

REVIEW

Recent advances and clinical application in point-of-care testing of SARS-CoV-2

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Abstract

The novel coronavirus 2019 (COVID-19) caused by SARS-CoV-2 spread rapidly worldwide, posing a severe threat to public life and health. It is significant to realize rapid testing and timely control of epidemic situations under the condition of limited resources. However, laboratory-based standardized nucleic acid detection methods have a long turnaround time and high cost, so it is urgent to develop convenient methods for detecting COVID-19. This paper summarizes the point-of-care testing (POCT) developed for novel coronavirus from three aspects: nucleic acid extraction, nucleic acid amplification, and detection methods. This paper introduces a commercial real-time detection system that integrates the abovementioned three steps and the matters needing attention in use. The primary purpose of this review is to provide a reference for emergency response and rapid deployment of COVID-19 and some other emerging infectious diseases.

KEYWORDS

COVID-19, instant detection, novel coronavirus, nucleic acid detection

1 | INTRODUCTION

COVID-19 has transmitted globally since its first appearance in late 2019, and now it has become a global pandemic, which poses a severe threat to the global economy and public health. Many studies have shown that SARS-CoV-2 is a single-stranded positive-strand RNA virus. At present, the gene targets used to detect SARS-CoV-2 include nucleocapsid (N), envelope (E), spike (S), RNA-dependent RNA polymerase (RdRP), and open reading frame 1ab (ORF1ab) genes.¹ The nucleic acid-based detection method has the advantage of high sensitivity, which is more sensitive and specific than the COVID-19 antibody detection method. Therefore, at present, reverse transcription-PCR (RT-PCR) of viral RNA is widely used in various countries to detect COVID-19 in the oropharynx

or nasopharyngeal swabs. RT-PCR is regarded as an essential basis for the diagnosis of COVID-19 by the WHO and China Health Construction Committee, and it has become the gold standard for the diagnosis of COVID-19. However, this method requires expensive large-scale instruments, specialized reagents, professional operators, and a complete standard laboratory that meets the biosafety conditions, making it unsuitable for timely detection.² As a result, it is impossible to detect suspected cases promptly and quickly in the first place, which delays the prevention and control of epidemic situations and has a potential negative impact on public safety.^{3,4} Therefore, a rapid and convenient method for detecting COVID-19 is urgently needed in the clinic with limited resources. Thus, the real-time detection of COVID-19 nucleic acid came into being.^{5,6} Point-of-care testing (POCT) is also known as bedside

detection and has become an excellent supplement to RT-PCR in standard laboratories.⁷ It refers to a detection method carried out at the sampling site and uses portable analytical instruments and matching reagents to obtain results quickly. Real-time detection is not only suitable for clinical laboratories but also performed by nonlaboratory personnel with simple training at the sample collection site. In addition, real-time detection requires only a few operation steps, and the results can be read intuitively. It has the advantages of accuracy, rapidity, portability, simplicity, and low cost, conducive to faster clinical decision-making and faster screening. Therefore, it is more likely to control the epidemic situation in time.

Although rapid detection has many advantages, it also brings some new problems and puzzles to the clinical laboratory at the same time. This paper introduces the technical principle of nucleic acid POCT products in novel coronavirus, the advantages and disadvantages of existing commercial integration instant detection methods, notes, and quality assurance of the POCT nucleic acid detection system for SARS-CoV-2, and the future development direction of nucleic acid POCT products.

2 | THE TECHNICAL PRINCIPLE OF NOVEL CORONAVIRUS NUCLEIC ACID POCT PRODUCTS

The core of nucleic acid POCT technology integrates nucleic acid extraction, amplification, and detection and automatically completes detection and result analysis.⁸

2.1 | Nucleic acid extraction

The extraction of nucleic acids is an extremely critical step in nucleic acid molecular detection (Figure 1). To realize on-the-spot rapid detection, simplified nucleic acid extraction, also known as nucleic acid extraction-free technology, is generally adopted in COVID-19's real-time nucleic acid detection products. The simplified nucleic acid extraction process is more straightforward than the classical kit method and fully automated nucleic acid extraction technology. The steps are faster, and no additional instruments or equipment are needed. Direct lysis based on high temperature and solution is the most commonly used method to simplify the process of nucleic acid extraction. The direct method based on high temperature can release nucleic acids in a few minutes. It is a common physical lysis method that destroys the capsid of the virus using a metal bath or boiling water and then roughly extracts DNA in a short time. Its obvious advantages are simple processing, simple instrumentation, and easy operation. More specifically, only a common heating device is needed to extract nucleic acids quickly. Analogously, the solution-based direct lysis method destroys pathogens and releases RNA by adding efficient lysis buffer into samples. Another effective nucleic acid extraction method is a one-step method based on solution lysis combined with high temperature.⁹ The one-step method is more straightforward than the multistep method, which reduces the possibility of opening the lid.¹⁰ Its advantages are no complicated sample processing and nucleic acid

extraction process, significantly shortened detection period, and simple operation. In addition, like Biousta, there are also classical methods of nucleic acid extraction by magnetic beads and nucleic acid amplification, realizing the rapid detection of POCT nucleic acids with "samples in and results out."

