Received: 2 June 2022 | Accepted: 9 August 2022

DOI: 10.1111/eci.13853

REVIEW

WILEY

A precise review on NAATs-based diagnostic assays for COVID-19: A motion in fast POC molecular tests

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Funding information
National Institute for Genetic Engineering and Biotechnology, Grant/Award Number: 99/1443; University of Isfahan

Abstract

Background: Diagnosis is one of the main strategies to deal with infectious and deadly diseases such as coronavirus disease 2019 (COVID-19). The global pandemic of COVID-19 has led to an immediate need to expand rapid diagnostic techniques. New isothermal-based methods are being developed for COVID-19 detection aiming to resolve the limitations related to the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) method through immediate samples processing and minimizing false-negative or ambiguous results. Advances in nucleic acid amplification techniques (NAATs) can provide affordable and easy-to-use diagnostic platforms with high sensitivity and specificity in order to be available to the public as approved commercial kits.

Aims: The development of point-of-care (POC) testing can assist in rapid clinical decision-making and mitigate burdens on health care facilities. Finally, we discussed the different diagnostic methods based on NAATs for COVID-19 in detail. Comparative parameters are addressed for all assays and Emergency Use Authorizations (EUA)-approved commercial tests are cited.

Conclusions: Isothermal-coupled methods and LAMP-based molecular methods have been suggested as suitable portable tests with high diagnostic speed for use in POC testing.

KEYWORDS

COVID-19, molecular diagnostic assays, nucleic acid amplification techniques, point-of-care testing, SARS-CoV-2

1 | INTRODUCTION

On 31 December 2019, an outbreak of unknown pneumonia was detected in Wuhan city, Hubei Province, China, with clinical symptoms such as dyspnoea and bilateral lung infiltrates.[1,2] It was soon discovered by deep analysis of metagenomic next-generation sequencing (mNGS) which was caused by a novel beta-coronavirus, the 2019 novel coronavirus (2019-nCoV), was ascribed to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[3] Then, as the coronavirus disease 2019 (COVID-19) pandemic spread to the rest of the world in a short time, it became a global health concern.[4,5] The genome structure of beta-coronaviruses (b-CoVs, subfamily Coronavirinae, order Nidovirales) possess an enveloped, nonsegmented, single-stranded positive-sense RNA with a single species of nucleocapsid (N) protein that is called ribonucleoprotein (RNP) core (Figure 1).[4] The main characteristic of viruses is club-like spikes projections of protein, which gives them a crown-like appearance under the electron

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microscope. Hence, their names are derived from the Latin word Coronam, called crown.[6] B-CoVs can infect an extended range of mammals, for example, humans and bats through the recruitment of angiotensin-converting enzyme II (ACE2) as a cell entry receptor.[7] Due to the very high prevalence of SARS-CoV-2, identifying people as soon as possible and quarantining them from other people is one of the most important and effective ways to tackle COVID-19.[8] This helps to differentiate people with the flu and cold from COVID-19 patients and prevent unnecessary quarantine and economic and psychological crises.[9] However, it is not possible to find all individuals infected with SARS-CoV-2 and the virus may be transmitted to other people by asymptomatic individuals, called silent carriers. Accordingly, an early and accurate diagnosis of COVID-19 is pivotal to curbing its spread and this emphasizes the importance of conducting and improving clinical tests.[10]

Current diagnostic methods are designed based on the viral RNA (molecular assays) and the antigen and antibodies associated with SARS-CoV-2 infection (serological assays). Although serology tests are inexpensive, fast and easy, the diagnostic value of this test is weak due to its low sensitivity and accuracy, high false-positive and false-negative rates and insufficient levels of serum antibodies including IgG and IgA against SARS-CoV-2 spikes at the outset incidence of symptoms in the blood samples.[8,11] Therefore, detection of these antibodies further indicates a history of previous infection and immunity to the virus and cannot be considered as a potential diagnostic assay alone.[9] Up to now, over 160 molecular diagnostic methods for the detection of 2019-nCoV have been approved by US Food and Drug Administration (FDA) and Emergency Use Authorizations (EUA).[12] Conforming to the World Health Organization (WHO), the instant precedence for emerging of COVID-19 clinical diagnostics methods should be based on detection at the rapid point-of-care (POC).[13] POC tests are affordable, hand-held molecular diagnostic assays operated at or near the sample collection site, and results are presented within minutes not hours.[14] Thereby, accurate POC diagnostic testing is designed to timely diagnose and control outbreaks of such infectious diseases.[15] Isothermal-based methods can be considered as a qualified option for POC detection tests due to no need for advanced equipment and reduced time for obtaining results. Therefore, focusing on these methods to improve the sensitivity and specificity in the diagnosis of SARS-CoV-2 can be valuable.

This review aimed to highlight the value of the molecular POC diagnostics system of COVID-19 for use in medical centres, airports and conferences and also improvement of their efficiency as usable home-based methods in the future is focused on the presentation of molecular diagnostics assays and their functional principles, comparison of their analytical approaches, benefits and drawbacks. Also, the current review is mentioned EUA-approved commercial test kits for SARS-COV-2 diagnostic methods.

HIGHLIGHTS

- COVID-19 due to high contagious converted into a global problem.
- The recent advances in molecular methods created a huge revolution in the diagnosis of COVID-19.
- Development of POC molecular tests can help rapid detection and mitigation of the outbreak of novel coronavirus.

2 | MOLECULAR DIAGNOSTIC TECHNIQUES FOR THE SASR-CoV-2

Since the first genetic information of SARS-CoV-2 was sequenced (January 2020) in GenBank (accession No. MN908947.3), diverse molecular methods have been hired for the detection of unique viral RNA sequences by nucleic acid amplification techniques (NAAT).[16] Employing isothermal amplification allowed the development of POC-NAATs for the detection of COVID-19. Isothermal amplification technology has been developed to exclude the requirement of a high-cost thermocycler in real-time-PCR assays.[17] The most common NAAT-based molecular assays are reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and isothermal nucleic acid amplification.[18] A number of these NAATs are available for diagnosis of SARS-COV-2. Each of these techniques, which is fully explained, can provide a rapid and feasible approach to the detection of SARS-COV-2.

3 | ROUTINE METHOD: RT-qPCR

WHO confirmed RT-qPCR as a golden standard method for detection of 2019-nCoV.[19] The RT-qPCR assay is a very efficient technology that has been frequently used when RNA is going to be investigated.[20,21] This method is a kind of PCR based on the NAAT. The most well-known of them is the TaqMan assay (the SYBR Green dye-based assay is also so popular).[19] To perform this method, a sample must first be taken from the COVID-19 suspected person's throat or nose using rhino-pharyngeal swabs and then RNA is extracted. The second step consists of the synthesis of the

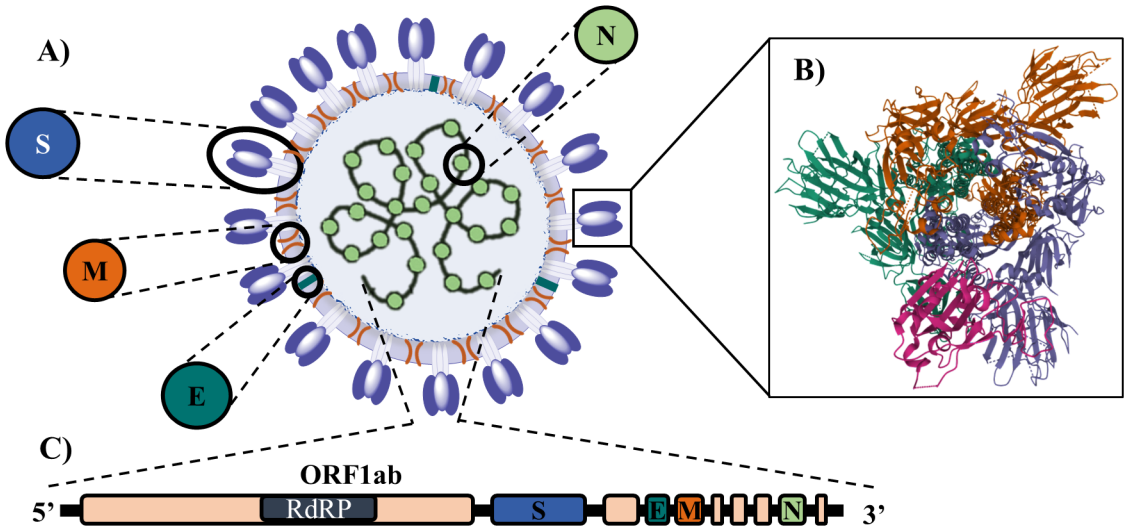

FIGURE 1 Structure of 2019-nCoV and its genomic and protein design. (A) Three pivotal structural proteins S, E and M are anchored on the viral envelope. Inside the envelope, there is a helical nucleocapsid that is composed of protein N protein with the viral RNA. The protein that is involved in the virus entering the host cell and causing the infection is S, which it does by binding to the ACE2. (B) 2019-nCoV spike protein structure in the prefusion state using electron microscopy and resolution 3.00 Å without nonstandard components. (C) The genes are arranged in the order 5′UTR-replicase-S-E-M-N-3′UTR-poly-A tail. The replicase was encoded by *ORF 1ab*, and about 60% of the entire RNA is included. 2019-nCoV, 2019 novel coronavirus; ACE2, Angiotensin-converting enzyme 2; E, Envelope; M, Membrane; N, Nucleocapsid; ORF, Open reading frame; PDB ID, 6ZOW; RdRP, RNA-dependent RNA polymerase; S, Spike; UTR, Untranslated region; NCBI Reference Sequence: NC_045512.

complementary DNA (cDNA) from extracted RNA by reverse transcription reaction using a reverse transcriptase (RT) enzyme.[22] Finally, amplification is done using designed primer-probe sets and the presence or absence of target nucleic acid is detected by the investigation of fluorescence level rather than the background signal and analysis of the obtained cycle threshold (Ct) of RT-qPCR.[9] Ct could be measured using different varieties of RT-qPCR machines, and overall, $Ct > 40$ and $Ct < 40$ are interpreted as negative and positive, respectively (i.e. using a chromo

4 system of Bio-Rad [USA]; authors' personal data, not published yet).[23] Various regions of the SARS-COV-2 sequence can be designed as possible viral targets for RT-qPCR. The most used gene targets are open reading frame 1ab (*ORF1ab*), the *N* gene. Also, *RNase P* (*RP*) is used for internal positive control.[22]

One-step assay and two-step assay are typically two ways to perform RT-qPCR. In a one-step reaction, the reverse transcription reaction and RT-qPCR occur in one single tube, and the RT enzyme is inactivated in the high-temperature DNA polymerase activation stage (Figure 2A). Hence, this assay is a so-called hot start. In two-step RT-qPCR, reverse transcription reaction and RT-qPCR occur in separate tubes with an optimized buffer for any reaction (Figure 2B).[19,24] Each assay has advantages and disadvantages that are shown in Table 1.

