



## Review article

## Laboratory detection of SARS-CoV-2: A review of the current literature and future perspectives

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## ABSTRACT

Nowadays, coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), whose infectivity is awfully strong, has been a major global threat to the public health. Since lung is the major target of SARS-CoV-2, the infection can lead to respiratory distress syndrome (RDS), multiple organ failure (MOF), and even death. The studies on viral structure and infection mechanism have found that angiotensin-converting enzyme 2 (ACE2), a pivotal enzyme affecting the organ-targeting in the RAS system, is the receptor of the SARS-CoV-2 virus. Currently, the detection of SARS-CoV-2 is mainly achieved using open plate real-time reverse-transcription polymerase chain reaction (RT-PCR). While open plate method has some limitations, such as a high false-negative rate, cumbersome manual operation, aerosol pollution and leakage risks. Therefore, a convenient method to rapidly detect SARS-CoV-2 virus is urgently and extremely required for timely epidemic control with the limited resources. In this review, the current real-time methods and principles for novel coronavirus detection are summarized, with the aim to provide a reference for real-time screening of coronavirus in areas with insufficient detection capacity and inadequate medical resources. The development and establishment of a rapid, simple, sensitive and specific system to detect SARS-CoV-2 is of vital importance for distinct diagnosis and effective treatment of the virus, especially in the flu season.

## 1. Introduction

The World Health Organization (WHO) has announced that coronavirus disease 2019 (COVID-19), an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as a worldwide pandemic on April 29, 2020 (Yang et al., 2020a,b). As of May 15, 2022, there have been totally 520,645,606 confirmed cases and 6287,235 deaths all around the world. It has been reported that the sequence of the major genes of SARS-CoV-2 is 5'-orf1a/b-s-e-m-n-3', including 5'-end methylated cap and 3'-end Poly A tail (Chen et al., 2020), which is in accordance with the structure of mRNA in eukaryotes. It has been reported that the virus specifically recognizes the receptor angiotensin-converting enzyme 2 (ACE2) on host cell membrane through the S protein on its surface (Wrapp et al., 2020; Letko et al., 2020), achieves fusion of virus and host cell membrane and then enters the host

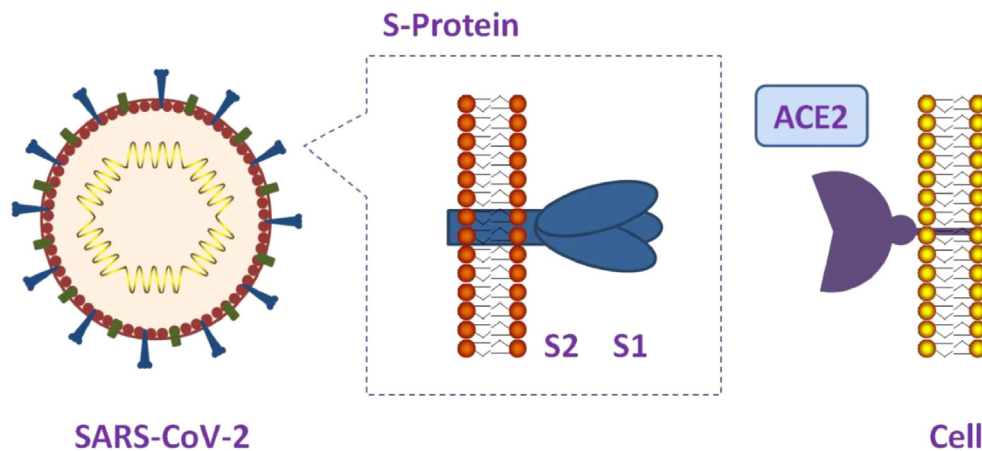
cell. After that, the protein translation system in host cell translates the 5'-end Orf1A/B gene of the virus and the RNA polymerase complex is produced. Via the genome of virus, the negative chain is generated, which serves as a template to generate the chain RNA sequence, thus finishing the duplication of the viral genome (Figure 1) (Mao et al., 2022). According to reports, normal or lowered white blood cell counts, reduced lymphocyte counts, elevated C-reactive protein (CPR) level and normal procalcitonin (PCT) appeared in most patients in the early phase of SARS-CoV-2 infection. Significant increases in proinflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor- $\gamma$  (IFN- $\gamma$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and typical cytokine storm have been found in patients infected with severe COVID-19 (Pan et al., 2020; Guo et al., 2019). The autopsy results of the first infested patient died of COVID-19 showed diffuse alveolar damage and lung hyaline membrane formation (Xu et al., 2020), which was in accordance with the symptoms

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**Figure 1.** Schematic diagram of interaction between SPIKE protein and ACE2 protein. The virus is able to specifically recognize ACE2 on the membrane of host cell through the S protein on its surface, achieves the fusion of virus and host cell membrane and then enters the host cell.

of acute respiratory distress syndrome and consistent with the pathological characteristics of SARS and MERS (Middle East Respiratory Syndrome). There is emerging information on histopathological variations in multiple organs, especially lungs (Xu et al., 2020; Tian et al., 2020; Zhang et al., 2020a,b). A recent research with single-cell RNA sequencing reported that ACE2 mainly expressed in germ cells, Leydig cells, and Sertoli cells in testis and ovaries, indicating that testis may be a potential target of SARS-CoV-2 infection (Yang et al., 2020a,b; Wang et al., 2020).