2.2 | Nucleic acid amplification (Figure 2)

2.2.1 | Reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR is the most common method to detect COVID-19 nucleic acid, and the number of kits developed using this principle is also the largest.¹¹ The principle of RT-PCR is that reverse transcriptase is used to convert RNA into its complementary cDNA, the specific region of the cDNA is amplified by polymerase chain reaction (PCR), chemiluminescent substances are added into the system for amplification, and the fluorescence signals are read in the processor at the endpoint to realize detection. The main obstacles of applying laboratory-based RT-PCR in real-time detection include the following: real-time fluorescence detectors are usually bulky and complicated to operate, professionals are required, and the turnaround time is extended. These limitations in real-time detection prompt researchers to explore ways to simplify and shorten the process while maintaining the sensitivity and specificity of conventional RT-PCR.¹² In the sample amplification stage of RT-PCR, a thermal cycler is needed to realize the program temperature change. Nevertheless, the cost of the thermal cycler is high, so it is difficult to equip it in large quantities in areas where resources are scarce.

2.2.2 | Isothermal amplification of nucleic acid

Isothermal amplification can rapidly amplify nucleic acids at a constant temperature without a thermal cycling step. It is the most promising alternative method of RT-PCR, which can detect viral RNA at a level similar to RT-PCR and is more suitable for real-time detection. At present, the commonly used isothermal amplification technologies for real-time detection include loop-mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and nicking enzyme-assisted reaction (NEAR).¹³

Reverse transcription loop-mediated isothermal amplification (RT-LAMP)

RT-LAMP first reverse transcribes the RNA genome of SARS-CoV-2 into cDNA, then designs four specially designed primers that can bind to six different regions of the target genome, and uses DNA polymerase with strand displacement activity instead of thermal denaturation to generate a single-stranded template. There were two internal primers and two external primers in the four-primer system. The 3' end of the forward primer initiates the synthesis of the initial DNA strand and is then replaced by synthesis initiated by the forward external primer. The reverse complementary sequence at the 5' end