3.1 | Benefits and drawbacks

The investigation of the presence of viral nucleic acid, even with a low viral load, is performed by the RT-qPCR technique with high sensitivity and acceptable accuracy. This technique has been used in many countries as a rather strong diagnostic technique to deal with the epidemic worldwide. This RT-qPCR is then considered as a time-consuming process, due to the need for complex and advanced equipment and skilled personnel. Moreover, the possibility of false-positive results due to the presence of some sort of contamination is another drawback of the current assay.[25]

Therefore, by turning to advanced methods, such as isothermal-based assays, many of these RT-qPCR problems are solved to a large extent. Also, these methods based on isothermal move in this direction so that people can perform this test at home comfortably and send the results to the doctor and so, visiting medical centres and as well as the spread of COVID-19 will be reduced. Therefore, the current review classifies the COVID-19 diagnostic assays based on isothermal conditions and elucidates them completely.

4 | ISOTHERMAL-BASED METHODS

4.1 | RPA assay

Recombinase polymerase amplification (RPA) is one of the isothermal NAAT-based methods of 2019-nCoV diagnosis that utilizes recombinase, single-stranded DNA binding proteins (SSBPs) and strand-displacing DNA polymerase to catalyse strand invasion of a primer into dsDNA.[26] The reaction starts with the formation of a nucleoprotein complex (recombinase-primer complex) through the pairing of the recombinase enzyme and the oligonucleotide primer.[17] This complex can invade the cDNA, derived from viral RNA, and bind the primers to their complementary sequences. Hence, this assay depends on RNA extraction and reverse transcription.[27] Primer extension and D-loop (ssDNA sites) formation is conducted by a strand-displacing DNA polymerase. SSBPs act as a stabilizer of displaced DNA strands by curbing the rebinding of the plus and minus strands. Finally, the template DNA structure is opened and DNA amplification is carried out by the DNA polymerase. New synthesized DNAs can act as template DNA for the following amplification cycles.[27,28] Calibration of RPA with lateral flow assay (LFA) can be presented as a visual simple-to-use method for POC diagnostic applications.[29] Here were explained two RPA-based methods for detection of the 2019-nCoV.

4.2 | L/RPA assay

Wang et al. established a combined isothermal assay known as ligation and recombinase polymerase amplification (L/RPA) for rapid detection of SARS-CoV-2. The performance of the assay relies on the viral RNA template, T4 DNA ligase and ligation probes set including probe A and probe B.[30] Notwithstanding the inefficiency of DNA ligases in RNA templates, this assay resolved the problem by optimizing the ligation protocol and employing the high sensitivity of RPA.[31,32] Each probe consists of a part annealing to the RNA and an 'amplification arm' to promote the RPA amplification. The diagnostic probes are base RNA fragments, termed biomarkers, on genes *N* and *ORF1ab* of the SARS-CoV-2 genome. In case of the presence of viral RNA, the probe set will pair to the targets and is ligated into a single-strand RNA (ssRNA) fragment by T4 DNA ligase activity (Step 1). Then, the produced fragment for each biomarker is separately amplified by RPA with the forward and reverse primers complementing the 'amplification arm' sequences (Step 2). Finally, the amplification signal can be detected in real-time using the binding of SYBR Green I fluorescence dye to dsDNA amplicons.[30] The procedure of this assay is shown in Figure 3.

4.2.1 | Benefits and drawbacks

The assay could be completed in <30 min without the requirement for such a piece of advanced equipment as a real-time PCR machine. Also, due to the avoidance of the RT procedure, the need for sample processing can conceivably be reduced by the current assay that, compared

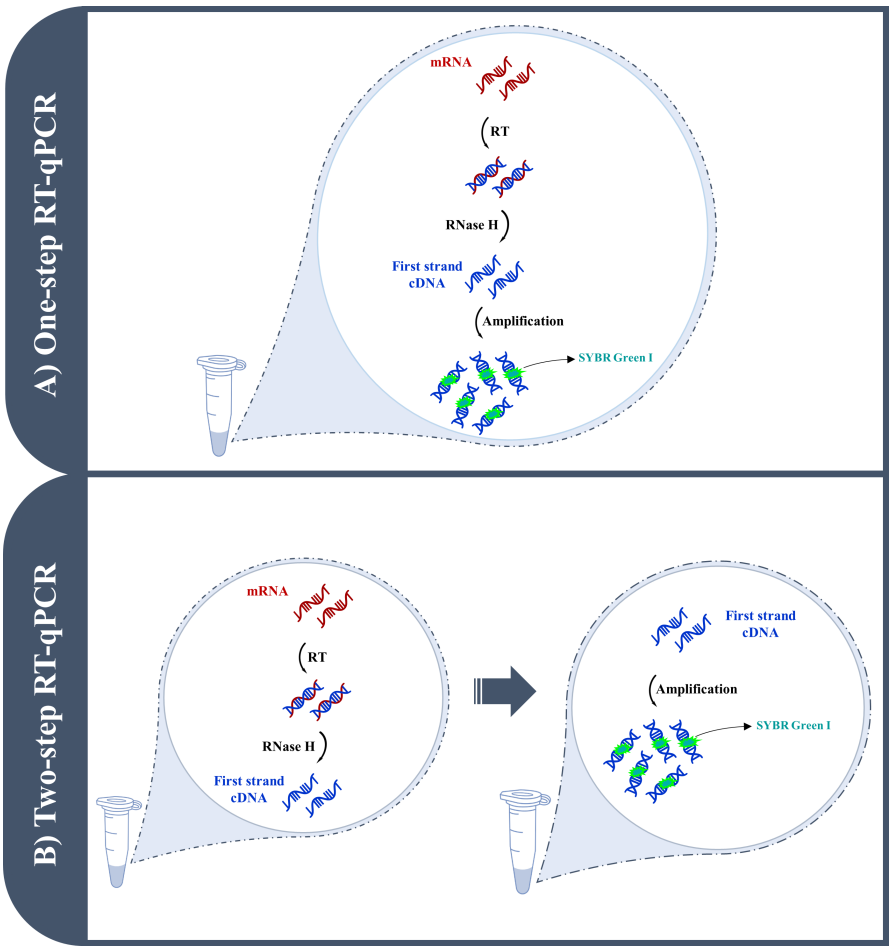

FIGURE 2 Schematic illustration of RT-qPCR mechanism. (A) One-step RT-qPCR laboratory stages. In a single tube, the RT and DNA polymerase are both present during reverse transcription simultaneously. RNA is first converted to cDNA by an RT, and then a DNA polymerase amplifies the cDNA, allowing quantitation through RT-qPCR. (B) Two-step RT-qPCR laboratory stages. In the two-step assay, reverse transcription and cDNA synthesis perform separately. This is then followed by RT-qPCR assays. RT-qPCR, reverse transcription-quantitative polymerase chain reaction.

TABLE 1 Investigation of characteristics of two RT-qPCR techniques.

Technique	Advantage	Disadvantage
One-step RT-qPCR	Contamination prevention	Increased risk of primer-dimer formation
	Convenience	cDNA is not always available
	Sensitivity	
	High-throughput sample screening	The condition is acceptable but not optimal
Two-step RT-qPCR	Enough cDNA for multiple real-time PCR reactions	Less convenient
	Sensitivity	Contamination risk
	Multiple targets	Likely to inhibit RT-qPCR by RT enzymes and buffers

with RT-based assays, has a potentially lower running requirement.[30,33] Another benefit of the L/RPA assay is no need for precise temperature control in both steps owing to the enzyme properties. Hence, the temperature of 37°C can even be afforded by body heat.[30] The L/RPA assay showed a sensitivity of 10^1 viral RNA copies per reaction, which was comparable to that of RT-qPCR and other nucleic acid detection assays for SARS-CoV-2.[30] Although an RT-qPCR machine was used in this study to read the fluorescence signal, battery-powered portable tube scanners are commercially available and ready to be applied to the fluorescence detection of the L/RPA assay.[34] Accordingly, this rapid and simple diagnostic assay is valuable for POC testing.

4.3 | LF-RPA

The systematic assessment of LFA during the outbreak of 2019-nCoV was launched at Oxford University in June 2020.[35] As shown in Figure 4, LF-RPA assay requires conventional forward primers, 5′ end tag marked reverse

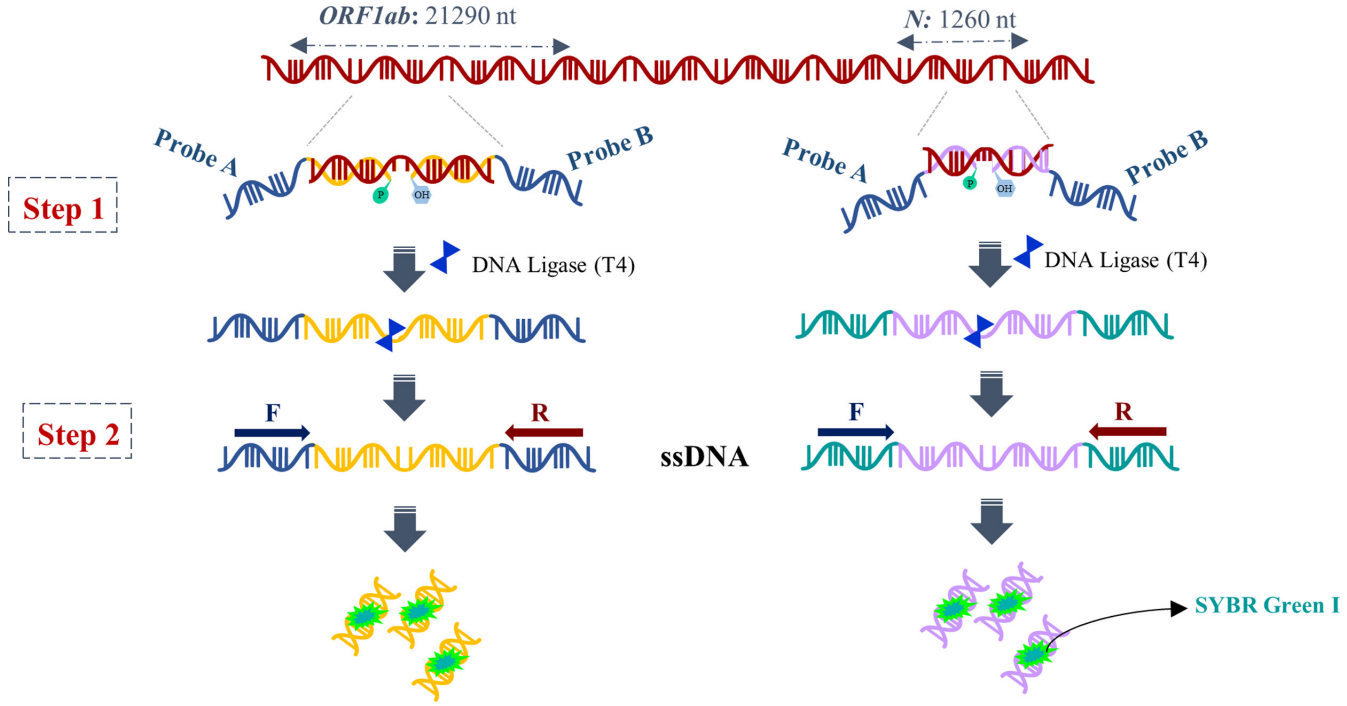

FIGURE 3 Schematic of the L/RPA assay for severe acute respiratory syndrome coronavirus 2 detection. ssRNAs will be generated via pairing biomarkers (probe set) to the targets and T4 DNA ligase activity if there is viral RNA. Then, the produced fragments are separately amplified by RPA and the forward and reverse primers. Finally, RT-qPCR is performed. NC_045512.2.