Nucleic acid testing (NAT) has a high sensitivity and low false negative rate, making it more sensitive and specific than other detection methods (such as antibody detection). At present, NAT with lab-based RT-PCR on oropharyngeal or nasopharyngeal swabs, the most common method used for detecting SARS-CoV-2, has been recognized as the gold standard for COVID-19 diagnosis by the National Health and Construction Commission of China (NHCCOC) and WHO. Nonetheless, this method shows some limitations including long time cost (about 1.5–2 h) for detection, and has specific and strict requirements for instruments, reagents, operators and laboratories, which may impede the effective prevention and control of the pandemic and exert potential adverse influence on public health.

Considering timely control of the pandemic, rapid and simple approaches for COVID-19 detection are urgently required. Point-of-care testing (POCT), also regarded as bedside testing, is a good supplement to standard laboratory testing. POCT is performed in the sampling area using portable analytical instruments plus matched reagents, making it possible for clinicians to quickly obtain the testing result.

The proper use of real-time detection could contribute to the rapid screening of virus and lessen the burden of standard laboratories. This paper summarizes the existing real-time methods for SARS-CoV-2 detection, hoping to provide useful information for medical institutions with insufficient detection capacity and scarce resources.

## 2. Methods of NAT

This part focuses on nucleic acid detection techniques, including RT-PCR, isothermal nucleic acid amplification (LAMP), gene editing and POCT detection. The advantages and disadvantages of these technologies are summarized in Table 1.

### 2.1. Reverse transcription polymerase chain reaction (RT-PCR)

Currently, RT-PCR is the most frequently-used method for SARS-CoV-2 detection, with various kits been exploited based on it. RT-PCR converts RNA into its complementary cDNA when reverse transcriptase exists, and then the specific regions of cDNA are amplified through PCR with chemiluminescent substances supplemented to the reaction system (Arumugam et al., 2020). During the process or at the end point for detection, fluorescent signals are read. Wang et al. (2021) evaluated the quality of 6 approved commercial nucleic acid assay kits, and the results showed that the lower limits of these kits differed greatly (Wang et al., 2020). When detecting SARS-CoV-2 with fluorescent RT-PCR, kits of poor detection limits and low sensitivity may produce false negative

**Table 1.** Advantages and disadvantages of nucleic acid detection techniques.

Testing methods	Testing time	LOD* (copies/ <i>ul</i> )	Advantage)	Disadvantage	Reference
Quantitative real-time PCR	1h	0.009–150	High sensitivity, specificity and accuracy, reducing product contamination and time of analysis	Difficulties in molecular probe design and quality of different batches of kits not ensured, leading to false-negative results	Wang et al. (2020)
Loop mediated isothermal amplification	30min	4.744	High specificity and sensitivity, simple operation, no need for PCR and expensive reagents, short reaction time, easy to detect the product, applicable in large-scale detection	Difficulty in primer design is challenging	Lu et al. (2020)
Gene editing	30min	NA	Fast detection speed, no complex laboratory equipment, lyophilized reaction reagents for long-term storage, high sensitivity and specificity, low detection cost, suitable for large-scale screening	Safety risk unclear	Sun et al. (2020)
Automated detection	<40min	4.6	No operator intervention, high throughput, standardized processes, reduction of labor cost	Higher equipment cost	Ding et al. (2020)
Nanopore amplicon sequencing detection	<15h	NA	Higher sensitivity and specificity based on the reference genome coverage in SARS-CoV-2 detection compared with RT-qPCR.	High costs and long detection time limited its application at this stage	Li et al. (2022)

\* Limit of detection (LOD), is defined as the amount of pathogen in a sample that would be missed at less than 5% of the time.

results. Therefore, laboratories should evaluate and compare kits produced by different manufacturers and batches before routine use so as to improve the detection accuracy. A multiplex RT-PCR method has been developed by Ishige et al. to detect Sarbecovirus specific E gene (E\_SAR-BECO) and specific gene (NIID-N) of SARS-CoV-2.

ABL proto-oncogene 1 (ABL1) can be used as the internal control to assess the quality of clinical samples and the detection quality of fluorescent RT-PCR (Ishige et al., 2020). ABL1 detects the SARS-CoV-2 RNA with low copy number (copy number <25), and its sensitivity to NIID-N is slightly higher than that of E\_SAR-BECO. By using NIID-N and E\_SAR-BECO as the target genes, SARS-CoV-2 detection can not only reach to a higher sensitivity but also save the use of reagents, the time and cost of clinical testing. Li et al. optimized the current fluorescent RT-PCR reaction system. The minimum dilution of viral RNA samples for SARS-CoV-2 detection was adjusted to 1/10 000 (initial value: 10 ng.  $\mu\text{L}^{-1}$ ) (Li et al., 2020a,b). The circulating value of clinical positive samples were all below 35 or 40, and the detection results were in line with those of the SARS-CoV-2 kits available on the market by and large. This optimized system is supposed to be a powerful tool for rapid clinical diagnosis.