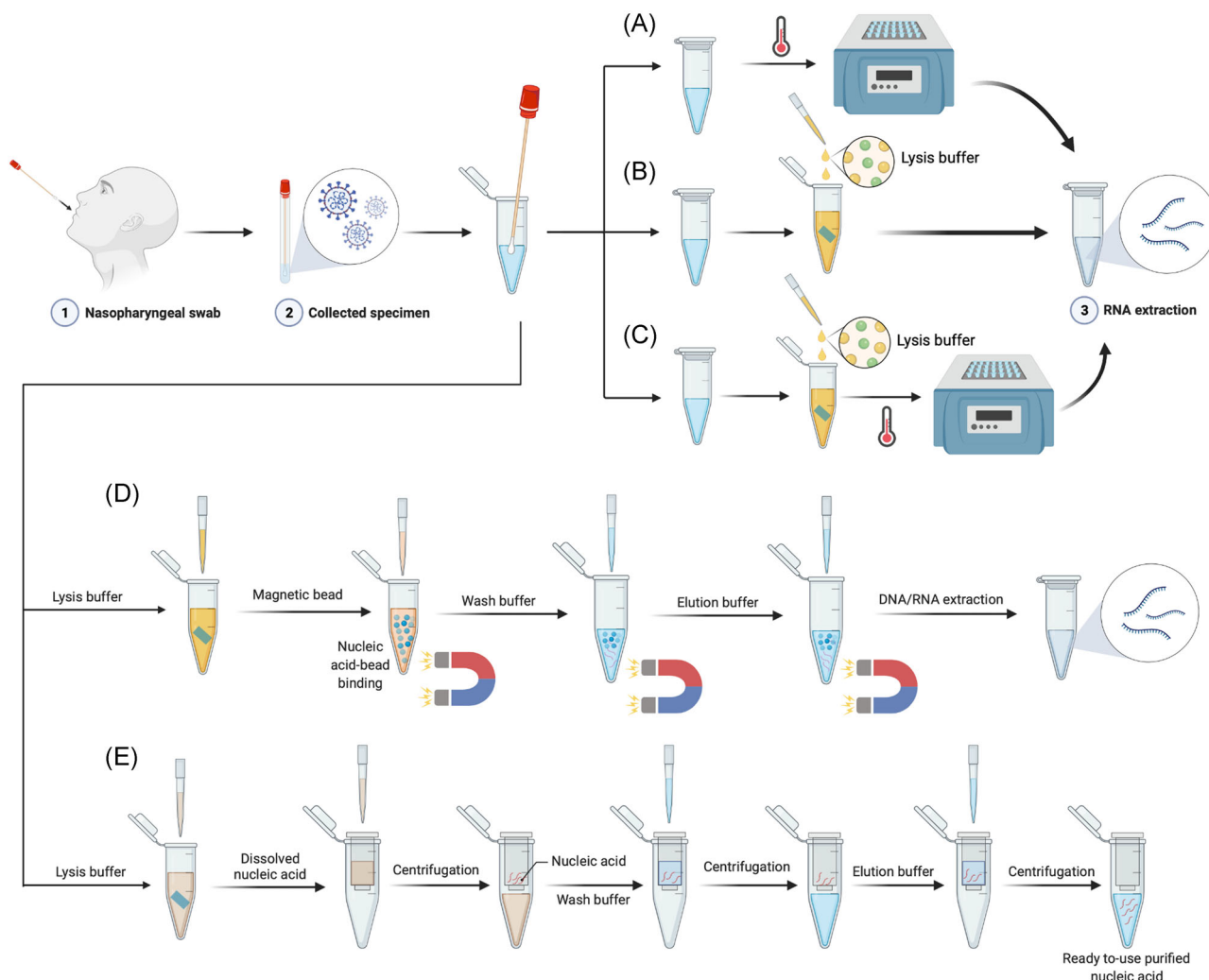


FIGURE 1 Principle of nucleic acid extraction in point-of-care testing of SARS-CoV-2. Schematic diagram of high temperature lysis (A), solution lysis (B), solution lysis combined with high temperature (C), magnetic bead extraction method (D), and spin-column-based method (E)

of the forward primer anneals with the downstream sequence in the substituted ssDNA chain to form a loop. Then, the ssDNA on each terminal loop acts as the start of the LAMP amplification cycle so that the target sequence is exponentially amplified. LAMP has high specificity and sensitivity and is easy to perform. It can be finished in less than 1 h at a constant temperature of 60–65°C, avoiding using a thermal cycler. The assay has a high specificity, sensitivity, and robust reproducibility. Its results can be monitored using a real-time PCR machine or visualized via a colorimetric change from red to yellow. The assay's limit of detection (LOD) is 118.6 copies of SARS-CoV-2 RNA per 25 µl reaction.¹⁴ The reaction can be completed within 30 min for real-time fluorescence monitoring or 40 min for visual detection when the template input is more than 200 copies per 25 µl reaction.¹⁴ However, because LAMP needs to be amplified at 60–65°C, it still needs external equipment such as a water bath and hot plate to provide constant temperature, which is not conducive to miniaturization and portability of the equipment. Therefore, it is more promising to develop isothermal amplification methods at room temperature in the future.

Reverse transcriptase-polymerase amplification (RPA)

RPA is also an isothermal amplification method of nucleic acids. Compared with LAMP, the reaction temperature RPA is lower, and the whole reaction can be realized at normal temperature without external heating components. RPA mainly depends on three enzymes: recombinase, which can bind single-stranded nucleic acids, single-stranded DNA binding protein (SSB), and strand displacement DNA polymerase. The protein–DNA complex formed by recombinant enzymes and primers targets the homologous sequence in template DNA. A strand displacement reaction occurs through strand displacement DNA polymerase, DNA synthesis is started, and the target region on the template is exponentially amplified. The substituted DNA single-strand binds to SSB to prevent further substitution from stabilizing the open duplex structure. Real-time fluorescence quantification was performed by combining the Exo probe. Furthermore, it can also be combined with an info probe to use lateral flow test strips to detect or add dye to visually detect and read, which will be more suitable for real-time detection. The single-tube RT-RPA method based on the Exo probe is 100% consistent with RT-qPCR.¹⁵ The detection limit can reach 7.74 copies per reaction. The