primer (usually 5′-biotin-DNA), a nuclease IV (nfo) and one end-labelled probe (usually 5′-FAM-DNA-3′ blocking group).[28] The blocking group acts as a polymerase extension inhibitor, and as a result, elongation does not occur at the 3′ end of DNA.[36] The modified 46-52-nucleotide probe includes a bulge, which is termed dSpacer, a substitute of a tetrahydrofuran residue with a standard nucleotide, which does not pair with any of the four DNA bases.[28] The nuclease IV nicks the dSpacer, only if the probe composes double-stranded DNA (dsDNA).[27] By cleavage in dSpacer, the 3′ end blocking group is released. Subsequently, a new 3′ OH is produced in the probe and DNA extension occurs. In other words, a modified oligonucleotide shifts the state from the probe into a primer.[28] A DNA amplicon tagged with FAM and biotin (FAM-DNA-Biotin) can bind to antibodies and streptavidin on dipsticks' surfaces, respectively. Therefore, the principle of LFA relies on the sandwich assay. There is a conjugation pad, and two lines (control and test) separately on LFA dipsticks where the specific antibodies are frozen on them. The test line is covered with streptavidin to capture the biotin-tagged amplicon, and the control line is coated with Ab-binding antibodies (Ab2) for catching FAM-antibodies-labelled gold particles (Ab1).[36–38] The LFA dipsticks are based on the capillary effect that transports fluid spontaneously. The liquid sample is run on the sample pad, and it flows across the conjugation pad containing Ab1. Thus, the Ab-amplicon will move towards the test line. In the line, the only biotin-tagged amplicon will be captured by the biotin-antibodies, showing a positive result.[27,37] Also, the free Ab1 antibodies (no biotin) move further from the test line, and finally, they are caught by the Ab2 in the control line. In other words, the control line is utilized to ensure the proper function of the lateral flow pad. If the sample is negative, no colour change (due to localized surface plasmon resonance in gold particles) is shown in the test line and we only see red colour in the control line. But if two red lines are seen, interpreted as a positive result. So, the LF-RPA assay shows a visual positive or negative result.[27,38]

4.3.1 | Benefits and drawbacks

Due to the usage of strand-displacing DNA polymerase and resulting in no need to initial denaturation of DNA template, RPA assay can be considered as a high-sensitivity POC-based test.[29] Since LF-RPA assay lasts 15 min, it can play an important role in the diagnosis of COVID-19.[39] The limit of detection (LOD) of the LF-RPA is determined as 1 copy/μl that can detect low viral load. By providing an LF-RPA system in the form of commercial kits, transportation and storage costs can be reduced and can be used by untrained personnel even at home.[27,37] Given that this method still requires RNA extraction and RT-steps, these issues increase the detection time. Another drawback is

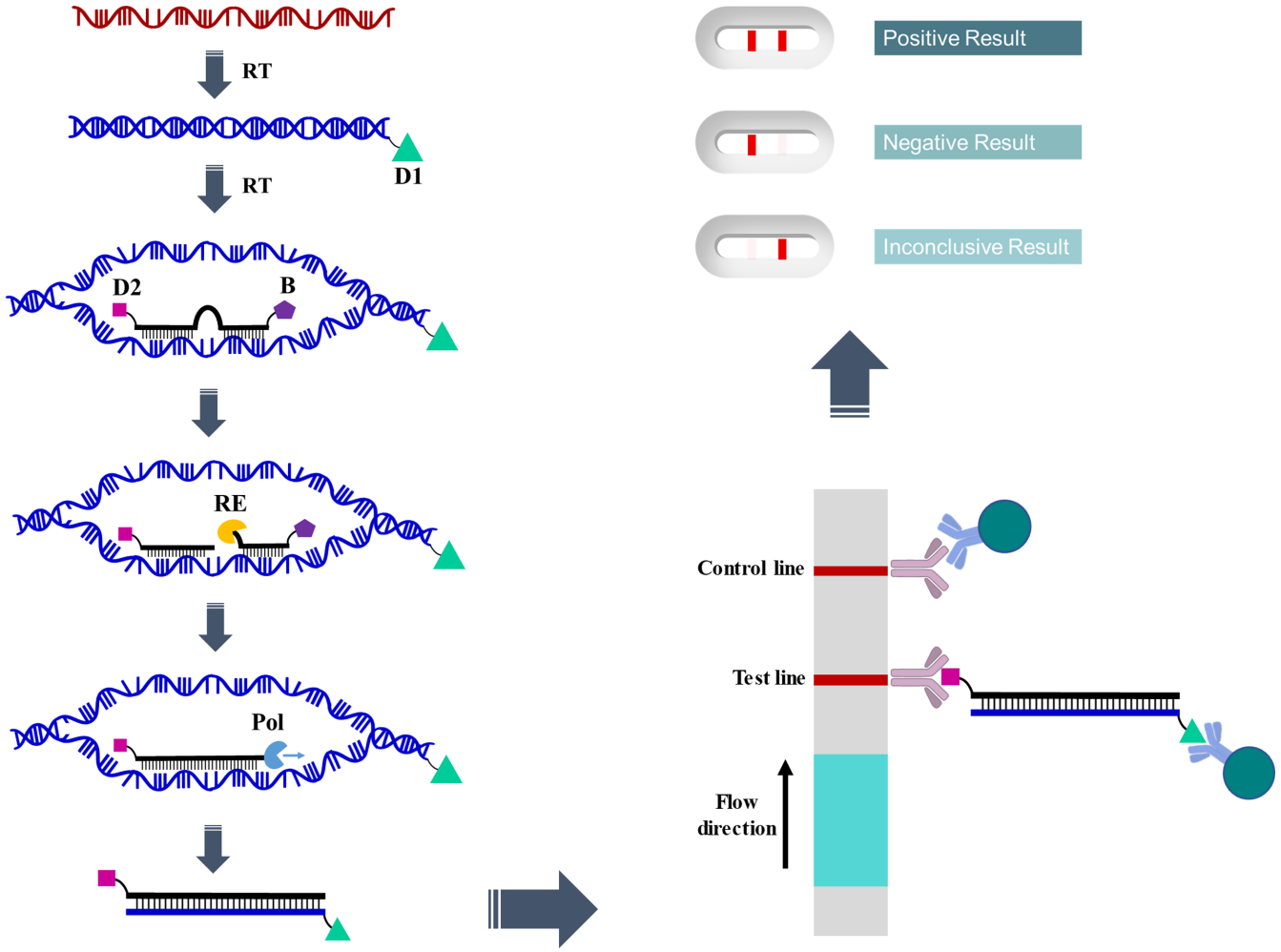

FIGURE 4 Lateral flow assay-based RPA for severe acute respiratory syndrome coronavirus 2 detection. During LF-RPA, the modified probe is marked with a 5′-specific antigenic tag and a 3′-polymerase blocking group. Also, the probe has a bulge, dSpacer. The nuclease IV nicks the dSpacer, only when the probe fully complements to target DNA. Thereby, DNA extension occurs and a dual-labelled amplicon is generated. A reddish band visible to a naked-eyes is generated on the test line if the labelled RPA amplicon was well produced. The control line confirms the validity of the test run. B, Blocking group; D1, Detector 1; D2, Detector 2; Pol, Polymerase; RE, Repair enzyme.

primer-dimer formation due to the utilization of a very low temperature (~42°C) for amplification of the target.[40]

4.4 | Colorimetric RT-LAMP assay

Reverse transcriptase loop-mediated isothermal amplification assay (RT-LAMP) is a rapid and one-step technique for amplifying nucleic acids at a constant temperature.[41] Since the onset and advancement of polymerase chain reaction (PCR), this NAAT-based method has been developed for extensive clinical use, particularly in the diagnosis of infectious diseases, for example, hepatitis infections and now COVID-19.[42] LAMP employs a primer-set (pairs of inner and outer primers, 4 or 6 primers), six or eight gene regions, and *Bacillus stearothermophilus* DNA polymerase (Bst DNA pol) strong strand displacement activity for DNA amplification that just the process is completed in 30 min and at constant temperature (60–65°C).[43]

The total operating time of RT-LAMP takes >1 h because of the simultaneous addition of an RT enzyme for the synthesis of cDNA from the extracted RNA. In other words, reverse transcription and loop-mediated amplification occur in one isothermal reaction. Bst DNA pol is generally resistant to inhibitors present in samples. Therefore, there is no need to be very careful in the sample processing such as RNA extraction.[22,41] As shown in Figure 5, designed primers are recruited for the reverse transcription and amplification that finally is produced loopback at DNA ends. DNA amplification can occur about10^9 times per 1 h.[11] Most studies demonstrate that SARS-CoV-2 targeting regions are *ORF1a* and *N* genes.[41,43] The incorporation of deoxynucleoside triphosphates (dNTPs) and the production of pyrophosphate during amplification causes a pH change in the reaction (PH reduction), which