The shortages of the application of laboratorial RT-PCR for real-time test are the large size of fluorescence detectors plus the complex operation (requiring professional personnel). These limitations have prompted researchers to optimize the detection procedure when maintaining the specificity and sensitivity of traditional RT-PCR method. During the amplification reaction of RT-PCR, thermal circulators are essential to perform the programmed temperature changes, but they are costly and therefore difficult to be equipped in areas with limited resources (Li et al., 2020a,b). Arumugam et al. have established a water bath heating instrument to replace the PCR circulator. It consists of two water baths: one for denaturation and the other for reverse transcription (Arumugam et al., 2020). The PCR tube is shuttled between the two water baths by a motor manipulator arm for the annealing and extension step. This equipment can process 96 samples at the same time with low cost and the reaction can be completed within 12 min via the thin film PCR reaction tube. Compared with the desktop PCR instrument, the whole procedure is significantly shortened, and the low cost makes it feasible in less developed areas. Table 2 lists some commercialized timely detection methods based on RT-PCR principle (Ye et al., 2022; NMPA, 2020).

## 2.2. Isothermal nucleic acid amplification technology

The loop-mediated isothermal amplification (LAMP) technique can achieve rapid amplification of nucleic acid under a constant temperature, which is the biggest difference between LAMP and PCR. The LAMP

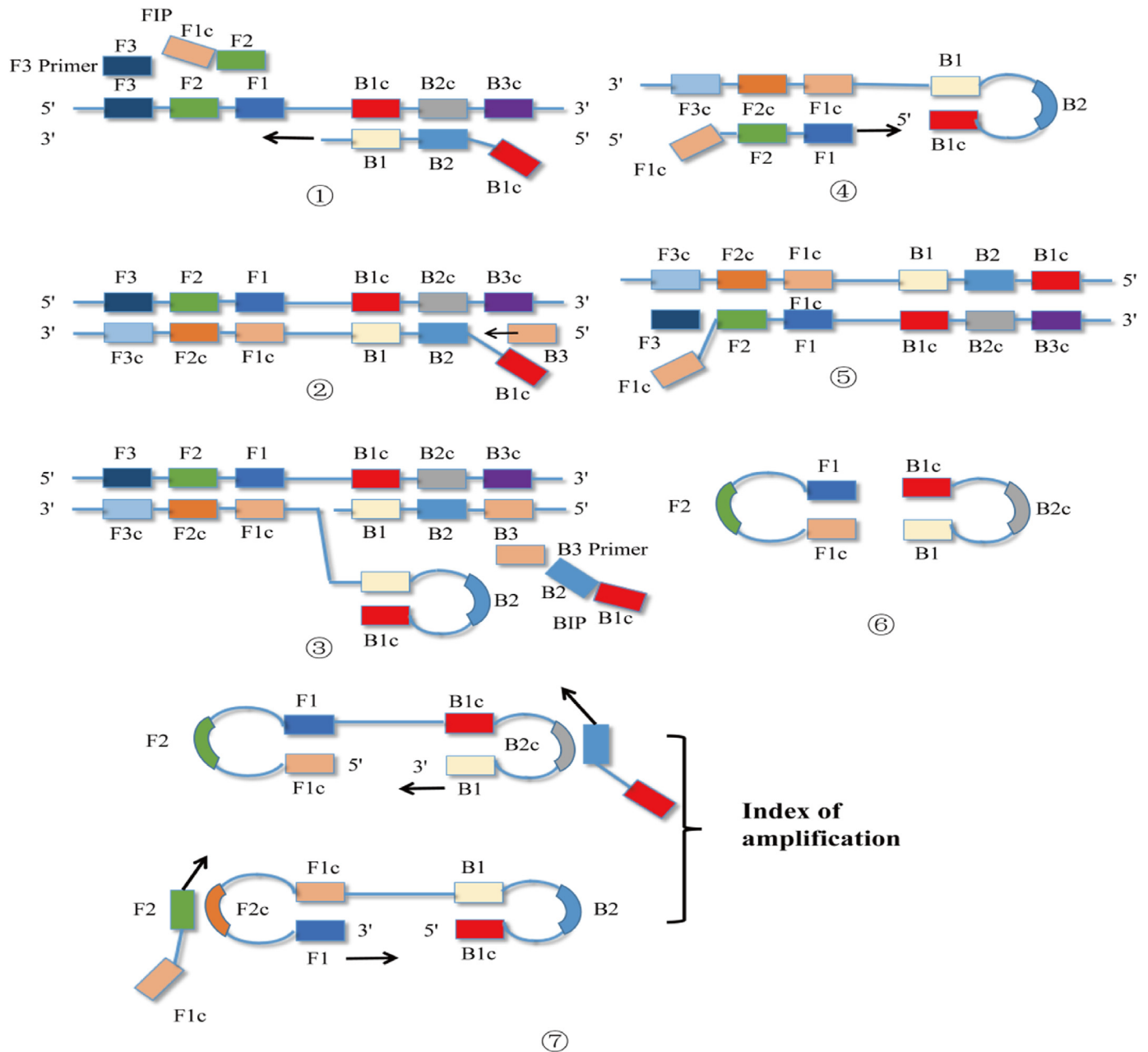
technique does not rely on an accurate temperature circulation system; thus, the amplification time of nucleic acid is shortened. The advantages of LAMP technology enable rapid detection and diagnosis of COVID-19, as well as a large-scale detection (Figure 2). LAMP is an efficient isothermal amplification technology (Shen et al., 2020). The amplification reaction relies on 4 primers to recognize 6 specific conserved DNA sequence and 1 strand replacement DNA polymerase at 65 °C, so that the synthesis of strand replacement DNA is in a continuous self-cycling process (Ackerman et al., 2020).

