo L, et al. (2020) Identification of a novel coronavirus causing severe pneumonia in human: A descriptive study. *Chinese Medical Journal* 133: 1015–1024.
Reuter JA, Spacek DV, and Snyder MP (2015) High-throughput sequencing technologies. *Molecular Cell* 58: 586–597.
Sandy Evangelista AB (2020) *Covid-19: Using Wastewater to Track the Pandemic*.
Seo G, Lee G, Kim MJ, Baek SH, Choi M, et al. (2020) Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor (vol 14, pg 5135, 2020). *ACS Nano* 14: 12257–12258.
Setianingsih TY, Wiyatno A, Hartono TS, Hindawati E, and Rosamarlina, et al. (2019) Detection of multiple viral sequences in the respiratory tract samples of suspected Middle East respiratory syndrome coronavirus patients in Jakarta, Indonesia 2015-2016. *International Journal of Infectious Diseases* 86: 102–107.
Shen MZ, Zhou Y, Ye JW, Al-Maskri AAA, Kang Y, et al. (2020) Recent advances and perspectives of nucleic acid detection for coronavirus. *Journal of Pharmaceutical Analysis* 10: 97–101.
Udugama B, Kadhiresan P, Kozlowski HN, Malekjahani A, Osborne M, et al. (2020) Diagnosing COVID-19: The disease and tools for detection. *ACS Nano* 14: 3822–3835.
Uhlenhaut C, Cohen JI, Pavletic S, Illei G, Gea-Banacloche JC, et al. (2012) Use of a novel virus detection assay to identify coronavirus HKU1 in the lungs of a hematopoietic stem cell transplant recipient with fatal pneumonia. *Transplant Infectious Disease* 14: 79–85.
van Dongen JE, Berendsen JTW, Steenbergen RDM, Wolthuis RMF, Eijkel JCT, et al. (2020) Point-of-care CRISPR/Cas nucleic acid detection: Recent advances, challenges and opportunities. *Biosensors & Bioelectronics* 166: 112445.
Wang CW, Yang XS, Gu B, Liu HF, Zhou ZH, et al. (2020a) Sensitive and simultaneous detection of SARS-CoV-2-specific IgM/IgG using lateral flow immunoassay based on dual-mode quantum dot Nanobeads. *Analytical Chemistry* 92: 15542–15549.
Wang J, Cai K, Zhang RQ, He XZ, Shen XX, et al. (2020b) Novel one-step single-tube nested quantitative real-time PCR assay for highly sensitive detection of SARS-CoV-2. *Analytical Chemistry* 92: 9399–9404.
Wang LY, Shen XY, Wang T, Chen PR, Qi N, et al. (2020c) A lateral flow strip combined with Cas9 nickase-triggered amplification reaction for dual food-borne pathogen detection. *Biosensors & Bioelectronics* 165: 112364.
Wang B, Jin S, Yan QS, Xu HB, Luo C, et al. (2021) AI-assisted CT imaging analysis for COVID-19 screening: Building and deploying a medical AI system. *Applied Soft Computing* 98: 106897.
Weissleder R, Lee H, Ko J, and Pittet MJ (2020) COVID-19 diagnostics in context. *Science Translational Medicine* 12: eabc1931.
Wong ML and Medrano JF (2005) Real-time PCR for mRNA quantitation. *BioTechniques* 39: 75–85.
Xia SM and Chen X (2020) Single-copy sensitive, field-deployable, and simultaneous dual-gene detection of SARS-CoV-2 RNA via modified RT-RPA. *Cell Discovery* 6: 37.

Yan C, Cui J, and Huang L (2020) Rapid and visual detection of 2019 novel coronavirus (SARS-CoV-2) by a reverse transcription loop-mediated isothermal amplification assay. *Clinical Microbiology and Infection* 26(6): 773–779.
Yang W, Dang X, Wang Q, Xu M, Zhao Q, et al. (2020) Rapid detection of SARS-CoV-2 using reverse transcription RT-LAMP method. *Microbial Biotechnology* 13: 950–996.
Yoo WS, Han HS, Kim JG, Kang K, Jeon HS, et al. (2020) Development of a tablet PC-based portable device for colorimetric determination of assays including COVID-19 and other pathogenic microorganisms. *RSC Advances* 10: 32946–32952.
Yousefi H, Mahmud A, and Chang D (2020) Detection of SARS-CoV-2 viral particles using direct, reagent-free electrochemical sensing. *Journal of the American Chemical Society* 143(4).
Yu L, Wu S, Hao X, Li X, Liu X, et al. (2020) Rapid colorimetric detection of COVID-19 coronavirus using a reverse transcriptional loop-mediated isothermal amplification (RT-LAMP) diagnostic platform: iLACO. *medRxiv* 66: 975–977.
Zamecnik CR, Rajan JV, Yamauchi KA, Mann SA, Loudermilk RP, et al. (2020) ReScan, a multiplex diagnostic pipeline, pans human sera for SARS-CoV-2 antigens. *Cell Reports. Medicine* 1: 100123.
Zava TT and Zava DT (2021) Validation of dried blood spot sample modifications to two commercially available COVID-19 IgG antibody immunoassays. *Bioanalysis* 13: 13–28.
Zhang Y, Odiwuor N, Xiong J, Sun L, Nyaruaba RO, et al. (2020a) Rapid molecular detection of SARS-CoV-2 (COVID-19) virus RNA using colorimetric LAMP. *medRxiv*.
Zhang NR, Wang LL, Deng XQ, Liang RY, Su M, et al. (2020b) Recent advances in the detection of respiratory virus infection in humans. *Journal of Medical Virology* 92: 408–417.
Zhang W, Du R-H, Li B, Zheng X-S, Yang X-L, et al. (2020c) Molecular and serological investigation of 2019-nCoV infected patients: Implication of multiple shedding routes. *Emerging Microbes & Infections* 9(1): 386–389.
Zhang B, Yue DY, Wang Y, Wang F, Wu SJ, et al. (2021) The dynamics of immune response in COVID-19 patients with different illness severity. *Journal of Medical Virology* 93: 1070–1077.
Zhao H, Liu F, Xie W, Zhou TC, OuYang J, et al. (2021) Ultrasensitive supersandwich-type electrochemical sensor for SARS-CoV-2 from the infected COVID-19 patients using a smartphone. *Sensors and Actuators B: Chemical* 327: 9.
Zhou P, Yang XL, and Wang XG (2020) A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 579(7798): 270–273.
Zhou B, Thao TTN, and Hoffmann D (2021) SARS-CoV-2 spike D614G change enhances replication and transmission. *Nature* 592(7852): 122–127.
Zhu X, Wang XX, Han LM, et al. (2020) Multiplex reverse transcription loop-mediated isothermal amplification combined with nanoparticle-based lateral flow biosensor for the diagnosis of COVID-19. *Biosensors & Bioelectronics* 166.