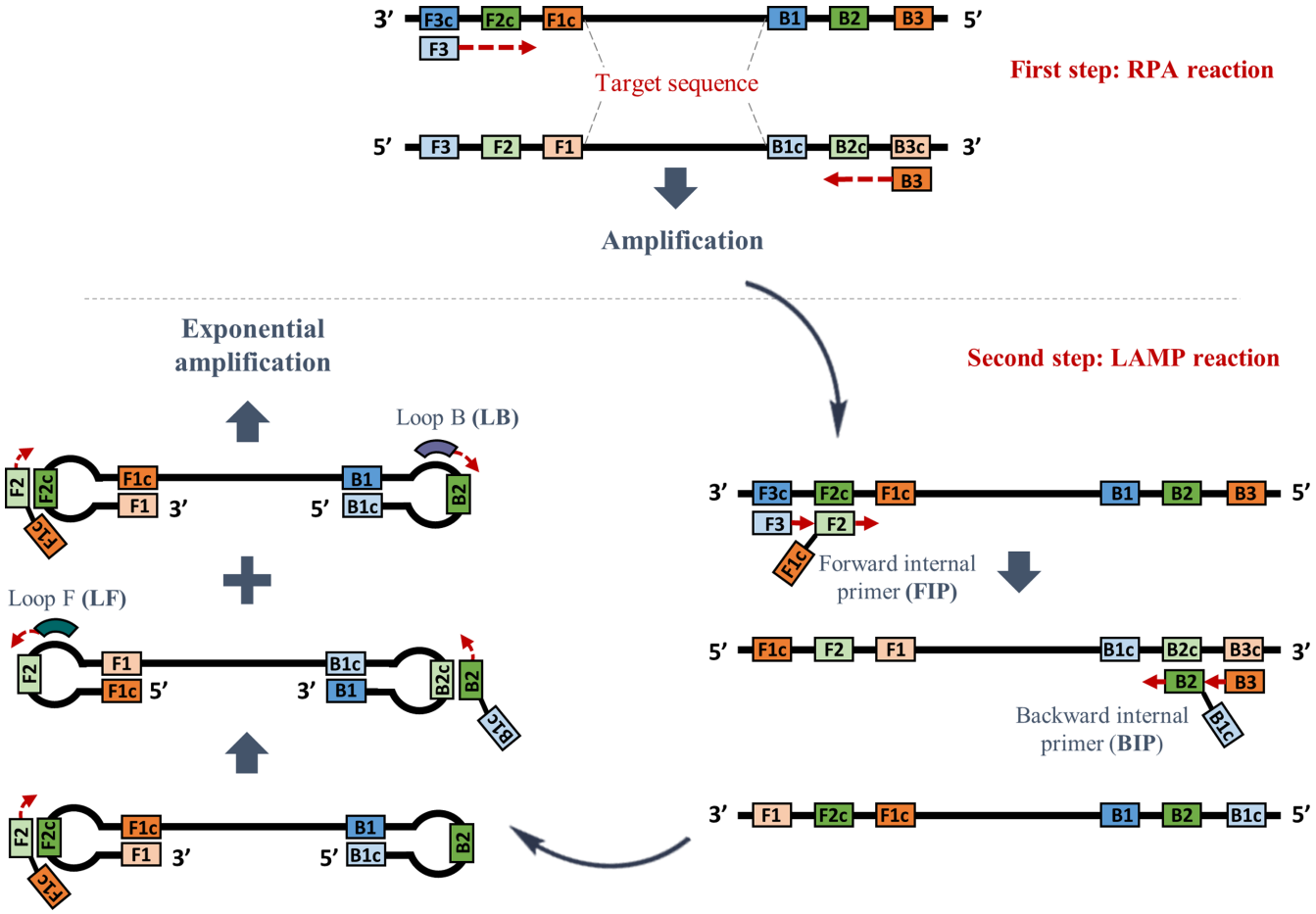

FIGURE 5 Visual diagnosis of COVID-19 using Colorimetric Penn-RAMP assay. Schematic depiction of procedure of Penn-RAMP assay. This assay employs two inner primers (FIP and BIP) and two outer primers (F3 and B3) that can recognize a total of six different regions in the target DNA. Also, two extra loop primers (LF and LB) are utilized to expedite amplification and diagnosis performance.

is visually detectable with pH-sensitive dyes (e.g. phenol red). The shift of colour from red to yellow indicates the presence of the target sequence present in the samples (positive results).[22] By RT-LAMP assay, the viral LOD is about 4.8 copies/μl with a sensitivity of 97.5% and a specificity of 99.7%.[44]

4.4.1 | Benefits and drawbacks

Unlike RT-qPCR, RT-LAMP assay does not require expensive complex equipment, and the colour change is detected easily using spectrophotometric or even simple mobile phone cameras. Also, minimal sample processing is required to get the final adequate results.[11,41] As mentioned, the total assay time is >1 h, so it is problematic to measure numerous samples. For unravelling this problem, Thi et al. developed a swab-to-RT-LAMP assay with high specificity (99.5%) but low sensitivity (86%) that used swab specimens directly without the requirement for an RNA extraction step.[45] Another drawback is the complexity of the primer design as it includes several primers with different varieties of features for each targeting region of SARS-COV-2.[22] Given the aforesaid benefit, this assay can be used as a fabulous rapid POC test for the detection of 2019-nCoV.

4.5 | Colorimetric Penn-RAMP assay

El-Tholoth et al.[46,47] have recently developed a combination NAAT-based method that consists of two isothermal processes of RPA and LAMP in a sealed single tube, in order to improve the sensitivity and speed of SARS-COV-2 diagnosis. In this technique, primers are designed for the *ORF1ab* gene of SARS-COV-2 RNA, which is a highly conserved sequence.[11] As shown in Figure 5, the RPA mixture (LAMP F3, B3 primers and buffer) and target sample are loaded inside of the tube's lid. Then, the LAMP mixture (FIP; forward internal primer, BIP; backward internal primer, LF; loop forward, LB; loop backward primers and Bst DNA pol) with leuco crystal

violet dye (LCV; an intercalating dye) is inserted within the tube itself.[48] Then, the tube's lid is closed, so-called sealed.

During this stage, outer LAMP primers (F3 and B3) are located in the targeted sequence of samples and all targets are amplified by the RPA enzyme. After the combination of the LAMP mixture, a specific two-step reaction initiates. The first step employs F3 and B3 primers at 38 °C, whereas FIP, BIP, LF and LB are used in the second stage at 63°C. Finally, loopback is created and exponential amplification occurs.[11,46,49] LCV dye is almost colourless and purple in the absence and presence of the dsDNA, respectively. Therefore, a colour change (Violet) will occur in the tube if the amplification process is carried out.[49]

4.5.1 | Benefits and drawbacks

The method is based on isothermal conditions. Therefore, no need for complicated equipment, high cost and energy.[50] El-Tholoth et al.[46] have shown that Penn-RAMP is an appropriate method with a LOD of 7 copies/μl, while LAMP and RT-PCR are not able to detect samples with <70 copies/μl of SARS-COV-2. Therefore, Penn-RAMP with 10-fold better detection sensitivity on purified processed nucleic acid can be more effective in reducing false-negative results than LAMP and RT-PCR methods.[15] Because time plays a very important role in the diagnosis of 2019-nCoV, they have investigated the rapid preparation of samples and their effect on diagnostic power. Results have shown that incubation at a temperature >65°C is sufficient to lyse the virus and confirm infection. The method has 100 times better sensitivity with respect to LAMP and RT-PCR. But due to the use of synthetic samples and the limited access to the actual samples at that time, the method requires confirming with actual samples.[46] Finally, Penn-RAMP can be defined as a rapid molecular POC test to detect 2019-nCoV.

4.6 | CRISPR assay

Another isothermal nucleic acid amplification method of 2019-nCoV detection relies on an adaptive RNA-guided prokaryotic defence system that innately cleaves foreign nucleic acids during infection using Cas (CRISPR-associated protein) nucleases.[51,52] The nucleases' activity depends on a specific short protospacer adjacent motif (PAM) that is an essential targeting component for distinguishing bacterial self from nonself DNA.[53] In clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system, virus DNA fragments (protospacers) are inserted into CRISPR repeat arrays at the first infection and subsequent transcription is utilized as guide CRISPR-associated RNAs (crRNAs) to cleave the cognate virus genome.[54] CRISPR/Cas9 system has been employed as a gene-editing technology since 2013,[55] but recently a unique feature was discovered in a group of Cas nucleases such as Cas12 and Cas13. These Cas enzymes promiscuously cleave collateral ssDNAs/ssRNAs that do not complement the guide RNA sequence. Therefore, this property can be used as an effective tool for viral pathogens detection, for example, SARS-COV-2.[56,57] CRISPR-COVID includes RNA-targeting CRISPR/Cas13a-based assay, termed SARS-CoV-2 DETECTR (DNA endonuclease-targeted CRISPR trans reporter), and a DNA-targeting CRISPR/Cas12-based assay, called SHERLOCK (specific high-sensitivity enzymatic reporter unlocking). In both cases, isothermal amplification occurs via RT-LAMP/RPA methods to produce dsDNA amplicons from the targeting CRISPR site.[58] DETECTR provides a Cas12a/crRNA-target dsDNA ternary complex through Cas12a/crRNA binary complex pairing to the target site that leads to activation of the nuclease domain of Cas12a. Finally, both target DNA strands and the fluorescent reporter ssDNAs are degraded by collateral cleavage activity of Cas12. Inversely, SHERLOCK relies on an RNA-targeting CRISPR/Cas13a system where DNA amplicons must be transcripted to ssRNA by T7 RNA polymerase first.[59] Then, Cas13a/crRNA recognizes target RNAs, is conformed to the Cas13a/crRNA-target ssRNA ternary complex and eventually cleaves the nontargeted ssRNA that is labelled at its 5′ and 3′ terminus (Reporter-Target-Quencher) in addition to the target RNA. In an LFA, the cleavage of the reporter leads to the appearance of a test line. To go towards a POC test, the detection speed of the current method should be improved using decrement in processing steps and a visual record of results on paper strips. Hence, the visual diagnostic method of All-In-One Dual CRISPR-Cas12a, termed AIOD-CRISPR, has been presented for SARS-CoV-2 detection.[60] The procedure of CRISPR-based methods is illustrated in Figure 6.

4.7 | AIOD-CRISPR assay

This assay managed to detect SARS-CoV-2 and human immunodeficiency virus type 1 (HIV-1).[61] The current method uses a one-pot reaction system. Thus, all substances required for amplification and CRISPR detection are mixed in a single step and incubated at 37°C.[62] Since the results of a rapid and robust AIOD-CRISPR assay