Compared with PCR, which requires three temperature cycles, LAMP can easily realize temperature control, reduce thermal energy consumption, and thus efficiently amplify nucleic acid within 15–60 min. The amplification process is shown in Figure 2. Yang et al. found that the ORF1Ab gene showed high specificity but low sensitivity, while the N gene exhibited high sensitivity but insufficient specificity. Simultaneous detection of ORF1ab, E and N genes insured the sensitivity and specificity of SARS-CoV-2 determination (Yang et al., 2020a,b). To improve the amplification efficiency and tolerance, Lu et al. developed a single-step single-tube mismatched amplification technique based on SARS-CoV-2 N gene using high-fidelity DNA polymerase, which could eliminate the mismatched base at the 3'-end of the primer during amplification, and has excellent tolerance with a detection limit of 118.6 copies each reaction (Lu et al., 2020). The detection speed of this assay is faster than that of normal LAMP, and the results can be obtained in less than 20 min. Isothermal amplification can be combined with microfluidic devices and used for the mass detection to provide more convenient, cheap, portable new crown detection method (Ganguli et al., 2020; Sun et al., 2020). A highly sensitive RT-LAMP detection kit compatible with the current reagents has been developed by Rabe and Cepko (2020) and can be used to read the detection results within 30 min by color method (Yang et al., 2020a,b). This kit can also be applied for SARS-CoV-2 detection in saliva. In order to further ameliorate the detection sensitivity while reduce the cost, virus RNA enrichment and sample handling methods were optimized in the aid of trichloroethyl phosphate (TCEP) and ethylenediaminetetraacetic acid (EDTA) to rapidly inactivate and lyse virus particles, while avoiding RNA destruction. The concentration of viral RNA is detected by a cheap and readily available silica particle suspension, which eliminates the need for specialized equipment and detection reagents. This method narrows the detection limit of RT-LAMP to 1 RNA copy per microliter at a cost of only US \$0.07 per sample. These improvements increase the availability of the SARS-CoV-2 test and facilitate the large-scale screening of the virus. Yan et al. designed a RT-LAMP detection method which targets the ORF1Ab and S genes in the open reading frame of SARS-CoV-2, and optimized the RT-LAMP detection method by comparing it with fluorescent RT-PCR (Yang et al., 2020a,b). Real-time turbidity monitoring as well as visual observation were utilized to evaluate the sensitivity and specificity of the detection method. Five primer sets were designed and LAMP real-time turbidimeter was performed to determine the amplification of ORF1ab and S genes, respectively. The results showed that ORF1AB-4 and S-123 amplified the genes within the minimum time. The optimal reaction temperature was 63 °C. This optimized detection method showed no cross-reaction with 60 other respiratory pathogens and exhibited a high specificity. To demonstrate the feasibility of this method, 130 samples from SARS-CoV-2 infected patients were collected. Among these samples, 58 cases were positively confirmed while 72 cases were tested negative. Thus, the sensitivity and specificity were both 100%.

Recently, El-Tholoth et al. (2021) and Song et al. (2021) described the protocol of a two-stage LAMP (COVID-19 Penn-RAMP) method, which can be performed using fluorescence or colorimetric assays in closed tubes. Although the operation of such design was similar to that of conventional RT-qPCR, they showed approximately 10-folds sensitivity for testing purified targets. However, even though significant progress has been achieved, these tests and approaches have not yet been applied to confirmed clinical specimens. Researches were relied on "mock" patient samples in which clinical swabs and blood samples were artificially

**Table 2.** Timely commercialized detection methods based on RT-PCR principle.

Trade name	Registered company	Target for detection	Detection time	Detectability
BioFire FilmArray	bioMérieux	S/M	45 min	500 copies/mL
Accula	Mesa Biotech	E	30 min	200 copy/reaction
QIAstat-Dx	QIAGEN	Orf1ab/E	1 h	1000 copies/mL
WizDx™ COVID-19 CrystalMix PCR kit	Wizbiosolutions	RdRP/E	1h	10 copies/ $\mu\text{L}$
Microchip RT-PCR COVID-19 Detection System	Lumex Instruments	N1/N2	50 min	$9 \times 10^3$ copies/mL
ARIES SARS-CoV-2	Luminex	ORF1ab/N1/N3	20 min	300 copy/reaction
COVID-19 Go-Strips	Biomeme	Orf1abS	1h	1.8 genome equivalent/microliter
Xpert Xpress SARS-CoV-2	Cepheid	E/N2	45 min	100 copies/mL