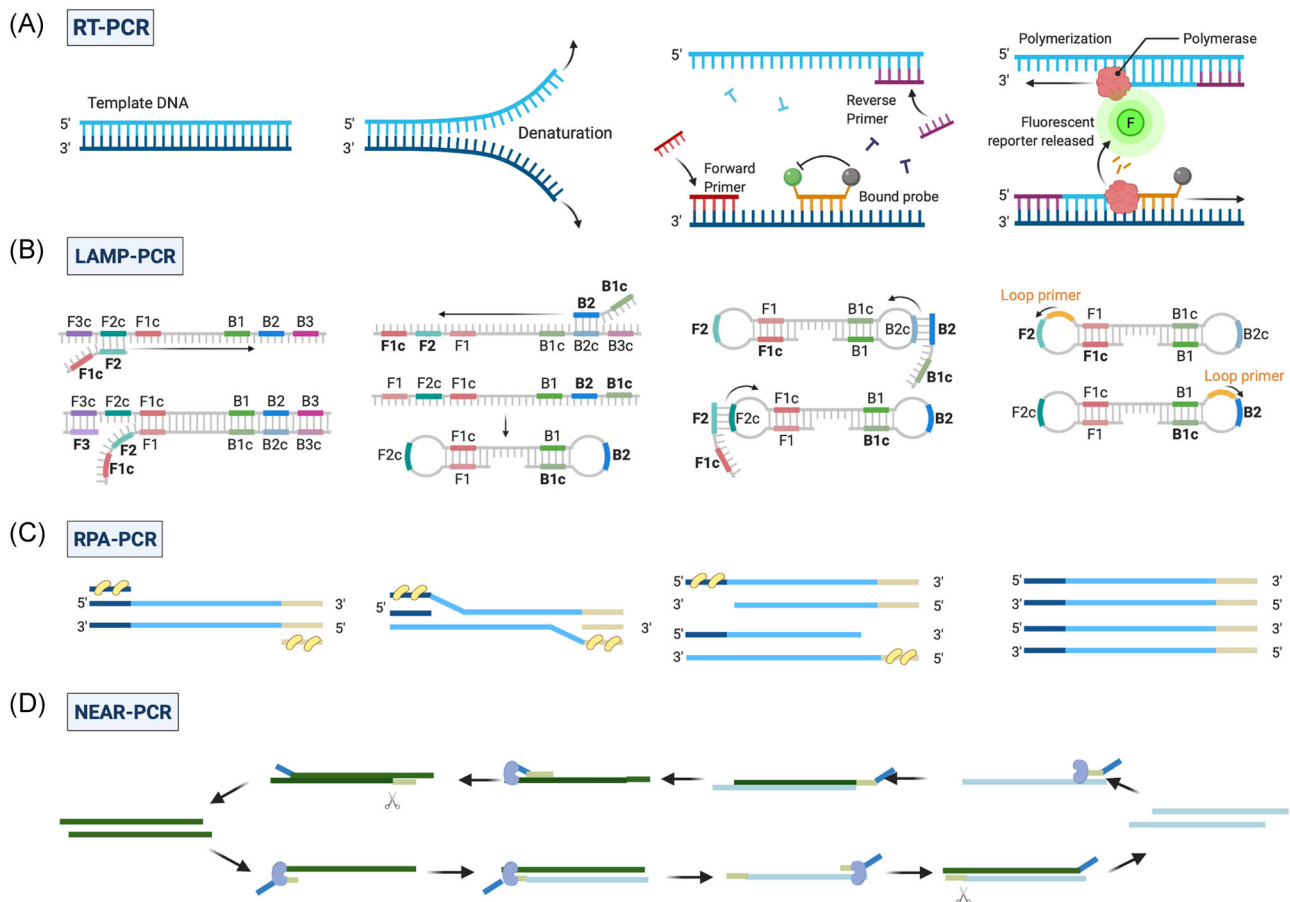


FIGURE 2 Principle of nucleic acid amplification in point-of-care testing of SARS-CoV-2. Schematic diagram of RT-PCR (A), LAMP-PCR (B), RPA-PCR (C), and NEAR-PCR (D)

results can be obtained within 7 min at the earliest, which is one of the fastest nucleic acid-based detection methods for SARS-CoV-2 thus far.¹⁶ RPA has high sensitivity and can be amplified at room temperature without heating parts, and the reaction speed is faster than that of LAMP. However, the application of this method in real-time detection is limited by patent protection.

Enzyme amplification reaction (NEAR)

NEAR is driven by reverse transcriptase, nicking enzymes, and isothermal amplification DNA polymerase. The template hybridizes with the primer, the extension product is replaced by the next template, the complementary strand of the replacement product is extended to form the nicking enzyme recognition site, and the nicking enzyme recognizes and cuts the specific short sequence of one strand in the double-stranded DNA to form a gap. Constant temperature-amplified DNA polymerase extends nucleotides from the 3' end of primers to synthesize short sequences, thus obtaining double-stranded NEAR amplification. The target DNA template is continuously amplified through cutting and extension cycles, and the molecular beacon is designed to generate fluorescent signals for quantification.¹⁷ NEAR is amplified exponentially, so this method has a high reaction speed and sensitivity. The disadvantage is that the design of short sequences increases the false positive rate.

2.3 | Detection method (Figure 3)

2.3.1 | Real-time fluorescence detection

The principle of real-time fluorescence detection is to add chemiluminescent substances to the system to amplify the fluorescence signals read in the process to realize quantification. The commonly used RT-PCR methods are the embedding fluorescent dye method and TaqMan probe method. The method of embedding fluorescent dyes refers to using fluorescent dyes such as SybrGreen, which can combine with DNA amplification products nonspecifically. The TaqMan probe method refers to designing a short oligonucleotide probe containing a 5' fluorophore and a 3' quenching group to anneal with the sequence in the DNA template, and Taq polymerase cleaves the fluorescent group on the annealed probe through its 5'-3' nucleic acid exonuclease activity to make it fluoresce. Quantification can be carried out by measuring the number of amplification cycles (C_t) when the fluorescence signal of amplification products reaches the fluorescence threshold. Fluorescence needs to be detected by a real-time fluorescence detector. The real-time fluorescence detector is miniaturized to integrate sample extraction and amplification and make the results easy to read, which can also be used in a real-time