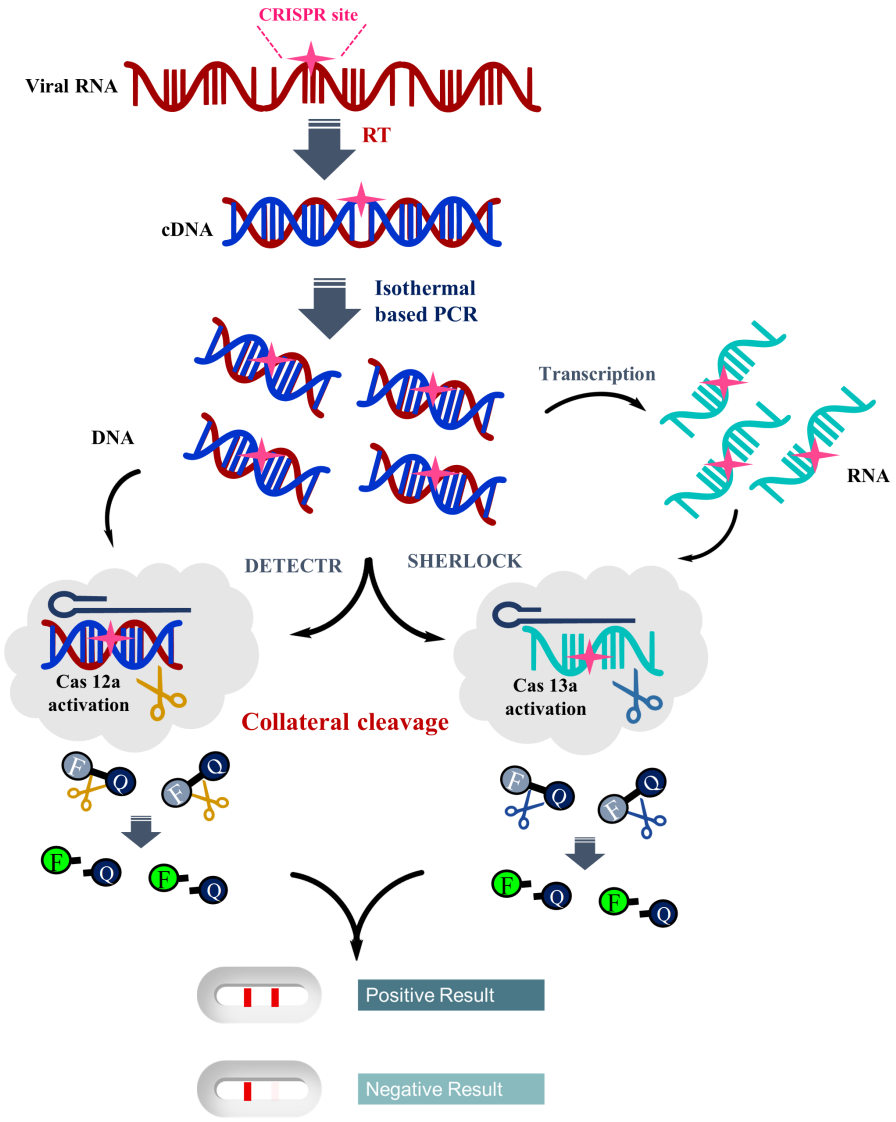

FIGURE 6 Schematic representation of SHERLOCK and DETECTR mechanisms involved in CRISPR-based diagnostic systems. The extracted RNA of patient specimens is converted to cDNA and amplified through isothermal procedures. The cDNA amplicons are either inserted directly into the CRISPR-Cas12 system (DETECTR) or transcribed to ssRNA first and then added to the CRISPR-Cas13 system (SHERLOCK). Cas12, 13 detect CRISPR targeting sequence to cut nucleic acid reporters, releasing fluorescence light. F, Fluorophore; RT, Reverse transcriptase; Q, Quencher.

can be observed visually, this CRISPR-based molecular diagnostic assay can further develop for the organization of a POC test. The AIOD-CRISPR system consists of two Cas12a-crRNA complexes (forward and reverse complexes) to bind two sites adjacent to recognition sites of primers on the *N* gene of the viral target sequence. Before the Cas12a-crRNA complexes are loaded into a solution, being individually made. The solution includes a pair of RPA primers, ssDNA-FQ reporters (Fluorophore, Quencher), recombinase, SSBP, strand displacement DNA polymerase and viral target sequences. During incubation, RPA amplification is first launched, revealing the binding sites of the Cas12a-crRNA complexes owing to the strand displacement activity of DNA pol.[60] According to Figure 7, if these complexes pair the target sites, the activity of Cas12a endonuclease will trigger and collaterally cut the adjacent ssDNA-FQ reporters to produce fluorescence.[63] For dual CRISPR-based nucleic acid detection, dual crRNAs act without the restriction of protospacer adjacent motif sites (PAM) sequence.

4.7.1 | Benefits and drawbacks

The DETECTR assay can detect 10 copies/μl in almost 30 min. Combining two methods of SHERLOCK and LFA (LF-SHERLOCK) creates the ability to detect 10 copies/μl of SARS-CoV-2 RNA with a sensitivity of 95% and a specificity of 100% that the total assay takes <1 h.[64,65] Although SHERLOCK is a comfortable, highly sensitive and specific diagnostic assay of viral RNA, the time-consuming process of reverse transcription is necessary to detect DNA sequences before the SHERLOCK assay.[59] In the AIOD-CRISPR method, all steps, from amplification to detection, are performed in a one-pot reaction system for quick, extremely sensitive and specific diagnosis of nucleic acids without requiring complex devices.[60] Due to the fluorescence and colour shift of reagents (from orange-yellow to green), the result of the diagnostic system will be seen by a naked-eyes even if the test is performed under an ambient light environment. These benefits enable detection in real-time and create great

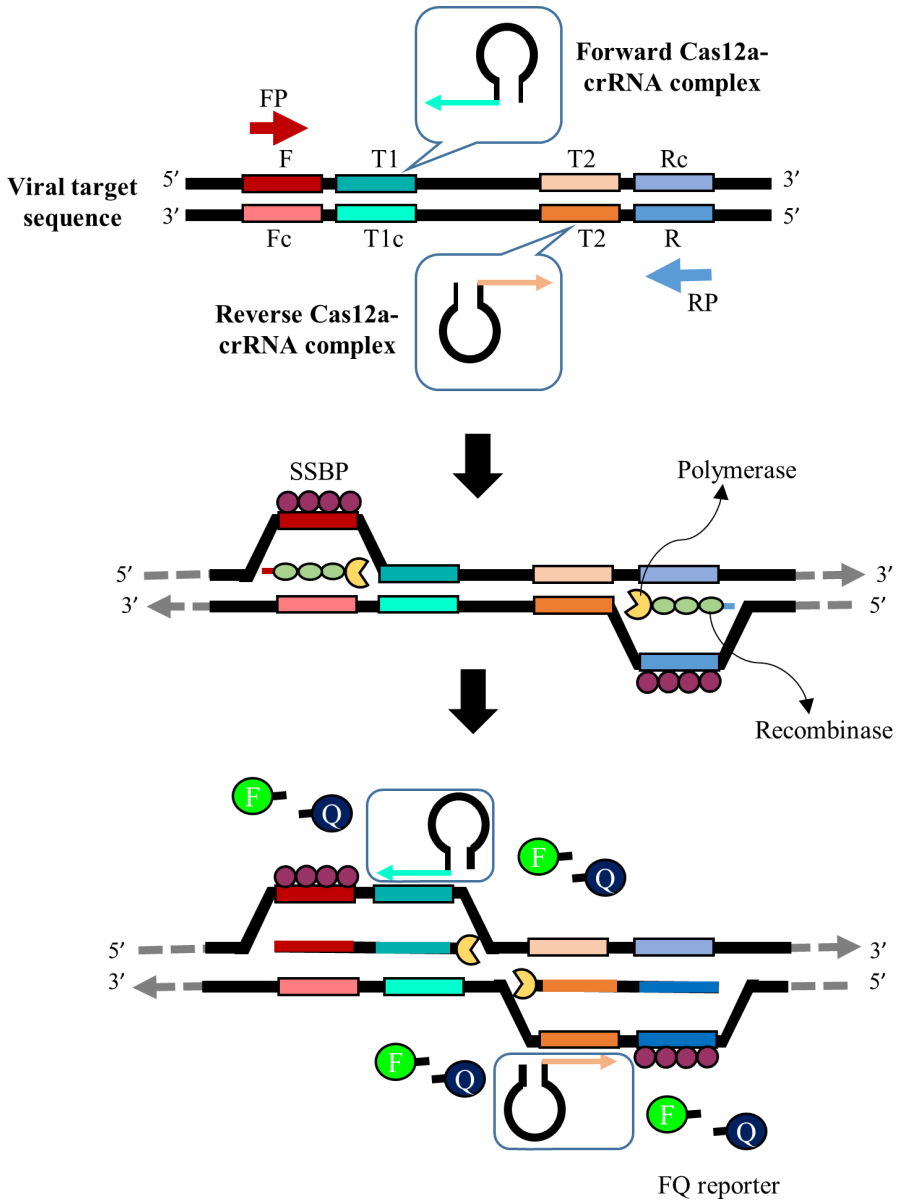

FIGURE 7 Fluorescence-based AIOD-CRISPR assay for severe acute respiratory syndrome coronavirus 2 detection. The procedure employs two Cas12a-crRNA complexes to pair two corresponding sites. These sites are proximate to the recognition regions of primers in the target sequence. Firstly, RPA is first initiated and reveals the binding sites of the Cas12a-crRNA complexes because of the strand displacement. By binding the Cas12a-crRNA complexes to the target sites, Cas12a cleaves the nearby ssDNA-FQ reporters to create fluorescence. AIOD-CRISPR, All-In-One Dual CRISPR-Cas12a; F, Fluorophore; FP, Forward primer; RP, Reverse primer; RPA, recombinase polymerase amplification; T, Target; Q, Quencher.

potential for POC diagnosis of COVID-19 due to the sensitivity of 100%.[60,63,65]

4.8 | qRT-NASBA assay

Nucleic acid sequence-based amplification (NASBA), also known as self-sustained sequence replications (3SR), is a continuous isothermal process for amplifying directly specific sequences of ssRNA even in the presence of genomic DNA.[66,67] The method has been modified as a molecular beacon-based multiplex quantitative real-time NASBA (qRT-NASBA) assay that can be used to detect a range of pathogens, from bacterial RNA to viral RNA in clinical samples, such as SARS-COV-2.[67–69] A conventional NASBA reaction involves NTPs (nucleoside triphosphates), two oligonucleotide primers (P1, P2), a molecular beacon probe (Fluorophore-DNA-Quencher), a proper reaction buffer and three enzymes including Avian myeloblastosis virus-reverse transcriptase (AMV-RT), T7 DNA-dependent RNA polymerase (T7-DdRp) and RNase H.[66,70]

The method consists of a two-step process of non-cyclic and cyclic phases that was defined as follows: (1) Denaturation of the target at 65°C. (2) Annealing heterogeneous forward primer (P1, Antisense) to the RNA analyte. P1 possesses a 3′ complementary sequence to the target RNA and a 5′ sequence that encodes the T7 RNA polymerase promoter. (3) Forming cDNA/RNA hybrids via reverse transcription activity of the AMV. (4) Hydrolysing RNA from cDNA/RNA hybrids using the action of RNase H. (5) Annealing reverse primer (P2, Sense) to newly synthesized cDNA. (6) Synthesis of dsDNA by the DNA-dependent DNA polymerase activity

of AMV-RT. The dsDNA has a coding site for T7 RNA polymerase promoter at one end that is a substrate for T7-DdRp. (7) Transcribing antisense RNA copies through T7-DdRp. (8) Amplification of enormous RNA by entering into the cyclic phase of the NASBA process.[66,70] All polymerase-dependent amplification is carried out in an isothermal condition (at 41°C) within 1.5–2 h. If there are target RNAs, the molecular beacon probe pairs with the complementary target sequence and is emitted a fluorescent signal by the fluorophore.[64,66] A schematic of the activities that take place in the NASBA mechanism is shown in Figure 8.