**Figure 2.** Schematic diagram of LAMP technique. The LAMP method does not rely on an accurate temperature circulation system; thus, the amplification time of nucleic acid is shortened. The advantages of LAMP technology enable rapid detection and diagnosis of COVID-19, as well as a large-scale detection ①- ⑥: Initial procedures to form dumbbell-like structure. ⑦: amplification of LAMP (Bao et al., 2020; Obande and Singh, 2020).

"adulterated" with SARSCOV-2RNA. Above all, Odiwuor et al. have presented the successful implementation of the LAMP strategy to detect SARS-CoV-2 viral RNA from patient cell lysis or purified RNA using visual colorimetric assays (Odiwuor et al., 2022).

In conclusion, LAMP detection method requires a shorter time for DNA amplification, strong specificity, and does not require a complex and precise temperature control system. Specialized equipment or trained professionals are not needed for result analysis, which can realize large-scale diagnostic testing and reduce the detection cost.

To achieve LAMP technology for amplification reaction, four specific primers, namely FIP, F3, BIP and B3, is designed mainly by the 3'-F1c, F2c, F3c region combined with the 5'-B1, B2, B3 region of the target gene. LAMP is mainly depended on the dynamic equilibrium state of DNA under constant temperature (about 65 °C), which involved with starting

step and cycling step. Target genes F1c and F1, F2c and F2, F3c and F3, B3c and B3, B2c and B2, B1c and B1 are complementary, respectively. F2 and F1c form FIP, while B1c and B1 complement each other to form BIP. LF is a forward loop primer, which can be combined with the loop structure between F1 and F2 to speed up the reaction. As a reverse loop primer, LB can bind to the loop structure between B1 and B2, which accelerates the reaction speed. The start-up time is very short. In the process of molecular binding replication, the target DNA is identified by isothermal amplification of nucleic acid through six specific sequences, which come from four specific primers. The normal nucleic acid amplification is initiated once these six specific sequences are completely matched. During the overall amplification, the cycle stage reaches >95%, and the recognition of target DNA depends on four specific sequences (FIP, BIP, LF and LB). Only when these four specific sequences are

completely matched and the constant cycle amplification can be carried out, thus ensuring the specificity and accuracy of isothermal amplification of nucleic acid (Bao et al., 2020; Obande and Singh, 2020).

### 2.3. CRISPR/Cas detection

#### 2.3.1. The CRISPR/Cas technique, a commonly-used gene-editing tool, can accurately recognize and cleave specific sequences

The principle of SHERLOCK (Specific Highsensitivity Enzymatic Reporter Unlocking) is shown in Figure 3. After reverse transcription of viral RNA into cDNA, isothermal amplification would be carried out. The DNA products are then transcribed back into RNA to amplify the target RNA sequence using T7RNA polymerase. Cas13a, a nonspecific RNase, is rapidly activated and binds to the targeted amplified RNA sequences. Meanwhile, nonspecific trans endonuclease cleavage (i.e., “side” cleavage) is performed on the nearby non-targeted RNA. Lysis of SSRNA reporter molecules help to discharge fluorescent dyes from quenching agents to amplify signal and detect nucleic acid. It also allows readout systems including fluorescence colorimetry and lateral flow to detect various targets quickly (Gootenberg et al., 2017). Zhang et al. reported the application of SHERLOCK in SARS-CoV-2 detection, which targeted the ORF1ab and S genes, followed by Cas13 cutting after isothermal amplification of RPA, and the results were read on commercial strips. The three-step detection only took about 1 h. However, the performance of CRISPR in detecting SARS-CoV-2 has only been assessed by synthetic RNA, with no patient samples used for detection. Hou et al. evaluated the sensitivity and specificity of SHERLOCK method targeting ORF1ab after RNA extraction (Hou et al., 2020). A 100% clinical sensitivity and good specificity of near single copy were achieved in 52 clinical samples with a turnaround time of only 40 min, demonstrating its diagnostic potential. SHERLOCK is a two-step reaction: RT-RPA amplification and then transcription back to RNA for Cas13 detection. Since two separate reaction steps are involved in the procedure, tube opening and solution handling adds not only the complexity of the method, but also the possibility of cross-contamination of samples; thus, it may not appropriate for detection without a well-controlled laboratory environment.