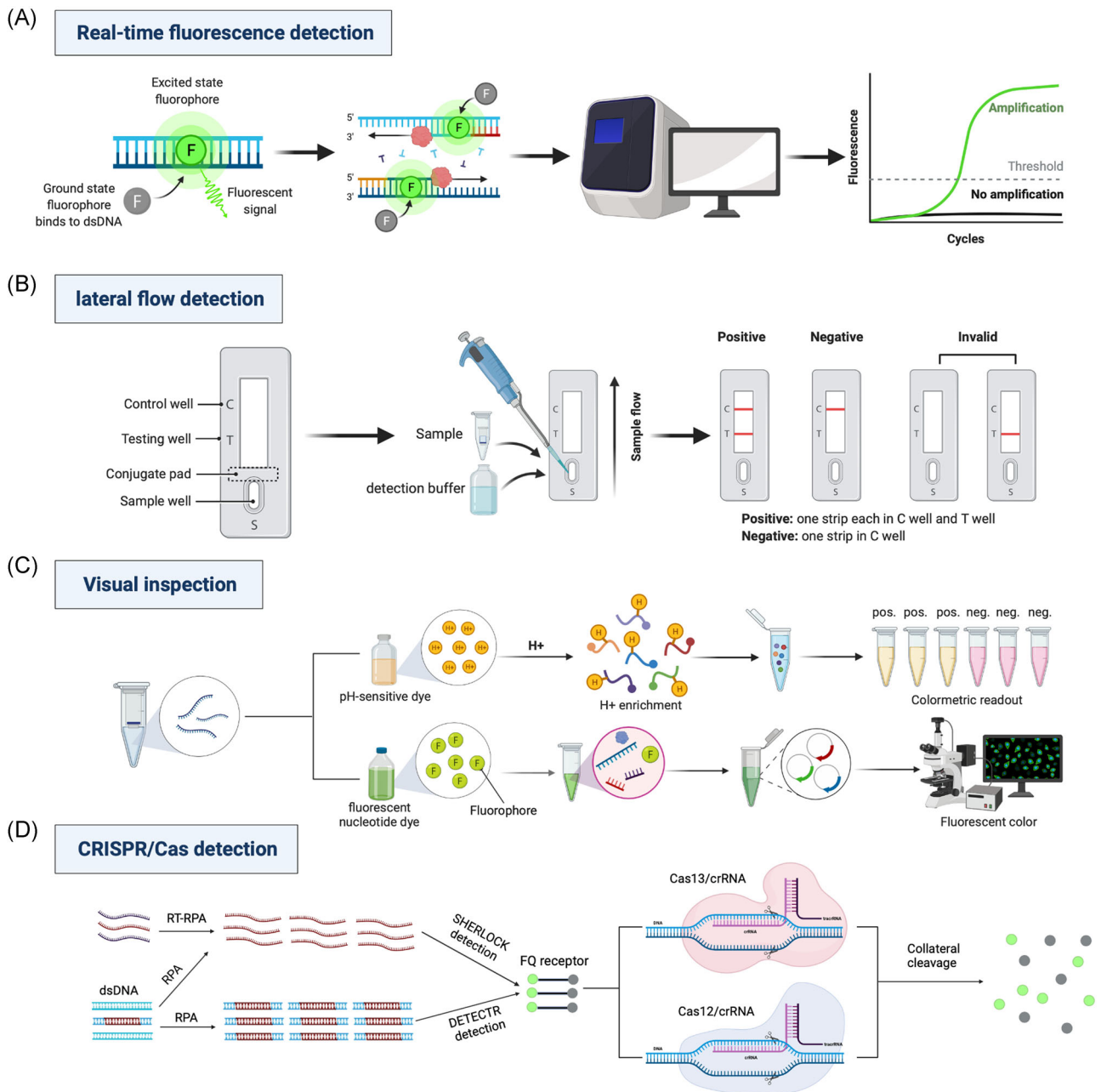


FIGURE 3 Detection method in point-of-care testing of SARS-CoV-2. Schematic diagram of Real-time fluorescence detection (A), Lateral flow detection (B), Visual inspection (C), and CRISPR/Cas detection (D)

detection environment. However, due to the need for specialized instruments, there are limitations in cost, popularization, and application.

2.3.2 | Lateral flow detection

Paper-based lateral flow analysis (LFA) is a potential tool for home detection or detection in resource-poor areas because of its low cost, easy manufacture, and full compatibility with real-time detection. The most common method is a colorimetric determination by color

change caused by the aggregation of AuNPs.¹⁸ At present, the Accula (MesaBiotech) company has developed commercial instruments using lateral flow technology as the detection method.