4.8.1 | Benefits and drawbacks

Forasmuch as 1000 copies of RNA can be synthesized in each transcription step of the qRT-NASBA process, fewer cycles, time and viral LOD (0.2 copies/μl) are required than RT-PCR or RT-LAMP. Also, RNA targets can be amplified 10^5-fold in 15 min and 10^9-fold in 2 h without the need for specialized heating equipment. It can be considered as a detection method of viral mRNA sequences without the risk of DNA contamination. Therefore, isothermal-based qRT-NASBA is 10–100 times more sensitive than RT-PCR, which can be a choice for utilization at POC.[70–72]

4.9 | TMA assay

Transcription-mediated amplification (TMA) is an isothermal, exponential auto-catalytic target amplification method that is almost identical to NASBA.[12,73] This method has been utilized to amplify cellular mRNA and viral RNA using RT and T7 RNA polymerase in a short time.[74,75] Two primers that are designed to perform the current method are a promoter primer with a 5′ flanking sequence for recognition of RNA polymerase and a nonpromoter primer. In the first step, hybridization occurs between the promoter primer and positive-sense target RNA.[74,76] The primer is extended via RT and subsequently, RNase H hydrolyses RNA in the DNA/RNA duplex (Formation of ssDNA).[77] The next step consists of binding a nonpromoter primer to ssDNA and production of dsDNA. This DNA/DNA duplex acts as a DNA intermediate for the rest of the steps called the exponential cycling phase.[74,76] In this phase, a negative-sense RNA amplicon is transcribed from the DNA intermediate that can be

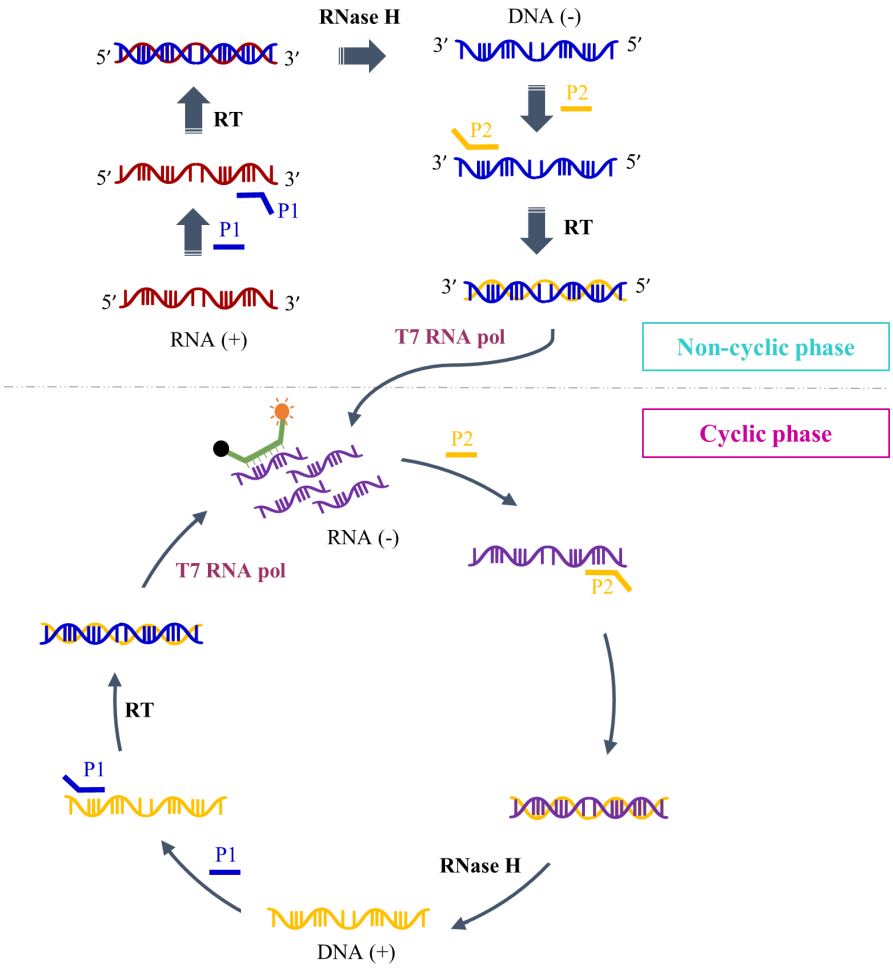

FIGURE 8 Schematic representation of nucleic acid sequence-based amplification containing molecular beacon detection. The two-step process carries out via noncyclic and cyclic phases. cDNA/RNA hybrids are synthesized by specific forward primer (P1, Antisense) and reverse transcriptase (RT). RNase H hydrolyses RNA, leading to the form ssDNA. The dsDNA is generated by reverse primer (P2, sense) and AMV-RT. The dsDNA consists of a coding site for T7 RNA polymerase promoter, transcribing antisense RNA copies through T7-DdRp. The molecular beacon probe pairs to the target sequence and emits fluorescence. P, Primer; RT, Reverse transcriptase.

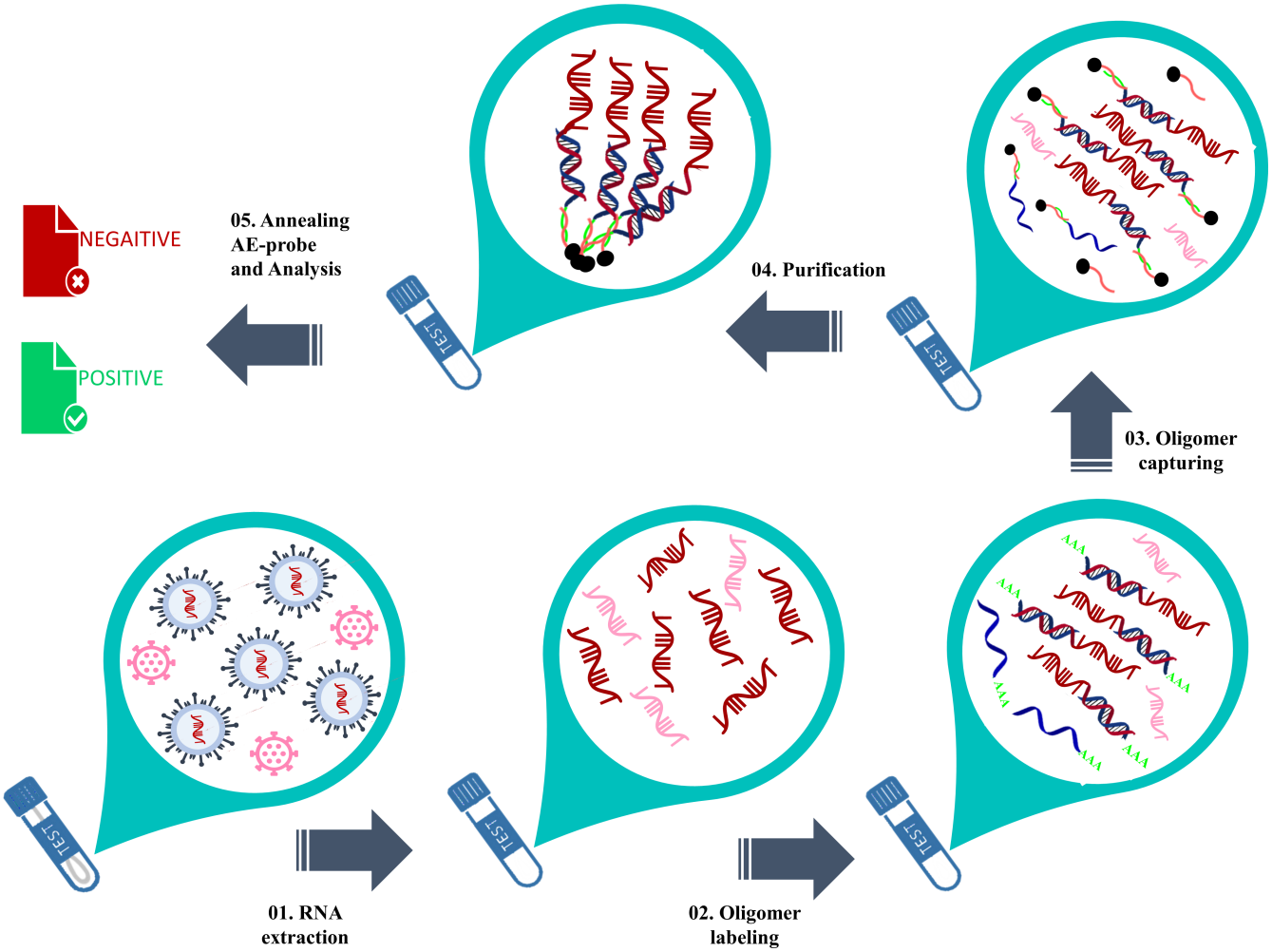

FIGURE 9 Working principle of Aptima severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) assay. Target RNA is purified using poly-dA-labelled capture oligomers. Poly-dA binds to a magnetic bead labelled poly-deoxythymidine (poly-dT). The complex is washed to remove inhibitors. Then, AE-labelled primers anneal to specific regions of SARS-CoV-2 RNA for detection of the produced amplicon. AE, Acridinium ester; RLU, Relative Light Units.

converted into a new dsDNA template.[77] Therefore, hundreds of copies of negative-sense RNA will be produced during the TMA assay. To detect the produced amplicon, a hybridization protection assay (HPA) is done using acridinium ester (AE)-labelled probes. If the probe is completely hybridized with the target area, the AE label will be resistant to hydrolysis, and subsequently, the signal will be produced. Otherwise, the probe is hydrolysed.[74,78]

4.9.1 | Benefits and drawbacks

The current assay is performed at 37–42°C and is no need for expensive thermocyclers.[64] All steps of this method, from amplification to diagnosis, are done in the same tube and with only reagent addition. Hence, the assay allows the detection of pathogens with minimized risks.[74] Results have shown that a TMA assay with a high sensitivity of 95.7% and LOD of 5.5 copies/μl has significant detection power compared to 96.25% on RT-PCR.[79]

4.10 | Aptima SARS-CoV-2 Assay

The Aptima SARS-CoV-2 assay, known as Panther system, is a NAAT-based detection method that is developed by Hologic Panther in May 2020.[12] It got FDA approval for testing COVID-19 patients for EUA only on 14 May 2020.[76] The Aptima assay targets two virus unique sequences located on the *ORF1ab* gene of the SARS-CoV-2 and is conducted on the automated Panther platform. For this reason, the method is also called the panther system.[80] The assay makes use of magnetic bead-based target capture, TMA techniques and AE-labelled probes for the extraction, amplification and two unique sequences within the *ORF1ab* region of SARS-CoV-2 RNA.[76,81]

According to Figure 9, the principles of Aptima assay procedure are as follows: (1) Invitro conditions, target RNA is extracted using poly-deoxyadenosine (poly-dA)-labelled capture oligomers. (2) These oligomers hybridize specific regions of the target RNA through specific complementary sequences to the target RNA. (3) Poly-dA binds to a magnetic bead labelled poly-deoxythymidine (poly-dT) at 25°C. (4) The complex (poly-dT/poly-dA/target RNA) is elicited using magnets. (5) Amplification occurs via annealing oligonucleotide primers to specific regions of SARS-CoV-2 RNA and enzymatic amplification. (6) An AE-labelled probe that binds to a region of the target amplicon is used for the detection of the produced amplicon. The covalently bound AE will produce chemiluminescence. (7) The emitted light is measured, and results are reported based on relative light units (RLU) and the kinetic curve. The assay amplifies two conserved regions of the *ORF1ab* gene in the same reaction. Remarkably, amplification of either or both regions of the *ORF1ab* gene leads to an RLU signal.[82–85]