Recently, Zhang Feng's team developed a new method called STOP (Sherlock Test in One Pot) based on the original SHERLOCK method. This simplified STOP technique (STOPCOVID) has a higher sensitivity and more convenient operation for SARS-CoV-2 detection compared with the SARS-CoV-2 detection kit based on fluorescent RT-PCR (Zhang et al., 2020a,b). In addition, the magnetic bead-based RNA extraction and purification technology allows for a minimum detection of the RNA virus samples by the optimal STOPCovid. V2 (STOPCovid version 2) platform and the minimum detection limit was 100 copies or 33 copies/mL. It can detect the positive samples within 15–45 min with sensitivity and specificity up to 93.1% and 98.5%, respectively (Joung et al., 2020).

The difference between the STOPCOVID and SHERLOCK is that the detection platform of STOPCOVID integrates LAMP and CRISPR-involved detection methods, and transforms the classical two-step SHERLOCK procedure into a one-step process. STOPCOVID can be carried out at a

single temperature and require no sample extraction. It can quickly determine SARS-CoV-2 within 1 h with a simple visual reading, and thus is suitable for point-of-care use and immediate detection.

#### 2.3.2. DETECTR Cas12, CRISPR-Cas effector family member, is a target activated non-specific single-stranded deoxy ribonuclease (SSDNase), an RNA-guided DNA endonuclease that cleaves SSDNA indiscriminately upon binding to a target sequence

The principle of DNA endonuclease targeted Crispr trans reporter (DETECTR) is shown in Figure 4. When using DETECTR method, viral RNA is firstly converted to DNA, then the specific targeted DNA sequence activates Cas12a after isothermal amplification, which in turn cleaves SSDNA reporter probe to discharge visible fluorescent molecules, thus achieving sensitive and specific DNA detection. Fluorescence based detection is relatively more sensitive and is able to achieve high throughput detection. Huang et al. used the one-step RT-RPA method to amplify the target region from the viral RNA extracted from nasal swab, and transferred the amplified products to the 96-well microtitration plate for fluorescence detection. The fluorescence plate reader was used to read the signal, which was easy to realize automation. The detection time was about 50 min, and the detection limit was 2 copies per reaction. The sensitivity was better than that of qPCR method (Huang et al., 2020). This method can be used in most well-equipped clinical laboratories in automation. For its application in small clinics with limited resources, specialized equipment for immediate testing needs to be designed. CRISPR can also detect signals using a side-flow dipstick, which reads the results quickly without any equipment. Therefore, it is more proper to be performed for real-time detection in a non-laboratory environment. For single-sample test, it is a preferable choice with the advantages of low cost and portability. However, the sensitivity of the method is reduced due to the use of lateral flow strip (Chen et al., 2018; Broughton et al., 2020; Vandenberg et al., 2021).

When using the methods mentioned above, samples must be opened for liquid manipulation. To avoid this problem, Ding et al., 2019 developed an all-in-one Dual CRISPR-Cas12 (AIOD-CRISPR) detection method (Ding et al., 2020). All components involved in nucleic acid amplification as well as CRISPR detection were mixed in one single tube reaction system with a sensitivity of 4.6 copies per microliter.

Recently, Sun et al. demonstrated that they had developed a single-tube detection platform (OR-DETECTR) based on RT-RPA and DETECTR for the detection of SARS-CoV-2 by side-flow analysis and the LoD was as low as 2.5 copies/ $\mu$ L, which were in accord with that of RT-qPCR in testing patient samples (Sun et al., 2021).

Guo et al. established a viral nucleic acid detection platform integrating sample processing formula, recombination enzyme assisted proliferation (RAA) and CRISPR detection, with a detection limit of  $1 \times 10^4$  copies per ml (Guo et al., 2020). To develop the platform more proper for real-time detection, a portable cassette with blue LED was built for visualization. Ackerman et al. developed a CRISPR-based virus diagnosis system called combinational array response for multiple nucleic acid assessment (CARMEN) (Ackerman et al., 2020). The multiple functional

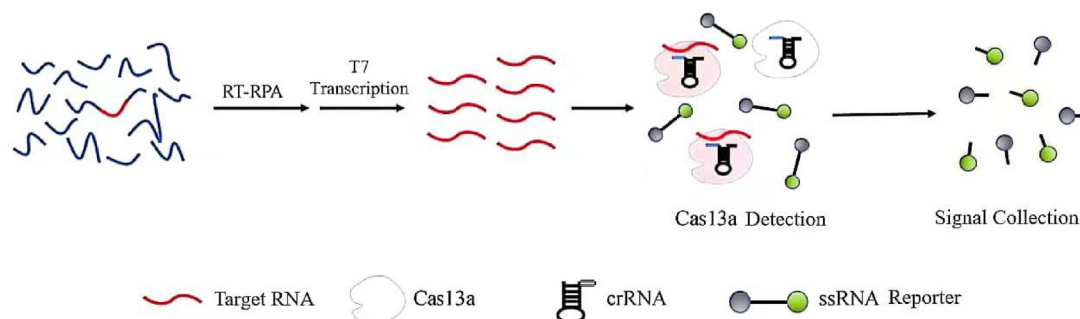


Figure 3. The illustration of SHERLOCK principle (Bao et al., 2020).