2.3.3 | Visual inspection

Compared with fluorescence detection, which requires special instruments to read signals, visual detection can be observed by the naked eye, making it more straightforward and intuitive to read the results and more suitable for real-time detection. Visualization

includes colorimetry with pH-sensitive dyes, turbidity methods, or fluorescent dye methods. Visualization is often used in isothermal amplification, which can further adapt to the use scene of real-time detection, reduce the professional requirements of operators, and do not need special equipment. However, when compared with fluorescence detection, the sensitivity of visual inspection decreases. Combining smartphone imaging and sensing platforms can realize the quantification of visual results, which is more objective than visual judgment and can improve detection accuracy.¹⁹

2.3.4 | CRISPR/Cas detection

The CRISPR/Cas system is a common tool for gene editing that can accurately identify and cut specific sequences. In contrast, the activated Cas enzyme can precisely cut target RNA and nonspecifically cut RNA or DNA in the surrounding environments, which can be used to detect a nucleic acid.

SHERLOCK

Specific high-sensitivity enzymatic reporter unlocking (Sherlock) reverse transcribes the viral RNA target into cDNA and then amplifies it by isothermal amplification technology. The amplified product is transcribed back to RNA by T7RNA polymerase to amplify the target RNA. Cas13a is a nonspecific RNase activated after recognition and combined with targeted amplified RNA products and performs nonspecific transendonuclease cleavage on nearby nontargeted RNA. Therefore, the ssRNA reporter molecule was split to release fluorescent dye from the quencher for signal amplification and nucleic acid detection. This method also allows reading methods to detect various targets quickly, such as colorimetry and lateral flow.²⁰ Traditional SHERLOCK involves two separate reaction steps, so it needs liquid treatment and tube opening, which increases the complexity and dramatically increases the possibility of sample cross-contamination. Hence, it is only suitable for standard laboratories. To make SHERLOCK more widely used in the field of real-time detection, Joung et al.²¹ combined LAMP with CRISPR-mediated detection steps, and the classic two-step SHERLOCK was transformed into a single-step reaction without sample extraction. The detection limit of this method can reach 100 copies, and the commercial lateral flow test strip or fluorescence reading test can complete the test within 1 h, making it suitable for real-time testing.²¹

DETECTOR

Cas12, another member of the CRISPR/Cas effector family, has the characteristic of target-activated nonspecific single-stranded deoxyribonuclease (ssDNase) and is an RNA-guided DNA endonuclease.²² Viral RNA is first transformed into DNA, and then the specific target sequence in isothermal amplified DNA activates Cas12a. Next, Cas12a sequentially cuts the ssDNA reporter probe to release macroscopic fluorescent molecules.²² This method can realize sensitive and specific DNA detection and can easily realize high throughput and automation. Meanwhile, the detection limit is two copies per reaction, and the sensitivity is better than qPCR.²³ However, there is

still a lack of related real-time detection equipment. Of course, CRISPR can also use lateral flow test strips to detect signals, but the detection sensitivity will be reduced.²⁴ An RNA-based CRISPR/Cas strategy combined with isothermal amplification can improve the sensitivity, specificity, and reliability of isothermal amplification. CRISPR detection can also be coupled with lateral flow reading, which is quite suitable for home detection, showing broad prospects for developing next-generation molecular diagnostic technology and the application of real-time detection.

3 | ADVANTAGES AND DISADVANTAGES OF COMMERCIAL INTEGRATED REAL-TIME DETECTION METHODS

3.1 | The current POCT tests for SARS-CoV-2 in the FDA site

Commercial real-time detection methods usually realize the integration of extraction, amplification, and detection processes by developing supporting equipment. All reagents are packed in a box. Automation can be basically realized using mechanical operation or microfluidic technology, manual operation can be minimized, total test time can be reduced, and turnover speed can be increased. Starting from March 2020, the U.S. Food and Drug Administration (FDA) has awarded EUA to several tests (<https://www.fda.gov/medical-devices/coronavirus-disease-2019-covid-19-emergency-use-authorizations-medical-devices/invitro-diagnostics-euas-molecular-diagnostic-tests-sars-cov-2#amendment>, accessed 1/8/2022). These tests are the Xpert Xpress SARS-CoV-2, Visby COVID-19, Roche Diagnostics Cobas 6800 SARS-CoV-2 test, Mesa BioTech Accula SARS-CoV-2, Abbott ID NOW, QIAstat-Dx, BioFire FilmArray, Cue COVID-19 Test, and Detect Covid-19 Test.

RT-PCR is the most commonly used real-time detection method and has been widely used in most commercial reagents for real-time detection. For example, Xpert Xpress SARS-CoV-2 from Cepheid, Visby COVID-19 from Visby Medical Inc., Accula SARS-CoV-2 from Mesa Biotech, Roche Cobas 6800 SARS-CoV-2 test from Roche Molecular Systems, Inc. and Abbott ID NOW from Abbott Diagnostics Scarborough, Inc. all adopt the detection principle of RT-PCR. More specifically, two targets of the E gene and N2 gene were detected through the detection principle of RT-PCR in Xpress SARS-CoV-2 from Cepheid company. The whole detection process was automated and completed in just 45 min. Moreover, it is worth mentioning that the test does not require special training.^{25,26} Xpert Xpress SARS-COV-2 has accurate and reliable test results. Many studies have confirmed excellent consistency (>99%) with laboratory-based RT-PCR. At the same time, the LOD of Xpert Xpress SARS-CoV-2 was low, with a specific value of only 8.26 copies/ml.^{27,28}