4.10.1 | Benefits and drawbacks

Aptima assay uses 37–41°C temperature with no requirement of expensive thermocyclers and has been used to detect different viral and bacterial pathogens.[83] This method has a sensitivity of 95.7% and a specificity of 100% for the detection of viral SARS-CoV-2 RNA. Also, Schneider et al. have shown that the Aptima test with a LOD of 0.15 copies/μl has a 95% detection possibility.[86] Aptima represents sensitivity comparable with the best RT-PCR methods

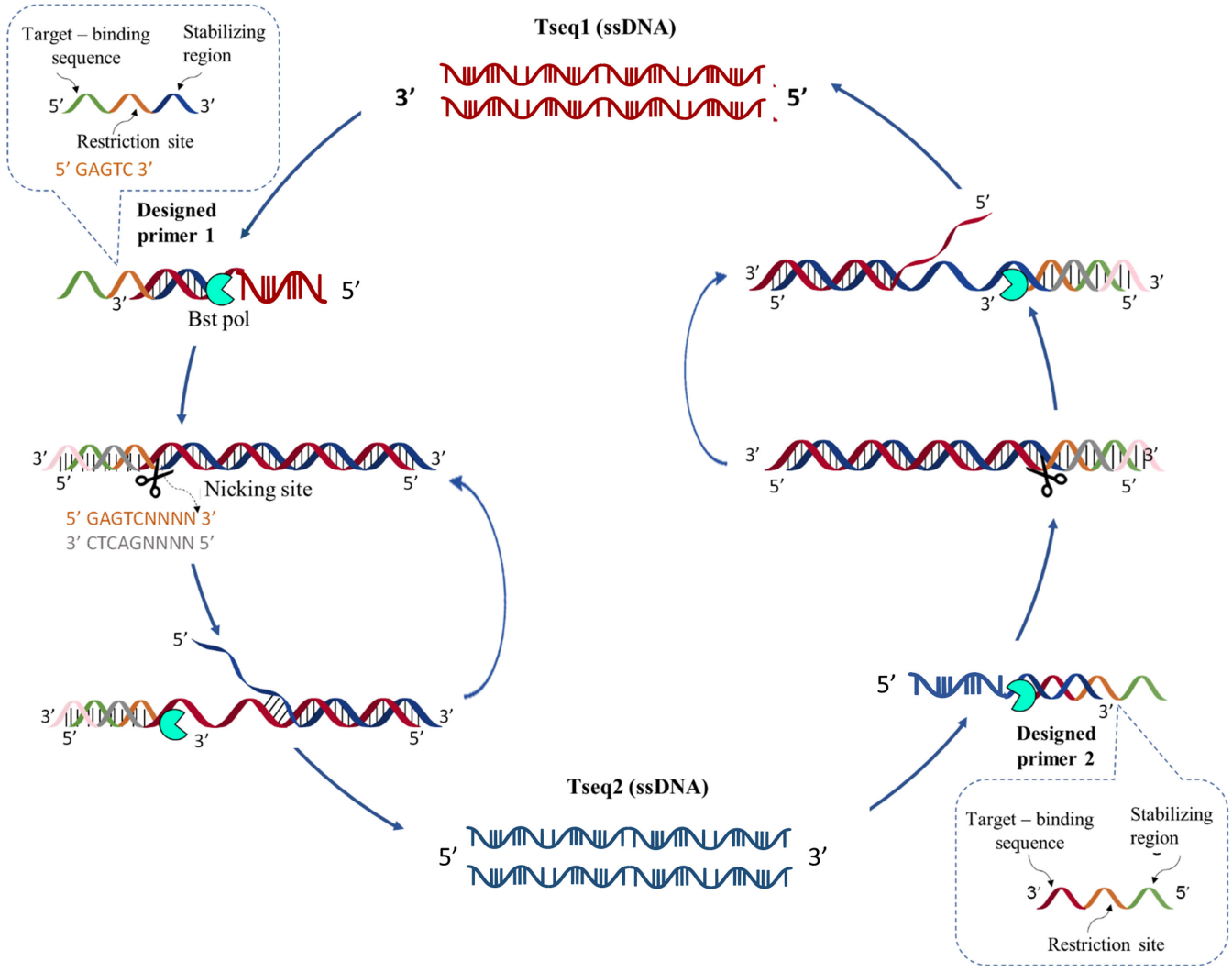

FIGURE 10 Schematic illustration of severe acute respiratory syndrome coronavirus 2 detection-based-ID NOW COVID-19 assay. The conjugated forward primer (P1) is paired to the template sequence. Bst DNA pol produces dsDNA, and the nicking endonuclease cuts the recognition sites in one strand of the dsDNA. Bst DNA pol extends the 3′ end at the nick and regenerates the double-stranded recognition site through strand displacement activity. Conjugated reverse primer (P2) pairs to produced ssDNA (Tseq2) and is elongated by DNA polymerase. Amplicons are cleaved again and so this approach is resumed. Tseq, Target sequence.

used.[76,81] Therefore, the assay with a performance rate of up to 1200 samples per day is considered as a high-throughput, fully automated molecular test.[12] Drawbacks for this assay include the need to train personnel and only use a nasopharyngeal swab for collecting samples. Negative results do not mean a lack of SARS-CoV-2 infection and should not be considered as final decisions. Also, infected individuals without symptoms may not have LOD of the virus and give a false-negative result.[82]

4.11 | NEAR assay

Nicking endonuclease amplification reaction (NEAR), which is also termed nicking enzyme-assisted amplification (NEAA) or nicking enzyme-mediated amplification (NEMA), is an isothermal method based on a nicking endonuclease and a strand-displacing DNA polymerase such as Bst pol.[87,88] The detection of pathogens and viruses (e.g. SARS-COV-2) is developed based on this technology.[73] Primers that are designed contain a stabilizing region, a nicking endonuclease recognition site, and a target-binding sequence, respectively.[88] This method is driven at 65°C by the following steps: (1) After the reverse transcription step, the conjugated forward primer (P1) is hybridized to the ssDNA template sequence (Tseq1). (2) Bst DNA pol extends from the 3′ end of the primer, forming a dsDNA. (3) The nicking endonuclease cleaves the recognition sites in one strand of the dsDNA.[89] (4) Bst DNA pol pairs to the recognition site of primer and extends the 3′ end at the nick, displacing the downstream strand that regenerates the double-stranded recognition site for the nicking enzyme.[89] (5) The separated ssDNA amplicon acts as a second template sequence (Tseq2) for binding of the conjugated reverse primer (P2) and is elongated by DNA polymerase. (6) Produced duplexes are cleaved again in the recognition sites, and extension can be initiated from the restriction site of P2.[87] The recognition site is regenerated with each displacement step. Therefore, target amplification is exponential to NEAR assay.[88] Abbott company used a united diagnostic system based on the NEAR assay called ID NOW COVID-19, which received FDA and EUA approval on 27 March 2020.[90] The assay relies on isothermal NAAT for sample processing that was designed to detect the RdRp region of the SARS-COV-2 genome.[91]

4.11.1 | Benefits and drawbacks

The unique reaction mechanism makes its amplification efficiency much higher than that of many other amplification systems.[92] Thousands of products can be generated from only one restriction site of primer, making NEAR a high-throughput technique. ID NOW COVID-19 with the ability to produce positive results in <5 min and stated LOD of 0.125 copies/μl is a rapid molecular diagnostic test

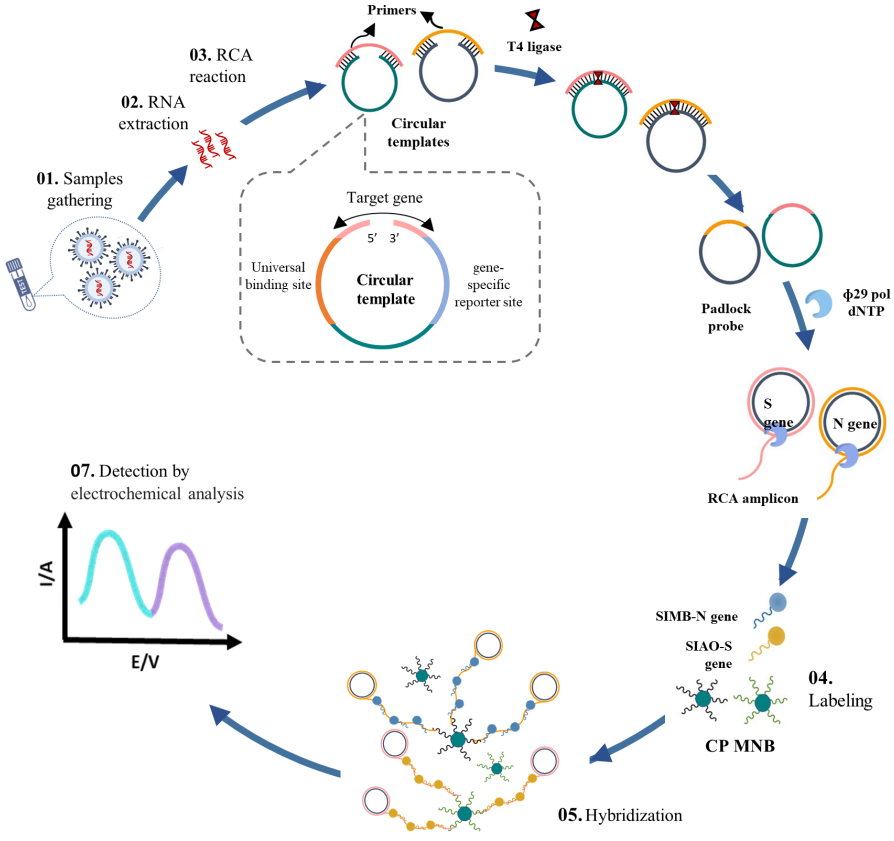

FIGURE 11 Principle workflow of the RCA assay by electrochemical analysis. First, RNA extraction is done from the samples. In the one-step strategy, designed primers hybridize into synthetic circular DNA templates. Then, the T4 ligase forms padlock probes, annealing viral N and S genes. Amplification of circular ssDNA product is performed by ϕ29 polymerase. Finally, CP-MNB, Si-RP (SiMB and SiAO) and the RCA amplicon are mixed in a single step, and CP-MNBs tether to targets, and electrochemical analysis is recorded. CP-MNB, Capture probe-conjugated magnetic bead particle; RCA, rolling circle amplification; SiAO, Silica-acridine orange; SiMB, Signified as Silica-methylene blue.

TABLE 2 Comparing the attributes of different molecular diagnostic assays of SARS-COV-2 and mentioning the methods approved by the EUA.