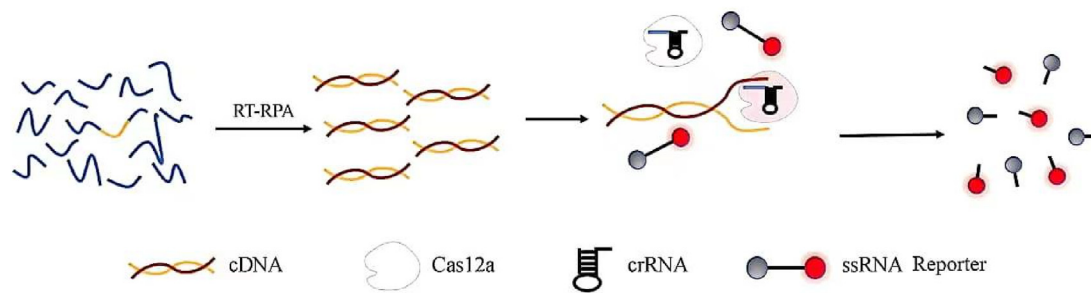


Figure 4. The illustration of DETECTR detection principle (Bao et al., 2020).

CRISPR-based CARMEN detection platform could scale up detection. In the CARMEN platform, a nucleic acid detection reagent mixture containing Cas13, sequence-specific CRISPR RNA (crRNA) was emulsified and a clipped reporter molecule was made into a droplet size. Similarly, the amplified samples to be tested were emulsified into droplet sizes, in which fluorescent dyes, sample droplets and a mixture of nucleic acid detection liquid were contained as identification symbols. Each micropore contained only 2 droplets collected through a single tube in the microfluidic system of the detection platform. If sequence-specific RNA detects its complementary target sequences, it will activate Cas protein cleavage cutting molecule to deliver fluorescence signal and thus to detect virus. Combined with CARMEN and Cas proteins, they are able to detect the virus causing human related diseases including SARS-CoV-2. The CARMEN testing platform can increase the amount of the sample being tested and therefore reduce the amount of test reagents and used samples. The sample can be tested in a larger dynamic range at a lower cost. This platform can realize large-scale clinical testing.

#### 2.4. Nanopore amplicon sequencing detection

Recently, Li et al. reported that nanopore amplicon sequencing (NAS), a detection on the basis of Oxford nanopore sequencing technology, exhibited improved sensitivity and accuracy. Meanwhile, this detection is time-saving demonstrated by involvement of viral genome amplification and nanopore sequencing in the detection of SARS-CoV-2. According to the coverage of reference genome, NAS showed not only high sensitivity (96.5%) but also high specificity (100%) when comparing with RT-qPCR (80.2% and 96.3%, respectively), even though the specimens were detected after storage for six months. The total test procedure costs less than 15 h, which was acceptable compared with that of RT-qPCR (~2.5 h). Above all, NAS approach could positively identify certain long-term stored specimens whose RT-qPCR analysis showed negative.

However, the application of NAS is limited at this stage due to its high costs and long detection time; thus, it is more proper to be posed as a supplementary test for RT-qPCR under the circumstances of the emergence of Omicron, an overwhelming variant. At the same time, the results of sequencing could be regarded as an indicator to monitor the dynamics of virus during infection. The study of Li et al. has demonstrated the wide application prospective of nanopore sequencing technology in SARS-CoV-2 detection and provided reference for the clinical identities of COVID-19. Some long-term stored specimens with negative RT-qPCR identification were examined positive via NAS method, which could also impetus the development of detection approaches for critical clinical pathogens in a more rapid and sensitive manner to further improve the diagnosis and control measurements of infectious diseases (Li et al., 2022).

### 3. The POCT platforms relied on the principle of RT-PCR to test SARS-CoV-2

Commercial point-of-care testing methods typically integrate the extraction, amplification and detection procedures through developing