Many studies have demonstrated that Visby COVID-19 meets the requirements of rapid testing of SARS-CoV-2 and has a high concordance with central laboratory-based RT-qPCR methods. When detecting SARS-CoV-2 RNA, the sensitivity and specificity of RT-PCR POC technology are 95% and 100%, respectively.²⁹ The detection limit was as low as or lower

than 500 copies/ml.³⁰ RT-PCR molecular diagnosis technology was combined with LFA technology in the Accula of Mesa Biotech company. The instruments used in this detection process are compact and easy to operate. It takes only half an hour to complete the detection and obtain the results. One hundred nasopharyngeal swab samples were detected in a clinical study, the results of which showed that the overall consistency and the positive consistency with RT-PCR were 84% and 68%, respectively. Low viral load samples are more likely to have false negatives.³¹ The above conclusion shows that the sensitivity of the Accula of Mesa Biotech company is not as high as we expected. Studies are evaluating the concordance between laboratory-developed RT-PCR detection and Roche Diagnostics Cobas 6800 SARS-CoV-2 detection. The results showed that the overall percent agreement was up to 95.8%, and Cohen's κ coefficient was 0.904 between the two platforms mentioned above.³² Abbott's ID designs directional amplification of the RdRp fragment of SARS-CoV-2 based on the NEAR principle, and now it has been widely used for commercial real-time detection. It takes only 5–13 min for this product to detect, but the positive coincidence rate between RT-PCR and ID NOW is approximately 80%, which is consistent with previous studies. These findings suggest that Abbott's ID may not be appropriate to detect weakly positive samples.^{33–41}

In addition, there are commercial platforms that can realize multiplex PCR, including QIAstat-Dx of QIAGEN company and BioFire FilmArray of bioMérieux company. In addition to supporting liquid transport media, QI-STAT-DX also has a unique dry swab direct loading procedure, which can obtain results within 1 h, and the manual operation takes less than 1 min. BioFire FilmArray uses nested multiplex PCR combined with microfluidic chip technology to realize 1-h rapid detection. Nested PCR technology can eliminate the interference of other nonspecific pathogen nucleic acid substances to the greatest extent and improve the detection sensitivity.³¹

Isothermal amplification integrated instruments are suitable for portable rapid detection in resource-deficient areas and crowded environments. At present, an isothermal nucleic acid amplification instrument suitable for real-time detection is urgently needed. Several assays use isothermal nucleic acid amplification technology for the qualitative detection of SARS-CoV-2 viral nucleic acids, including the Cue COVID-19 Test, Detect Covid-19 Test, and Talis One COVID-19 Test System.

SARS-CoV-2 viral nucleic acids can also be detected qualitatively by the cue COVID-19 test method, utilizing isothermal nucleic acid amplification technology. The positive and negative percent agreement between the Cue COVID-19 test and the reference SARS-CoV-2 detection were 91.7% and 98.4%, respectively.⁴² The positive percent agreement and the negative percent agreement of the Cue COVID-19 detection method were demonstrated to have a high degree of consistency with that of central laboratory detection. Meanwhile, accurate POC testing can be helpful in situations where strict control of suspected COVID-19 patients is required. One way to detect nucleic acids is to read the open reading frame 1ab, RT-LAMP, and lateral flow strip technologies all use the above method to accomplish COVID-19 detection. The Talis One COVID-19 detection method uses real-time isothermal amplification technology, and a Talis One instrument is used when the extracted nucleic acid is amplified and detected. However, clinical validation data for the Talis One

COVID-19 Test System and the Detect COVID-19 Test are lacking because these two detection methods have just completed development.

Some convalescent homes in the United Kingdom have recently tried a new model called POKITTM Central. It is a benchtop molecular detection system that integrates insulated isothermal PCR amplification/detection based on fluorescence, magnetic bead-based nucleic acid extraction, and liquid handling technologies. It offers a straightforward protocol for nucleic acid detection. When RT-PCR is used as the reference standard, POKITTM Central has acceptable sensitivity and specificity, especially for cases with symptoms. To be more specific, eight specimens can be detected at once, and the results are displayed on the monitor no more than one and a half an hour.⁴³