Method	Condition	EUA-approved commercial test kit	attribute	Company	Sample source
RT-qPCR	Regular PCR	DASH SARS-CoV-2/S Test	Real-time RT-PCR, Multiple Targets	Minute Molecular Diagnostics, Inc.	NP/OP
LF-RPA	Isothermal	NA	–	–	NP
RT-LAMP	Isothermal	MobileDetect Bio BCC19 (MD-Bio BCC19) Test Kit	RT-LAMP, Multiple Targets	MobileDetect Bio Inc.	NP
Penn-RAMP	Isothermal	NA	–	–	NP
DETECTR	Isothermal-coupled method	DETECTR BOOST SARS-CoV-2 Reagent Kit	RT-LAMP, CRISPR, Multiple Targets	Mammoth Biosciences, Inc.	NP/OP
SHERLOCK	Isothermal-coupled method	Sherlock CRISPR SARS-CoV-2 Kit	RT-LAMP, CRISPR, Multiple Targets	Sherlock BioSciences, Inc., USA	NP/OP
AIOD-CRISPR	Isothermal	NA	–	–	T/OP/NP
RT-NASBA	Isothermal	NA	–	–	T
TMA	Isothermal	Procleix SARS-CoV-2 Assay	TMA, Single Target	Grifols Diagnostic Solutions Inc.	NP
Aptima	Isothermal	Aptima SARS-CoV-2/Flu assay	Real-time TMA, chemiluminescent, Multi-analyte, Multiple Targets	Hologic, Inc	NP/OP
ID NOW COVID-19	Isothermal	ID NOW COVID-19 2.0	RT, Isothermal amplification, Single Target	Abbott Diagnostics Scarborough, Inc.	NP
Electrochemical RCA	Isothermal	NA	–	–	NP

Abbreviations: LOD, Limit of detection; NP, Nasopharyngeal swab; OP, Oropharyngeal swab; T, Throat swab.

designed for POC testing.[90,91] Also, the study has shown the ID NOW COVID-19 with a sensitivity of 87.7% is considered as an appropriate diagnostic system by florescent.[91] An influential drawback of NEAR is the generation of nonspecific products, which can restrict the sensitivity and specificity of the method and subsequently increase the risk rate of detection. So, the best reaction condition should have been taken for the improvement of this drawback.[22,88] Figure 10 shows the mechanism of the ID NOW COVID-19 assay.

5 | Electrochemical RCA assay

Rolling circle amplification (RCA) is another isothermal enzymatic NAAT-based method to produce long nucleic acid from shorter nucleic acid and could be considered as advanced biotechnology for the diagnosis of infectious human diseases such as viruses and bacteria.[93] Extended RCA-based systems are capable of detecting a variety of targets, including RNA, DNA, protein and even microRNAs.[93,94] The assay consists of ligation and amplification steps that are described as follows: (1) designed primers hybridize with synthetic circular DNA templates.[95] (2) Ligation and formation of padlock probes occurred using T4 ligase. (3) Padlock probes can anneal to complementary sequences of viral *N* and *S* genes. (4) ϕ29 polymerase with strand displacement activity amplifies continuously circular ssDNA product.[52] The RCA amplicon is a concatemer with multiple repetitive units, which correspond to the circular DNA template. The current assay can produce a 10^9-fold amplicon per circle with LOD of 1 copy/μl of viral *N* or *S* genes within 1.5 h.[96,97] This assay was discovered in the late twentieth century, and in 2003, an RCA-based SARS-COV detection was reported for the first time.[93,96] However, this detection was based on gel electrophoresis for analysis of numerous samples is hard and time-consuming.[98] Therefore, this approach is not suitable and efficient for the detection of SARS-CoV-2. To rapidly detect the RCA amplicons, Chaibun et al. used

Target	Operating temperature (°C)	Template	Detection time (min)	LOD (copies/μl)	EUA number	Sensitivity (%)	Specificity (%)	Literature
ORF1ab, N	65–97	DNA	2–4 h	5.5	EUA210603	96.25	NA	24
RdRP	~42	DNA	15	1	NA	97	100	27,39
ORF1ab, N	60–65	DNA	30	4.8	EUA201215	97.5	99.7	44
ORF1ab	RPA: 38 LAMP: 63	DNA	RPA: 15–20 LAMP: 40	7	NA	100	NA	49
N	Amplification:62 CRISPR/Cas12 (detection):37	DNA	~30	10	EUA210625	90	100	65
ORF1ab/N	37	DNA	<60	10	EUA200466	95	100	65
N	41	DNA	40	1.2	NA	100	NA	60,63
N	41	RNA	90–120	0.2	NA	98	100	70,72
ORF1ab	37–42	RNA	240	5.5	EUA201734	95.7	NA	79
ORF1ab	37–41	DNA	~270	0.15	EUA202959	95.7	100	83,86
RdRp	56	DNA	<5	0.125	EUA210517	87.7	99.6	90,91
N	37	DNA	<120	1	NA	99	NA	96

an electrochemical biosensor based on differential pulse voltammetry (DPV) techniques for the *N* and *S* genes of SARS-CoV-2. The assay relies on one-step hybridization of amplicons with redox-active labelled probes.[96] In this approach, the circular templates consist of three parts containing the target gene, a universal capture probe and gene-specific reporter probe binding regions. Moreover, two redox dyes, methylene blue (MB) and acridine orange (AO), are coated onto silica nanoparticles (SiNPs), which are signified as Silica-methylene blue (SiMB) for *N* gene and Silica-acridine orange (SiAO) for *S* gene, respectively. Then, the coated nanoparticles are labelled with specific reporter probes, which are complementary to gene-specific reporter probe binding regions on circular templates. Labelled probes are denoted as the SiMB-RP-*N* gene and SiAO-RP-*S* gene that can target the capture probe-conjugated magnetic bead particle (CP-MNB). In the one-step strategy, CP-MNB, Si-RP (SiMB and SiAO) and the RCA amplicon are mixed in a single step, followed by a single washing step. Finally, CP-MNBs tether to a cDNA or RNA target, which is conjugated to labelled probes, and electrochemical analysis is recorded by using a portable potentiostat device connected to a laptop.[96] A schematic of the target RNA amplification mechanism via RCA assay is shown in Figure 11.

5.1 | Benefits and drawbacks

A notable benefit of the electrochemical RCA is that it can be done rapidly by utilizing a water bath or heating block. Also, the assay prevents the production of false-positive results, which can decrease accuracy and sensitivity.[97] In one-step hybridization strategies, there were found no significant differences compared with step-wise sandwich hybridization. Hence, the one-step strategy was used in the study due to its high speed, reduction in contamination and pipetting steps and also convenience.[96] Moreover, this approach has become a worthy method owing to its cost-effective, ultrasensitive and adaptability with a liquid sample and high surface

area for hybridization. Due to the low complexity of the RCA assay compared with other isothermal-based methods, it can be easily used by nonskilled users.[99] Therefore, this method is valuable for the POC system.

6 | CONCLUSION AND FUTURE PERSPECTIVE

Although COVID-19 vaccination is underway, our combat against this deadly virus is by no means over and one of the most powerful approaches for fighting against SARS-CoV-2 is to develop several simple-to-use tests for POC detection. The current molecular diagnostic tests provide variable levels of accuracy, sensitivity and specificity for the detection of SARS-CoV-2. Hence, we discussed different molecular diagnostic methods of SARS-CoV-2. RT-qPCR is the conventional molecular diagnostic assay for COVID-19 in many countries, so that results can be taken in a few hours with satisfying sensitivity and specificity. However, there are some limitations of RT-qPCR in meeting the current needs, including the demand for expensive thermocycling equipment and expert personnel to conduct the assay and interpretation of results. Some of these limitations can be dominated by isothermal-based alternative diagnostic assays, and the characteristics of these diagnostic techniques are summarized in Table 2.

Since isothermal amplification techniques only require a simple heating block, they are considered as rapid, effective-cost and easy-to-use SARS-CoV-2 detection methods, which can be useful for outbreak areas and areas with poor laboratory equipment and be marketed in the form of commercial test kits. Nevertheless, the principal problem correlated with molecular and serological tests for the SARS-CoV-2 detection is the analytical action, leading to the creation of false-negative and false-positive results. On the contrary, although isothermal-based methods are a more affordable and more comfortable alternative to RT-PCR, optimization of parameters such as primers and reaction conditions remains an issue. Hence, these challenges open a gaping hole in the accurate detection of disease. To moderate these problems, the simultaneous use of a couple of different techniques like colorimetric Penn-RAMP assay has been regarded as a valuable alternative to be performed at the POC. Since going to the clinical setting for testing can raise the outbreak risk of COVID-19 and subsequently the burden on the health care system, providing home-based advanced diagnostic kits plays a vital role in treating viral infection.

Paper-based RNA diagnostic tools, such as LF-RPA and LF- SHERLOCK, have the potential to link with a smartphone application, opening a new perspective for developing POC COVID-19 detection. In this way, the infectious specimen is gathered by people suspected of COVID-19. Next, by adding the nasal swab to specific reagents of diagnostic kits, the colorimetric result can be perceived on paper. By recording the colorimetric shift through a smartphone, the result could be sent to clinicians via the internet for analysis. Since all processes perform at home, the burden, strain and cost of care are reduced for both individuals and the government. Even using paper-based RNA diagnostic tools in public places such as airports and conferences can be very practical and important. Therefore, according to the efficiency of these tools in different situations, it can be very valuable for governments to make these kits available. Currently, developed countries have turned to these kinds of diagnostic tools and are improving the specificity and sensitivity of these kits and the diminution of viral LOD.

We expect that RNA-based diagnostic systems of POC like LAMP and CRISPR will be used worldwide, in near future. This review has tried to provide a new perspective on the functional efficiency of POC diagnostic systems for COVID-19 by comparing molecular approaches and recounting their advantages and disadvantages. However, more studies for the comparison between newly developed methods in respect of accuracy, sensitivity, specificity and reliability are still needed.

AUTHOR CONTRIBUTIONS

Zohreh Hojati conceived the idea. Bahareh Maleki and Zohreh Hojati performed all the literature reviews. Bahareh Maleki wrote the manuscript and designed the figures. Zohreh Hojati edited the manuscript and supervised the study. All authors read and approved the final manuscript.

ACKNOWLEDGEMENTS

This work was supported by a joint project by the University of Isfahan (Research Chancellor; Project NO.:348383/3) and the National Institute for Genetic Engineering & Biotechnology (NIGEB) (Project NO.: 99/1443).

FUNDING INFORMATION

This research was financially supported by the University of Isfahan (Research Chancellor; Project NO.:348383/3) and the National Institute for Genetic Engineering & Biotechnology (NIGEB) (Project NO.: 99/1443).

CONFLICT OF INTEREST

The authors have no relevant financial or nonfinancial interests to disclose.

CONSENT TO PARTICIPATE

Not applicable.

CONSENT TO PUBLISH

Not applicable.

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How to cite this article: Maleki B, Hojati Z. A precise review on NAATs-based diagnostic assays for COVID-19: A motion in fast POC molecular tests. *Eur J Clin Invest*. 2022;00:e13853. doi: 10.1111/eci.13853