complementary instrumentation, packing all reagents in one single box, and using mechanical manipulation or microfluidic technology to achieve basic automation, minimize manual operation, reduce total test time, and improve turnover velocity. Since March 2020, the U.S. Food and Drug Administration (FDA) has granted EUAs for several tests. It is noteworthy that an increasing number of commercialized point-of-care tests based on RT-PCR principles are being developed (FDA, 2020). Current point-of-care assays for SARS-CoV-2 testing that are exempt from the Clinical Laboratory Improvement Amendment Quality Standards (CLIA) are Xpert Xpress SARS-CoV-2 from Cepheid and Accula SARS-CoV-2 from Mesa Biotech. Xpert Xpress SARS-CoV-2 (Cepheid) detects both E and N2 gene targets by RT-PCR, and results can be obtained in about 45 min, automating the entire process and requiring no special training for the user (Moran et al., 2020). Many teams have compared Xpert Xpress SARS-CoV-2 with laboratory-based RT-PCR in clinical samples. The results showed excellent agreement (>99%) and reliability even in detecting lower levels of virus. Mesa Biotech's Accula combines RT-PCR molecular diagnostics and side-flow analysis to produce results in just 30 min, and is portable for that its size is merely similar to the palm of your hand. To estimate the performance of the Accula assay, Hogan et al. compared its testing results in 100 nasopharyngeal swab samples and found an overall agreement of 84.0% and a positive agreement of only 68.0% (Hogan et al., 2020). For samples with low viral load, positive consistency is low and false negatives are prone to occur. This suggests that the Accula instant test is not highly sensitive and the balance between the potential advantages of point-of-care testing and reduced diagnostic accuracy should be carefully considered. There are also some commercial platforms that enable multiplex PCR (mPCR), such as BioFire FilmArray (bioMerieux) who combines nested multiplex PCR with microfluidic chip technology to realize quick test within 1 h. Nested PCR technology can minimize the interference of other non-specific pathogen nucleic acid substances, while improving the sensitivity of the detection (NMPA, 2020). Besides, DVBOX (Detect Virus get BOX), also known as poison BOX, is a test equipment with fully closed, highly sensitive decay flow control, which is a candidate for coronavirus POCT nucleic acid detection. The equipment consists of a microfluidic chip integrated with reagents, analytical software and instrument platform by independent research and development. Among them, there is a programmable digital control linear motor with high speed and precision positioning, which is debut in China. DVBox demonstrated many advantages including low operating environment requirements, high sensitivity and low false negative rate, low testing cost, no need for sample transportation, BSL-2 laboratory environment, P3 level protection, fewer manual operation steps and shorter testing time. They could achieve the phenomenon called "after the sample input, the result is output" without a large number of professional protection and experimental operation training of technical personnel. Compared with the existing novel Coronavirus nucleic acid detection platform (Obande and Singh, 2020), the LOD value of the existing novel Coronavirus nucleic acid detection platform is much lower than other products. The minimum detection is 10 copies/mL Virus content while sensitivity is an order of magnitude higher than other products (Table 3).

**Table 3.** Comparison of technical parameters of high-throughput novel coronavirus nucleic acid detection Platforms.

Brand and Products	DVBOX	Cepheid GeneXpert	Bio Merieux FilmArray	GenMark	Accula
Detection principle	Fluorescence quantitative PCR	Fluorescence quantitative PCR	Fluorescence quantitative PCR	Fluorescence quantitative PCR	Fluorescence quantitative PCR
Extraction technology	Centrifugal column	After pyrolysis, RT-PCR was performed by magnetic bead enrichment	After pyrolysis, RT-PCR was performed by magnetic bead enrichment	After pyrolysis, RT-PCR was performed by magnetic bead enrichment	RT-PCR for molecular diagnosis and lateral flow analysis
Laboratory requirements	Fully enclosed chip, no laboratory, 10 square meters of sampling room can be tested	It needs to be operated in a BSL-2 environment and nucleic acid extraction is required	It needs to operate in a BSL-2 environment, manually adding reagents and samples	Need to operate in a BSL-2 environment	NA
Running time	50–60min	30–45min	50–70min	60–80min	30 min
Flux	1–24	4–80	1–12	4–80	NA
Detection of targets	ORF1ab/N	E/N2	S/M	ORF1ab/N	E
Detection limits of matching reagents	10 copies/mL	250 copies/mL	330 copies/mL	250 copies/mL	200 copies/reaction

#### 4. Conclusion and prospects

POCT testing should be of high sensitivity to infections while inexpensive in price, and should be repeatedly performed per week to help preventing viral transmission by asymptomatic individuals (Mina et al., 2020). Although RT-qPCR is of preeminent sensitivity and specificity and is regarded as the "gold standard" for distinct COVID-19 detection, its application as POCT in remote areas is limited because of the need of complicated equipment, trained personnel, and matched reagents. In recent years, the LAMP method has emerged as an alternative to PCR owing to its convenience and simplicity, low expenditure, short analysis time, and no need for complex equipment (Van dongen et al., 2020). However, low temperature may lead to nonspecific amplification and false-positive results, and POCT testing still faces challenges in the reliability of clinical pathogen detection. CRISPR platforms with highly specific sgRNAs for target sequences can prevent the identification of untargeted products without affecting the results. The establishment of SHERLOCK, DETECTR and other nucleic acid detection technologies on the basis of CRISPR/Cas system has the characteristics of high specificity, high sensitivity, simple preparation, short time consumption and multiple detection, which can be used for field deployment and rapid diagnostic testing, and can also be used for virus identification and detection, which also promotes the development of the next generation of nucleic acid detection technology. The first SARS-CoV-2 diagnostic assay relied on SHERLOCK has been approved by FDA. With the continuous in-depth research on these rapid detection technologies, more rapid nucleic acid detection technologies on the basis of CRISPR/Cas system will be applied in the COVID field in the future (Huang et al., 2020).

In short, each nucleic acid detection technology has its own advantages and disadvantages, which should be coordinated in practical application to improve the efficiency of diagnosis more accurately.

#### Declarations

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Data included in article/supplementary material/referenced in article.

#### Declaration of interests statement

The authors declare no conflict of interest.

#### Additional information

No additional information is available for this paper.

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