3.2 | The current POCT tests for SARS-CoV-2 in China

China has also made outstanding contributions to the research and development of POCT nucleic acid detection systems for SARS-CoV-2. Commercial real-time detection devices based on the principle of RT-PCR in China include Sansure iPonatic and the 2019 novel coronavirus nucleic acid detection kit of Shanghai Toujing Life Technology Co., Ltd. (card fluorescence PCR method). IPonatics adopts one-step nucleic acid extraction-free technology combined with a rapid nucleic acid detection system, completing one-stop sample lysis, nucleic acid extraction, PCR amplification, and result analysis. The magnetic bead method is used to extract nucleic acids in the detection cassette of life through the scene, which realizes the whole process automation of extraction and amplification. However, this method needs to be manually loaded with magnetic beads and samples, so it should be carried out in a standard laboratory environment. Isothermal amplification can significantly reduce equipment volume because it does not need a thermal cycler and can realize amplification and visual detection at a constant temperature. Therefore, it is more suitable as an instant detection method. Biousta's EasyNAT instant molecular diagnostic system is an approved integrated isothermal amplification instrument. The whole process took 79 min, and the entire process was closed for detection without a professional PCR laboratory. The reagent was premixed in a fully automatic reaction tube by vitrification technology. The processes of cracking, magnetic bead extraction, purification, elution, and amplification are automatically completed under external magnetic conductance.

4 | NOTES AND QUALITY ASSURANCE OF THE POCT NUCLEIC ACID DETECTION SYSTEM FOR SARS-COV-2

Because rapid detection at this stage requires opening the cover of the sample sampling tube and then adding samples, the rapid detection of 2019-nCoV nucleic acid should be carried out in a second-level biosafety laboratory. The detection process's opening times, inactivation, and complexity vary according to different rapid detection platforms. Based on biosafety risk assessment, appropriate

personal protective measures (including gloves, masks, and isolation gowns) should be taken. In principle, the PCR laboratory should set up the following areas: reagent storage and preparation area, specimen preparation area, amplification, and product analysis area. A rapid detection platform integrates nucleic acid extraction and amplification detection. The sample preparation area, amplification area, and product analysis area can be combined. If the laboratory room is limited, a set of PCR rapid inspection workstations with three regional functions can be placed in a well-ventilated room. The three functional areas of the device should have three spaces with enough instruments and equipment for the corresponding functional areas, one of which should be a reagent preparation area similar to a clean workbench. The second is the specimen preparation area with an A2-type secondary biosafety cabinet function, and the third is the amplification area for air discharge. These three areas are completely independent of each other in physical space, and there can be no direct communication with air.

The laboratory should use the highly sensitive (detection limit ≤ 500 copies/ml) detection reagent approved by the National Medical Products Administration. The rapid detection reagent manufacturer must provide the analytical performance of the whole rapid detection system, including but not limited to the detection limit and precision. The laboratory should establish standard operating procedures for the entire process of detection operation according to the reagent instructions. Before conducting clinical tests, the laboratory should verify the performance of the whole rapid detection system. For performance verification, pseudovirus positive quality control products and negative clinical specimens packed with corresponding virus RNA sequences with known concentrations can be used. Performance indicators include but are not limited to detection limit and precision. If the laboratory is equipped with multiple rapid detection instruments, the laboratory should evaluate the reproducibility of different instruments.

The laboratory should carry out indoor quality control. Indoor quality control products should include negative quality control products (normal saline) and weak positive quality control products whose concentration is 1.5–3 times the detection limit. Every time you turn on the machine, first detect weak positive quality control products and negative quality control products. After quality control was qualified, clinical specimen detection started. In addition, it is necessary to participate in ventricular interstitial assessment regularly.

The laboratory should analyze and explain the results according to the reagent instructions. Comprehensive judgment should be made in combination with the original amplification curve for fast detection equipment that can automatically report the results and display the original amplification curve. When the detection limit of the rapid detection system is ≤ 500 copies/ml, the detection result is negative, and the negative result can be directly reported. The test result is positive. The original sample should be rechecked with another 1–2 kinds of conventional nucleic acid detection reagents that are more sensitive, preferably for amplifying different target areas. If necessary, the patient can be resampled for re-examination, and the results can only be reported if the re-examination is positive.

Although the rapid detection platform integrating nucleic acid extraction and amplification detection is simple to operate, the whole experimental process still involves many aspects, such as “anti-contamination” nucleic acid detection, performance verification, equipment maintenance, indoor quality control, result analysis and reporting, laboratory biosafety, and so forth. Nucleic acid rapid detection laboratory personnel still need to go through the on-the-job training of the clinical gene amplification detection laboratory, obtain corresponding qualifications, and can only go on the job after the basic training of 2019-nCoV nucleic acid rapid detection and the evaluation of working ability.

5 | SUMMARY AND PROSPECT

At present, most of the existing nucleic acid detection platforms in novel coronavirus use open RT-PCR or isothermal amplification and still need to operate in a BSL-2 laboratory environment, which is not a real POCT device. Ideal POCT products need to be characterized by miniaturization, automation, visualization of results, rapidity, high precision, and high throughput detection.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Shiqiang Shang and Jianhua Mao had the idea for and designed the study and took responsibility for the integrity of the data and the accuracy of the data analysis. Qing Ye contributed to the writing of the report. Qing Ye, Shiqiang Shang, Dezha Lu, Ting Zhang, and Jianhua Mao contributed to the critical revision of the report. All authors contributed to data acquisition, analysis, or interpretation and reviewed and approved the final version.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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